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IMPLEMENTATION OF ISO/IEC 17025 PRACTICES
IN SMALL AND ACADEMIC LABORATORIES

by

Eric Layne Oliver

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
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Major: Food Science and Technology

Under the Supervision of Professor Jayne E. Stratton

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IMPLEMENTATION OF ISO/IEC 17025 PRACTICES IN SMALL AND ACADEMIC LABORATORIES

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University of Nebraska, 2018

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Food Manufacturers are facing challenging times due to regulations such as the Food Safety Modernization Act (FSMA) requiring them to provide evidence they are producing safe foods. Food testing laboratories aid in the mitigation of food safety issues providing evidence that a manufacturers food safety system is acceptable. To perform these activities laboratories are required to adhere to certain standards such as ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories. However, implementation of ISO/IEC 17025 practices is challenging, especially for small and academic laboratories, due to lack of available guidance. A long-term goal of the University of Nebraska Food Processing Center Laboratory Services (UNL-FPCLS) has been to prepare for ISO/IEC 17025 accreditation and provide accredited testing services to the food industry. This project included implementation of a quality management system including organizational structure, policies, support programs, and standard operating procedures. Over 63 SOPs, 103 forms, 19 manuals and lists, and 6 support programs were developed and implemented in this project. Media qualification verification procedures were developed for non-selective solid (Tryptic Soy

Agar), non-selective liquid (Tryptic Soy Broth, Buffered Peptone Water), and selective liquid (Neogen Reveal[®] 20 Hour *E. coli* O157:H7, Romer RapidChek[®] *Listeria*) media to evaluate growth and quality parameters over the shelf life of the media. These procedures serve as a guide for implementing a media control program. Shelf life at room temperature and 2-8°C was determined for TSA (7 and 60 days), TSB/BPW (2 and 13 weeks), RapidChek[®] *Listeria* (3 and 12 hours), and Reveal[®] 20-Hour (6 hours both), respectively. Method verification of qualitative in-scope methods Neogen Reveal[®] 20-Hour for detection of *E. coli* O157:H7, Romer RapidChek[®] for detection of *Listeria* spp., and BioMérieux VIDAS[®] UP *Salmonella* SPT for detection of *Salmonella* spp. was also performed. All methods gave results of 100% for sensitivity. This project provides academic and small laboratories with methods and procedures that may be used as guides for implementing quality management systems and verifying methods to become ISO compliant and pursue ISO/IEC 17025 accreditation. Finally, the FPCLS completed all ISO compliance requirements and is positioned to pursue ISO/IEC 17025 accreditation.

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Dedication

This dissertation is dedicated to my wife, Madelynn H Oliver,
for always being there for me even when times were tough
and helping me stay focused in reaching the finish line.
I am eternally grateful for your understanding, friendship, and love.

I love you!

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It is my honor to be able to acknowledge all of the individuals who guided and assisted me during my journey in completing my PhD. This dissertation would not have been possible without their encouragement and involvement.

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INTRODUCTION

Foodborne pathogens are one of the main causes of disease for consumers both in the United States of America (USA) and abroad accounting for over 15.6 Billion dollars in medical and other expenses, over 53,000 cases of illness, and approximately 2,300 deaths each year in the USA alone (Flynn 2014). These statistics highlight the public health impact that foodborne pathogens have on society and the need to continuously improve the safety of the food supply. Furthermore, food is essential to survival and foodborne pathogens such as *Escherichia coli* (*E. coli*) O157:H7, *Salmonella* spp., and *Listeria monocytogenes* (*L. monocytogenes*), can be present without us even knowing that they are there as they are too small to see with the naked eye, approximately 0.3-5 μm in length, and do not necessarily affect the physical attributes (color, tastes, etc.) of the food products being consumed (Holt and others 2000). As human beings we inherently value the preservation of life and the well-being of family members, friends, neighbors, and colleagues.

Because of the value placed on life, and the fact that the presence of foodborne pathogens in the foods we consume can cause so much harm, emphasizes the importance to not only study foodborne pathogens and figure out ways to eradicate them from the food supply, but also to determine how to detect foodborne pathogens in food products before they ever reach commerce. Conducting product analysis with accredited laboratories will help to mitigate the potential for contaminated food products from ever reaching consumers at restaurants, on grocery store shelves, and in their homes thus minimizing the impact that foodborne pathogens have on the health of human beings.

The following will be discussed in this dissertation:

1. Guidelines/standards that accredited testing laboratories must follow to evaluate food products for foodborne pathogenic microorganisms
2. Discuss what those laboratories must do in order to meet the guidelines on a continuous basis and why having guidelines and standards are important
3. Evaluate a method for qualifying the effectiveness of microbiological media that has been prepared in-house
4. Define procedures for verifying in-scope qualitative methods for laboratory accreditation

This dissertation will begin with a literature review (Chapter 1) of the International Organization for Standardization (ISO). The review will focus on who ISO is, why they were developed, and why accreditation bodies such as ISO are used for accrediting food testing laboratories. The types of guidelines/standards they produce to help aid many different industries, including the food industry, in producing better higher quality results will also be highlighted. An overview of the major ISO guidance documents that affect the food industry will be explored including looking at ISO documents/standards ISO 22000, ISO 9001, and ISO/IEC 17025.

In addition to the overview of ISO/IEC 17025, this chapter will go deeper into the requirements necessary to achieve ISO/IEC 17025 accreditation as these standards and guidelines govern food testing facilities. The steps necessary to achieve accreditation, the development of a functioning quality management system, and the various programs, procedures, documentation, and verification activities that must be put into place to become ISO/IEC 17025 compliant will be examined to gain a better understanding of how a laboratory becomes ISO accredited.

As an addition to this section we will look at the differences between ISO/IEC 17025:2005 and ISO/IEC 17025:2017 as the standards have been updated. Finally, we will take a look at some recalls that have occurred due to foodborne pathogens being present in the food supply chain and will discuss the challenges facing laboratories in meeting ISO requirements and providing evidence they are capable of performing ISO standardized procedures to help prevent these issues from occurring.

After the literature review, the ISO implementation and research activities that were performed for this dissertation will be discussed in Chapter 2. First, we will take a look at the implementation of ISO guidelines and practices in the Food Processing Center Laboratory Services (FPCLS) food testing laboratory at the University of Nebraska – Lincoln (UNL) for the purpose of obtaining ISO accreditation. A brief overview of the FPCLS including who they are and what they do will proceed the implementation information. Gaps in industry and the reasoning for the UNL FPCLS to obtain ISO accreditation including the benefits for both UNL and for the food industry will be discussed.

ISO implementation activities included: the development of a quality management system unique to the UNL FPCLS; development of all necessary documentation including analysis methods, SOPs, and forms for guiding, directing and capturing all of the activities within the laboratory; and implementation of support programs such as environmental monitoring, training, and temperature monitoring programs to provide the laboratory with control over its activities. The implementation of these programs, documents, and procedures, will provide the structure that the UNL

FPCLS needs to meet all of the requirements in the ISO/IEC 17025 standard and to pursue ISO accreditation from an accreditation body.

Following the chapter on the implementation of ISO practices and guidelines in the FPCLS, we will look at a proposed method for determining the effectiveness and quality of microbiological media from commercial sources in Chapter 3. A method was developed to evaluate the acceptability of several different types of media for use within the FPCLS food testing laboratory including, non-selective solid, selective solid, non-selective liquid, and selective liquid media types. These analyses will tell us if the media we have selected for evaluating various food samples for foodborne pathogens is of acceptable quality for use in testing, suitable for its intended purpose, and how long the media is good for while still producing an acceptable result in order to meet ISO/IEC 17025 guidelines for in-house preparation of microbiological media.

As part of this analysis the quality (color, pH, contamination, etc.) as well as the growth acceptability of each media type was evaluated. This portion of the dissertation is important because it is required under ISO/IEC 17025 guidelines that the microbiological media that is used for sample analysis purposes be acceptable in order to demonstrate that results obtained by the laboratory are consistent, accurate, and reliable (AOAC International 2015). In order for a laboratory to obtain ISO/IEC 17025 accreditation they must provide evidence that their microbiological media is prepared correctly and that it produces a consistent result to demonstrate that results obtained from test procedures can be trusted.

The final research chapter of this dissertation, Chapter 4, contains verification testing performed on in-scope laboratory methods (AOAC approved methods) for the

detection of foodborne pathogens in food samples. Three methods of foodborne pathogen detection were selected for verification including; Neogen Reveal[®] 20 H test kit for detecting *E. coli* O157:H7; BioMérieux VIDAS[®] UP *Salmonella* SPT test kit for detecting *Salmonella* spp.; and Romer Labs RapidChek[®] *Listeria* test kit for detecting *Listeria* spp. Food samples of various types were inoculated with foodborne pathogens, both positive and negative control organisms, and tested against the kits to determine the sensitivity of each kit and the ability of the FPCLS to perform the kits according to manufacturer's guidelines.

Verification of in-scope methods will provide us with evidence that the FPCLS is capable of performing standardized methods as well as demonstrate that the methods are fit for the purpose that the FPCLS intends to use them for. Additionally, the study results will provide information on the effectiveness and of the kits in detecting foodborne pathogens in various food matrices while providing the sensitivity of each kit in obtaining the expected result. This final part of the dissertation is important as it is required under ISO/IEC 17025 requirements that all methods utilized by the laboratory are verified to be fit for their intended purpose (AOAC International 2015) and that the laboratory is capable of obtaining an expected result under controlled conditions.

In conclusion, foodborne pathogen contamination and disease are always going to be a major concern for all individuals in the food industry and for consumers. However, the implementation of ISO practices and the accreditation of food testing laboratories by ISO/IEC 17025 standards will help provide the food industry with trusted test results ensuring that the foods they are producing are safe for commerce. We must continue to

advance and to grow in order to meet the demands of the consumer and to facilitate the safety of food products that are available for everyone to eat.

Therefore, having more ISO/IEC 17025 accredited laboratories to serve the food industry will aid in the mitigation of food safety issues and provide standardized food product analyses which in-turn will help minimize the risk of foodborne illness from contaminated products. The activities in this dissertation may also serve as a guide/tool to other establishments, specifically small and academic laboratories, in helping them to achieve ISO/IEC 17025 accreditation or simply as a blueprint for improving their laboratory operations.

CHAPTER 1

LITERATURE REVIEW

**“An Introduction to
The International Organization for Standardization”**

INTRODUCTION TO THE INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

Producers and manufactures in the food industry are facing a challenging road due to consumer pressure and ever-changing regulations requiring them to provide evidence that they are producing safe foods for commerce. Since the implementation of the Food Safety Modernization Act (FSMA) in 2011 (FDA 2011), food producers and manufacturers have come under even more pressure to provide safer food to consumers while providing United States (US) governmental agencies such as the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) and the Food and Drug Administration (FDA) with proof that their food products are safe for consumption (USDA 2015; USDA 2018a; FDA 2018b).

FSMA regulations indicate that food manufacturers must implement measures within their processes that are capable of “*significantly minimizing or preventing the occurrence of identified hazards...through the use of...product testing programs...*” (FDA 2011). Although there are other ways in which product safety can be determined, product analysis is the most efficient way in identifying microbiological hazards and provides the strongest evidence that the food is safe for consumption.

Food producers and manufacturers must submit samples to an accredited laboratory to verify that their products are safe for commerce (FDA 2011). These testing facilities must meet certain criteria in order to be accredited and for the results that they generate to be trusted by the food industry and by governmental agencies that regulate the food industry in the United States of America (USDA 2015; FDA 2018b). The most widely accepted and recognized set of guidelines for verifying the acceptability of testing

and calibration laboratories comes from the International Organization for Standardization and International Electrotechnical Commission (ISO/IEC) standard ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories (ISO and IEC 2005; ISO and IEC 2017; ISO 2018c). Although other bodies develop standards for various processes such as the European Committee for Standardization, ISO/IEC standards/guidelines are recognized and accepted worldwide (Romero et al. 2007; ISO 2018c) making them one of the primary set of guidelines utilized by accreditation bodies when accrediting a laboratory for food product analysis.

Since food testing establishments are required to meet ISO/IEC or other standardized guidelines for competence before they are allowed to test food products for their release into commerce, it is important to understand who is generating those standards, who is using them, the different types of laboratories that are ISO accredited, and which standards affect the food industry. Furthermore, it is also important to understand in detail the main ISO standard that dictates the requirements for food testing facilities and evaluate what those requirements are, how to implement ISO guidelines/standards within a laboratory regardless of size, and why it is important to follow these standards in the food industry to facilitate the safety of food that is being consumed in the US and abroad.

BACKGROUND OF ISO

Who is ISO and What is Their Purpose

ISO, or the International Organization for Standardization, is a non-government affiliated independent non-profit organization that has “member bodies” (member bodies

are standards developing organizations) in 163 countries (one per country) around the world including the US, Canada, Brazil, Mexico, Argentina, China, Russia, England, Australia, and South Africa to name a few (ISO 2016; ISO 2017; ISO 2018a). ISO provides a platform for developing international standards, with the help of member bodies, to ensure that procedures and processes are performed the same regardless of geographical location. ISO headquarters are located in Geneva, Switzerland and are coordinated by a Central Secretariat that oversees all of its operations and coordinates the development and publication of new standards (ISO 1997; ISO 2016; ISO 2017). Today ISO is recognized as a leader in international collaboration and the development of standardized methods worldwide (ISO 2017; ISO 2018a).

Forming the Organization Known as ISO

The International Organization for Standardization that we recognize today did not begin as ISO. In 1926 the International Federation of the National Standardizing Associations (ISA) was formed to begin working on issues related to standardizing processes and equipment in mechanical engineering related to issues such as screw threads, rolling bearings, shafts, and pipe sizes (ISO 1997; Martincic 1997). In 1942, the ISA was disbanded due to safety and other issues related to WWII in Europe (ISO 1997; Martincic 1997).

After WWII ended in 1945, it was determined that work could safely resume on coordinating international standards. In October 1946 the ISA and the United Nations Standards Coordinating Committee (UNSCC) met at the Institute of Civil Engineers in London, England to reorganize and form the International Organization for

Standardization effectively ending the existence of the ISA and UNSCC (ISO 1997; Martincic 1997; ISO 2018a). The purpose of this meeting was to develop a new internationally recognizable organization that was supported by all major countries and that could “*facilitate the international coordination and unification of industrial standards*” (ISO 2018a). Based on the agreements made at the 1946 meeting, ISO was formed and was recognized as an official international organization on the 23 February 1947 (ISO 1997; Martincic 1997; ISO 2018a). Since then, ISO has continued to grow and adapt into the internationally recognized standardization body that many industries rely on and utilize today.

Abbreviation not Acronym – Naming ISO

ISO is not an acronym for the International Organization for Standardization but is instead an abbreviated name that was adopted by the organization to reduce confusion (ISO 2018a; Wikipedia 2018). In fact, it was found during the developing of the International Organization for Standardization that the name for the organization was different in the three main languages associated with ISO; English, French, and Russian. In French ISO is referred to as the “*Organisation Internationale de Normalisation*” with the abbreviation OIN, and in Russian ISO is referred to as “*Mezhdunarodnaya Organizatsiya po Standartizatsii*” or MOS (ISO 2018a; Wikipedia 2018).

Therefore, the International Organization for Standardization adopted “ISO” to be their recognized and official abbreviated name in 1946 when they convened in London to form ISO from the ISA. The abbreviation was chosen due to its roots in the Greek language coming from the word “isos” which is translated as “equal” (Martincic 1997;

ISO 1997; ISO 2018a; Wikipedia 2018) so that the abbreviated name for the organization would be the same regardless of the language being used or the country the standard was developed in (ISO 2016; ISO 2018a; Wikipedia 2018). ISO stated that, “*Whatever the country, whatever the language, we are always ISO*” (ISO 2018a).

Accreditation Bodies – Necessities for Quality, Equality, and Improvement

Organizations, such as ISO, are necessary because they produce standards that help guide and direct institutions in performing high quality consistent work worldwide. In food testing facilities and other industries, using standards creates an environment where the institution can demonstrate that the analysis or process they performed was reliable, of the highest quality, and that the results they produce can be trusted (ISO 1997; Martincic 1997). ISO standards help provide necessary guidelines that can be utilized as a reference or resource for companies to continue to improve and meet the ever-growing demands of their cliental.

ISO also helps to facilitate collaboration between all member countries on which rules/guidelines will be put into place that affect how companies do business (ISO 2016). Having an organization like ISO ensures that regardless of whether you test a sample, run a process, or produce a product in the United States, Europe, or Asia that you can be confident that it was performed to a specific level of acceptability and the result or product can be trusted (ISO 1997; ISO 2018c). Martincic et. al. stated that having standards produced by organizations such as ISO helps to “*provide clear identifiable references... and encourage fair competition in free market economies*” (Martincic 1997). Since ISO is a global network of member bodies and works with over 700

organizations and 100,000 field experts worldwide (ISO 2016), it can be assured that the standards and guidelines produced are truly a world effort and reflect the progress and desires of the majority of the world to improve and advance in their respective fields.

ISO STANDARDS AND GUIDELINES

What are ISO Standards

ISO standards are documents that help provide guidelines and specifications to ensure that no matter where you are in the world a specific test, process, material, or procedure is performed/produced in the same way and will give approximately the same result (within the accepted range) while also being fit for its intended purpose (ISO 2017; ISO 2018c). ISO defined an international standard as “*a document containing practical information and best practice...an agreed upon way of doing something or a solution to a global problem*” (ISO 2016). In testing laboratories, internationally recognized standards provide the backbone for controlling all aspects of the laboratory from management to analysis procedures.

Importance of ISO Standards

Standards are important internationally do their effect on trade markets. Having standards or guidelines that all markets must follow helps to facilitate or at minimum “*encourage fair competition in free-market economies*” (Martincic 1997). Without these standards there would be no guidelines or rules that would direct markets around the world to produce items under the same conditions or meet the same level of quality for a

specific item. In food analyses, food samples tested in one country might pass testing requirements but fail in another leading to a biased or unfair market.

Viewing standards as an “agreement” helps to clarify why having standards is so important when performing food sample analysis and other procedures. Without standards, there would be no way to demonstrate that the results obtained from a food testing laboratory were accurate which could result in adulterated food products being released into commerce and ultimately causing a foodborne illness outbreak within the United States or even worldwide.

Benefits of ISO Standards

Having standards that dictate and provide guidance on how a process should be set up, how a management system should be defined, or how testing procedures should be verified and conducted, provides many benefits to production, testing, and other processes in all industries. International standards provide the consumer with confidence that the certified products they are purchasing were made in a controlled environment and meet minimum standards set internationally to demonstrate the product is “*safe, reliable, and of good quality*” (ISO 2017; ISO 2018c; Wikipedia 2018).

These standards also help establishments minimize the amount of errors that they have as well as reduce the amount of waste they see through standardizing their processes and procedures (Wikipedia 2018) meanwhile governing their management structure to demonstrate documentation and other aspects of business are also handled in the same manner. ISO standards help to control data and increase the interoperability and compatibility (Martincic 1997) of data internationally allowing for better communication

and data sharing. ISO stated that “*All players in the food supply chain, be they farmers, manufacturers or retailers, can benefit from the guidelines and best practice contained in ISO standards*” (ISO 2017).

Other benefits that may be realized from following ISO standards include; becoming more competitive by offering products/services accepted internationally, reduced costs through managing available resources better, and increased revenue leading to self-sustainable business (ISO 2014; ISO 2016; ISO 2018c). Society benefits from establishments using standards through receiving safer, reliable services and products, and by organizations addressing global challenges that affect society such as sustainability and climate change (ISO 2016).

For food testing and other laboratories, implementing standards improves the quality of work being performed, results being obtained, training and competency of staff, and increases the reliability and trust in the data being generated by those laboratories (ILAC 2001; Halevy 2003; Rodima et al. 2005; ISO 2018c). Additionally, implementing standardized practices and guidelines would provide necessary structure to food testing and other laboratories potentially leading to their continued growth and improvement (Kohl 1998; Honsa and McIntyre 2003).

Regulators also benefit from the use of standards as they see an increase in the similarities from country to country which boosts trade, stabilizes the supply chain, and makes it easier for establishments worldwide to outsource their processes and services (ISO 2014; ISO 2016; ISO 2017). Governmental agencies rely on ISO standards as they utilize them to help develop better regulations since they are formed from global experts and are accepted as sound methods (ISO 2017; ISO 2018c). Finally, implementing ISO

standards provides establishments with international recognition (ILAC 2001) indicating that they are a reliable trustworthy option further strengthening relationships within their respective industries and with governmental agencies.

Types of ISO Standards

Now that the importance and the benefits of utilizing ISO standards has been established, let's take a look at what types of standards are available and what types of institutions are certified to use them. ISO standards cover a wide variety of process across various industries such as medical device, energy management, risk management, and testing laboratories (ISO 2018a). However, there are a few exceptions that should be noted prior to discussing the industries ISO covers in detail.

One of these exceptions is the electrical and electronic engineering standards which are developed and controlled by the International Electrotechnical Commission (IEC) (ISO 1997; Martincic 1997) which works closely with ISO and in some cases releases joint standards with them. Other types of standards not covered by ISO are the telecommunication standards which are developed and distributed by the International Telegraph Union (ITU), and the information technology (IT) standards which are technically covered by the JTC1 although they are a committee comprised of both ISO and IEC (ISO 1997; Martincic 1997; ISO 2018b) and release standards with both identifiers as will be seen later on.

Standards that are covered by ISO, and in many cases ISO/IEC, can be found for almost all other industries. ISO has released over 22000 recognized standards (ISO 2017; ISO 2018b) that are available and are being used in almost every country worldwide.

Some examples of these ISO standards and the areas they are commissioned for can be seen in Table 1.1.

Examples of these standards include ISO 13485 which helps to evaluate the quality of medical devices throughout their life cycle, ISO 4217 Currency Codes helping institutions avoid confusion and mistakes when working with different world currencies by standardizing the currency nomenclature, and ISO 45001 Occupational Health and Safety which is aimed at workplace safety and helping establishments create a safer work environment for their employees (ISO 2018b). There is even an ISO standard that is designed to help organizations deal with bribery, ISO 37001 Anti-Bribery Management Systems, which describes how to detect and address issues with bribery as they arise (ISO 2018b). ISO standards are fundamental tools in helping all industries advance, grow, and improve so that they can produce the best services, processes, and products possible.

Accreditation Bodies

Behind all of the different standards that are available are the different industries that utilize them. There are establishments both large and small, private and public, and universities/academic institutions that have achieved ISO/IEC accreditation for various processes and procedures. Accrediting institutions such as the International Laboratory Accreditation Cooperation (ILAC) certifies accreditation bodies such as the American Association for Laboratory Accreditation (A2LA), American National Standards Institute - American Society for Quality National Accreditation Board (ANAB), Perry Johnson

Laboratory Accreditation, Inc. (PJLA), and the National Voluntary Laboratory Accreditation Program (NVLAP) to name a few (ANSI 2018).

These accreditation bodies audit and accredit businesses and academic institutions under ISO guidelines and standards. The American National Standards Institute (ANSI) stated that “*accreditation is the process of evaluating the competence of a conformity assessment body*” (ANSI 2018) indicating that all ISO accredited laboratories and business establishments have demonstrated that the services they provide, management systems they have within their establishments, or products that they produce meet specified requirements.

Types of ISO Accredited Laboratories

ISO accreditation is important to many industries especially those that utilize testing services. ISO/IEC 17025 was developed as the international standard for all testing and calibration laboratories and is utilized as the primary standard for ISO compliance in the food testing industry. There are several private and governmental laboratories such as Eurofins, Medallion, IEH, Silliker (Merieux NutriSciences), Vanguard and several Department of Agriculture laboratories (A2LA 2018; IEH 2018; PJLA 2018) that have been given ISO accreditation in the United States. These laboratories provide testing services to the food industry, helping to demonstrate that the food products released into commerce are safe for consumption.

When reviewing the lists of accredited institutions, it is found that there are very few academic laboratories due to the immense challenge of becoming ISO/IEC accredited and issues with maintaining such a laboratory. In fact, when reviewing one of

the major accreditation bodies lists of ISO/IEC 17025 accredited facilities it was found that only one university facility was on that list in the United States (A2LA 2018).

However, ISO accredited facilities can be found throughout the world in the private and public sectors as well as at academic institutions for all types of industries including the food testing industry for the various ISO standards. One university laboratory achieved ISO accreditation for several methods within their Environmental Radiology Laboratory for gamma emitters in milk, honey, vegetables, and meat products (Zapata-García et al. 2007), while another was granted accreditation for methods within their Nuclear Analytical Laboratory for alpha/beta emitting nucleotides and other methods (Chung et al. 2006).

A method for geosmin and 2-methyl-i-borneol analysis by closed loop stripping and gas chromatography was validated for ISO/IEC accreditation by a private institution in Spain (Romero et al. 2007). Another institution in Spain was granted ISO/IEC accreditation for their sensory quality evaluation methods for cheeses and other food products (Elortondo et al. 2007), while a forensic genetic laboratory was granted ISO/IEC 17025 accreditation for their single nucleotide polymorphism typing assay for human identification (Børsting et al. 2009).

Other institutions throughout the world that have obtained accreditation and implemented ISO level quality management systems include the Federal University of Rio Grande do Sul in Brazil (Grochau and ten Caten 2012) and the University of Tartu in Estonia (Rodima et al. 2005). Wineries in Greece have implemented ISO 9000 accredited quality management systems in order to reduce defective product, improve communication, and increase customer satisfaction (Aggelogiannopoulos et al. 2007).

Medical laboratories in Canada have also utilized ISO standards and implemented QMSs to improve communication and patient safety (Guzel and Guner 2009). Drug use and doping are major issues for all countries and in 2012 the Society of Hair released guidance documents for analyzing hair samples for drugs recognizing ISO/IEC 17025 and other regulatory standards (Cooper et al. 2012). Another doping issue being addressed utilizing ISO/IEC 17025 standards is for analyzing the blood and urine samples of race horses by the Horseracing Forensic Laboratory in Europe (Maynard et al. 2003).

More recently in 2018, a laboratory in the United States was granted ISO accreditation for analyzing nitroaromatic explosives in radiologically contaminated soil for forensic purposes (Boggess et al. 2018). This diverse grouping of examples of ISO accredited facilities from forensics to food analysis just goes to show that ISO/IEC accreditation is being utilized throughout the world for the improvement and advancement of all types of processes and plays a major role in providing a backbone for many industries to lean on for guidance and support.

ISO Standards that Affect the Food Industry

Within the food industry there are several ISO and ISO/IEC standards that are utilized for accreditation. These standards provide the guidelines that dictate what types of management systems must be in place, types of documentation that is necessary, and in the case of testing facilities what laboratory items/processes need to be addressed, tracked, and trended in order for an establishment to become ISO or ISO/IEC accredited. These standards including ISO 22000, ISO 9000/9001, and ISO/IEC 17025 affect all aspects of the food industry and will be discussed in more details.

First, ISO 22000 Food Safety Management is a family of standards that are dedicated to setting the requirements necessary for food establishments throughout the entire food chain from farm or primary production to the table of the consumer to demonstrate their ability to control food safety hazards while providing the ground work for HACCP (hazard analysis critical control point) principles to be enforced (Escanciano and Santos-Vijande 2014; ISO 2017; ISO 2018b). These standards are unique because they cover the entire organization providing guidance at all parts of the food supply chain (ISO 2017; ISO 2018b). ISO 22000 standards and principles are necessary to ensure that regardless of the type of food supply chain step either it be farming practices or manufacturing, that the food produced is free of hazards and safe for consumption.

Areas that are focused on within ISO 22000 include: planning, implementing, and maintaining a food safety management system; demonstrating compliance to governmental regulations such as the Food Safety Modernization Act (FSMA) (FDA 2011); and enhancing customer satisfaction by communicating with and meeting customer food safety requirements (Escanciano and Santos-Vijande 2014; ISO 2017; ISO 2018b). Additionally, the standards also aide the food industry in seeking certification or self-declaration to ISO 22000 requirements (Escanciano and Santos-Vijande 2014; ISO 2018b). In addition to ISO 22000, ISO 22005 was put into place to give further guidance specifically for design and development of feed and food traceability systems providing producers and manufactures with a tool/guide to enhance the traceability of their products throughout their own systems (Olsen and Borit 2013; ISO 2018b).

Another set of standards that are directly utilized by, but not produced for, the food industry are the ISO 9000 series. These standards involve the implementation of a

quality management system (QMS). It was stated that “*quality management principles are a set of fundamental beliefs, norms, rules and values...accepted as true...as a basis for quality management*” (ISO 2015) with the seven quality management principles being; customer focus, leadership, engagement of people, process approach, improvement, evidence-based decision making, and relationship management (Sampaio et al. 2009; ISO 2015). Within this family of standards, ISO 9001 is the standard that directly focuses on quality management system development and is the only standard that establishments can be certified against within the 9000 series (Sampaio et al. 2009; ISO 2018b).

These standards can be utilized by establishments regardless of their size, type of process or products produced, or their geographical location. Areas that are focused on within ISO 9001 include: implementing a fully functional QMS; meeting customer needs and requirements while being customer focused; management structure and leadership in achieving quality objectives; meeting management regulatory requirements; and focus on continual improvement of processes, performance, and organizational capabilities (Sampaio et al. 2009; ISO 2015; ISO 2018b).

Finally, the last ISO standard that will be discussed which is utilized in the food industry for the accreditation of food testing facilities is ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories. These standards provide testing facilities with a guide on how to properly manage a laboratory and all other aspects of testing and calibration laboratories that affect samples from arrival to when the final results go to the client regardless of the company’s size or how many employees they have (ISO and IEC 2005; ISO and IEC 2017; ISO 2018b).

Additionally, being ISO/IEC 17025 compliant allows establishments to demonstrate that they are competent and can produce valid acceptable results that can be trusted worldwide providing them recognition within the industry (ILAC 2001; AOAC International 2015; ISO 2018b).

Some areas that are focused on within ISO/IEC 17025 include: implementing a quality management system; handling of customer feedback and communicating with clients; method verification and validation; record keeping and document control; equipment management; and implementation of support programs such as environmental monitoring, temperature monitoring, and training programs (A2LA 2001; ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017; ISO 2018b). ISO/IEC 17025 is a very important standard utilized for assessing the competence of all testing and calibration laboratories in the food industry for accreditation (AOAC International 2015; ISO 2018b). Therefore, it is necessary to discuss this standard in greater detail which will be accomplished in the next section of this review.

ISO/IEC 17025 STANDARD FOR TESTING AND CALIBRATION LABORATORIES Government Requirements in Food Industry

Producers and manufactures in the food industry within the United States of America are required to implement measures within their processes that meet all of the regulations released in 2011 as the Food Safety Modernization Act (FDA 2011). These regulations require that food industry establishments demonstrate, through various methods such as product analysis, that their products are safe for consumption (free of foodborne pathogens) and that their processes are under control, to US governmental

agencies such as the USDA-FSIS and the FDA (USDA 2015; USDA 2018a; FDA 2018b).

FSMA regulations indicate that food manufacturers must implement measures within their processes that are capable of “*significantly minimizing or preventing the occurrence of identified hazards, including through the use of environmental and product testing programs and other appropriate means...*” (FDA 2011). Although there are other ways in which product safety can be proven, and product testing is not specifically required under FSMA regulation, product testing is still the most efficient way in identifying microbiological hazards and provides the strongest evidence that the food is safe for consumption.

Food producers and manufacturers that submit samples for microbiological analysis must submit them to a laboratory that meets a certain level of acceptability to verify that their food safety system is functioning appropriately and that the results generated by the testing facility can be trusted (FDA 2011; USDA 2015; FDA 2018b). Although ISO accreditation is not specifically required in the United States under FSMA regulations (FDA 2011), it is one of the most effective ways for a food testing facility to demonstrate that they meet regulatory guidelines and show they are competent and capable of producing reliable trusted results (A2LA 2001; AOAC International 2015). Additionally, as more laboratories become ISO compliant, it may eventually lead to regulations requiring food testing laboratories to meet even higher standards or even obtain accreditation prior to performing product release testing.

The most widely accepted and recognized set of guidelines for verifying the acceptability of food testing laboratories is ISO standard ISO/IEC 17025 General

Requirements for the Competence of Testing and Calibration Laboratories (ISO and IEC 2005; ISO and IEC 2017; ISO 2018c). Although there are other guidelines/standards available, ISO/IEC guidelines are recognized and accepted worldwide (Romero et al. 2007; ISO 2018c) making them one of the primary standards utilized in and accredited against in the food industry by laboratories performing product release.

Since ISO/IEC 17025 is so important to the food industry, it becomes important to understand all of the aspects about what it is and the requirements contained within its pages which food testing facilities must follow. As mentioned in the previous section, ISO/IEC 17025 standards provide testing facilities with a guide on how to properly manage their laboratory and all other aspects of product analysis that affect samples from arrival to when the final results go to the client (ISO and IEC 2005; ISO and IEC 2017; ISO 2018b).

Also, being ISO/IEC 17025 compliant allows establishments to demonstrate that they are competent and capable of providing clients with valid acceptable results that can be trusted worldwide allowing them to perform product release testing in the United States (A2LA 2001; AOAC International 2015; ISO 2018b). The following paragraphs will discuss the details pertaining to the contents of the ISO/IEC 17025 standard, steps necessary to obtain ISO/IEC 17025 accreditation, and what requirements must be met in order to become ISO/IEC 17025 compliant.

Steps to Obtaining ISO/IEC 17025 Accreditation

In order for any food testing laboratory in the United States to obtain ISO/IEC 17025 accreditation they must first meet certain requirements, have a functioning

laboratory in place that already complies with ISO/IEC 17025 guidelines/requirements, and submit certain pre-audit documents to the accrediting body of choice (A2LA 2015b; A2LA 2016b). Since all accrediting bodies are slightly different in their requirements for accreditation, even though they are accrediting against the same standard, we will be using the steps required for ISO/IEC 17025 accreditation primarily from the American Association of Laboratory Accreditation (A2LA) which is a non-profit, non-governmental accrediting body/system (A2LA 2016b), as a guide for this section – keeping in mind that it is a very lengthy process and only a few of the major steps will be discussed. For further details on the steps to obtaining ISO/IEC 17025 accreditation through A2LA refer to “Table 1.4 List of Steps for Obtaining ISO/IEC 17025 Accreditation” in Appendix 1 of this review.

Becoming accredited is not a simple task, and there are many steps, program requirements, and conditions that must be met prior to and during the accreditation process in order for any food testing laboratory to ultimately achieve accredited status. We will begin by discussing some of the required steps in obtaining ISO/IEC 17025 accreditation utilizing A2LA, followed by the program requirements necessary to ultimately complete the accreditation process.

Achieving ISO/IEC 17025 accreditation begins just like any other process by looking into what you want to accomplish, learning more about it, and determining what is required to complete that task. With accreditation, that involves obtaining a copy of the conformity assessment standard, in this case ISO/IEC 17025 (ISO and IEC 2005; ISO and IEC 2017) and reviewing the standard and any other supporting documentation that

you might need to gain a better understanding of what will be required of your laboratory to meet ISO requirements for accreditation (A2LA 2015a; A2LA 2016b).

Next, the laboratory must obtain the conformity checklist that details all of the requirements within the ISO/IEC 17025 standard that must be met prior to scheduling an initial assessment. The laboratory must then provide evidence to the accrediting body that they have obtained copies of the standard and the checklist to guide them through their laboratory compliance process (A2LA 2015a; A2LA 2016a) which ensures the accreditation body that they are implementing the appropriate standards.

Although other accreditation bodies such as Perry Johnson Laboratory Accreditation (PJLA) list other steps to perform first (PJLA 2009), almost all of the steps to accreditation are the same just in a different order culminating in an assessment of the laboratory against the standard to achieve accreditation status. Therefore, we will continue following the A2LA system throughout this review.

After taking the initial steps for accreditation the food testing laboratory must now begin the hardest part of becoming accredited. The laboratory must develop a unique quality management system (QMS) that fits its needs by altering or updating all current processes, policies, and procedures to meet ISO/IEC 17025 requirements prior to submitting for an initial assessment (A2LA 2001; PJLA 2009; A2LA 2015a). This is by far the most challenging part of becoming accredited as it can take months or even years, depending on available resources and current laboratory capabilities, to implement all parts of the standard into the laboratory's current system. As part of this process the laboratory must:

1. Develop a draft scope of accreditation detailing what food analysis application(s) they intend to become accredited for
2. Implement all parts of the QMS including management structure, employee policies, quality assurance, laboratory procedures, records and other documentation, support programs, and a quality manual (if applicable)
3. Perform internal audits against the standard/checklist to verify that the laboratory meets all of the ISO/IEC 17025 requirements and is ISO compliant
4. Conduct management review meetings to discuss issues and track/trend available laboratory data to show improvement and predict areas of concern
5. Translate all available documentation into English (if applicable)

(A2LA 2015a; A2LA 2016a)

Once the QMS is in place and the laboratory has completed all required pre-accreditation tasks, then the laboratory may submit an application to the accrediting body to begin the accreditation process. During this stage of the process the laboratory will be required to submit examples (if not all) of its current documentation, standard operating procedures (SOPs), equipment lists, records, internal audit findings, corrective actions taken, a completed conformity assessment checklist, and other supporting information as proof that they are currently meeting ISO standards (PJLA 2009; A2LA 2015a; A2LA 2016b).

Additionally, the laboratory's authorized representative and deputy representatives must sign an agreement that "*all statements made on the application are correct to the best of his/her knowledge and belief*" and understand they are "*responsible for ensuring that all of the relevant conditions for accreditation are met*" (A2LA 2016b). After the submittal of the application, the laboratory will be assigned an assessor that will conduct the pre-assessment (desk audit of the laboratory's documentation) followed by

an initial assessment (onsite assessment) to determine if the laboratory should receive ISO/IEC 17025 accreditation for its intended scope (PJLA 2009; A2LA 2015a; A2LA 2016b).

If the laboratory passes these assessments then they will become accredited for the scope of work they submitted achieving the goal they set of obtaining ISO/IEC 17025 accreditation. This is a major milestone for any food testing facility, but these establishments must remember that this is not the end of the road as there will be surveillance assessments and annual renewal assessments (A2LA 2015a; A2LA 2016b) in which the food testing laboratory will have to continually demonstrate that they still meet all guidelines within the ISO standard that they worked so hard to obtain or their accreditation status may be forfeited.

Finally, there are several conditions that an accredited laboratory must meet in order to maintain ISO accredited status. Some of these conditions include:

1. Provide accommodation to the accrediting body giving access to documentation, the laboratory or laboratories where the applicable analyses are taking place, and to all equipment, personnel, records, complaints, and past assessments
 2. Must comply at all times with the standard
 3. Maintain impartiality and integrity in all of its dealings
 4. Retention of records, both quality and technical, for required time frames and making them accessible to an auditor within a reasonable amount of time
 5. Only claim accreditation status for methods on its scope of accreditation
 6. Pay all fees associated with maintaining accreditation to the accrediting body
 7. Never mislead clients or use accreditation in a misleading manner
 8. Inform the accrediting body of any changes to the organization, management, personnel, accredited methods on scope, or any other changes that could affect the laboratory's accreditation status
- (A2LA 2015b)

As long as all of these conditions, and other conditions not mentioned here, are met then the laboratory may retain its ISO accreditation to ISO/IEC 17025 and continue to receive all of the benefits that come with being an accredited laboratory.

Accreditation Requirement – Quality Management Systems

When preparing for ISO/IEC 17025 accreditation, a laboratory must develop a quality management system (QMS) that meets its establishments needs and conforms to the ISO standards and principles in ISO 9001 (ISO and IEC 2005; Sampaio et al. 2009; AOAC International 2015; ISO 2015; ISO and IEC 2017). Quality management systems can be defined as “*the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management*” and “*control how quality policies are implemented and quality objectives are achieved*” (A2LA 2001; Allen 2013).

Implementing a QMS is a challenging task that can take food testing laboratories months or even years to accomplish as it involves all parts of the management structure, policies, and procedures needed to meet the standard (A2LA 2001; A2LA 2015a).

In order to be compliant to the ISO/IEC 17025 standard, a food testing laboratory must have policies and procedures that cover many areas of concern from the accrediting body prior to attempting accreditation. Some of these areas include management requirements such as:

1. Organizational structure including proof that the establishment can be held legally responsible and that the establishment has both managerial and technical personnel with the authority to carry out their duties
2. Document control procedures for both internally and externally generated documents such as regulations, standards, test methods, equipment manuals, etc.

3. Review of requests, tenders, and contracts providing evidence that the laboratory has the capability and resources to perform agreed upon test procedures and that they are using appropriate approved methods
4. Purchasing services and supplies policies and procedures for selecting external contract services and approved suppliers, and vendor lists and procedures for purchasing, receiving, and storing of critical supplies
5. Service to the customer including proof of communication with customers as well as policies and procedures for a complaint and feedback system
6. Improvement and progress shown through changes made due to audit findings, customer feedback, management reviews, and corrective/preventive action reports
7. Corrective and preventive action procedures for handling issues of nonconformance to policies or procedures and to aid in continual improvement of the laboratory
8. Control of records for the purpose of identifying, collecting, filing, storing, and disposal of quality and technical records
9. Internal audit procedures and records for verifying that the laboratory's operations are compliant with the standard
10. Management reviews for ensuring that management system policies and procedures are suitable for their intended purpose, reviewing corrective/preventive action reports, reviewing proficiency sample test results, reviewing customer feedback and complaints, and making recommendations for changes and improvement to the quality management system

(ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017)

Putting these policies and procedures in place provides the necessary structure to the laboratory to be successful in not only obtaining ISO/IEC 17025 accreditation but also for maintaining ISO accreditation status into the future.

Industries Utilizing Quality Management Systems

Many establishments have implemented QMSs utilizing ISO standards such as ISO 9001, ISO 15189, and ISO/IEC 17025 within their organizations in order to gain the benefits of having the structure and stability that QMSs provide. It was shown that the implementation of a QMS utilizing ISO 15189 standards in Canadian hospitals provided a structural foundation for quality in the hospital laboratories and that the safety of patients was positively impacted by preventing patient safety issues (Allen 2013). In Europe, quality management systems have been put into place to help improve food composition databases by the European Food Information Resource Network (EuroFIR). It was discovered that having a QMS in place help to reinforce EuroFIR's quality procedures and were "*fundamental to improving quality of data exchanged across Europe and beyond*" (Castanheira et al. 2009).

University laboratories have also implemented QMSs according ISO/IEC 17025 such as the Federal University of Rio Grande do Sul in Brazil and University of Tartu in Estonia (Rodima et al. 2005; Grochau et al. 2010). Rodima et al. stated that implementing quality management systems at universities "*gives significant added value to the university by helping to...broaden the minds (quality awareness) of the students*" (Rodima et al. 2005). Yet another example can be seen in a small winery in Greece where quality management system practices according to ISO 9000 standards have been utilized in order to reduce defective product, improve communication, and increase customer satisfaction (Aggelogiannopoulos et al. 2007).

Quality management systems have been studied and found to have a positive impact on logistics, customer service, and even the overall quality of food supply chains

(Zimon 2017). QMSs adopted according to ISO/IEC 17025 standards have even been integrated into the management systems of national institutes such as the National Metrology Institute of Montenegro providing them with advantages as well as recognized confidence and reliability in their procedures (Asanovic et al. 2018). All of these examples go to show that developing and integrating ISO standard quality management systems is not only possible but beneficial whether the task involves hospital work and patient safety or managing food supply chains helping to lead to and drive continuous improvement.

Other Accreditation Requirements – Technical Requirements

Along with developing a comprehensive quality management system to govern over and provide structure/support to the laboratory, food testing laboratories seeking ISO/IEC 17025 accreditation must also meet other requirements. These requirements involve the development and implementation of “technical requirements” that include everything from the generation of laboratory records, test methods, and SOPs, to the implementation of support programs such as environmental monitoring, training, equipment, and temperature monitoring programs that affect sample results in the laboratory for methods that are part of the scope of accreditation (A2LA 2001; ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017). Some of these technical requirements include:

1. Understanding and monitoring the factors (human error, environmental conditions, etc.) that contribute to the measurement uncertainty of a test result

2. Personal records, including training records, displaying the technical competence and abilities of staff to perform in-scope analyses as well as a training program with procedures on how to properly train personnel on laboratory procedures
3. Laboratory should be arranged to minimize potential cross contamination events. A temperature monitoring program for all laboratory supplies and samples must be in place to verify accuracy of test parameters and proper storage of test items. Laboratory must demonstrate that the area where sample analysis is being performed is suitable for that purpose
4. Use of “only” approved recognized methods for performing accredited tests, and verifying of test methods that are part of the scope of accreditation to demonstrate that the laboratory can perform them according to approved methods to a level of acceptability
5. Obtaining the measurement of uncertainty for all test methods to verify the accuracy of test results (if applicable)
6. Control of all data generated by the laboratory and having procedures for the acquisition, processing, reporting, storage, and retrieval of all customer information and data/results
7. Implementing an equipment program ensuring all equipment is approved for use with procedures for operating and maintaining all equipment within the laboratory with records to track the cleaning and maintenance of the equipment
8. Laboratory must have procedures for calibration of laboratory equipment and policies to demonstrate that they are calibrated prior to being used for any scope of accreditation test method
9. Detailed procedures and policies for the handling of test items including the receipt, storage, retention, and disposal of all test items to demonstrate that the integrity of test samples is not compromised – sample items must be traceable
10. Laboratory must have controls in place to verify the validity of test results and the data from those controls should be trended to look for issues within the system
11. Laboratory must have SOPs for media qualification and quality testing procedures

12. All personnel should participate in proficiency testing to demonstrate that they are capable of performing the test methods appropriately and producing acceptable results
 13. Results obtained by the laboratory should be reported to the client accurately, unambiguously, objectively, and in accordance to the standards on a test report that has been signed by the individual who authorized the sample analysis
- (A2LA 2001; ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017)

It is important to recognize that when attempting to become accredited to ISO standards it is the expectation of the accrediting body that the laboratory has already implemented to the best of their ability all of the management and technical requirements within the standard (A2LA 2015a; A2LA 2015b). The technical requirements listed within this section and in the management requirements section above are only a few of the items that need to be addressed to obtain accreditation (A2LA 2001; AOAC International 2015). It is the responsibility of the testing laboratory seeking accreditation to understand all of the requirements for accreditation and develop a comprehensive QMS that includes all management policies, flow charts, SOPs, records, programs, and all other items that are necessary to achieve accreditation and operate a successful laboratory utilizing ISO standards.

Differences Between ISO/IEC 17025:2005 and ISO/IEC 17025:2017

New information is constantly being generated impacting the food industry and leading to improvements in the way food products are being harvested, produced, or in how they are being tested for foodborne pathogens. These improvements are sometimes large enough that updates to the ISO standards may be necessary in order to facilitate

change and improvement across the entire industry. In 2017, ISO released an updated version of the ISO/IEC 17025 standard going from version ISO/IEC 17025:2005 to ISO/IEC 17025:2017 (ISO 2018b). Although the majority of the content stayed the same, there were some notable changes that can be discussed including the structure of the document, changes to the scope of the standard, and a change in the standard from providing detailed steps to focusing on results (Eurolab 2017; SADCAS 2018). A few of these changes will be addressed in the following paragraphs.

First, the structure of the ISO/IEC 17025 standard has changed dramatically. It is no longer based on just two sections consisting of “Management” and “Technical” requirements (ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017) but is rather broken up into five main sections entitled; General Requirements, Structure Requirements, Resource Requirements, Process Requirements, and Management System Requirements (Eurolab 2017; ISO and IEC 2017; SADCAS 2018). These new sections still contain all of the original content from the previous version but in a different order to better facilitate the flow of information within the standard (Eurolab 2017).

Some of the sections that have been moved to new sections include: personnel moving from sections 4.1.5 f-h and 5.2 in the 2005 standard to 6.2 in the 2017 standard; accommodations of environmental conditions being moved from section 5.3 to section 6.3 and renamed to facilities and environmental conditions; equipment moving from section 5.5 to section 6.4 in the new standard; measurement traceability moving from section 5.6 to section 6.5 and being renamed to metrological traceability; and review of requests, tenders, and contracts moving from section 4.4 to section 7.1.1 in the new standard (Eurolab 2017; SADCAS 2018). A comparison between the section titles and

the document structure of ISO/IEC 17025:2005 and ISO/IEC 17025:2017 can be found in Appendix 1 Table 1.2. For a breakdown of the section changes within ISO/IEC 17025:2017 and the cross-reference sections from ISO/IEC 17025:2005 see Appendix 1 Table 1.3.

Other changes that should be noted for accreditation include the addition of a “risk-based approach” section to the standard. This section requires that all laboratories seeking accreditation implement procedures and practices to address “risk” and “opportunities” throughout their processes to demonstrate the management system is effective in helping the laboratory obtain trusted results, prevent or reduce the impact that potential failures have on the management system and ability of the laboratory to obtain results through testing procedures, and aid in the continual improvement and success of the laboratory (Eurolab 2017; SADCAS 2018).

The scope of the standard has also changed along with the definition of what a laboratory is. In the 2017 standard a laboratory is defined as “*an organization that can perform testing, calibration and/or sampling associated with subsequent testing or calibration*” (Eurolab 2017). This new definition places an emphasis on sampling as being a part of laboratory activities instead of just testing and calibration which implies that all ISO/IEC 17025 accredited establishments will now need to address sampling whenever laboratory activities are mentioned within each section of the standard (Eurolab 2017; SADCAS 2018).

Finally, there is a new emphasis on obtaining results from each process instead of providing a detailed description of how to obtain those results. The ISO/IEC 17025:2017 standard has been altered to remove descriptions of individual processes and instead

focus on performance making them more open to interpretation and leaving it up to each laboratory on how they will meet the requirement (Eurolab 2017). With the performance-based requirements laboratories will now have much more freedom to design and develop procedures and systems that meet their unique needs while still adhering to and meeting the requirements set forth in the ISO/IEC 17025 standard and maintaining their accreditation status.

CHALLENGES IN OBTAINING ISO ACCREDITATION

Despite all of the benefits that can be realized from obtaining ISO accreditation, there are many things that have been discussed throughout this review that must be accomplished by a laboratory or other establishment before that can become a reality. Implementation of ISO practices involves the development of a quality management system (QMS) and creation of documentation (Zapata-García et al. 2007; Grochau et al. 2010; Grochau and ten Caten 2012), implementation of in-house control programs such as environmental monitoring, training (Honsa and McIntyre 2003), and media qualification programs, and the verification of all in-scope methods used within the laboratory (A2LA 2001; AOAC International 2015). This process can be problematic for any laboratory, whether it be privately held, part of public institution, or run by a local or federal government.

Implementing all of these things in order to meet the standard and obtain accreditation can be a very challenging task. Some of the other challenges that face accreditation seeking laboratories include the time and difficulty of developing and implementing the quality management system, documents, forms, policies, and programs

to meet the requirements, and how well prepared they are when they begin the process (Vlachos et al. 2002; Zapata-García et al. 2007; Hullihen et al. 2009; MDT 2016). It was stated that “*the length of time and the ease or difficulty of the accreditation process depends on your team’s experience and preparation*” (MDT 2016).

There is also a large financial commitment that an establishment must make in order to implement all of the requirements of the standard as well as pay the fees to the accrediting body (Zapata-García et al. 2007; Hullihen et al. 2009; MDT 2016). This is especially true in developing countries where financial and human resources are not as abundant (Massoud et al. 2010). Other challenges that could occur include:

1. Issues developing a QMS and proper organizational structure that meets the needs of the establishment and also the requirements in the standard
2. Developing procedures with risk-based decision making
3. Having appropriate leadership and management commitment to obtaining accreditation
4. Coordinating policies, documentation, processes, and procedures between management and departments and from one department to another
5. Issues meeting all quality requirements in the standards
6. Ensuring the timing of obtaining accreditation fits the business model of the establishment
7. Maintaining the QMS and continuing to improve

(Vlachos et al. 2002; Zapata-García et al. 2007;
Hullihen et al. 2009; DQS et al. 2016; Rahmat et al. 2016)

Obtaining ISO accreditation can be especially challenging for academic laboratories that are primarily focused on research activities and student development. Grochau et al. in regards to academic institutions stated that, “*Testing services are not a priority, the performance of professionals is measured based on their teaching activities*

and publications, and the laboratories are shared with research and teaching activities”

(Grochau et al. 2010). Other institutions have mentioned similar issues due to teaching and research taking priority when attempting accreditation (Zapata-García et al. 2007). These challenges make it very difficult to implement a QMS and develop/maintain a laboratory whether it be in a private, government, or academic environment, which is functional and sustainable to meet industry needs in order to obtain ISO accreditation.

RECALLS AND THE NEED FOR STANDARDS

ISO accredited food testing laboratories provide product testing services to the food industry including; chemical and nutritional compositions, residues and contaminants, speciation, allergen, packaging, and sensory analysis (Intertek 2018). However, one of the main functions of food testing laboratories is to test for foodborne pathogens verifying that food safety systems are functioning appropriately and that food products that producers and manufacturers are providing to commerce are safe for consumption. Despite having food testing facilities, recalls still occur and in some cases cause disease and harm to our friends, family, and colleagues. Several recalls related to food safety have already surfaced in 2018 from both FDA and USDA FSIS regulated establishments which will be mentioned briefly.

Recalls for food pathogen concerns are not as common as other recalls such as mislabeling or foreign material, but they do occur and are cause for concern. One such recall by the Evershing International Trading Company was initiated in 2018 due to their shredded coconut product containing *Salmonella* spp. (FDA 2018a). Another recall initiated in December 2017 and pushing into 2018 by the Springfield Smoked Fish

Company was due to *Listeria monocytogenes* being found in pre-sliced salmon (FDA 2018a).

Other examples of FDA regulated products that have been recalled for foodborne pathogen concerns include Organic Amaranth Flour for *Salmonella* spp. contamination, and cream cheese contaminated with *Listeria monocytogenes* (FDA 2018a). But, it is not just FDA products that are a concern as many USDA FSIS regulated products have also been recalled in 2018. SMI Holdings recalled 484,400 lbs. of boneless beef top sirloin due to potential *Salmonella* spp. contamination (USDA 2018b). Olli Salumeria Americana initiated a recall for several pepperoni, chorizo, and other salami products due to *Listeria monocytogenes* contamination (USDA 2018b), while yet another company, Triple T Specialty Meats Inc. recalled 20,630 lbs. of chicken salad due to *Salmonella* spp. contamination (USDA 2018b).

Although not certain, these recalls may have been potentially avoided if sample analysis and other control measures had been utilized prior to these products entering commerce. Food testing facilities help to verify that the manufacturers food safety management system is functioning correctly and that their production process is acceptable. In addition, results obtained from these testing activities provide necessary evidence in support of the safety of food products before they ever reach restaurants, grocery stores, and people's homes.

Due to their role in the food industry, food testing laboratories are on the front lines of preventing or at least minimizing the amount of recalls that arise as they provide the data needed for monitoring the effectiveness of food safety preventive control programs and continuous improvement efforts, leading to the improved safety of the food

supply chain. Since the analysis of food is so important to the safety of food products, it also becomes important for those laboratories to meet certain guidelines and a level of acceptability so the results they generate can be trusted – which can be achieved through ISO accreditation.

Literature Review Tables

This section contains tables in support of the sections within this literature review for obtaining ISO accreditation. These tables consist of the section differences and the cross references between ISO/IEC 17025:2017 and ISO/IEC 17025:2005 as well as a comprehensive breakdown of the steps necessary to obtain ISO accreditation.

Tables contained in this appendix:

Table 1.1: List of Common ISO and ISO/IEC Standards

Table 1.2: Section differences between ISO/IEC 17025:2005 and ISO/IEC 17025:2017

Table 1.3: Cross references between ISO/IEC 17025:2017 and 17025:2005

Table 1.4: General Steps for Obtaining ISO/IEC 17025 Accreditation

Table 1.1: List of Common ISO and ISO/IEC Standards. Table displays a list of commonly used ISO and ISO/IEC standards for various industries (ISO 2018b).

Standard Name	ID Number	Standard Name	ID Number
Quality Management	ISO 9001	Medical Device	ISO 13485
Information Security Management	ISO/IEC 27001	Language Codes	ISO 639
Environmental Management	ISO 14001	Currency Codes	ISO 4217
Testing and Calibration Laboratories	ISO/IEC 17025	Social Responsibility	ISO 26000
Risk Management	ISO 31000	Sustainable Events	ISO 20121
Energy Management	ISO 50001	Occupational Health and Safety	ISO 45001
Food Safety Management	ISO 22000	Anti-Bribery Management Systems	ISO 37001
Date and Time Format	ISO 8601	Country Codes	ISO 3166

Table 1.2: Section Differences Between ISO/IEC 17025:2005 and ISO/IEC 17025:2017. Table displays the section differences between ISO/IEC 17025:2005 and ISO/IEC 17025:2017 that must be addressed for accreditation (ISO and IEC 2005; AOAC International 2015; ISO and IEC 2017).

ISO/IEC 17025:2005	ISO/IEC 17025:2017
<i>4. Management Requirements</i>	<i>4. General Requirements</i>
4.1 Organization	4.1 Impartiality
4.2 Management System	4.2 Confidentiality
4.3 Document Control	<i>5. Structural Requirements</i>
4.4 Review of Requests, Tenders and Contracts	<i>6. Resource Requirements</i>
4.5 Subcontracting of Tests and Calibrations	6.1 General
4.6 Purchasing Services and Supplies	6.2 Personnel
4.7 Service to the Customer	6.3 Facilities and Environmental Conditions
4.8 Complaints	6.4 Equipment
4.9 Control of Nonconforming Testing and/or Calibration Work	6.5 Metrological Traceability
4.10 Improvement	6.6 Externally Provided Products and Services
4.11 Corrective Action	<i>7. Process Requirements</i>
4.12 Preventive Action	7.1 Review of Requests, Tenders and Contracts
4.13 Control of Records	7.2 Selection, Verification, and Validation of Methods
4.14 Internal Audits	7.3 Sampling
4.15 Management Reviews	7.4 Handling of Test and Calibration Items
<i>5. Technical Requirements</i>	7.5 Technical Records
5.1 General	7.6 Evaluation of Measurement Uncertainty
5.2 Personnel	7.7 Ensuring the Validity of Results
5.3 Accommodation and Environmental Conditions	7.8 Reporting of Results
5.4 Test and Calibration Methods and Method Validation	7.9 Complaints
5.5 Equipment	7.10 Nonconforming Work
5.6 Measurement Traceability	7.11 Control of Data and Information Management
5.7 Sampling and Subsampling	<i>8. Management System Requirements</i>
5.8 Handling of Test and Calibration Items	8.1 Options (General / Option A / Option B)
5.9 Ensuring the Quality of Test and Calibration Results	8.2 Management System Documentation (Option A)
5.10 Reporting the Results	8.3 Control of Management System Documents (Option A)

Annex A Nominal Cross References to ISO 9001

Annex B Guidelines for Establishing Applications for Specific Fields

Appendices to ISO/IEC 17025 2005/2017

Appendix A: Equipment

Appendix B: Microbiology

Appendix C: Chemistry

Appendix D: Pharmaceutical Analysis and Legal Standards

Appendix E: Legal Samples

8.4 Control of Records (Option A)

8.5 Actions to Address Risks and Opportunities (Option A)

8.6 Improvement (Option A)

8.7 Corrective Actions (Option A)

8.8 Internal Audits (Option A)

8.9 Management Reviews (Option A)

Annex A. Metrological Traceability

A.1 General

A.2 Establishing Metrological Traceability

A.3 Demonstrating Metrological Traceability

Annex B. Measurement System Options

Table 1.3: Cross References Between ISO/IEC 17025:2017 and 17025:2005. Table shows the cross references from ISO/IEC 17025:2017 standard to previous sections in ISO/IEC 17025:2005 standard for initial or renewal accreditation, adapted from (ISO and IEC 2005; ISO and IEC 2017; Eurolab 2017).

ISO/IEC 17025:2017		ISO/IEC 17025:2005	
4. General Requirements			
4.1	Impartiality	4.1.4, 4.1.5	Organization
4.2	Confidentiality	4.1.5 c	Organization
5. Structural Requirements			
5.0	Structural Requirements	4.1	Organization
6. Resource Requirements			
6.2	Personnel	4.1.5 f-h, 5.2	Organization, Personnel
6.3	Facilities and Environmental Conditions	5.3	Accommodation and Environmental Conditions
6.4	Equipment	5.5	Equipment
6.5	Metrological Traceability	5.6	Measurement Traceability
6.6	Externally Provided Products and Services	4.5, 4.6	Subcontracting of Tests and Calibrations, Purchasing Services and Supplies
7. Process Requirements			
7.1	Review of Requests, Tenders and Contracts	4.4	Review of Requests, Tenders and Contracts
7.2	Selection, Verification, and Validation of Methods	5.4.1, 5.4.2	Test and Calibration Methods and Method Validation, General, Selection of Methods
7.3	Sampling	5.7, 5.8 Note 2, 5.10.2 h, 5.10.3.2	Sampling
7.4	Handling of Test and Calibration Items	5.8	Handling of Test and Calibration Items
7.5	Technical Records	4.13.2	Technical Records
7.6	Evaluation of Measurement Uncertainty	5.4.6	Estimation of Uncertainty of Measurements
7.7	Ensuring the Validity of Results	5.9	Assuring the Quality of Test and Calibration Results
7.8	Reporting of Results	5.10	Reporting of Results
7.9	Complaints	4.8	Complaints
7.10	Nonconforming Work	4.9	Control of Nonconforming Testing and/or Calibration Work
7.11	Control of Data and Information Management	4.13	Control of Records
8. Management System Requirements			
8.1	Options (General / Option A / Option B)	N/A	N/A

ISO/IEC 17025:2017		ISO/IEC 17025:2005	
8.2	Management System Documentation (Option A)	4.2	Management System
8.3	Control of Management System Documents (Option A)	4.3	Document Control
8.4	Control of Records (Option A)	4.13.1	Control of Records, General
8.5	Actions to Address Risks and Opportunities (Option A)	N/A	N/A
8.6	Improvement (Option A)	4.7.2, 4.12	Service to the Customer, Preventive Action
8.7	Corrective Actions (Option A)	4.11	Corrective Action
8.8	Internal Audits (Option A)	4.14	Internal Audits
8.9	Management Reviews (Option A)	4.15	Management Review
<i>Annex A. Metrological Traceability</i>			
A.1	General	N/A	N/A
A.2	Establishing Metrological Traceability	N/A	N/A
A.3	Demonstrating Metrological Traceability	N/A	N/A
<i>Annex B. Measurement System Options</i>			
B	Measurement System Options	N/A	N/A

Table 1.4: General Steps for Obtaining ISO/IEC 17025 Accreditation. Table shows the general list of steps for obtaining ISO/IEC 17025 accreditation from A2LA accreditation body for food microbiology laboratories (A2LA 2015a; A2LA 2016b).

Step #	Process or Step Required	Applicable Documents/Forms	Comments/Requirements
<u>Preparing for Initial Accreditation</u>			
1	Obtain official copy of ISO/IEC 17025 standard and/or AOAC guidelines for laboratories performing microbiological and chemical analysis of Food, dietary supplements, and pharmaceuticals	Current 17025 Standard	Establishment will be accredited against these guidelines
2	Review ISO/IEC 17025 standard and general requirements for accreditation documents (A2LA R101)	17025:2005 R101	
3	Estimate cost of accreditation and submit form (F119 Estimate Request) to A2LA	F119	
4	Obtain conformity checklist (C204 – Specific Checklist: Combined ISO/IEC 17025 and Food & Pharmaceutical Testing Laboratory Accreditation)	C204	May be used for internal audits to help verify laboratory is meeting guidelines
	A2LA form F102 Ownership Confirmation for ISO documentation and checklist	F102	Submit by email or fax
5	Complete a “Selection List” or “Draft Scope” of accreditation	A2LA Website	Scope of accreditation is the fundamental document attesting to organization’s competence, official listing of tests laboratory is competent to perform, scope identified by internationally recognized standard test methods (include date, version, edition, etc.), must use current versions and show competency in method, if not performing entire method denote exclusions on the scope, will be reviewed by the assessor during audit
6	Generate a Quality Management System (including policies and procedures in accordance with C204)	All QMS Documents and Quality Manual	This includes all SOPs, work instructions, protocols, forms, program documents, etc. that are in-scope
7	Internal Audit	Audit Checklists and Reports	Internal audits must be completed according to and internal audit schedule and completed prior to assessments
8	Management Review	Annual Management Report and KPIs	Must be completed prior to assessments
9	Translate all supporting documents and materials into English	All Documents	Assessments will be conducted in English only; all documents must be in English and establishment must provide someone to the auditor that speaks English to communicate with
10	Assign a laboratory representative responsible for upholding accreditation requirements	FPCLS Position Description	
11	Assign an individual responsibility over Quality Management System (Quality Manager)	FPCLS Position Description	

Step #	Process or Step Required	Applicable Documents/Forms	Comments/Requirements
	Management authorized representatives review and agree to A2LA R102 Conditions for Accreditation	A2LA R102	Must conform and agree to conditions of accreditation (R102) or accreditation will not be granted by A2LA
12	Complete A2LA Application of Accreditation for ISO/IEC 17025	A2LA F101	Assessment must be completed within 1 year of submittal or laboratory forfeits payment and must start the process over
13	Submit the following to A2LA: <ul style="list-style-type: none"> Completed A2LA application for accreditation Completed conformity checklist All supporting (bench audit) documents including; organization charts, proficiency plan, summary of proficiency results, equipment list, quality manual, all SOPs, certification certificates for calibration companies used (identity, location, accreditation status), staff matrix, selection list or draft scope of accreditation, Payment 	A2LA F101 C204, C101 A2LA I109 A2LA R102 A2LA F117 Organizational Charts Proficiency Testing FPCLS Equipment Inventory List Quality Manual All SOPs	Proposed scope of testing must be included on the application including field/area, testing technologies, and methods and/or relevant standards to be used Uncertainty is not required for out laboratory because we are only doing qualitative methods Laboratory location must also be noted on the scope of accreditation Payments are non-refundable, unless assessment is not completed
	Receive A2LA CAB portal credentials	Log-In Name and Password	The conformity assessment bodies (CAB) portal will be used for uploading all documentation and handling all parts of the accreditation process
	Upload supporting documents not sent with application		Laboratory may upload supporting documents after receiving credentials if they are too large to submit with application
14	Receive name of assessor and that assessors' bio-sketch from A2LA		1 or more assessors may be assigned to your accreditation team, assessors cannot provide consultation Assessors utilize a provided instruction manual and checklists to conduct the assessment in order to standardize the audit
15	Pre-assessment (Optional) or Initial Assessment A2LA assessment by designated assessor, review of the following: <ul style="list-style-type: none"> Quality Management System for implementation and compliance Check against conformity standard Review technical activities (if requested) 		Assessed as a Main/Permanent laboratory as we only have 1 laboratory in a fixed location Objective of assessment is to establish if laboratory complies with A2LA requirements for accreditation Conducted on site
16	Assessor schedules and performs pre-assessments and/or Initial Assessment	A2LA R102 A2LA R105 A2LA R103 A2LAP102 A2LA TAC Consensus Documents Quality Manual Quality SOPs	A2LA will certify that establishment; <ul style="list-style-type: none"> is competent to perform in-scope tests management system addresses/conforms to all elements of ISO/IEC 17025, is documented and is fully operational is operating in accordance with its management system conforms to any additional requirements of A2LA Assessments may last 1 to several days

Step #	Process or Step Required	Applicable Documents/Forms	Comments/Requirements
		Equipment Inventory List	Assessment team will request copies or access to quality documents and SOPs to conduct the audit
		Training Documents	Involves
		Proficiency Test Results	<ul style="list-style-type: none"> Initial briefing with laboratory management Interviews with staff Demonstrations of test methods
		Confidentiality and Conflict of Interest	<ul style="list-style-type: none"> Examination of equipment and calibration records
		All other technical documents associated with scope	<ul style="list-style-type: none"> Audit of quality management system Evaluation of compliance to A2LA documents Written report of assessor's findings Exit briefing and discussion of deficiencies
	Corrective Actions and Deficiencies	CAPA Documents	Laboratory is expected to respond to all "Initial Assessment" audit findings and deficiencies within 1 month (30 Days) from date of exit briefing and resolve all findings within 4 months
		Root Cause Analysis	Must include root cause analysis and a copy of corrections or sufficient objective evidence proving correction has been made
			Corrective action reviews that take longer than 2 hours by assessor may require payment
<u>Surveillance Assessment Preparation</u>			
1	6 months prior to midpoint of accreditation; <ul style="list-style-type: none"> Alerted of surveillance assessment Confirm CAB information Agree to A2LA R102 Conditions of Accreditation Upload to CAB portal all surveillance assessment supporting documents/information Payment 	A2LA R102 Quality Manual Quality Documents	Reassessment of laboratory to verify it is still meeting A2LA requirements and follow accreditation guidelines
2	Upload to CAB portal; <ul style="list-style-type: none"> Up-to-date organizational charts (name and function of key personnel) Highlight any changes since initial assessment 	Organizational Charts Management Reviews	Any changes within the organization that occurred after the initial assessment must be noted on the organizational chart
3	Upload to CAB portal any other applicable or requested documentation Surveillance assessment will occur 1 year following initial accreditation Renewal assessment will occur 2 years after initial assessment Renewal or reaffirmation of accreditation will only be granted after establishment has submitted proper payment in full and resolved all deficiencies from the surveillance assessment		Laboratory is expected to respond to all audit findings and deficiencies within 1 month (30 Days) from date of exit briefing

Step #	Process or Step Required	Applicable Documents/Forms	Comments/Requirements
<u>Annual Review of Accreditation</u>			
1	3 months prior to midpoint of renewal accreditation cycle; <ul style="list-style-type: none"> Establishment alerted of annual review Confirm CAB information Agree to A2LA R102 Conditions of Accreditation Upload to CAB portal all annual review supporting documents/information Payment 	A2LA R102 Quality Manual Quality Documents	Laboratory must <ul style="list-style-type: none"> Pay annual fees Pay assessor fees Under go 1-day surveillance audit Audit to confirm laboratory is still in compliance with accreditation requirements
2	Upload to CAB portal; <ul style="list-style-type: none"> Up-to-date organizational chart (name and function of key personnel) Highlight any changes since initial assessment Also provide separate UNL organizational chart (if necessary) 	Organizational Charts	Must update all records to include current personnel and technical capabilities, any changes must be addressed
3	Upload to CAB portal; <ul style="list-style-type: none"> Most recent internal audit results Most recent management review Annual reviews occur at the midpoint of each 2-year accreditation renewal cycle	Internal Audit Checklists and Reports Management Reviews	Internal audits must be conducted prior to beginning of assessment
4	Reaffirmation of accreditation by A2LA – <ul style="list-style-type: none"> Reaffirmation good for 1 year When reaffirmation expires establishment will be prompted to submit appropriate renewal information and fees Annual review assessment begins (see initial assessment for process requirements)	Scope of Accreditation A2LA R102 See Initial Assessment Documents/Forms	 Audit to confirm laboratory is still in compliance with accreditation requirements
<u>Renewal of Accreditation</u>			
1	6 months prior to expiration of current accreditation; <ul style="list-style-type: none"> Establishment alerted of accreditation renewal Confirm CAB information Agree to A2LA R102 Conditions of Accreditation Upload to CAB portal all renewal of accreditation supporting documents/information Payment 	A2LA R102 Quality Manual Quality Documents Scope of Accreditation	Full reassessment every 2 years , or when significant changes to scope have been made
2	Review all documents located on the CAB portal for accuracy and completeness. Upload/change out any documents that have been updated with the current version		

Step #	Process or Step Required	Applicable Documents/Forms	Comments/Requirements
3	Upload to CAB portal; <ul style="list-style-type: none"> Up-to-date organizational chart (name and function of key personnel) Highlight any changes since initial assessment Also provide separate UNL organizational chart (if necessary) 	Organizational Charts Management Reviews	Must update all records to include current personnel and technical capabilities, any changes must be addressed
4	Complete and upload conformity assessment checklist – this will be provided on the CAB portal	C204/C101	Checklist must be completed to demonstrate compliance
5	Upload to CAB portal; <ul style="list-style-type: none"> Quality manual (uncontrolled) Any supporting documentation from the assessor's checklist (SOPs) Accreditation Status 	R105	Assessor will also check to verify laboratory is properly referencing A2LA accreditation
6	A2LA notifies establishment of name of assessor and provides assessors' bio-sketch Renewal assessment begins (see initial assessment for process requirements)	A2LA R102 See Initial Assessment Documents/Forms	Laboratory is expected to respond to all "Initial Assessment" audit findings and deficiencies within 1 month (30 Days) from date of exit briefing and resolve all findings within 60 days If there are no deficiencies, or only minor deficiencies with sufficient objective evidence for corrections, then renewal is automatically granted Failure to correct deficiencies, or major deficiencies found, will result in withdrawal of the accreditation

Bibliography

A2LA. 2001. *A2LA Food Microbiology Program Requirements*. "AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing." The American Association for Laboratory Accreditation. <https://www.a2la.org/>.

A2LA. 2016a. *A2LA: C204 Specific Checklist - Combined ISO/IEC 17025 and Food and Pharmaceutical Testing Laboratory Accreditation Program Requirements*. The American Association for Laboratory Accreditation. <https://www.a2la.org/>.

A2LA. 2018. *A2LA: Currently Accredited Organizations*, The American Association for Laboratory Accreditation. <https://www.a2la.org/>.

A2LA. 2015a. *A2LA: I105 – Typical Steps in Preparing for the Accreditation Process*, 1-6. American Association for Laboratory Accreditation. <https://www.a2la.org/>.

A2LA. 2016b. *A2LA: R101 - General Requirements: Accreditation of ISO/IEC 17025 Laboratories*, The American Association for Laboratory Accreditation. <https://www.a2la.org/>.

A2LA. 2015b. *A2LA: R102 – Conditions for Accreditation*, 1-3, American Association for Laboratory Accreditation. <https://www.a2la.org/>.

Aggelogiannopoulos, D., E. H. Drosinos, and P. Athanasopoulos. 2007. "Implementation of a Quality Management System (QMS) According to the ISO 9000 Family in a Greek Small-Sized Winery: A Case Study." *Food Control* 18 (9): 1077-1085. doi:10.1016/j.foodcont.2006.07.010.

Allen, Lynn C. 2013. "Role of a Quality Management System in Improving Patient Safety – Laboratory Aspects." *Clinical Biochemistry* 46 (13): 1187-1193. doi:10.1016/j.clinbiochem.2013.04.028.

ANSI. "Resources: U.S. Accreditation Bodies." American National Standards Institute, accessed 4/16/2018. 2018. <https://www.standardsportal.org>.

AOAC. 2015. *AOAC International: Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals*. AOAC International.

Asanovic, Vanja, Milena Raonic, Naina Cuturic-Knezevic, Goran Vukoslavovic, Milena Popovic, and Vladan Jestrovic. 2018. *Quality System Implementation in the National Metrology Institute of Montenegro*. Vol. 2, 2387-96 Springer Berlin Heidelberg, Accred Qual Assur. 0949-1775,14320517.

Bogges, Andrew, Stephen Crump, Clint Gregory, John Young, and Glen Kessinger. 2018. "Analytical Method for Nitroaromatic Explosives in Radiologically Contaminated Soil for ISO/IEC 17025 Accreditation." *Forensic Chemistry* 7: 26-32. doi:10.1016/j.forc.2017.12.001.

Børsting, Claus, Eszter Rockenbauer, and Niels Morling. 2009. "Validation of a Single Nucleotide Polymorphism (SNP) Typing Assay with 49 SNPs for Forensic Genetic Testing in a Laboratory Accredited According to the ISO 17025 Standard." *Forensic Science International: Genetics* 4 (1): 34-42. doi:10.1016/j.fsigen.2009.04.004.

Castanheira, Isabel, Mark Roe, Susanne Westenbrink, Jayne Ireland, Anders Møller, Simonetta Salvini, Hedwig Beernaert, Marine Oseredczuk, and Maria Antónia Calhau. 2009. "Establishing Quality Management Systems for European Food Composition Databases." *Food Chemistry*, 113 (3): 776-780. doi:10.1016/j.foodchem.2008.05.091.

Chung, Kun, Geun Choi, Wanno Lee, Young Cho, and Chang Lee. 2006. "Implementation of ISO/IEC 17025 Standard in a Nuclear Analytical Laboratory: The KAERI Experience." *Accreditation and Quality Assurance*, 10 (11): 603-605. doi:10.1007/s00769-005-0060-1.

Cooper, Gail A. A., Robert Kronstrand, and Pascal Kintz. 2012. "Society of Hair Testing Guidelines for Drug Testing in Hair." *Forensic Science International*, 218 (1-3): 20-24. doi:10.1016/j.forsciint.2011.10.024.

DQS, Gulo, Sara and Rao, Ganesh. "Top 10 Challenges to Overcome for Implementing ISO 9001:2015 and ISO 14001:2015.", last modified Aug 1, accessed 4/16/2018. 2018. <https://dqsus.com>.

Elortondo, F. J. Pérez, M. Ojeda, M. Albisu, J. Salmerón, I. Etayo, and M. Molina. 2007. "Food Quality Certification: An Approach for the Development of Accredited Sensory Evaluation Methods." *Food Quality and Preference*, 18 (2): 425-439. doi:10.1016/j.foodqual.2006.05.002.

Escanciano, Carmen and María Leticia Santos-Vijande. 2014. "Reasons and Constraints to Implementing an ISO 22000 Food Safety Management System: Evidence from Spain." *Food Control*, 40: 50-57. doi:10.1016/j.foodcont.2013.11.032.

Eurolab, aisbl. 2017. *Handbook ISO/IEC 17025:2017*, 1-32. Eurolab. Belgium. <http://www.eurolab.org/documents/>.

FDA. 2011. *FDA Food Safety Modernization Act: Public Law 111-353, 111th Congress, an Act*. U.S. Government Publishing Office.

FDA. "Food and Drug Administration "Recalls, Market Withdrawals, and Safety Alerts." last modified Apr 12, 2018. accessed 4/12/2018. <https://www.fda.gov/>.

FDA. "U.S. Food & Drug Administration DHHS: "Food"." U.S. Government Publishing Office, last modified Apr 11 2018, accessed Apr 16, 2018.
<https://www.fda.gov/Food/default.htm>.

Flynn, Dan. "USDA: U.S. Foodborne Illnesses Cost More than \$15.6 Billion Annually." Food Safety News - Marler Clark, last modified Oct 9 2017, accessed Apr 16, 2018.
<http://www.foodsafetynews.com/2014/10/foodborne-illnesses-cost-usa-15-6-billion-annually/#.WtTCiIjwZPa>.

Grochau, Inês, Carlos Ferreira, Jane Ferreira, and Carla ten Caten. 2010. "Implementation of a Quality Management System in University Test Laboratories: A Brief Review and New Proposals." *Accreditation and Quality Assurance*, 15 (12): 681-689.
doi:10.1007/s00769-010-0713-6.

Grochau, Inês and Carla ten Caten. 2012. "A Process Approach to ISO/IEC 17025 in the Implementation of a Quality Management System in Testing Laboratories." *Accreditation and Quality Assurance*, 17 (5): 519-527. doi:10.1007/s00769-012-0905-3.

Guzel, Omer and Ebru Ilhan Guner. 2009. "ISO 15189 Accreditation: Requirements for Quality and Competence of Medical Laboratories, Experience of a Laboratory I." *Clinical Biochemistry*, 42 (4): 274-278. doi:10.1016/j.clinbiochem.2008.09.011.

Halevy, Avner. 2003. "The Benefits Calibration and Testing Laboratories may Gain from ISO/IEC 17025 Accreditation." *Accreditation and Quality Assurance*, 8 (6): 286-290.
doi:10.1007/s00769-003-0632-x.

Holt, John G., Noel R. Krieg, Peter H. A. Sneath, James T. Staley, and Stanley T. Williams. 2000. *Bergey's Manual of Determinative Bacteriology*, edited by William R. Hensyl, Linda E. Forlifer. 9th ed. Philadelphia, PA USA: Lippincott Williams and Wilkins, a Wolters Kluwer Company.

Honsa, Julie D. and Deborah A. McIntyre. 2003. "ISO 17025: Practical Benefits of Implementing a Quality System." *Journal of AOAC International*, 86 (5): 1038-1044.
<http://www.ncbi.nlm.nih.gov/pubmed/14632407>.

Hullihen, Karen, Verna Fitzsimmons, and Michael R. Fisch. 2009. "Establishing an ISO 17025 Compliant Laboratory at a University." *International Journal of Modern Engineering*, 10 (1): 55-64.

IEH. "IEH Laboratories and Consulting Group "Food Microbiology Testing Services - ISO/IEC 17025 Scope." IEH Laboratories and Consulting Group, accessed 4/12/2018. 2018.

ILAC. 2001. *Why Become an Accredited Laboratory*. IALC Publications, International Laboratory Accreditation Cooperation.

Intertek. "Testing and Analysis Food Services." Intertek Group plc, accessed 4/12/2018. 2018. <http://www.intertek.com/>.

ISO. 1997. *Friendship among Equals*, "Recollections from first fifty years." International Organization for Standardization. Geneva, Switzerland.

ISO. 2016. *ISO in Brief*. International Organization for Standardization. Geneva, Switzerland.

ISO. 2015. *ISO Quality: Quality Management Principles*. International Organization for Standardization. Geneva, Switzerland.

ISO. "ISO: All about ISO." International Organization for Standardization, accessed <https://www.iso.org/>, <https://www.iso.org/about-us.html>.

ISO. 2014. *ISO: Economic Benefits of Standards*. International Organization for Standardization. Geneva, Switzerland.

ISO. 2017. *ISO: ISO and Food*. International Organization for Standardization. Geneva, Switzerland.

ISO. "ISO: Popular Standards." International Organization for Standardization, accessed 4/12/2018. 2018. <https://www.iso.org/>.

ISO. "ISO: The Main Benefits of ISO Standards." International Organization for Standardization, accessed 4/12/2018. 2018.

ISO and IEC. 2005. *ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories*. 2nd ed. Geneva, Switzerland: International Organization for Standardization.

ISO and IEC. 2017. *ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories*. 3rd ed. International Organization for Standardization. Geneva, Switzerland.

Kohl, Herfried. 1998. "The New ISO 17025 - Basic Idea." *Accreditation and Quality Assurance* 3: 422-425.

Martincic, Cynthia. 1997. *IT Standards: A Brief History of ISO*. University of Pittsburgh. Pittsburgh, Pennsylvania.

Massoud, May A., Rabih Fayad, Rabih Kamleh, and Mutasem El-Fadel. 2010. "Environmental Management System (ISO 14001) Certification in Developing Countries: Challenges and Implementation Strategies." *Environmental Science and Technology*, 44 (6): 1884-1887. <http://www.ncbi.nlm.nih.gov/pubmed/20151632>.

Maynard, S., S. Foster, and D. J. Hall. 2003. "ISO 17025 Application within Racing Chemistry: A Case Study." *Technovation* 23 (9): 773-780. doi:10.1016/S0166-4972(03)00002-6.

MDT. "Discovering ISO/IEC 17025: The Challenges and Benefits." Medical Design Technology Magazine Online, Plastics Color Corporation, last modified Aug 4, <https://www.mdtmag.com/article/2016/08/discovering-iso-iec-17025-challenges-and-benefits>.

Olsen, Petter and Melania Borit. 2013. "How to Define Traceability." *Trends in Food Science & Technology*, 29 (2): 142-150. doi:10.1016/j.tifs.2012.10.003.

PJLA. "PJLA's Listing of Accredited Labs." Perry Johnson Laboratory Accreditation, Inc., accessed 4/12/2018. 2018. <http://www.pjllabs.com/>.

PJLA. 2009. *Steps to ISO/IEC 17025 Accreditation*. Perry Johnson Laboratory Accreditation, Inc. Troy, MI.

Rahmat, Suharni, Chew Boon Cheong, and Hamid, Mohd Syaiful Rizal Bin Abd. 2016. "Challenges of Developing Countries in Complying Quality and Enhancing Standards in Food Industries." *Procedia - Social and Behavioral Sciences*, 224: 445-451. doi:10.1016/j.sbspro.2016.05.418.

Rodima, Ako, Martin Vilbaste, Olev Saks, Erko Jakobson, Eve Koort, Viljar Pihl, Lilli Sooväli, et al. 2005. "ISO 17025 Quality System in a University Environment." *Accreditation and Quality Assurance*, 10 (7): 369-372. doi:10.1007/s00769-005-0011-x.

Romero, J., I. Manero, and J. Laso. 2007. "Validation of Geosmin and 2-Methyl-i-Borneol Analysis by CLSA-GC-FID Method to Obtain ISO-17025 Accreditation." *Journal of Chromatographic Science*, 45 (7): 439-446. doi:10.1093/chromsci/45.7.439.

SADCAS. 2018. *Sadcas Policy – ISO/IEC 17025:2017 Transition*, 11-16. Southern American Development Community Accreditation Service, TR14.

Sampaio, Paulo, Pedro Saraiva, and Antonio G. Rodrigues. 2009. "ISO 9001 Certification Research: Questions, Answers and Approaches." *International Journal of Quality & Reliability Management*, 26 (1): 38-58. doi:10.1108/02656710910924161.

USDA, FSIS. "Food and Nutrition: United States Department of Agriculture." U.S. Government Publishing Office, accessed Apr 12, 2018, <https://www.usda.gov/topics/food-and-nutrition>.

USDA, FSIS. "Recalls and Public Health Alerts: Current Recalls and Alerts." United States Department of Agriculture Food Safety and Inspection Service, last modified Feb 14 2018, accessed Apr 12, 2018, <https://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/current-recalls-and-alerts>.

USDA, FSIS. "United States Department of Agriculture Food Safety Inspection Service "Regulatory Compliance." U.S. Government Publishing Office, last modified Mar 24, accessed 4/12/2018. 2018. <https://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance>.

Vlachos, N. A., C. Michail, and D. Sotiropoulou. 2002. "Is ISO/IEC 17025 Accreditation a Benefit Or Hindrance to Testing Laboratories? the Greek Experience." *Journal of Food Composition and Analysis*, 15 (6): 749-757. doi:10.1006/jfca.2002.1097.

Wikipedia. "International Organization for Standardization." Wikimedia Foundation, Inc., last modified Apr 12, 2018, accessed Apr 16, 2018, https://en.wikipedia.org/wiki/International_Organization_for_Standardization.

Zapata-García, D., M. Llauradó, and G. Rauret. 2007. "Experience of Implementing ISO 17025 for the Accreditation of a University Testing Laboratory." *Accreditation and Quality Assurance*, 12 (6): 317-322. doi:10.1007/s00769-007-0274-5.

Zimon, Dominik. 2017. "The Impact of Quality Management Systems on the Effectiveness of Food Supply Chains." *TEM Journal*, 6 (4): 693-698. doi:10.18421/TEM64-07.

CHAPTER 2

**EXPERIENCE IMPLEMENTING ISO/IEC 17025 STANDARDS IN A
SMALL ACADEMIC SERVICE LABORATORY**

**“Implementation of ISO/IEC 17025 Practices for the
Food Processing Center Laboratory Services
at the University of Nebraska-Lincoln
Food Processing Center”**

INTRODUCTION TO ISO

Why Have Accredited Laboratories

Food is a fundamental aspect of life! But, what if the food is not safe to consume containing foodborne pathogens that potentially could lead to disease and possibly even death? This is a major problem faced by the food industry today with the heavy burden of providing consumers with food products that are safe to consume. Food industry establishments are also faced with the challenge of complying with ever-changing regulations that now require them to provide even more evidence that the food products they are producing are manufactured in an acceptable environment and are free of physical, chemical, and biological hazards ensuring the safety of consumers.

Regulations such as the Food Safety Modernization Act (FSMA), which was released in 2011 (FDA 2011), have been enforced over the last several years by governmental agencies such as the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) and the Food and Drug Administration (FDA) (USDA 2015; USDA 2018a; FDA 2018b). FSMA regulations require that food industry establishments provide US governmental agencies with proof that their food products are safe for consumption. These food safety regulations affect all establishments in the food industry from farmers to manufacturers and must be met prior to releasing product into commerce.

So, how do food industry establishments acquire the evidence they need to verify their products are safe for consumption? One way this may be accomplished is through submitting samples to food testing laboratories that meet a certain criteria/level of acceptability or that are accredited (FDA 2011). This helps to demonstrate that the results

food establishments are using for releasing their products into commerce are reliable and can be trusted by not only the food industry but also by governmental agencies such as the FDA or USDA-FSIS (USDA 2015; FDA 2018b).

International Organization for Standardization

One of the most respected and widely recognized organizations that establishes the criteria that must be met for food testing laboratories to perform product release testing is the International Organization for Standardization (ISO) (ISO 2016; ISO 2018a). ISO is a non-government affiliated independent non-profit organization located in Geneva, Switzerland that has member bodies in 163 countries around the world including the United States of America, Canada, Mexico, Brazil, England, China, and Australia (ISO 2016; ISO 2017; ISO 2018a). They produce internationally recognized standards to ensure that procedures and processes are performed the same regardless of geographical location and are considered a leader in international collaboration and the development of standardized methods worldwide (ISO 2017; ISO 2018a).

ISO/IEC 17025 Standard

Within the food testing industry, ISO accredited laboratories are relied upon by food producers and manufacturers to provide trustworthy results proving the food producer's products are indeed safe to release into commerce. ISO, in conjunction with the International Electrotechnical Commission (IEC), created guidelines for verifying the acceptability of food testing laboratories which is now the basis for all food testing laboratory accreditations from ISO/IEC. This standard is known as ISO/IEC 17025

General Requirements for the Competence of Testing and Calibration Laboratories (ISO and IEC 2005; ISO and IEC 2017; ISO 2018c).

The ISO/IEC 17025 standard contains all of the requirements necessary for a food testing laboratory to become ISO accredited and provides guidelines and specifications on how to properly manage a food testing facility from the time samples arrive to when the final results go to the client regardless of the company's size or how many employees they have (ISO and IEC 2005; ISO and IEC 2017; ISO 2018b). Following these guidelines also helps to ensure that no matter where you are in the world a specific test, process, material, or procedure is performed in the same way and will give approximately the same result while also being fit for its intended purpose (ISO 2017; ISO 2018c).

Some areas included in the ISO/IEC 17025 standard are: implementing a quality management system; handling of customer feedback and communicating with clients; method verification and validation; record keeping and document control; equipment management; and implementation of support programs such as environmental monitoring, temperature monitoring, and training programs (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017; ISO 2018b). All of these requirements must be met prior to any laboratory submitting for or obtaining ISO accreditation status. Implementation of these standards within a food testing laboratory allows them to demonstrate that they are competent and can produce valid acceptable results that can be trusted worldwide providing them recognition within the industry (ILAC 2001; AOAC 2015; ISO 2018b).

Benefits and Challenges of Accreditation

Implementing the ISO/IEC 17025 standard within a food testing laboratory provides structure for the laboratory to gain ISO accredited status while also affording many benefits that can be realized by the laboratory. Some of these benefits include: increase in the reliability and trust in the data being generated; minimization of errors in laboratory analyses, sample processing, and reporting of results; reduced costs; improved quality of work being performed; improved training and competency of staff; and provides recognition within the food industry (Martincic 1997; ILAC 2001; Halevy 2003; Rodima et al. 2005; ISO 2014; ISO 2016; ISO 2017; ISO 2018c).

Even though there are many benefits of obtaining ISO/IEC 17025 accreditation for food testing laboratories, not very many small or academic laboratories attempt to obtain accreditation because it is very challenging and requires a lot of resources. Implementation of ISO practices involves the development of a quality management system (QMS) and creation of documentation (Zapata-García et al. 2007; Grochau et al. 2010; Grochau and ten Caten 2012), implementation of in-house control programs such as environmental monitoring, training (Honsa and McIntyre 2003), and media qualification programs, and the verification of all in-scope methods used within the laboratory (A2LA 2001; AOAC 2015).

The main challenges associated with implementing all of the requirements to obtain ISO accredited status include the time and difficulty of developing and implementing the quality management system, documents, forms, policies, and programs to meet the requirements, and how well prepared the food testing laboratory is when they begin the accreditation process (Vlachos et al. 2002; Zapata-García et al. 2007; Hullihen

et al. 2009; MDT 2016). Obtaining accreditation status is a financially burdensome task that can take several months or even years to accomplish and requires not only management commitment but also the coordination of policies, procedures, and departments within the establishment, all while maintaining and continuing to improve the quality management system (Vlachos et al. 2002; Zapata-García et al. 2007; Hullihen et al. 2009; MDT 2016; DQS et al. 2016; Rahmat et al. 2016). This is not necessarily in the best interests of academic laboratories which are primarily focused on research and teaching activities (Zapata-García et al. 2007; Grochau et al. 2010) and should be discussed in great detail prior to attempting ISO accreditation.

Small/Academic Laboratories and Accreditation

Despite the challenges facing small (less than 10 employees) and academic laboratories in obtaining accreditation, it is possible for these laboratories to successfully implement a QMS, generate all required documentation, implement support programs, and obtain ISO/IEC 17025 accreditation. One university laboratory achieved accreditation for several methods within their Environmental Radiology Laboratory for gamma emitters in milk, honey, vegetables, and meat products (Zapata-García et al. 2007), while another was granted ISO accreditation for methods within their Nuclear Analytical Laboratory for alpha/beta emitting nucleotides and other methods (Chung et al. 2006).

A method for geosmin and 2-methyl-i-borneol analysis by closed loop stripping and gas chromatography was validated for ISO/IEC accreditation by a private institution in Spain (Romero et al. 2007). Another institution in Spain was granted ISO/IEC

accreditation for their sensory quality evaluation methods for cheeses and other food products (Elortondo et al. 2007). Other institutions around the world that have obtained ISO accreditation include the Federal University of Rio Grande do Sul in Brazil (Grochau and ten Caten 2012) and the University of Tartu in Estonia (Rodima et al. 2005).

These successes show that it is possible to obtain ISO/IEC accreditation at small and academic institutions despite some of the challenges that may exist given that these laboratories are fully committed to complying with ISO/IEC standards. However, when reviewing one of the major accreditation bodies lists of ISO/IEC 17025 accredited facilities in the United States of America (USA), it was found that only one university facility, University of Nebraska-Lincoln (UNL) Food Allergy Research and Resource Program (FARRP) laboratory was on the list (A2LA 2018). This goes to show just how few university laboratories have taken the steps to become ISO/IEC compliant to better service the food industry in the USA.

The following sections will begin by describing the objectives that the Food Processing Center Laboratory Services (FPCLS) set for preparing for ISO accreditation followed by who the FPCLS is and what role they play in the food industry, why they are obtaining ISO/IEC 17025 accreditation, and some of the benefits that the university and industry will gain from the FPCLS acquiring accreditation status. Following the discussion on the FPCLS, the processes used to implement a unique laboratory management system to serve as the backbone for the FPCLS will be reviewed. This will involve taking a more in depth look at how the documentation, such as standard operating procedures (SOPs) and forms, were developed. This discussion will be followed by a detailed look at the support programs implemented to provide stability to the laboratory

management system and facilitate the success of the laboratory in meeting all ISO/IEC 17025 requirements prior to seeking accreditation.

OBJECTIVES OF PROJECT

The Food Processing Center Laboratory Services (FPCLS) is part of the Food Processing Center (FPC) located at the Food Innovation Center (FIC) on the University of Nebraska-Lincoln (UNL) campus. The FPCLS set a goal to meet all requirements determined by ISO for obtaining accreditation to ISO/IEC 17025 standards. This was a very long and challenging process and the experiences that the FPCLS had and the processes that they followed may be used as a guide for other small and academic laboratories who wish to improve their processes or prepare for obtaining ISO accreditation status.

The long-term goal of this project is to establish and maintain a fully functioning food testing laboratory that is ISO/IEC 17025 compliant, adequately prepared to obtain and maintain ISO/IEC 17025 accreditation, and capable of providing food testing services, guidance, and training to the food industry. Along with becoming ISO compliant, the goal will be to provide professional experiences and training opportunities to FPCLS graduate students while enhancing the portfolio of the UNL Food Processing Center in the UNL Food Science and Technology Department (FDST). To achieve this long-term goal four primary objectives were addressed within this project.

Objective 1. Establish a quality management system (QMS) unique to the FPCLS that implements the proper organizational structure, policies, programs, and detailed standard operating procedures (SOPs) to govern all aspects of the FPCLS in order to meet ISO/IEC 17025 management requirements for accreditation and to integrate this

system with the UNL management system. This quality management system was developed in accordance with ISO/IEC 17025 requirements and guidelines (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017) in order to better facilitate the transition to ISO compliance when the laboratory is ready to pursue ISO/IEC 17025 accreditation. A quality manual detailing all aspects of the QMS was developed including sections for; document and record control; employee policies; purchasing services and supplies; corrective and preventive action; internal audits; handling customer feedback and communication with clients; and management reviews. The organizational and management structure/hierarchy of the FPCLS was also determined to ensure the success of the quality management system.

Objective 2. Develop a system for generating all of the forms, lists, manuals, and SOPs necessary to capture data, record laboratory functions, and guide/direct all laboratory processes and procedures. A unique naming system was developed in order to better identify all of the documents generated as part of the ISO/IEC accreditation preparation process and to allow for the tracking of different version numbers of those documents. Along with the document identification system, a unique sample identification system was generated to facilitate the tracking and traceability of laboratory samples from the point they enter the system to when the final report goes to the client. Finally, templates were generated to control the format of all of the documents within the QMS ensuring control and consistency of all documents throughout the FPCLS.

Objective 3. Generate all of the forms, lists, manuals, and SOPs necessary to meet all of the technical requirements for obtaining ISO/IEC 17025 accreditation in accordance with ISO/IEC 17025 requirements and guidelines (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). Forms were generated to capture all data from client samples and record laboratory information such as temperatures on incubators and refrigerators. All types of SOPs were generated including; equipment use and maintenance, media preparation, storage and retrieval of culture stocks, laboratory

cleaning and housekeeping procedures, and test methods for performing sample analyses for foodborne pathogens.

Objective 4. Develop and implement all necessary support programs that will help to aid in the implementation of the quality management system and demonstrate that all processes and procedures in the FPCLS are working correctly. All programs were developed in accordance with ISO/IEC 17025 requirements and guidelines (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). Support programs that were developed include; environmental monitoring, temperature monitoring, and training programs. All programs were implemented in support of the FPCLS laboratory management system and will be utilized to ensure that the FPCLSs testing facility is adequate for its intended purpose of testing client samples.

Note: All SOPs, forms, lists, manuals, and programs that were developed for the FPCLS were created with the intention of pursuing ISO/IEC 17025 accreditation and therefore were generated in a fashion that would make them ISO/IEC 17025 compliant. All documentation, procedures, and forms generated throughout this process were put into place and are currently being utilized by the FPCLS to improve the laboratory and meet ISO/IEC 17025 requirements in order for the laboratory to be ISO compliant prior to submitting for accreditation. The FPCLS has not scheduled its initial assessment with the accrediting body and is currently continuing to improve its QMS but intends to submit for accreditation in the near future.

FOOD PROCESSING CENTER LABORATORY SERVICES OVERVIEW

The Food Processing Center and FPC-Laboratory Services

As part of the Institute of Agriculture and Natural Resources (IANR) at the University of Nebraska-Lincoln (UNL), the Food Processing Center (FPC) has played a major role as a leader in bridging the gap between academia and the food industry and has been a destination for food processing and applied research (FPC 2018). Established in 1983, the FPC has grown into a “*multi-disciplinary resource*” that provides

“consulting, educational, technical, and business development services” to the food industry for all types of products such as “grains...fruits, vegetables, dairy products,” and meat products (FPC and Flores 2015; FPC 2018). Some of these services include; applied and engineering research, labeling and regulatory compliance, pilot plant services for product development, and sensory analysis (FPC and Flores 2015; FPC 2018).

As part of the FPC, laboratory services are also offered for analyzing food products. This group is known as the Food Processing Center Laboratory Services or FPCLS (FPC and Flores 2015; FPC 2018). Located on Innovation Campus at UNL, the FPCLS is dedicated to performing analysis procedures for the presence of microorganism including aerobic plate count, anaerobic plate count, lactic acid plate count, yeast and mold, and probiotic testing (FPC 2018). The FPCLS also tests for foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*. Other test procedures offered include pH, water activity, water analysis, mycotoxin analysis, and many others (FPC 2018) for all food types.

Additionally, the FPCLS provides other services to the food industry such as performing complex research projects and product validation studies, shelf life testing, and workshops to help educate the food industry on important aspects of food safety (FPC 2018). All of these capabilities make the FPCLS a good option for food industry establishments looking for the services they need to improve the safety of their products while at the same time getting expert advice from the staff at the Food Processing Center to address their food safety issues and concerns.

Why Attempt ISO Accreditation and Gaps in Industry

Since the FPCLS has so many food testing capabilities and is already working closely with the food industry on many different projects to help improve the safety of food products, the next logical step is to pursue ISO/IEC 17025 accreditation so that they can provide an even greater level of service, guidance, consulting, and training to the food industry. Food recalls due to the presence of pathogenic microorganisms are occurring all too often. The Springfield Smoked Fish Company initiated a recall in 2017 for pre-sliced salmon due to the presence of *Listeria monocytogenes*, while another company had to recall Organic Amaranth Flour due to *Salmonella* spp. contamination (FDA 2018a).

Other recalls in 2018 included SMI Holdings recalling 484,400 lbs. of boneless beef top sirloin due to potential *Salmonella* spp. contamination, and Olli Salumeria Americana being recalling several pepperoni, chorizo, and other salami products due to *Listeria monocytogenes* contamination (USDA 2018b). Recalls such as these justify the importance of having laboratories that can be relied upon not only for product analysis services, but advice and guidance when food safety issues arise.

There are also other reasons why becoming ISO/IEC 17025 accredited is a growing need for the UNL-FPCLS. Small and very small food establishments are not always aware of the regulations and guidelines that they must follow to produce safe food products, and there are many of these companies in the Midwestern United States near UNL. University laboratories are accustomed to working with small processors and helping them design and develop their processing parameters to meet current standards and guidelines (Rodima et al. 2005). The FPCLS at UNL is no different. ISO stated that

“All players in the food supply chain, be they farmers, manufacturers or retailers, can benefit from the guidelines and best practice contained in ISO standards” (ISO 2017).

By becoming ISO/IEC 17025 accredited the FCPLS would be able to better serve these establishments by providing them an avenue for evaluating not only their environmental and quality samples but also meeting their product release sample testing needs. Additionally, by partnering with the FPC food establishments would have access to consulting on food safety issues, advice on product production parameters and HACCP development, sanitation program improvement, and many other areas where they may need help to improve their food safety systems.

The FPCLS would also like to grow and improve, becoming a leader in the food industry by providing guidance to not only food industry manufacturers, but also to other small food testing facilities and academic institutions. Through obtaining ISO/IEC 17025 accreditation they will be more capable of providing other establishments with the help they need to improve their processes, programs, and methods in providing more reliable research and laboratory service data. Many of the UNL-FPCLS procedures and practices being developed including the quality management system structure, support programs, and other standard practices/procedures may serve as a guide for other academic institutions or small laboratories. By following procedures established by the FPCLS other laboratories may meet the expectations of obtaining ISO/IEC 17025 accreditation or simply improve their processes and procedures to better serve the food industry.

Benefits to ISO Accreditation for FPCLS

This project will have an immediate impact not only on the UNL-FPCLS but also the UNL Food Science Department and the food industry in the Midwestern United States. In the Midwest, the food industry would benefit in multiple ways if the FPCLS were to add accredited testing capabilities to its list of services. The FPCLS would be able to not only test client products, as mentioned previously, ensuring food safety has been achieved and that the food is safe for commerce reducing potential recalls, but also better serve small and very small food companies by understanding all guidelines and industry standards while continuing to build lasting relationships (Halevy 2003), thus helping to improve the food industry as a whole.

Additionally, research and other academic facilities would also benefit from these practices and could utilize the processes put into place by the FPCLS for ISO/IEC accreditation as a guide for improvement to enhance their laboratory capabilities and the reliability of their data regardless of whether they are trying to obtain ISO/IEC accreditation or not.

Multiple benefits will also be realized by the FPCLS due to the goals of this project. Implementing ISO/IEC 17025 standards would improve the quality of work being performed and results being obtained, improve training and competency of staff, and increase the reliability and trust in the data being generated by the FPCLS (ILAC 2001; Halevy 2003; Rodima et al. 2005; ISO 2018c). Additionally, implementing ISO/IEC 17025 practices and guidelines would provide necessary structure to the laboratory for growth and improvement (Kohl 1998; Honsa and McIntyre 2003).

Finally, implementing ISO/IEC standards and becoming accredited would provide the FPCLS with international recognition (ILAC 2001) that the laboratory is a reliable trustworthy option strengthening relationships with the food industry, other academic institutions, and other laboratories. This would further solidify the FPCLS as a main source of knowledge and testing services that is trusted and relied upon for industry improvement and growth in the Midwestern United States.

Initial ISO Compliance Preparation Steps – Before You Begin

In order for the FPCLS to adequately prepare for obtaining ISO/IEC 17025 accreditation a plan of action had to be initiated before developing or implementing a quality management system or other documents and support programs. First, a time table was generated to help guide in the preparation process and keep the process on task. The timetable developed for completing all steps in preparation for ISO/IEC 17025 accreditation was designed to incorporate all accreditation requirements according to the chosen accreditation body A2LA (A2LA 2015; A2LA 2016b) and can be found in the tables section at the end of the chapter – Table 2.T1.

Next, before beginning the preparation process, it was necessary to determine all parts of the ISO/IEC 17025 standard that needed to be addressed within the FPCLS for accreditation and what needed to be done to meet those requirements. To accomplish this and provide a guide in preparing for accreditation, a table was generated consisting of all of the sections from the ISO/IEC 17025 standard checklist (AOAC 2015; A2LA 2016a) for the ISO/IEC 17025:2005 standard (ISO and IEC 2005) available from the accrediting body. Then all necessary information, forms, policies, and procedures that needed to be

developed or implemented to meet the requirements in the standard were inserted into the table – see Table 2.T2.

These two aforementioned tables were vital in the success of the FPCLS in preparing to begin the accreditation process, may serve as a guide to other establishments who wish to know what is required to obtain accreditation, and may also be useful as internal audit tools when preparing for accreditation. Once these two tasks were completed, the process of developing/implementing all policies, procedures, and programs necessary to meet ISO accreditation requirements for obtaining ISO/IEC 17025 accreditation status were initiated.

FPCLS STRUCTURE AND MANAGEMENT SYSTEM

To be successful in obtaining ISO accreditation, the FPCLS had to develop a unique fully functional quality management system (QMS) that not only met all of the requirements to the ISO/IEC 17025 standard but also the needs of the FPCLS. Developing and implementing a QMS can be very challenging but is the most important part of preparing for ISO accreditation as it encompasses all functions and operations within the laboratory.

Quality management systems are defined as “*a collection of business processes focused on...meeting customer requirements*” that are “*aligned with an organization's purpose and strategic direction*” (Wikipedia 2018). They can be further defined as “*the quality, administrative, and technical systems that govern the operations of a laboratory*” (AOAC 2015) and “*the organizational structure, responsibilities, procedures, processes and resources for implementing quality management*” (A2LA 2001). With these

definitions in mind, the FPCLS developed and implemented a QMS that met its needs as well as the ISO accreditation requirements defined by the ISO/IEC 17025 standard (ISO and IEC 2005). The following sections will detail the overall structure of the FPCLSs quality management system and review some of the policies and procedures that were implemented as part of the development process.

Management Structure and Organization

As part of developing a QMS it is important to understand the management structure within the laboratory. It is required that any laboratory attempting to obtain accreditation must be able to be held “legally responsible” for the services it provides and have an organizational chart detailing which individuals are responsible for laboratory activities (AOAC 2015). Academic institutions have an interesting management layout that is unique to universities as there are many layers to the overall management structure. Not only do you have the management structure of the laboratory attempting accreditation, but also the department the laboratory belongs to, institution the department belongs to, and the University the institution belongs to with each layer containing another level of management. Within the UNL-FPCLS this is no different which can be seen in Figure 2.1 below.

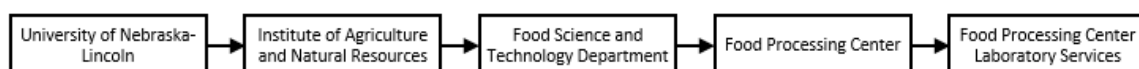


Figure 2.1: Overall Management Structure at the University of Nebraska-Lincoln. Figure shows the management structure at UNL. Only the overall hierarchy is shown not the titles or positions of the individuals responsible for those areas.

However, to meet ISO accreditation requirements it is only necessary to show the immediate management structure containing the individuals who are responsible for managing and maintaining the laboratory. The FPCLSs management structure includes the department head of the Food Science and Technology Department (director of the FPC), director of the FPCLS, technical management, and laboratory personnel (Figure 2.2). The overall departmental organization chart for the FPC can be seen in the figures section – Figure 2.F1. All of these individuals play an important role in the ability of the laboratory to function appropriately and help maintain the QMS on a daily basis. It is always important to understand which individuals are responsible and determine what roles they play before developing a management system to verify that all parts of the QMS are properly implemented and maintained during operation.

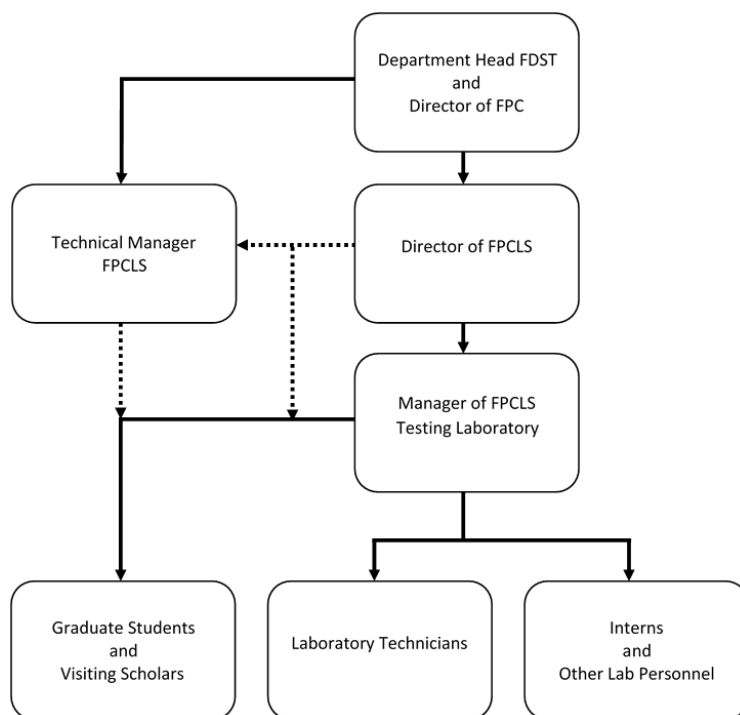


Figure 2.2: General Management Structure of Food Processing Center Laboratory Services. Figure shows the general management structure with position titles for Food Science and Technology Department (FDST), Food Processing Center (FPC), and Food Processing Center Laboratory Services (FPCLS). Names of responsible individuals have been omitted. Solid lines represent direct reports while dashed lines represent authority over, but not directly managing for laboratory purposes.

Management System Support

Along with understanding the structure of the organization and ensuring that each part of the system has individuals responsible for maintaining it, there are many other parts of developing a quality management system and ensuring that the laboratory has all of the policies and procedures necessary for success that must be discussed. These “management” and “technical” requirements as directed by the ISO/IEC 17025 standard include developing and implementing policies and procedures for: document and record control, reviewing of contracts, laboratory improvement, corrective/preventive actions,

personnel, equipment, handling of samples, and many more areas within the organization (ISO and IEC 2005; AOAC 2015). Table 2.1 contains a list of management and technical requirements that must be addressed by any laboratory prior to attempting ISO accreditation. The following paragraphs will discuss some of the areas that were addressed and the policies and procedures that were implemented in the FPCLS to support the quality management system.

Table 2.1: ISO/IEC 17025 Management and Technical Requirements. Table displays a list of “management” and “technical” requirements that must be addressed to obtain ISO/IEC 17025 accreditation for testing and calibration laboratories (ISO and IEC 2005; AOAC 2015).

Management Requirements	Technical Requirements
Organization	General Requirements
Management System	Personnel
Document Control	Accommodation and Environmental Conditions
Review of Requests, Tenders, and Contracts	Test and Calibration Methods and Method Validation
Subcontracting of Tests and Calibrations	Equipment
Purchasing Services and Supplies	Measurement Traceability
Service to the Customer	Sampling
Complaints	Handling of Test and Calibration Items
Control of Nonconforming Testing and/or Calibration Work	Ensuring the Quality of Test and Calibration Results
Improvement	Reporting the Results
Corrective Action	Appendices
Preventive Action	Appendix A: Equipment
Control of Records	Appendix B: Microbiology
Internal Audits	Appendix C: Chemistry
Management Reviews	Appendix D: Pharmaceutical Analysis and Legal Standards
	Appendix E: Legal Samples

DEVELOPING A MANAGEMENT SYSTEM: MANAGEMENT AND TECHNICAL REQUIREMENTS

Documentation and Document Control

Any good management system has proper document and record control policies. The FPCLS developed policies on the control of documents and records as part of the preparation process for obtaining ISO accreditation. As part of these standard operating procedures (SOP) the FPCLS put into place requirements that all documents and records are to be formatted in the same manner, have a unique identification number, are tracked for changes, and are secure. So, how does any establishment accomplish all these requirements to meet the standard? The next few paragraphs discuss how the FPCLS accomplished these requirements in the process of achieving a well-defined and controlled management system.

Developing FPCLS Forms

When developing a management system to meet all management and technical requirements for ISO/IEC 17025 accreditation it is important that the laboratory's system have structure within its controlling documentation. One way that this task may be accomplished is through the use of document templates. Templates provide the necessary structure to all forms, lists, and other types of documents to demonstrate that there is continuity throughout the system. If templates are not utilized then each document generated could have a completely different format making them hard to use, maintain, and update as well as making the system as a whole less stable and lacking control.

The FPCLSs templates for forms and lists are very basic but still provide the structure necessary to show control over the system and allow for the ease in generating

new documents, reviewing documents for changes, and maintaining the document control system. FPCLS “form” templates were generated for both Microsoft® Word and Excel programs in both portrait and landscape styles containing the same basic elements which can be seen in Table 2.2 and in Figures 2.3 and 2.4.

Table 2.2: FPCLS Template Requirements. Table shows the template requirements established for use on forms, lists, logs, etc. by the UNL-FPCLS.

-
1. Header
 - a. Left – University Name, Laboratory Name, Location
 - b. Middle – Unique ID Number
 - c. Right – Page X of X
 2. Body
 3. Footer
 - a. Left – Authorizing Individual
 - b. Middle – Controlled Copy
 - c. Right – Approval Date
-

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Name of Form XXX

Figure 2.3: FPCLS Template Header Example. Figure shows example displaying a header template for a form, list, log, and other documentation utilized by the FPCLS at UNL.

Authorized By: Laboratory Manager	Controlled Copy	Approval Date: MM/DD/YYYY
-----------------------------------	-----------------	---------------------------

Figure 2.4: FPCLS Template Footer Example. Figure shows example displaying a footer template for a form, list, log, and other documentation utilized by the FPCLS at UNL, date is in month/day/year format.

Along with having all of the same basic elements, each template is preformatted to include specific font styles, font sizes, margin requirements, etc. This allows for further structure within the system making for easier development and review of documents at the FPCLS. To ensure that these templates are utilized, and all formatting requirements are met, an SOP for the Control of Documents was generated to implement these requirements and provide guidance on where to get the templates and how to use them. As part of the preparation process for ISO/IEC 17025 accreditation the FPCLS generated forms, lists, manuals, and other documentation to meet all of the requirements for controlling documents in the ISO standard. Examples of these documents include:

1. Data record forms for sample data collection including; general enumeration, Burkholderia chamber, pathogen isolation, pathogen screen testing, etc.
2. Laboratory forms for all types of processes including; laboratory analysis reports, sterile batch records, culture access logs, balance verification logs, temperature monitoring, environmental monitoring, measuring device verification, pH and conductivity measurement, media preparation, etc.
3. Management forms to document QMS processes including; corrective/preventive action and root cause analysis, client satisfaction surveys, personnel signature and initials forms, employee access logs, technician and trainer evaluation sheets, approved technician/trainer lists, employee position descriptions, etc.
4. Training forms for evaluating competency of staff including; personnel training form (generic), good laboratory practices, introductory, core competency, quality assurance competency modules, test method training modules (in-scope methods), etc.
5. Quality assurance auditing forms including internal audit checklist and report forms for all ISO/IEC management and technical requirement sections

Not all forms, lists, and logs that were generated for use in the FPCLS are listed here. Many forms, lists, and other documentation types are needed to meet all of the requirements for ISO/IEC 17025 accreditation.

Developing FPCLS Standard Operating Procedures

As discussed previously with forms, templates are extremely valuable in providing structure to these documents, as well as lists, and other types of documentation. This statement is also true for standard operating procedures. These procedures help provide the guidance and structure necessary for any management system to be successful. Without SOPs there would be no way to verify that each task within the organization whether it is storing files, reviewing employee performance, or performing sample analyses for pathogenic microorganisms, is being done correctly or even the same way each time by different individuals creating an unstable management system.

At the FPCLS, standard operating procedures are utilized and contain information, policies, and step by step instructions to prepare a document, receive supplies or samples, or even perform testing procedures ensuring each task is performed the same way each time. This level of control is absolutely necessary and allows the FPCLS to have confidence that all of its processes are performed correctly and that any results or data generated from the laboratory are of high quality and can be trusted which provides further confidence to its cliental.

Since standard operating procedures are so important to the success of the management system, each SOP should be formatted to provide the structure necessary to show control over the system and allow for the ease in generating new documents, reviewing documents for changes, training employees, providing structured guidance to

all processes/personnel, and maintaining the document control system. FPCLS standard operating procedure templates were generated in Microsoft® Word containing the elements which can be seen in Table 2.3. For a visual example of these elements refer to the figures section at the end of the chapter – Figure 2.F2.

Along with having all of the needed elements, the SOP template includes specific font styles, font sizes, margin requirements, etc. to ensure that all SOPs are generated in the same way and that they contain all the information required to provide guidance to the FPCLS staff. Some of these further formatting requirements can be seen in Table 2.4, while the section requirements can be found in Table 2.T3 in the tables section at the end of the chapter.

Table 2.3: FPCLS Template Requirements for SOPs. Table shows SOP template title page and structural requirements established for use by the UNL-FPCLS.

-
1. Header
 - a. Left – University Name, Laboratory Name, Location
 - b. Right – Unique ID Number
 2. SOP Title Page
 - a. University or Company Logo
 - b. Name of Institution and Department
 - c. Unique SOP # and Revision #
 - d. Date Last Reviewed MM/DD/YYYY and Effective Date
MM/DD/YYYY
 - e. SOP Title
 - f. Authorizing Signatures
 - i. Author: Name, Title, Signature, Date MM/DD/YYYY
 - ii. Reviewer: Name, Title, Signature, Date MM/DD/YYYY
 - iii. Approver: Name, Title, Signature, Date MM/DD/YYYY
 3. Body Sections
 - a. Purpose
 - b. Scope
 - c. Prerequisite Documents
 - d. Responsibilities
 - e. Materials and Media
 - f. Equipment
 - g. Definitions
 - h. Procedures
 - i. References
 - j. Revisions – Table
 - i. Revision Date, Changes Made, Approved by Initials, and
Supersedes Version Number (##)
 4. Footer
 - a. Left – Controlled Copy
 - b. Right – Page X of X
-

Note: Not all SOP sections are contained within each SOP. Sections are determined based on the type of SOP being generated and its purpose. e.g. SOP for conflict of interest policies would not have an equipment section whereas a SOP for analyzing client samples for pathogenic microorganisms by Qualicon BAX® would have an equipment section.

Table 2.4: FPCLS SOP Template Formatting Requirements. Table shows SOP template formatting requirements established for use by the UNL-FPCLS.

-
1. Fonts – Arial or Calibri
 2. Font Size – 10-12 pt. font
 3. Document Justification – Left or Block
 4. Section Numbers
 - a. Use only number designations such as 1., 1.1., 1.1.1., 1.1.1.1., etc.
 5. Section Headers – **Bold** and Underlined
 6. Sub-Headers – **Bold**
 7. Notes within Document – *Italicized*
 - a. Should be accompanied by Δ symbol (in front of note)
 8. Margins and Spacing
 - a. Document Margins – standard 1” all (sides)
 - b. List Indent Alignment of Section Levels (1.1, 1.1.1, etc.)
 - i. Level 1 (1.) – Align 0”, Text Indent 0.25”
 - ii. Level 2 (1.1.) – Align 0.25”, Text Indent 0.65”
 - iii. Level 3 (1.1.1.) – Align 0.65”, Text Indent 1.15”
 - iv. Level 4 (1.1.1.1.) – Align 1.15”, Text Indent 1.75”
 - v. Level 5 (1.1.1.1.1.) – Align 1.75”, Text Indent 2.5”
-

It might not seem necessary to control all of these formatting elements but doing so allows for further structure within the document control system making for easier development and review of SOPs at the FPCLS. To ensure that the SOP template is utilized and that all formatting requirements are met, SOPs for the Writing of Standard Operating Procedures and Control of Documents were generated to implement these requirements and provide guidance on where to find the template and how to properly prepare an SOP for use in the FPCLS. Each SOP contains all sections necessary to provide the important relevant information to review and direct policies, perform laboratory tasks, or conduct testing procedures – see Table 2.T3 in the tables section at the end of the chapter for SOP section details.

As part of the preparation process for ISO/IEC 17025 accreditation the FPCLS generated many SOPs to meet all of the requirements in the ISO standard for controlling

documents and providing support to procedures and personnel. Examples of the SOPs generated for use in the FPCLS are:

1. Equipment SOPs for use operation and maintenance including; general microscopy, operation and maintenance of BioMérieux miniVIDAS® systems, biosafety cabinets, autoclave usage and maintenance, reference standards and materials, calibration of pipettors, balances and working weights usage and maintenance, calibration of pH meters, etc.
2. Laboratory operation SOPs including; media preparation, cleaning glassware and dishwasher use, pipetting methods, measuring device verification, environmental monitoring, temperature monitoring, eyewash checks, housekeeping schedule, etc.
3. Test method SOPs detailing step by step testing instructions for; general enumeration, determination of foodborne pathogens by BAX® PCR, Burkholderia chamber, determination of *E. coli* O157:H7 in food and sponge samples, pathogen screen by miniVIDAS®, API proficiency testing, etc.
4. Quality assurance SOPs to meet accreditation management requirements including; protection of confidential information, conflict of interest and impartial services, control of documents, purchasing services and supplies, corrective and preventive action, internal audits, laboratory safety, training program, management reviews, etc.

Not all SOPs generated for use in the FPCLS are listed here. Many SOPs and other documents are needed to meet all of the requirements for ISO/IEC 17025 accreditation.

Standard Operating Procedures are vital to the success of any laboratory as they provide necessary structure and guidance to all staff members within the organization on how to properly perform all process, procedures, and how to conduct policies determined by the organization. The FPCLS has greatly benefited from the use of SOPs by seeing increased stability within the management system and more reliability in results being

generated by the technical staff. Utilizing a template for the generation of SOPs has also benefited the FPCLS by making it easier to train personnel and allowing for easier updating of the SOPs when errors are found or new processes are added. Without SOPs, the FPCLS would not be capable of pursuing ISO/IEC 17025 accreditation and would not be able to benefit from the structure that these documents have provided.

Document Identification System

Having templates to make sure all formatting is the same is extremely valuable, but it is also important to be able to determine what documents are being utilized and be able to locate them quickly. This was accomplished by the FPCLS through giving each document a unique identification number (ID). Document identification numbers were determined using a system that works best for the FPCLS and meets the needs of its staff. These unique identifiers account for the type of document being generated, the number the document is in the FCPLS document master list, and the version number of the document to better aid in locating, referencing, and utilizing them for FPCLS purposes. A list of different document types can be found in Table 2.5.

To generate unique identification numbers for each document within the FPCLS, a Control of Documents SOP was put into place that details all of the steps necessary to provide each new document a unique ID. To provide a document with a unique ID it is first determined what function the document will have in the FPCLS management system. This involves deciding if it is a (1) quality assurance document (QA), such as management forms, training documents, internal audit forms, or data record forms; (2) Food Processing Center document (FPC) such as master lists, inventories, protocols,

manuals, or the quality manual; or (3) a standard operating procedure (SOP) for equipment, laboratory procedures, test methods, or quality assurance. Based on these categories the first portion of the unique identifier is selected utilizing either a 2 or 3 letter function code (FPC, SOP, QA) followed by a 2-3 letter code for the document type such as QLM, LIT, PRO, EQ, LP, TM, QA, FRM, TRN, IAF, and DRF depending on the processes or procedures it most closely aligns with (Table 2.5).

After the letter codes have been established then the document can receive its unique 3 number code (XXX) and its appropriate 2 number version code (V-XX) depending on if it is a new document or a revised document. For example, a form generated for use in taking temperatures on a refrigerator might have the FPCLS unique identifier of QA-FRM-025-V-01 while an SOP created for performing maintenance on the autoclaves might have a FPCLS unique identifier of SOP-EQ-025-V-01. To better understand the unique identification system the example of QA-FRM-025-V-01 can be broken down into descriptive parts: QA (Quality Assurance Document), FRM (Form), 025 (25th form on the master list), V (version), and 01 (current version number).

This unique document identification system allows the FPCLS to easily identify which type of document they are using during all operations within the laboratory. Additionally, having these unique identifiers allows the FPCLS to easily locate the document within the master document list and allows easy retrieval of all SOPs or forms from their secure storage locations on UNL BOX, which is only accessible by FPCLS staff, as they are kept in numerical order in folders by function and type.

Table 2.5: FPCLS Unique Document Identifiers. Table shows unique document identifiers and types utilized by the ULN-FPCLS for forms, lists, manuals, SOPs, etc.

Document Identifier	Document Type
FPC-QLM	Quality Manual
FPC-LIT	Lists, Manuals, and Inventories
FPC-PRO	Protocols for client procedures
SOP-EQ	Standard Operating Procedure for Equipment
SOP-LP	Standard Operating Procedure for Laboratory Procedures
SOP-TM	Standard Operating Procedure for Testing Methods
SOP-QA	Standard Operating Procedure for Quality Assurance Processes
QA-FRM	Laboratory Forms
QA-TRN	Training Documents and Forms
QA-IAF	Internal Audit Forms and Documentation
QA-DRF	Data Record Forms

Traceability of Samples

Having unique identification numbers for traceability purposes is not only important for documentation but is also valuable and necessary for client samples. If a laboratory does not have a way to track client samples throughout the testing process then they will not be able to provide evidence that the sample was tested according to ISO/IEC 17025 standards and that the result is acceptable and can be trusted. The FPCLS sample identification system allows for the traceability of client samples from the point that they are received until they are disposed of after testing or retention. This allows the FPCLS to properly manage all client samples and helps to better identify issues with analyses or results when they arise which further assists in providing good service to the client.

To implement a sample tracking system, the laboratory needs to recognize what is required to track its samples based on the type of samples received, sample load, and laboratory capabilities. The FPCLS for instance, being a smaller laboratory, does not currently have a need for a laboratory information management system (LIMS) or other automated sample tracking and data management software. Therefore, the FPCLS

implemented a paper-based tracking system that fits its needs but still meets the requirements set forth in the ISO standard. Some of the basic aspects of the FPCLS sample tracking system allowing for the traceability of client samples will be discussed in further details including receiving, processing, and reporting.

First, samples are received by the shipping and receiving department at the UNL-FDST Food Processing Center and an email notification is sent to the laboratory that they have arrived (some samples may also be hand delivered to the laboratory by the client). FPCLS staff members retrieve the samples, transport them to the laboratory, and enter them into the laboratory's sample system. Samples are received with a FPCLS Sample Submittal form that has been previously filled out by the client detailing the sample information (ID, type, lot etc.), client information (name, address, contact information, etc.) and tests being requested for the samples (pathogen screen, coliforms, general enumeration, pH, etc.).

Sample submittal forms are vital to the sample system as they detail all of the important information related to the sample(s) and what analyses need to be performed for the client. On the FPCLS sample submittal form there is a column for the staff member to assign a laboratory identification number (001-XXX) to each sample which is next to the sample ID (Figure 2.5). This begins the tracking process as this number will be associated with the sample for the remainder of the analyses and reporting procedures.


UNL-FPC Laboratory Services Sample Submittal Form				 University of Nebraska - Lincoln The Food Processing Center Laboratory Services Institute of Agriculture and Natural Resources												
Attention: Lab Manager				Send Report To:					Date Submitted:							
Address				Company Name and Address:					Bill To Name and Address: (If Different)							
Address																
City, State, Zip Code				Phone Number:					Copy Report To: (Email)							
Phone: (XXX) XXX-XXXX				Email:												
Email: samplesubmission@unl.edu																
<div style="border: 1px solid black; padding: 5px;"> Lab Use Only Date Received: _____ Job Entry #: _____ </div>				Analysis Requested - Please Mark Boxes for All Testing Needed												
				General Tests				Pathogen Tests				Packages		Other		
Lab Use Only	Sample ID	Lot # or Description		Aerobic Plate Count	Coliforms Gen. & coli	Lactic Acid Bacteria Count	Yeast & Mold	Bacillus cereus	E. coli O157H7	Listeria species	Salmonella	Staph. aureus	Enumeration Package	Food Screen	pH	Water Activity

Figure 2.5: FPCLS Sample Submittal Form Example. Figure displays example of a FPCLS Sample Submittal form that is provided to the FPCLS by the client with their samples.

After review of the sample submittal form, client sample information is added to the Sample Submission Logbook. At this point the samples are given a job entry number (JE-XXX) that will be associated with those samples throughout the testing process. The combination of the job entry number and the assigned identification number from the submittal form make up the unique laboratory sample ID used for tracking the sample on all data record forms and for reporting results. An example of a sample ID number might be JE-001-001 which indicates that this is the first job entry and the first sample within that job entry.

These ID numbers are never duplicated as job entry numbers are sequential increased with each new sample submission to the laboratory and therefore are unique to these samples not just during analysis but for the existence of the system. In cases where samples must be retested a letter code system is used (B, C, D...) indicating that the sample has already been evaluated (letter code not required on original sample).

Finally, after analysis is completed a final report is compiled and it is important that the correct results be provided to the client. Sample IDs (job entry/sample number) are used to identify the samples and their results for the final report. Final reports also receive a unique ID number that is used to store the final results in the FPCLS data archive. FPCLS unique report numbers consist of a 2-digit year code (YY), job entry number (JE-XXX), a unique 3 letter client code (AAA) representing the client name, and finally a 4-number month/day code (MMDD) representing the date the samples were submitted for testing to ensure that each report is unique and that no report identification number is ever repeated.

An example of a unique FPCLS report ID might look like 18-JE-025-FPC-0101. Each unique sample ID is then captured along with its corresponding data/results on the report. Having unique report and sample IDs allows the FPCLS to easily track sample data throughout the testing process, identify issues when they arise, ensure that the correct information and results are being sent to each client, and allow the FPCLS to easily retrieve results from secure storage if it becomes necessary for audits or other purposes.

Management System Support Programs

Laboratory management systems are not successful on their own and require additional support programs in order to function correctly and verify that the laboratory is meeting ISO/IEC 17025 accreditation requirements. In order to implement a fully functioning management system the FPCLS developed several support programs to meet all of the ISO standard requirements. These programs are vital to the success of the

laboratory and provide evidence that the FPCLS is capable of performing sample analysis according to ISO practices and that the results being generated by the laboratory are of high quality and can be trusted.

Since these programs are so vital to the success of any laboratory, it is important to understand what support programs have been implemented to give a better understanding of how to meet ISO requirements and be ISO/IEC 17025 compliant for obtaining accreditation. The support programs that were implemented by the FPCLS that will be discussed include: Equipment Maintenance and Calibration, Environmental Monitoring, Temperature Monitoring, Proficiency Testing, Training, and Microbiological Media Control programs. An overview of what laboratory functions are covered within each of the FPCLS support programs is provided:

UNL-FPCLS Support Programs

1. *Equipment Maintenance and Calibration Program* – Equipment is extremely important to the success of any laboratory and any testing procedure. If the equipment being utilized is not fit for its intended purpose or is not properly calibrated for use then the results of the analysis might be impacted greatly. FPCLS procedures help to make sure that all equipment is installed correctly, performs to an acceptable level, properly calibrated, maintained through its use, and even retired appropriately when it is no longer needed.

To further aid in ensuring the acceptability of all FPCLS equipment, an Equipment Maintenance and Calibration Schedule is in place to help guide the FPCLS as to when calibration and cleaning of equipment is to take place. Additionally, a Laboratory Equipment Maintenance Log captures all of the maintenance procedures to verify that they have occurred allowing the FPCLS to verify that no equipment maintenance or calibration events are missed. Finally, to make sure that all equipment is utilized correctly, SOPs are in place for all major

pieces of equipment detailing how to properly operate and maintain them, ensuring that they are used correctly during laboratory operations.

2. *Environmental Monitoring Program* – Laboratory cleanliness is essential to all laboratory operations and test procedures. If the laboratory is not suitable for analyzing client samples then there is no way to know if a failing result is due to a client sample or from contamination from the testing environment. The FPCLS has an environmental monitoring program that helps to verify that the laboratory is fit to perform sample analysis. Procedures are in place that determine what types of locations are monitored and what constitutes a passing or failing mark for bacterial load. The laboratory is separated into testing zones and swab/settle plates are used to monitor the testing environment.

All data generated from the environmental monitoring program is captured on data record forms and treated just like a client sample being given a sample ID with the final results being reviewed and stored in archives. In order to make sure that the same laboratory locations are not tested from month to month, and to capture when monitoring activities are performed, an Environmental Monitoring Log is utilized by the FPCLS. Environmental monitoring is a key program that provides the laboratory with additional proof that client samples were tested correctly meeting ISO requirements.

3. *Temperature Monitoring Program* – Monitoring the temperature of all areas that might affect client samples is crucial to the success of all laboratory procedures. If the temperature of sample storage is excessively warm or cold it could dramatically affect the results seen during analysis. The FPCLS temperature monitoring program consists of monitoring activities for all incubators and refrigerators as well as the testing environment to demonstrate that all samples and testing supplies are stored or utilized in an appropriately controlled environment.

To facilitate the temperature monitoring program SOPs are in place that determine what should be monitored, how often monitoring takes place (typically

twice each day), and what the acceptable temperature ranges are for all equipment or areas being checked. The FPCLS only uses calibrated thermometers for performing temperature monitoring activities that have been calibrated externally and verified internally using Thermometer Verification Forms to ensure the accuracy of each reading. To capture monitoring activities, the FPCLS uses a Temperature Monitoring Log which is reviewed for temperature fluctuations or deviations allowing the laboratory to make the appropriate adjustments to maintain the temperature of its' test and storage environments.

4. *Proficiency Testing Program* – Verifying that all employees of the FPCLS are capable of performing sample testing to an acceptable level is critical in providing the client with results that can be trusted. Each FPCLS employee undergoes proficiency training for the testing procedures they perform. Although there are several ways this may be accomplished, one way the FPCLS accomplishes proficiency training is by using American Proficiency Institute (API) samples to verify the competency of the staff members on different procedures and laboratory techniques.

Even though using an outside provider is not required, it makes proving proficiency of each technician much easier since an outside sample is evaluated by each technician and API evaluates the results providing an independent review and report. Having technicians that are proficient in each testing procedure helps to provide the laboratory with consistency in all test methods and give clients confidence in the results obtained.

5. *Training Program* – Without proper training there is no way to guarantee that testing procedures will be done the same way or that the same result will be obtained from one technician to the next. Training is the single most important support program as no other program can work correctly without properly trained personnel. The FPCLS has a fully functional training program and all laboratory personnel are required to undergo training prior to performing laboratory analyses. To demonstrate the success of training sessions and provide evidence

that the FPCLS staff are capable of performing each lab function, training forms and modules are utilized.

Personnel Training Forms are used to capture training activities and show that each technician has been signed off on a particular laboratory procedure or function. Training modules are utilized for more important laboratory functions requiring that certain criteria be met in order for a laboratory technician to be considered proficient in a specific procedure or function. Some of these modules include training such as; laboratory introductory activities, CORE competency, in-scope microbiological methods for foodborne pathogens, general enumeration, quality assurance, and internal audit training.

To provide further evidence of the competency of the FPCLS staff, a Master Training Checklist is used to show all laboratory functions that each employee is trained on and when the training took place. To control training and testing activities within the FPCLS laboratory an Approved Test Method Trainers List is available as well as an Approved Technician List detailing which staff member can train others and which ones are approved to perform each test method. Finally, all technicians and trainers in the FPCLS laboratory are evaluated each year to see where they have improved and what areas they might need additional training.

6. *Microbiological Media Control Program* – Microbiological media control is probably the next most important support program after employee training. Almost all laboratory test methods require some type of media during sample processing or analysis. It is critical to all testing facilities that the media being utilized for analyzing samples is made correctly, supports the growth of the target organism at an acceptable level, and is of good quality to verify the media does not have an adverse effect on the sample results. The FPCLS has established several SOPs to guide the preparation of media as well as the testing of that media to verify its' acceptability during analysis.

When media arrives at the FPCLS, it is quarantined until analysis shows that it is acceptable for use following the FPCLS procedures for Media and Assay

Kit Acceptable Quality Limits. During media evaluation checks are done to determine if the American Type Culture Collection (ATCC) positive and negative control organisms pass testing. All media is prepared per Media Preparation SOPs and all preparation activities are captured in the Media Preparation Log to make sure all microbiological media prepared for in-scope testing was made correctly. Sterile Batch Records are produced for all microbiological media that is autoclaved. Quality control samples are prepared for each batch of media to confirm that it is acceptable and meets the FPCLS microbiological media standards for growth acceptability and quality. Finally, all media (powdered base and prepared) is stored in appropriate locations/temperatures that are monitored to make sure the integrity of the media is maintained before it is used for analyzing client samples.

Support programs are crucial to the success of any management system as they provide the necessary basis to allow the management system to reach its full potential. At the FPCLS the support programs help maintain the management system and allow it to function at an optimal level. Without support programs the management system would be obsolete as there would be no way to demonstrate that all of the management system functions were working correctly. The integration of well put together support programs into the laboratory management system is absolutely necessary if a laboratory is attempting to obtain ISO accreditation. Without support programs that provide additional structure to the FPCLS, ISO/IEC 17025 accreditation would not be a possibility for the FPCLS at the University of Nebraska-Lincoln Food Processing Center.

EXPERIENCES AND CONCLUSIONS

ISO accreditation is not a one-time thing. It is a living breathing ever changing system that requires the attention of everyone involved in the running and maintaining of the laboratory at all times. Preparing for ISO/IEC 17025 accreditation is extremely challenging and takes a lot of hard work and dedication in order to develop and implement all of the necessary policies and procedures to meet ISO accreditation requirements.

However, achieving accreditation is not the final step, as it takes just as much effort to maintain accreditation status from year to year as for the initial accreditation process. For the FPCLS, it took several years to structure the laboratory so that it could even begin the preparation process for ISO/IEC 17025 accreditation. After the process began, it still took 1 ½ years to complete the development of all support programs and the implementation of the quality management system. This would not have been possible without having individuals who were dedicated to developing programs and implementing all of the processes and procedures in the laboratory.

As mentioned, this was not an easy task even with having dedicated personal focused on preparing the laboratory for ISO accreditation. Along the way, many struggles were experienced while trying to implement all of the requirements necessary in preparing to pursue ISO/IEC 17025 accreditation, which made the process take even longer and even more challenging to accomplish. One of these struggles was a reflection of the type and size of the laboratory and included financial challenges. Becoming ISO accredited can be very expensive as it takes time (personnel salaries) and money for supplies to develop and implement support programs and verify testing methods. This

can be a major problem for small and academic laboratories, such as the UNL-FPCLS, that do not have a lot of funding that can be used for these types of activities.

Another challenge for the FPCLS was lack of available personnel. The FPCLS currently does not have a laboratory manager and therefore the laboratory director had to perform both director and laboratory manager duties along with faculty duties at UNL. This added a burden to the process as it reduced the amount of time that could be spent on preparation activities. Finally, the FPCLS experienced some issues with personnel not being receptive to ISO practices as they were implemented. Most of the staff in the FPCLS laboratory are graduate students and it was difficult to help them understand the importance of why things were changing as well as getting them to comply with those changes. This issue slowed the preparation process as it required a lot of additional training and verification that all activities were being performed according to the ISO standard in the FPCLS testing laboratory.

Despite these challenges, the FPCLS was able to achieve the goals of this project by mitigating many of these issues through various means. For instance, the financial burden was lightened by purchasing supplies in bulk in order to receive discounts. Additionally, having graduate students perform the majority of the work help to reduce the personnel costs associated with ISO compliance and implementation. To reduce issues associated with laboratory personnel not complying with ISO practices, training was conducted for all laboratory personnel and ISO implementation was restricted to certain individuals showing competence in ISO practices.

Therefore, in developing a quality management system fit for the UNL-FPCLS and developing/implementing all of the documentation, SOPs, and programs necessary

for ISO/IEC 17025 accreditation was very challenging but also rewarding. In preparation for ISO accreditation the UNL-FPCLS developed/implemented over 63 SOPs, 103 forms, 19 manuals/lists, 6 support programs, and a comprehensive quality manual. All of these policies, procedures, forms, etc. were necessary to become ISO/IEC 17025 compliant despite the size of the laboratory as all programs and processes must meet ISO standards regardless of whether the laboratory is large or small.

Despite being a small academic laboratory, as each of these programs, policies, and procedures was put into place, it led to improvements in the performance of the laboratory. Some of the improvements that have been realized by the FPCLS due to the ISO/IEC 17025 accreditation preparation process include:

1. Quality of work being performed within the laboratory
2. Better control of documentation and records
3. Increased awareness by staff regarding issues when they arise
(corrective/preventive action)
4. Improved sample handling and tracking capabilities
5. Increased control and confidence in data/results that are sent to clients
6. More consistent training of staff with increased confidence in their testing abilities
7. Improved work environment and ability to maintain laboratory

In conclusion, although this was a challenging task, the improvements seen in the FPCLS were worth the effort it took to overcome the struggles encountered. ISO/IEC 17025 accreditation is not for all laboratories but the improvements that can be seen and the benefits that can be realized were definitely worth preparing the FPCLS to pursue ISO accreditation. So, after completing the development and implementation of the

quality management system and all of the policies, procedures, documentation, and support programs, the question remains – is the UNL-FPCLS ready to submit an application to the accrediting body and attempt accreditation? The answer is YES!

Lists of Media Qualification Verification Tables and Figures

Chapter 2 Tables

Table 2.T1: FPCLS ISO/IEC 17025 Accreditation Timeline

Table 2.T2: A2LA C204 Checklist Requirements for ISO/IEC 17025 Accreditation

Table 2.T3: Standard Operating Procedure Sections and Content Requirements

Chapter 2 Figures

Figure 2.F1: UNL-FPC Organizational Chart

Figure 2.F2: FPCLS SOP Template Example

Tables Supporting FPCLS ISO/IEC 17025 Accreditation

Table 2.T1: FPCLS ISO/IEC 17025 Accreditation Timeline. Table shows the FPCLS timeline to obtain ISO/IEC 17025 accreditation according to A2LA accreditation requirements (A2LA 2015; A2LA 2016b).

Step #	Process or Step Required
1	Obtain official copy of ISO/IEC 17025 standard and/or AOAC guidelines for laboratories performing microbiological and chemical analysis of food, dietary supplements, and pharmaceuticals
2	Obtain conformity checklist (C204 – Specific Checklist: Combined ISO/IEC 17025 and Food & Pharmaceutical Testing Laboratory Accreditation) from A2LA
3	Obtain C101 – General Checklist: ISO/IEC 17025 Laboratory Accreditation Program from A2LA
4	Review and complete A2LA form F102 Ownership Confirmation for ISO documentation and checklist for application
5	Review ISO/IEC 17025 standard and general requirements for accreditation documents (A2LA R101)
6	Estimate cost of accreditation and submit form (F119 Estimate Request) to A2LA (submit through website)
7	Generate a Quality Management System and finalize all quality system documents including; forms, SOPs, lists, and organization charts; make any necessary changes and revisions (first draft only)
8	Conduct an Annual Management Review (repeat yearly and when preparation process completed)
9	Verify in-scope methods for accreditation
10	Complete a “Selection List” or “Draft Scope” of accreditation
11	Complete Internal Audit of all sections including updates and necessary changes for compliance to the standard
12	Assign a laboratory representative responsible for upholding accreditation requirements
13	Assign an individual responsibility over Quality Management System (Quality Manager)
14	Generate proficiency testing 4-year plan
15	Start A2LA application process for ISO/IEC 17025 accreditation – fill out application and start reviewing application sections
16	Complete C101 – General Checklist: ISO/IEC 17025 Laboratory Accreditation Program from A2LA for application process
17	Management authorized representatives review and agree to A2LA R102 Conditions for Accreditation for application process

Step #	Process or Step Required
18	Fill out A2LA F117 – Technical Staff Matrix for Accreditation ISO/IEC 17025
19	Gather all documents necessary for bench audit to be submitted with application (<i>see required documents below</i>)
20	Complete A2LA application of accreditation for ISO/IEC 17025 and review all sections for completion
21	Submit application for ISO/IEC 17025 accreditation to A2LA to initiate accreditation process, receive CAB account, obtain an assessor, and schedule initial assessment for accreditation
22	Submit payment for accreditation application and to proceed for initial assessment
23	Initial assessment (audit) of compliance to ISO/IEC 17025 for FPCLS testing facility

Table 2.T2: A2LA C204 Checklist Requirements for ISO/IEC 17025 Accreditation.

Table shows the C204 checklist requirements for obtaining ISO/IEC 17025 accreditation according to ISO/IEC 17025:2005 guidelines (A2LA 2001; ISO and IEC 2005; AOAC 2015; A2LA 2016a).

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
4 Management Requirements	
4.1 Organization (4.1.1 - 4.1.6)	<ul style="list-style-type: none"> • FPCLS is part of UNL • Quality Manual • All testing is performed onsite • Organizational Chart • Designated personnel with authority to conduct activities, FPC Director, Laboratory Manager, deputies appointed as needed • Position descriptions and job responsibilities • Training Program and SOPs • Conflict of interest training • Training records kept • Confidentiality and conflict of interest SOPs • Management reviews
4.2 Management system (4.2.1 - 4.2.7)	<ul style="list-style-type: none"> • Quality Manual • Quality Policy Statement, management commitment, purpose • QA policies and procedures • CAPA activities • Internal audits • Management reviews • Training program – all employees trained on QMS • Roles and responsibilities defined • Management verifies the integrity of the QMS
4.3 Document control (4.3.1 - 4.3.3.4)	<ul style="list-style-type: none"> • Control of Documents SOP • Master list of all documents • Document review (every 2 years) • SOPs and policies available to all personnel (hard and electronic copy) • Only approved controlled documents available • Document and Record Retention Table • Documents contain a revision history • SOP for writing SOPs • Handwritten amendments not allowed • All documents tracked by unique ID and version number
4.4 Review of requests, tenders, and contracts (4.4.1 - 4.4.5)	<ul style="list-style-type: none"> • Quality Manual • SOP for Review of Requests Tenders Contracts and Subcontracting Tests • UNL-FPCLS Sample Submittal Form • Laboratory is capable of performing all tests offered to clients (technical staff) • Communication with clients on tests performed • Records maintained in “Testing Requests Folder”
4.5 Subcontracting of tests and calibrations (4.5.1 - 4.5.4)	<ul style="list-style-type: none"> • Quality Manual • Communication with clients on tests performed • Subcontracting Work • Approved vendor list, only with A2LA accredited establishments
4.6 Purchasing services and supplies (4.6.1 - 4.6.4)	<ul style="list-style-type: none"> • Quality Manual • SOP Purchasing Services and Supplies • Approved vendor list • SOP Quality Control Media and Assay Kit Acceptable Quality Limit Verification • COAs • Signed invoices, approved POs, signed packing slips, kept in business office • EShop records • Management review meetings to approve vendors

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
4.7 Service to the customer (4.7.1 - 4.7.2)	<ul style="list-style-type: none"> • Quality Manual • Confidentiality agreements • FPCLS website – services provided • FPCLS Client Satisfaction Survey (web based) • FPCLS final reports • Feedback solicited on the laboratory report
4.8 Complaints	<ul style="list-style-type: none"> • FPCLS Client Satisfaction Survey (web based) • Folder in Lab Directors Outlook used to file feedback and complaints • SOP Complaints and Control of Nonconforming Tests
4.9 Control of nonconforming testing and/or calibration work (4.9.1 - 4.9.2)	<ul style="list-style-type: none"> • Quality Manual • SOP Complaints and Control of Nonconforming Tests • Designated responsible personnel, laboratory management handles nonconformances • Corrective actions, CAPA • Root Cause Analysis
4.10 Improvement	<ul style="list-style-type: none"> • Quality Manual • Quality Policy, objectives, audit results, analysis of data, CAPA, and records
4.11 Corrective action (4.11.1 - 4.11.5)	<ul style="list-style-type: none"> • Quality Manual • SOP for Corrective and Preventive Action • CAPA activities • Root Cause Analysis • Monitoring activities • Internal audits, checklists and reports
4.12 Preventive action (4.12.1 - 4.12.2)	<ul style="list-style-type: none"> • Quality Manual • SOP for Corrective and Preventive Action • CAPA activities • Monitoring activities
4.13 Control of records (4.13.1 - 4.13.2.3)	<ul style="list-style-type: none"> • Quality Manual • Procedures for identification, collection, filing, accessing, storing, and disposing of records • SOP Control of Records and Data • SOP Control of Documents • Records are password protected • Folders where records are kept are password protected • UNL BOX – backup files • All records held secure • Document and Record Retention Table • All records filed, maintained, and held secure • All records complete and contain necessary information – identification of technician, performance of test activities/calibrations, and results • All media has unique identification • Media and Reagent Receiving Logbook • Media and Reagent Preparation Logbook • Autoclaves and incubators mapped • Pipettors, thermometers, balances certified • Master Training List • All corrections initialed and dated
4.14 Internal audits (4.14.1 - 4.14.4)	<ul style="list-style-type: none"> • Quality Manual • CAPA Records • SOP Corrective and Preventive Action • SOP Internal Audits • Master Internal Audit Schedule • Internal audit reports and checklists • Management review meetings
4.15 Management review (4.15.1 - 4.15.2)	<ul style="list-style-type: none"> • Quality Manual • Management support • SOP Management Reviews

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
	<ul style="list-style-type: none"> • Management review meetings (scheduled) • Management review forms • CAPA forms, reports • Internal audit reports • KPIs and evaluation of laboratory progress • Customer feedback, satisfaction surveys • Personnel review
5 Technical requirements 5.1 General (5.1.1 - 5.1.2)	<ul style="list-style-type: none"> • FPCLS accounts for all factors that determine correctness and reliability of test results • A2LA P103b– Annex: Policy on Estimating Measurement Uncertainty for Life Sciences Testing Labs - CAT I and CAT II • Qualitative methods only
5.2 Personnel (5.2.1 - 5.2.5)	<ul style="list-style-type: none"> • Qualified personnel – resumes, work experience, training records • Equipment SOPs • SOPs test methods • Training program, Training SOP, training records, and forms, training modules, ongoing competency of personnel documented • Position descriptions, personnel files • Training Master Checklist • Training records filed in training binder • New employees not allowed to test samples until deemed competent • Technician and Trainer Evaluation Sheets • Annual performance reviews • Proper management in place, Laboratory Supervisor
5.3 Accommodation and environmental conditions (5.3.1 - 5.3.5)	<ul style="list-style-type: none"> • FPCLS has appropriate accommodations and facilities for testing samples • Environmental monitoring program, performed monthly • SOP Environmental Monitoring • Environmental Monitoring Log • Laboratory Maintenance Schedule • Autoclave Room Monthly Maintenance • Monitoring is consistent with industry standards • All reagents/media stored in temperature monitored locations • FPCLS defines use of acceptable water sources for media – SOP Use of Nanopure Water System • Media preparation and testing activities are performed at separate times • FPCLS laboratory has badge access, controlled • SOP Housekeeping Schedule and Eyewash Checks • SOP Cleaning and Maintenance of Incubators, Refrigerators, and Freezers • In-house qualification of media, Quality Control of Media and Assay Kits • Acceptable Quality Limit Verification SOP • Purchasing Services and Supplies SOP • Approved Vendors List • Sterile Batch Records
5.4 Test and calibration methods and method validation (5.4.1 - 5.4.7.2)	<ul style="list-style-type: none"> • FPCLS SOPs for test methods, handling samples, storage, preparation of media, calibrating balances and thermometers, etc. • Validated in-scope methods, only qualitative methods used so no uncertainty measurements needed • Equipment SOPs • Laboratory Processes SOPs • SOPs and other relevant documents are available (hard copy and electronic) • Deviations approved by management, CAPA activities and records • Only approved test methods that have been validated/verified will be used for in-scope methods • All standard methods (AOAC) kept up-to-date, only newest versions used in laboratory • Client communication, discussions on testing methods, protocols used if necessary • Proficiency testing and method validation • Document control SOP

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
	<ul style="list-style-type: none"> • SOP for Writing SOPs, version number updated when changes made, only controlled versions of most recent version available • Accuracy of results verified – detection limit, selectivity of method, linearity, limit of repeatability, etc. all suitable for intended use • Estimate of Uncertainty – only qualitative methods • Control of Records and Data SOP • Data is kept secure, password protected folders, hard copies kept in locked office file cabinets • Software used by FPCLS meets its needs and is suitable for intended purpose • Computers and equipment are maintained • Integrity of data and test information is maintained and controlled
5.5 Equipment (5.5.1 - 5.5.12)	<ul style="list-style-type: none"> • FPCLS testing facility is equipped with appropriate equipment and supplies • Equipment SOPs • Test Method SOPs • No equipment is used outside the control of the FPCLS • Calibration of equipment SOPs, thermometers, balances, incubators, pipettors • All equipment is either calibrated or calibration is performed by external service provided before it is used • Maintenance Schedules and performance checks on equipment, monitoring records • Performance Evaluations of Measuring Equipment Using Reference Standards • Installation Operation Performance Evaluation of Equipment (IQ, OQ, PQ) • Balance Verification Tests • Incubator Temperature Mapping • Measuring Device Verification • Thermometer Verification • Eyewash Station Checking • Test protocols and safe guards are in place to protect against adjustments that might invalidate test results (alarms, lock outs)
5.6 Measurement traceability (5.6.1 - 5.6.3.4)	<ul style="list-style-type: none"> • All equipment is calibrated, or certified • Equipment SOPs and manuals • Only certified establishments are used for external calibrations • ISO Certificates on file for external calibration companies • Calibration certificates are maintained and filed from external companies – include measurement uncertainty, statement of compliance, etc. • FPCLS verifies that all equipment used can provide the level of uncertainty needed by its clients • FPCLS will use SI units • All reference standards (weights, thermometer) are certified by an accredited establishment, and are only used for calibration purposes • All reference standards are stored appropriately, secured in locked room (Laboratory Directors office) • All calibration and verification records both internal and external are kept in a secure location and are available for review
5.7 Sampling (5.7.1 - 5.7.3)	<ul style="list-style-type: none"> • FPCLS does not conduct sampling for clients • Protocol system is in place for procedures requiring customer requested deviations • Protocol Title Page Template
5.8 Handling of test and calibration items (5.8.1 - 5.8.4)	<ul style="list-style-type: none"> • Submission of Laboratory Samples SOP • Laboratory test method SOPs • Environmental Monitoring SOP • Monthly Maintenance Schedules • Temperature Monitoring Records • Equipment Inventory List • Equipment receives a unique identification for calibration and reference items and other special items, other items tracked by serial number • Proper labeling used in laboratory • Any abnormalities or departures from normal conditions are noted on the Sample Submittal Form, Sample Receiving Log, and testing paperwork • Test method SOPs in place to control sample handling and processing • Refrigerators, freezers, and storage cabinets dedicate to sample storage • Training Program, training records • Sample retention periods and disposition after testing defined

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
5.9 Assuring the quality of test and calibration results (5.9.1 - 5.9.2)	<ul style="list-style-type: none"> • Quality Assurance SOPs • Test Method SOPs • Equipment SOPs • Control of Records and Data • Quality control – positive and/or negative controls • Submission of Laboratory Samples SOP • Proficiency Testing, FPCLS conducts proficiency testing with API (2 times per year) • Testing of API Proficiency Samples SOP • FPCLS has completed, successfully, proficiency testing on all in scope methods of analysis • Internal proficiency testing of technicians • CAPA activities, Corrective and Preventive Action SOP • Evaluate proficiency results and make changes/issue corrective actions where necessary
5.10 Reporting the results (5.10.1 - 5.10.9)	<ul style="list-style-type: none"> • Writing a Laboratory Analysis Report • FPCLS Personnel Signatures and Initials Form • Laboratory Analysis Report Template • FPCLS reports all results on testing paperwork, handwritten, and then transfers the data to an electronic final report • All reports and data are reviewed for errors • CAPA Activities as required • All test reports include; a title, name and address of the laboratory performing the testing, client information, unique identification number to track the report, test code, sample ID information, dates of testing and reporting, test results, and signature of authorized individual, etc. • When results are amended, a Supplemental Report is sent to the client with the correct information
Appendices Appendix A Table 1: Calibration and Verification of Equipment	<ul style="list-style-type: none"> • Equipment SOPs • Autoclave Usage and Maintenance SOP • Autoclave mapping • Sterile Batch Records, media verified weekly or every 5 batches • EHS Autoclave checks • Daily Balance Verification Log • Balance Verification Tests (linearity, etc.) • Conductivity Log, DI/Nanopure water tested weekly • Automatic Dispenser Usage Log • Fume hoods and biosafety cabinets certified annually • pH Meter Log • Temperature Monitoring Log – refrigerators, freezers, deep freezers, incubators (high/low) • Incubator Temperature Mapping • Temperature Mapping Hot Cold Spot Template • Thermometer Verification Form • FPCLS timers are certified against a NIST traceable timer • Pipettors are certified by an external provided • Water activity meter is verified when used • Working weights are verified against master weights yearly, master weights are calibrated every 5 years
Appendix B: Microbiology Appendix B: Organisms (1)	<ul style="list-style-type: none"> • Storing, Freezing, and Retrieval of Cultures From -80°C Freezer SOP • Frozen Culture Access Log • Frozen Culture Addition Log • Test Method SOPs
Appendix B: Media (2.1 - 2.1.3)	<ul style="list-style-type: none"> • Quality Control Media and Assay Kit Acceptable Quality Limit Verification SOP • Control Media and Assay Kit Acceptable Quality Limit Test • Media receives a unique identification number upon arrival and receives a unique code when it passes QA/QC testing • Media and Reagent Preparation Logbook • Media and Reagent Receiving Logbook • Media Preparation SOP

Audit Checklist Section	Information, Documents, and Forms Providing Compliance
	<ul style="list-style-type: none"> • COAs of dehydrated media are kept on file • All media is quarantined until passing QA/QC testing
Appendix B: Reagents/Kits/Identification Systems (3)	<ul style="list-style-type: none"> • Quality Control Media and Assay Kit Acceptable Quality Limit Verification SOP • Control Media and Assay Kit Acceptable Quality Limit Test • Media and Reagent Receiving Logbook • COAs of assay kits are kept on file • Assay kits receive a unique identification number upon arrival and receive a unique code when they pass QA/QC testing • All assay kits are quarantined until passing QA/QC testing
Appendix B: Sterilization (4)	<ul style="list-style-type: none"> • Autoclave SOP • Sterile Batch Records • Weekly sterility checks or with every 5th batch • EHS checks
Appendix C: Chemistry	<ul style="list-style-type: none"> • Not Applicable to the FPCLS
Appendix D: Pharmaceutical Analysis and Legal Standards	<ul style="list-style-type: none"> • Not Applicable to the FPCLS
Appendix E: Legal Samples	<ul style="list-style-type: none"> • Not Applicable to the FPCLS

Table 2.T3: Standard Operating Procedure Sections and Content Requirements.
 Table shows a list of SOP section headers and what should be contained within those sections. Not all sections are used for each SOP (depends on SOP type).

SOP Section	Description of Contents
Purpose	Why the document is being generated
Scope	What is the overall reason for having the document and who does the document apply to
Prerequisites/Documents	Any documents associated with the SOP such as other SOPs, forms, and manuals, etc.
Responsibilities	Who is responsible for implementing or following the procedures
Materials and Media	Any necessary materials or media needed to properly perform the procedures
Equipment	Any special equipment needed for performing the procedures
Definitions	Definitions of terms or words used that may be confusing including the definitions of acronyms so that the wording in the SOP may be better understood
Procedures	All of the steps and information necessary to complete the procedure
References	Any external documents utilized for the procedure to be used effectively, or that were used in the creation of the SOP
Revisions	Any changes made to the SOP. Changes made will cause a change in the version number

Figures Supporting FPCLS ISO Accreditation

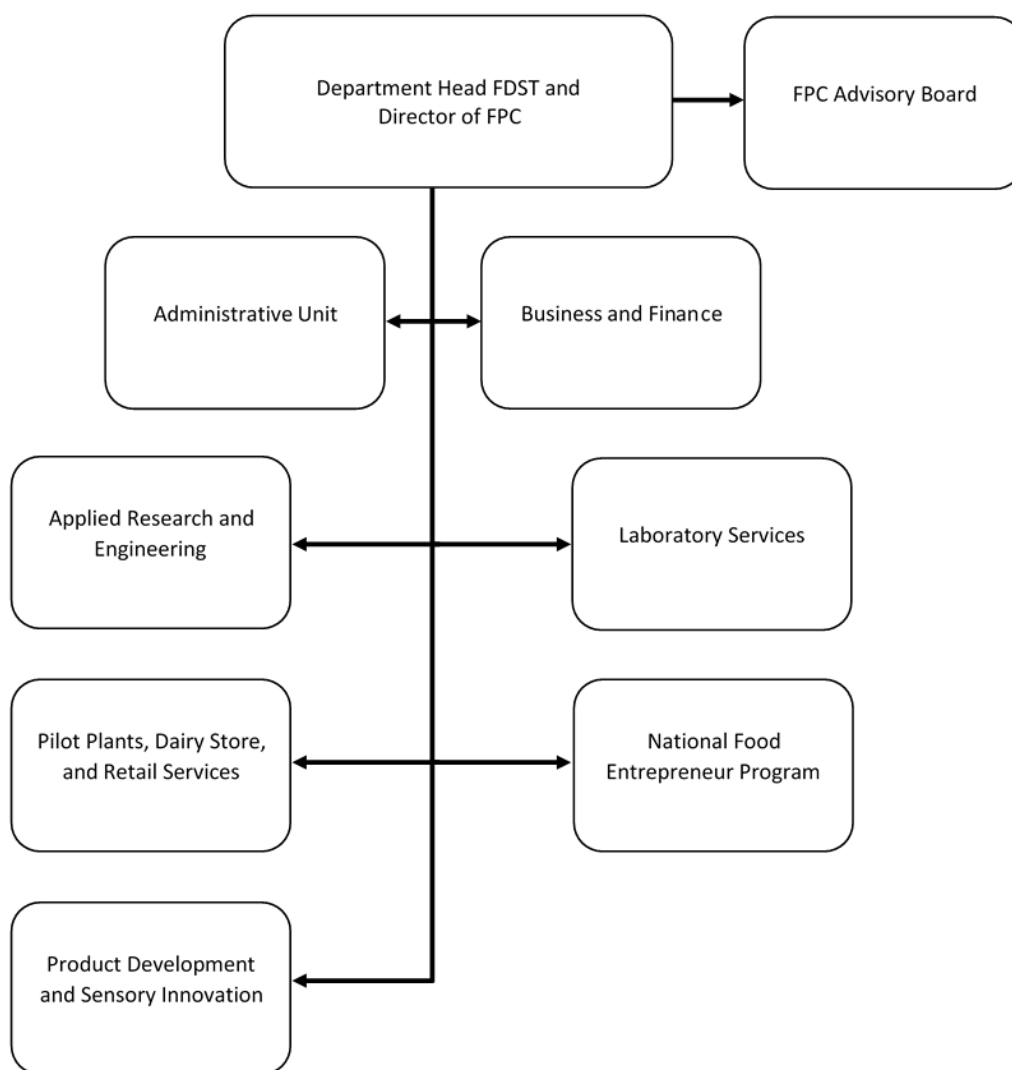



Figure 2.F1: UNL-FPC Organizational Chart. Figure displays the Food Processing Center organization chart consisting of the different departments within the FPC at UNL. Names of responsible individuals have been omitted, adapted from UNL FPC organizational chart (FPC 2017).

	University of Nebraska–Lincoln Food Science and Technology Department Food Processing Center Laboratory Services	SOP #	SOP-XX-XXX
		Revision #	V-XX
		Last Reviewed	MM/DD/YYYY
		Effective Date	MM/DD/YYYY
SOP Title: Insert Title Here...			

	Name	Title	Signature	Date
Author				MM/DD/YYYY
Reviewer				MM/DD/YYYY
Approver				MM/DD/YYYY

1. **Purpose:**
 - 1.1. **Subheadings**
 - 1.2. XXXXXXXXXX
 - 1.2.1. XXXXXXXXXX
2. **Scope:**
3. **Prerequisites/Documents:**
4. **Responsibilities:**
5. **Materials and Media:**
6. **Equipment:**

Figure 2.F2: FPCLS SOP Template Example. Figure displays example of a SOP template containing the document header, title page, and body with section headers and formatting utilized by the FPCLS at UNL.

Bibliography

- A2LA. 2001. *A2LA Food Microbiology Program Requirements*. "AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing." The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- A2LA. 2016a. *A2LA: C204 Specific Checklist - Combined ISO/IEC 17025 and Food and Pharmaceutical Testing Laboratory Accreditation Program Requirements*. The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- A2LA. 2018. *A2LA: Currently Accredited Organizations*. The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- A2LA. 2015. *A2LA: I105 – Typical Steps in Preparing for the Accreditation Process*, 1-6. American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- A2LA. 2016b. *A2LA: R101 - General Requirements: Accreditation of ISO/IEC 17025 Laboratories*. The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- AOAC. 2015. *AOAC International: Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals*. AOAC International.
- Chung, Kun, Geun Choi, Wanno Lee, Young Cho, and Chang Lee. 2006. "Implementation of ISO/IEC 17025 Standard in a Nuclear Analytical Laboratory: The KAERI Experience." *Accreditation and Quality Assurance*, 10 (11): 603-605. doi:10.1007/s00769-005-0060-1.
- DQS, Gulo, Sara and Rao, Ganesh. "Top 10 Challenges to Overcome for Implementing ISO 9001:2015 and ISO 14001:2015.", last modified Aug 1, accessed 4/16/2018. 2018. <https://dqsus.com>.
- Elortondo, F. J. Pérez, M. Ojeda, M. Albisu, J. Salmerón, I. Etayo, and M. Molina. 2007. "Food Quality Certification: An Approach for the Development of Accredited Sensory Evaluation Methods." *Food Quality and Preference*, 18 (2): 425-439. doi:10.1016/j.foodqual.2006.05.002.
- FDA. 2011. *FDA Food Safety Modernization Act: Public Law 111-353, 111th Congress, an Act*. U.S. Government Publishing Office.
- FDA. Food and Drug Administration, "Recalls, Market Withdrawals, and Safety Alerts." last modified Apr 12, accessed 4/12/2018. 2018. <https://www.fda.gov/>.

FDA. "U.S. Food & Drug Administration DHHS: "Food." U.S. Government Publishing Office, last modified Apr 11 2018, accessed Apr 16, 2018, <https://www.fda.gov/Food/default.htm>.

FPC, UNL. "The Food Processing Center." Web Developer Network, University of Nebraska–Lincoln, Institute of Agriculture and Natural Resources, accessed 4/20/2018. 2018. <https://fpc.unl.edu/>.

FPC, UNL. 2017. *The Food Processing Center Organizational Chart*. University of Nebraska Lincoln Food Processing Center. Lincoln, NE.

FPC, UNL and Rolando A. Flores. 2015. *Semiannual Report: Impact on Nebraska and the Global Food Industry*. University of Nebraska Lincoln.

Grochau, Inês, Carlos Ferreira, Jane Ferreira, and Carla ten Caten. 2010. "Implementation of a Quality Management System in University Test Laboratories: A Brief Review and New Proposals." *Accreditation and Quality Assurance*, 15 (12): 681-689. doi:10.1007/s00769-010-0713-6.

Grochau, Inês and Carla ten Caten. 2012. "A Process Approach to ISO/IEC 17025 in the Implementation of a Quality Management System in Testing Laboratories." *Accreditation and Quality Assurance*, 17 (5): 519-527. doi:10.1007/s00769-012-0905-3.

Halevy, Avner. 2003. "The Benefits Calibration and Testing Laboratories May Gain from ISO/IEC 17025 Accreditation." *Accreditation and Quality Assurance*, 8 (6): 286-290. doi:10.1007/s00769-003-0632-x.

Honsa, Julie D. and Deborah A. McIntyre. 2003. "ISO 17025: Practical Benefits of Implementing a Quality System." *Journal of AOAC International*, 86 (5): 1038-1044. <http://www.ncbi.nlm.nih.gov/pubmed/14632407>.

Hullihen, Karen, Verna Fitzsimmons, and Michael R. Fisch. 2009. "Establishing an ISO 17025 Compliant Laboratory at a University." *International Journal of Modern Engineering*, 10 (1): 55-64.

ILAC. 2001. *Why Become an Accredited Laboratory*. IALC Publications, International Laboratory Accreditation Cooperation.

ISO. 2016. *ISO in Brief*. International Organization for Standardization. Geneva, Switzerland.

ISO. "ISO: All about ISO." International Organization for Standardization, accessed <https://www.iso.org/>, <https://www.iso.org/about-us.html>.

ISO. 2014. *ISO: Economic Benefits of Standards*. International Organization for Standardization. Geneva, Switzerland.

ISO. 2017. *ISO: ISO and Food*. International Organization for Standardization. Geneva, Switzerland.

ISO. "ISO: Popular Standards." International Organization for Standardization, accessed 4/12/, 2018, <https://www.iso.org/>.

ISO. "ISO: The Main Benefits of ISO Standards." International Organization for Standardization, accessed 4/12/2018. 2018.

ISO and IEC. 2005. *ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories*. 2nd ed. Geneva, Switzerland: International Organization for Standardization.

ISO and IEC. 2017. *ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories*. 3rd ed. International Organization for Standardization. Geneva, Switzerland.

Kohl, Herfried. 1998. "The New ISO 17025 - Basic Idea." *Accreditation and Quality Assurance*, 3: 422-425.

Martincic, Cynthia. 1997. *IT Standards: A Brief History of ISO*. University of Pittsburgh. Pittsburgh, Pennsylvania.

MDT. "Discovering ISO/IEC 17025: The Challenges and Benefits." Medical Design Technology Magazine Online, Plastics Color Corporation, last modified Aug 4, <https://www.mdtmag.com/article/2016/08/discovering-iso-iec-17025-challenges-and-benefits>.

Rahmat, Suharni, Chew Boon Cheong, and Hamid, Mohd Syaiful Rizal Bin Abd. 2016. "Challenges of Developing Countries in Complying Quality and Enhancing Standards in Food Industries." *Procedia - Social and Behavioral Sciences*, 224: 445-451. doi:10.1016/j.sbspro.2016.05.418.

Rodima, Ako, Martin Vilbaste, Olev Saks, Erko Jakobson, Eve Koort, Viljar Pihl, Lilli Sooväli, et al. 2005. "ISO 17025 Quality System in a University Environment." *Accreditation and Quality Assurance*, 10 (7): 369-372. doi:10.1007/s00769-005-0011-x.

Romero, J., I. Manero, and J. Laso. 2007. "Validation of Geosmin and 2-Methyl-i-Borneol Analysis by CLSA-GC-FID Method to Obtain ISO-17025 Accreditation." *Journal of Chromatographic Science*, 45 (7): 439-446. doi:10.1093/chromsci/45.7.439.

USDA, FSIS. "Food and Nutrition: United States Department of Agriculture." U.S. Government Publishing Office, accessed Apr 12, 2018. <https://www.usda.gov/topics/food-and-nutrition>.

USDA, FSIS. "Recalls and Public Health Alerts: Current Recalls and Alerts." United States Department of Agriculture Food Safety and Inspection Service, last modified Feb 14 2018, accessed Apr 12, 2018, <https://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/current-recalls-and-alerts>.

USDA, FSIS. "United States Department of Agriculture Food Safety Inspection Service "Regulatory Compliance"." U.S. Government Publishing Office, last modified Mar 24, accessed 4/12/2018. 2018. <https://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance>.

Vlachos, N. A., C. Michail, and D. Sotiropoulou. 2002. "Is ISO/IEC 17025 Accreditation a Benefit or Hindrance to Testing Laboratories? the Greek Experience." *Journal of Food Composition and Analysis*, 15 (6): 749-757. doi:10.1006/jfca.2002.1097.

Wikipedia. "Quality Management System." Wikimedia Foundation, Inc., last modified Apr 11 2018. accessed Apr 26, 2018. https://en.wikipedia.org/wiki/Quality_management_system.

Zapata-García, D., M. Llauradó, and G. Rauret. 2007. "Experience of Implementing ISO 17025 for the Accreditation of a University Testing Laboratory." *Accreditation and Quality Assurance*, 12 (6): 317-322. doi:10.1007/s00769-007-0274-5.

CHAPTER 3

METHOD VERIFICATION OF MEDIA QUALIFICATION AND

CONTROL PROGRAMS

“Qualification of Microbiological Media from Commercial Sources”

INTRODUCTION

Media Qualification and ISO Guidelines

International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC) standards exist to help guide laboratories in performing higher quality testing producing results that can be trusted worldwide (Romero et al. 2007; ISO 2018b). ISO has developed a standard that directly affects the food testing industry known as ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017; ISO 2018b).

Preparing a laboratory to obtain ISO/IEC 17025 accreditation is an extremely challenging task that takes months or even years to accomplish and is especially challenging for academic laboratories that are focused on research and teaching activities (Grochau et al. 2010). This preparation process involves the implementation of various programs, creation of documents and records, and the verification of all laboratory functions (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). These functions include sample receipt, sample processing and testing, training, data collection, and reporting of results to clients.

However, one of the areas that may be overlooked is the verification of in-house microbiological media qualification and control procedures. Proper microbiological media preparation is vital to the success of a laboratory as it directly affects the outcome of all testing procedures that utilize prepared solid or liquid media to garner results (Sutton 2006; Sandle 2016). Sandle stated “*media is of fundamental importance...to obtain pure cultures, to grow and count microbial cells, and to cultivate and select*

microorganisms...without high-quality media...achieving accurate, reproducible, and repeatable microbiological test results is reduced” (Sandle 2016). In order to develop an in-house microbiological media qualification program, the laboratory must: (1) decide what characteristics need to be assessed to determine if a particular media passes or fails qualification; (2) properly design a study that will evaluate the media and demonstrate its efficacy; and (3) utilize the data gained from those tests to determine what constitutes failing or passing throughout the media shelf life.

So, why perform media qualifications? Because it is required to become ISO compliant and for obtaining ISO accreditation. Media qualification must be performed for in-house prepared microbiological media prior to the media ever being utilized for testing purposes, and a procedure to verify the continued quality of that media must be established to meet ISO/IEC 17025 guidelines (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). It was stated that “*microbiological media are critical materials that shall be calibrated/verified as to their performance*” (AOAC 2015).

Media quality control procedures must be in place to verify the “suitability” of each media type, both liquid and solid, selective and non-selective, for each laboratory method and require that all media created internally or purchased be examined (A2LA 2001; ISO and IEC 2005; AOAC 2015). This examination requires that each batch of media be evaluated for productivity, selectivity (if necessary), and sterility (A2LA 2001; AOAC 2015) to provide evidence that the microbiological media being utilized by the testing facility is not adversely affecting the test results obtained from client samples.

Many types of microbiological media can be utilized by food testing laboratories in order to generate results for their clients. There are several different types of media

including non-selective/selective agars (solid media) and non-selective/selective liquid (broth) media types. Microbiological media are important to laboratory procedures because they support the growth/survival of microorganisms and can be utilized to segregate different types of microorganisms when using selective or differential media types (Sandle 2016).

Examples of media utilized in the food industry include: Sorbitol MacConkey Agar for isolating and differentiating the presence of *Escherichia coli* O157:H7 from generic *Escherichia coli* strains (March and Ratnam 1986; Church et al. 2007; Possé et al. 2008; BD 2009); CHROMagar for the isolation and detection of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria* spp., *Staphylococcus aureus*, and *Candida albicans* (BD 2009; Church et al. 2007; Perry and Freydière 2007); Oxford and Modified Oxford media for the detection and selection of *Listeria* spp. (BD 2009; FDA 2017); XLT4 media for the selection and differentiation of *Salmonella* spp. (BD 2009); and general purpose media such as Tryptic Soy Agar and Tryptic Soy Broth for general enumeration and growth promotion (BD 2009; Neogen 2017).

Even though the majority of microbiological media utilized by laboratories are commercially prepared and meet ISO 11133:2014 requirements (ISO 2014), it is still required that these media (liquid broths, solid agars, powdered base) be qualified by the establishment (A2LA 2001; AOAC 2015). This is due to the fact that all media, despite the being quality control tested at the manufacturing facility, have been shown to occasionally provide poor quality or failing results (Jones et al. 2002; CLSI 2004). For example, Sorbitol MacConkey Agar has a potential failure rate of about 1.04% while Tryptic Soy Agar has a potential failure rate of about 2.00% (Jones et al. 2002) with the

most common causes of failure being no growth of the quality control organisms, selective media not properly inhibiting organisms, and contamination (CLSI 2004).

These failure rates may not seem significant but for a high-volume laboratory that goes through a lot of media, test failure due to media performance could potentially become a major problem. Therefore, it becomes very important to perform qualification procedures on in-house prepared microbiological media from commercial sources, and to develop procedures for properly verifying purchased media. These procedures should demonstrate that the microbiological media is not affecting laboratory results and is performing as expected.

Although media qualification is required according to ISO/IEC 17025 guidelines (A2LA 2001; AOAC 2015), a qualification of in-house media protocol is difficult if not impossible to find. Many establishments that choose to go through accreditation are privately held, and even public institutions do not seem to have an interest in publishing their media qualification processes or results. Fortunately, there are at least some sources available as guidance for qualifying media, but not for performing media qualification verification studies. One such organization, the Australian Society for Microbiological (ASM), has developed a process for verifying in-house media for use (ASM 2012) but not for verifying media preparation processes or the utilization of those media in specific methods.

Other papers have been published detailing different aspects of media qualification without sharing a lot of results. One such institution, the Clinical and Laboratory Standards Institute (CLSI formally NCCLS), released a report (M22-A3) detailing quality characteristics of media that should be considered such as cracking,

unequal filling of agar plates, changes in color, bubbles, etc. and general inoculation instructions with recommended control microorganisms (CLSI 2004). Another group released a paper detailing both characteristics to consider (cracking, unequal filling of agar plates, bubbles, pH, etc.) as well as growth determination using classic and ecometric methods sharing some data and referencing both the ASM 2012 and the CLSI M22-A3 reports (Basu et al. 2005). Lastly, other establishments have also released information on qualifying microbiological media but they typically reference the ASM 2012 report, CLSI M22-A3 report, and/or Basu 2005 et al. publication so the information is not new or additive just repetitive (PARN 2009).

ISO has also released standards to help aid in the verification of media providing insight on how to test microbiological media and determine if it is of high quality and acceptable. This standard is known as ISO 11133:2014 Microbiology of food, animal feed and water – preparation, production, storage and performance testing of culture media (ISO 2014; ISO 2018a). However, this ISO standard is designed for commercial producers of media, non-commercial establishments providing media to other laboratories, and laboratories that are preparing microbiological media from ingredients for their own use (ISO 2014; ISO 2018a). ISO 11133:2014 is not designed for standard food testing laboratories that are utilizing media purchased from a commercial source (ISO 2014; ISO 2018a) and therefore should not be considered the only reference used when verifying in-house media qualification programs.

OBJECTIVES OF PROJECT

The Food Processing Center Laboratory Services (FPCLS) located at the University of Nebraska-Lincoln (UNL) Food Processing Center (FPC) has set a goal to meet all of the requirements set forth by ISO for obtaining accreditation to ISO/IEC 17025 standards. This included the verification of microbiological media qualification for media prepared from commercial sources, and the implementation of a quality procedure to guarantee the acceptability of all media prepared within the FPCLS. The experiences that the FPCLS had and the processes that they developed may be used as a guide for other small and academic laboratories who wish to improve their processes or prepare for obtaining ISO accreditation status. The in-house media qualification verification procedure from this project, adapted from Basu et al. 2005 and ASM 2012, will provide the FPCLS and other laboratories with a verified method for ensuring that the media qualification program is effective and that the microbiological media utilized by the FPCLS meets specific standards for growth, quality, and shelf life.

The goal of this project is to establish and maintain a fully qualified microbiological media control program that provides evidence showing all media used for in-scope analyses meet FPCLS requirements for acceptability. Additionally, this program will include a comprehensive media preparation and control procedure for the ongoing verification of all in-scope media types to demonstrate all microbiological media utilized by the FCPLS for client sample analysis is of high quality, acceptable for testing, and performing to its intended purpose throughout its shelf life. Establishing this program and implementing media control procedures will further position the FPCLS to become ISO/IEC 17025 compliant and accomplish one of the many requirements necessary to

obtain ISO/IEC 17025 accreditation. To achieve this goal four primary objectives were addressed within this project.

Objective 1. Develop and carry out a method for the verification of qualification procedures for in-house prepared microbiological media from commercially available dehydrated microbiological media base and evaluate its acceptability throughout the media shelf life. This method was designed to accommodate the capabilities of the FCPLS while still meeting the requirements set forth in the ISO/IEC 17025 standard (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). A study was designed to test the quality and growth parameters throughout the shelf life of the media that the FPCLS determined were critical for its media types being utilized in testing client samples. Three categories of media (non-selective solid, non-selective liquid, selective liquid) were evaluated including 5 media types (Tryptic Soy Agar, Tryptic Soy Broth, Buffered Peptone Water, Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour for *E. coli* O157:H7) for various quality characteristics and growth acceptability at multiple time points to determine the shelf life and acceptability of each media type.

Objective 2. Determine, in conjunction with the microbiological media qualification method, the variation that could be expected in quality characteristics and growth acceptability responses from different lot numbers and/or different brands of each media type over the shelf life of the media. As part of media qualification verification testing several different lot numbers of each media type were evaluated representing different brands and different ages (varying expiration dates) to aid in determining the acceptable quality parameters and shelf life the FPCLS could expect regardless of the brand, lot, or age of microbiological media being utilized for testing.

Objective 3. Establish acceptable quality limit parameters for all in-house prepared microbiological media based on the results obtained from the first two objectives. Results for quality characteristics and growth acceptability from the media qualification shelf life studies were utilized to determine which quality characteristics

are most affected over time and can be utilized to determine if the media is no longer acceptable for use in client sample testing. Additionally, the data was utilized to determine at which point in the shelf life the media no longer provided an acceptable growth response hindering the media ineffective for FPCLS testing. The parameters necessary to properly evaluate and qualify in-house prepared media for use in the FPCLS were then determined and added to the FPCLSs media qualification standard operating procedure.

Objective 4. Implement an in-house media qualification procedure for the preparation and control of all commercially purchased media types utilized for in-scope testing of client samples based on the results generated from the media qualification verification process. The FPCLS developed and implemented a media qualification procedure to evaluate the efficacy of all commercially prepared dehydrated media base prior to entering the laboratory system and being utilized for testing client samples. This process is based on the techniques utilized from the media qualification verification process and ensures that all media being used for testing purposes meets the requirements of the FPCLS for quality and adheres to the requirements in the ISO/IEC 17025 standard of media being fit for its intended purpose.

Note: Media qualification verification procedures are vital in providing evidence that the various media types being utilized by the food testing laboratory are fit for their intended purpose and meet all FPCLS and ISO/IEC requirements for acceptability. Media qualification verification should not be confused with standard media qualification (screening) of commercially prepared media types upon arrival to the laboratory. These are separate processes as media qualification verification is an intensive testing procedure designed to fully vet each media type and is a “one time” procedure, while standard media qualification of commercially purchased media is for doing a screening check to determine acceptability of each media lot “every time” a new lot is purchased and is an “ongoing” process. Other publications only describe media qualification and not the full verification process as described in this project.

METHODS AND MATERIALS

Selection of Media Types for Evaluation

Media verification and qualification are vital activities in preparing the FPCLS to obtain ISO accreditation. In accredited laboratories microbiological media must meet certain standards before being used. Although verification procedures are available for use or adaptation (Basu et al. 2005; ASM 2012), verification of in-house media qualification methods are difficult if not impossible to find. Using an adapted version of Basu et al. 2005 and ASM 2012, five types of microbiological media from three different media categories were selected for the development of media qualification verification testing in the FPCLS. A list of the media included in the testing may be found in Table 3.1.

Only media types that are utilized for performing test methods that are part of the scope of accreditation were selected for media qualification verification testing. This included non-selective and selective liquids, as well as non-selective solid media. Selective solid media were not included in this procedure as they are not typically utilized for any of the test methods that will be part of the scope of ISO accreditation within the FPCLS testing laboratory at this time but may be included later on as the FPCLS expands the scope of its accreditation.

Table 3.1: Media Categories and Types. Table shows the microbiological media selected for evaluation by the FPCLS for media qualification verification testing in order to meet ISO/IEC 17025 accreditation requirements.

Non-Selective Liquid Media (QNL) Tryptic Soy Broth (TSB) Buffered Peptone Water (BPW)	Non-Selective Solid Media (QNS) Tryptic Soy Agar (TSA)
Selective Liquid Media (QSL) Romer RapidChek [®] <i>Listeria</i> (LRC) Neogen Reveal [®] 20-Hour <i>E. coli</i> O157:H7 (REC)	Selective Solid Media (QSS) Not Tested

Method Alterations in Comparison to References

Performing media qualification verification is very laborious and requires the analysis of many samples in order to demonstrate the efficacy of each media type being evaluated. When preparing the test protocol, both the ASM 2012 report and the publication by Basu et al. 2005 were reviewed for guidance in what the basic procedure for media qualification should be. Most of the baseline procedures, which were used for the FPCLS media qualification standard operating procedure (SOP), are derived from these two publications with a few changes that should be noted.

To meet the needs of the FPCLS, only qualitative recovery testing was developed for batches of media. It is recommended that each batch be tested against a previously prepared media batch (ASM 2012). However, since the purpose of this study was to compare different lot numbers of media types to determine the actual acceptable quality characteristic values and growth parameters this was not performed.

During inoculation preparation, Basu et al. 2005 recommended growing the cultures for 4 hours to obtain a 0.5 McFarland's Standard equivalent to guarantee

consistency in the amount of inoculum being transferred to each media sample. In the protocol described here, the cultures were grown overnight (18-24 hours) and then adjusted to a 0.5 McFarland's Standard equivalence by transferring a portion of the inoculum (typically 900µl or 1.0 ml) to sterile tryptic soy broth (TSB). This was found to be more effective at providing a consistent inoculum level. The FPCLS also chose to use American Type Culture Collection (ATCC) and FPCLS culture collection microorganisms that were selected to be part of the FPCLS quality control program, better fitting the needs of the FPCLS.

Another difference between the protocol described here and the references utilized was the physical characteristic or quality parameters that should be evaluated. Basu et al. 2005 recommends checking “*bubbles or pits, unequal filling of plates (uniform leveling), cracked medium in plate and freezing or crystallization,*” and also evaluating the pH of the media (Basu et al. 2005), while the Australian Society of Microbiology recommends the evaluation of “*colony size, colony morphology, biochemical responses, volume loss (by weight), gel strength, gas, turbidity, clarity, and hemolysis*” in shelf life studies for microbiological media (ASM 2012).

For simplicity, the FPCLS wanted to evaluate only simple characteristics that could be determined visually or by using basic procedures so that they could be easily utilized later on for everyday media qualification. This is allowed since there are no actual recommendations for which characteristics to consider in the ISO standard (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). Therefore, the chosen parameters for evaluation were; cracks, drying/thinning, color, contamination, pH, volume loss (by weight), and acceptable morphology depending on media type.

Compared to the published references, the most significant change that was made for the success of the qualification verification procedure, was altering the final step in the selective liquid media protocol. According to the ASM 2012 report, after incubation of the inoculum in the selective liquid media a portion should be transferred to selective and non-selective agars for evaluation using a 10 µl loop (ASM 2012). The point of this step is to determine if a certain number of organisms (< or >10 CFU) are present in the media. However, streak plating may not give the best opportunity for individual organisms to separate out for quantification, so the method was altered to transfer 10 µl to the plate using a pipettor and then spread plate the solution. This was much more effective at providing clear separation of the colonies during the growth evaluation stage.

Finally, one advantage of the FPCLS protocol over the ones used as references is the full-scale media qualification verification rather than focusing on everyday media qualification procedures only. Samples of each media type were tested against the qualification verification protocol at two storage temperatures (20-25°C and 2-8°C), across 6-7 time points for shelf life testing, for 3-4 lot numbers of each media type, and for a minimum of 3 replications. The details for performing media qualification verification for each of the media types selected for this project by the FPCLS including inoculation preparation, shelf life determination, test protocols, and results from analyses will be discussed in further details in the following sections.

Determining Quality Characteristics

There are several characteristics that may be assessed when determining if microbiological media, either liquid or solid, is suitable for its intended use. Although

there are no regulations depicting what characteristics must be evaluated when qualifying in-house media, there are guidelines that are available to help assist in the process.

Characteristics that have been recommended by international organizations include: drying and/or cracking, color changes, uneven filling or insufficient amount, growth, colony size and morphology, turbidity, volume, excessive moisture or dehydration, noticeable precipitants, biochemical responses, and contamination (CLSI 2004; ASM 2012). Basu et al. 2005 determined a quality control method for culture media for the detection of pathogens in infected patients evaluating many of the recommended characteristics. More specifically, it including pH, excessive bubbles, evaluation of additives (blood for hemolysis), unequal filling of plates, cracking, growth, colony size and morphology, gel strength, and contamination (Basu et al. 2005).

Based on these guidelines and previous studies, certain characteristics were chosen to be compatible to the FPCLSs laboratory size, capabilities, and needs. Therefore, in-house prepared media was separated into three distinct categories (non-selective solid, non-selective liquid, and selective liquid), and then assessed based on the category by specific characteristics for both quality and growth. The characteristics that were evaluated for non-selective solid media (Tryptic Soy Agar – TSA) included: cracking, drying/thinning of agar, color, pH, and contamination. Acceptable growth for non-selective solid media was evaluated based on the ecometric method of growth determination utilizing absolute growth index (AGI) scores. How to determine AGI scores will be discussed later on in this chapter.

The characteristics for non-selective liquid media were established for Tryptic Soy Broth (TSB) and Buffered Peptone Water (BPW) and included color, approximate

volume loss, pH, and contamination. As with non-selective solid media growth was evaluated but instead of AGI scores a different method was used. Here a tube score based on turbidity was applied to measure acceptable growth since the inoculated media is not plated. To accomplish this, absorbance values were obtained at 600 nm to determine the absorbance values against determined acceptance criteria for growth. The results were then compared to the tube score results to reduce the subjectivity of the method and to verify that this score system can be used effectively in evaluating growth.

Finally, characteristics for evaluating selective liquid media were determined for Romer RapidChek® *Listeria* (LRC) and Neogen Reveal® 20-Hour *E. coli* O157:H7 (REC). The quality characteristics chosen for evaluation included: color, volume loss, pH, and contamination. Growth was assessed by spread plating 10 µl of the overnight culture to provide a quantitative measure of growth. Bacterial growth was then evaluated against a pass/fail (qualitative result) criteria based on number of cells (CFU) present on the plates – see table 3.2 for growth criteria. Additionally, any growth observed was evaluated for proper morphology to verify that results only included the organisms of interest and not contaminants.

Each of the mentioned characteristics, both quality and growth, for determining if the media is acceptable or if it fails qualification were evaluated over the shelf life of each media type chosen by the FPCLS. Failure criteria were primarily based on growth acceptability, lack of contamination, pH, and volume (depending on media type) and have been derived from the acceptance criteria in “Guidelines for the Quality Assurance of Medical Microbiological Culture Media” (ASM 2012). Quality characteristics for each media type (cracking, contamination, color, etc.) were evaluated using an acceptability

score based on a ratio of passing and failing results derived from the overall mean acceptability.

Acceptability ratios were derived by averaging the number of passing and failing results from each replication (e.g. two replications passing acceptance criteria and one replication failing criteria would be a score of 67). Lot number acceptability ratios were then averaged together to obtain the overall mean acceptability score (e.g. one lot acceptability ratio of 67 and two lot acceptability ratios of 100 would give an overall mean acceptability score of 89). Acceptability scores of < 100 were considered alert levels (approaching unacceptable) with scores of < 90 being unacceptable. See Table 3.2 for the acceptance criteria determined by the FCPLS for all characteristics evaluated throughout the media qualification verification shelf life study.

Table 3.2: Media Qualification Acceptance Criteria. Table displays media qualification verification shelf life acceptance criteria for characteristics evaluated for non-selective solid, non-selective liquid, and selective liquid media types by the FPCLS.

-
1. Non-selective solid media (TSA)
 - a. AGI Score ≥ 70
 - i. FPCLS target ≥ 80
 - b. No visible cracks
 - c. No drying or thinning of agar
 - d. No visible contamination
 - e. No noticeable color change (darker or lighter)
 - f. pH within range 7.3 ± 0.2 (TSA)
 2. Non-selective liquid media (TSB, BPW)
 - a. Tube turbidity score “2”
 - b. Absorbance score @ 600 nm ≥ 0.500
 - c. No visible contamination
 - d. No noticeable color change (darker or lighter)
 - e. pH within range 7.3 ± 0.2 (TSB), 7.2 ± 0.2 (BPW)
 - f. No significant volume loss (< 0.25 g)
 3. Selective liquid media (LRC, REC)
 - a. Positive quality control (QC) culture (≥ 10 CFU) on selective agar and 50:50 plates
 - b. Negative QC culture (< 10 CFU) on selective agar, 50:50 plates, and non-selective agar
 - c. Acceptable morphology of colonies
 - d. No visible contamination in negative control tube or on non-selective agar
 - e. No noticeable color change (darker or lighter)
 - f. pH consistent with historical data
 - g. No significant volume loss (< 0.25 g)
-

Note: Acceptance criteria will be discussed within the methods and results sections for each media category with details on how to properly evaluate each media type against the acceptance criteria noted in Table 3.2. Acceptability scores for quality characteristics are < 100 (alert) and < 90 (unacceptable).

Designing an Appropriate Shelf Life Study

Similar to determining characteristics for evaluating media types, there are no set regulations on exactly which parameters must be followed or sampling requirements for a shelf life study. In fact, the standard the FPCLS is attempting accreditation against,

ISO/IEC 17025, does not mention any guidelines for media qualification (ISO and IEC 2005; ISO and IEC 2017). The AOAC guidelines that many labs utilize for audit preparation simply state that, “*every batch of media...shall be examined to ensure it is suitable for use*” (ISO and IEC 2005; AOAC 2015).

However, there are guidelines available that may help in the process of establishing such testing and many shelf life studies have been conducted that could be utilized in the experimental design. For example, one study that was reviewed determined the shelf life of chicken fillets related to the presence of *Salmonella* Enteritidis after product processing using high pressure pasteurization which may not seem to be relevant while developing testing procedures for media shelf life or qualification (Argyri et al. 2018). However, the study design was helpful because it utilized multiple pressure levels, across three-time points, and evaluated two storage temperatures.

For the FPCLS shelf life study, multiple characteristics were evaluated, across six to seven-time points (depending on media type), and two storage temperatures. Although the aforementioned study was conducted on food, a similar design can be implemented for use in evaluating media over time. In reviewing shelf life studies for food products, it was possible to build a shelf life study for microbiological media by merging information from studies more related to microbiological media, to determine with other guidelines which characteristics to evaluate.

Just as shelf life for food is defined as “*the time period within which the food is safe to consume and/or has an acceptable quality to consumers*” (Erickson and Hung 1997), shelf life for media might be defined as – the time period within which the media maintains its original quality attributes at an acceptable level and continues to provide

acceptable growth for quality control organisms. Just like foods, media will deteriorate over time leading to lower quality and reduced growth which could adversely affect testing results in a laboratory setting.

When designing the shelf life study, “Guidelines for Assuring Quality for Medical Microbiological Culture Media” (ASM 2012) were also taken into consideration.

Although this document provides examples of what a shelf life study should look like, they continually highlight that each laboratory is ultimately responsible for determining “*its own acceptance and rejection criteria*” and that each laboratory needs to determine the testing strategy that best fits their laboratory’s needs (ASM 2012). More specifically the “Validation of Shelf Life Example: Method 1” (ASM 2012) was followed along with analysis parameters determined by Basu et al. 2005 except that the procedures were modified to better capture the media characteristics that were chosen by the FPCLS to evaluate and to accommodate the testing capabilities of the laboratory – see Table 3.3.

Based on other shelf life studies (Ulisse et al. 2006) analysis for all non-selective media were conducted for about 3-months (98 days). For selective media the length of the study was based on manufactures’ recommendations for use. In any case, media was tested longer than the expected shelf life to provide a guaranteed acceptability at the final shelf life time point (i.e. 3-month). Romer Labs recommends the use of rehydrated LRC within 3 hours if stored at room temperature (20-25°C) and within 24 hours if stored in the refrigerator (~4°C) (Romer 2016a; Romer 2016b); while Neogen Corporation recommends REC, media be used within 6-hours of rehydration (Neogen 2016). Based on these time recommendations the FPCLS conducted the shelf life testing for LRC and REC media for 48 hours taking samples at various time points.

In order to account for variations in media between not only suppliers, but also from batch to batch preparation, non-selective media were prepared (three replications) from different lot numbers across multiple suppliers. Three lots of BPW were evaluated across two suppliers, while four lots each of TSA (two suppliers) and TSB (one supplier) were tested. For selective liquid media for the detection of *Listeria* spp. and *E. coli* O157:H7, three lots were evaluated but only across one supplier each as these are specialty media and are not provided by multiple suppliers – Table 3.3 displays the total number of lots and suppliers evaluated during analysis for each media type.

Media samples were prepared for shelf life testing independently for each lot and replication, with enough being prepared to conduct all the evaluations required for the entire shelf life study. Media tubes and plates were selected at random and separated into either room temperature (20-25°C) or refrigerated (2-8°C) storage for the duration of the shelf life testing. Sample plates or tubes of each lot number and replication were randomly selected for evaluation from both the room temperature and refrigerated storage locations at each time point.

Once the characteristics under evaluation were predetermined, it is then important to design a shelf life study that will capture all of those characteristics at specific intervals to provide the most useful data set and truly capture the point at which the media is no longer acceptable.

Non-selective solid media (TSA) were sampled at 0, 3, 7, 14, 30, 60, and 98 days to account for immediate use and storage shelf life options. Non-selective liquid media (TSB, BPW) were sampled at 0, 2, 4, 6, 8, 10, and 14 weeks (98 days). Selective liquid media (LRC, REC) were sampled at 0, 6, 12, 24, 36, and 48 hours due to their inherently

short shelf life. These sampling intervals provided enough data points to properly track the quality and growth characteristics of the media over time and allowed the FPCLS to make informed decisions on the actual shelf life of each media type based on the quality and growth characteristics evaluated throughout this study – see Table 3.3.

Table 3.3: Overview of Media Qualification Testing Parameters. Table shows an overview of FPCLS media qualification verification shelf life testing parameters including media category, type, characteristics examined, and shelf life time points tested.

Media Category	Media Type ¹	# Lots Tested	# Suppliers ²	Quality Characteristics ³	Shelf Life Time Points
Non-Selective Solid	TSA	4	2	growth (GI score), cracks, dry/thin, contamination, color, pH	0, 3, 7, 14, 30, 60, and 98 days
Non-Selective Liquid	TSB	4	1	turbidity (tube score), absorbance, contamination, color, pH, volume loss	0, 2, 4, 6, 8, 10, and 14 weeks (98 days)
	BPW	3	2		
Selective Liquid	LRC	3	1	growth (positive, negative, 50:50 QC cultures), colony morphology, contamination, color, pH, volume loss	0, 6, 12, 24, 36, and 48 hours
	REC	3	1		

¹Media Types are tryptic soy agar (TSA), tryptic soy broth (TSB), buffered peptone water (BPW), Romer RapidChek® *Listeria* media (LRC), and Neogen Reveal® 20-Hour *E. coli* O157:H7 media. ²Suppliers included Acumedia, BD Difco, and Cole Palmer. Supplier information for generic media types was kept confidential by using codes for lot numbers evaluated. ³Characteristics GI (growth index score or % relative growth index must be ≥ 70), volume loss is by weight in grams (criteria < 0.25 g loss), turbidity scores are 0-2 with 2 being good growth, absorbance is at 600 nm ≥ 0.500 , acceptability of selective liquid media growth is ≥ 10 CFU for positive quality control (QC) cultures and < 10 cfu for negative QC cultures, 50:50 are plates containing both positive and negative QC cultures, other quality characteristic acceptability scores < 100 alert level and < 90 unacceptable.

Analyzing the Data

Data collected from the shelf life study for all media evaluated at both storage temperatures was analyzed to determine the actual shelf life of each media type.

Evaluating the growth parameters is the most effective method in determining when the media begins to fail throughout the shelf life study as it is the most important factor for analyzing laboratory samples. However, each media type will be viewed slightly different since acceptable growth rate determination and other characteristics of interest may vary from media to media.

Refer to Table 3.2 for a list of acceptance criteria for each characteristic evaluated. Also, refer to each media types methods and materials section for details pertaining to the processes and procedures used to collect all of the data necessary to determine the acceptability of each media type.

Non-Selective Solid Method

Bacterial Cultures and Inoculum Preparation

Two non-pathogenic microorganisms, positive and negative FPCLS quality assurance designated cultures, were used for evaluating acceptable growth (AGI score) of non-selective solid media, TSA, as part of the media qualification verification test procedure. An *Escherichia coli* strain, *E. coli* (Migula) Castellani and Chalmers ATCC 25922, was chosen for testing as the positive control and a *Staphylococcus* strain, *Staphylococcus aureus* Rosenbach ATCC 6538P, was selected as the negative control. Both strains were acquired from the American Type Culture Collection (ATCC) and verified for purity.

Strains were individually reactivated from -80°C freezer stocks (20% glycerol in tryptic soy broth) by aseptically transferring a loop full of frozen culture to a tryptic soy agar plate (TSA, Acumedia Neogen Corporation, Lansing, MI, USA), streaking for isolation, and incubating at 35-37°C for 18-24 hours. Overnight cultures were then prepared by aseptically transferring 1 colony from the plate to a 9-ml tube of tryptic soy broth (TSB, Acumedia Neogen Corporation, Lansing, MI, USA) and incubating at 35-37°C for 18-24 hours.

After incubation, inoculum was prepared by vortexing the overnight tubes for 5-10 seconds to ensure they were homogenous, and then transferring 900 µl (*E. coli*) and 1 ml (*S. aureus*) of the overnight culture to a 5 ml tube of TSB to create a solution that is equivalent to a 0.5 McFarland's Standard (approximately 1.5×10^8 CFU/ml). Adjustments were made when necessary. Tubes (5-ml each) were then vortexed for 5-10 seconds to ensure inoculum was homogenous. Inoculum was made fresh each time and used within 4 hours of preparation (inoculum prepared for each lot number and replication separately).

Preparation of TSA

Tryptic Soy Agar from two manufacturers (Acumedia, BD Difco) was selected for analysis (two lot numbers each) and given generic names to protect the manufacturers identities – TSA-1, TSA-2, TSA-3, and TSA-4. All media was prepared per manufacturer's instructions by adding 40 grams of dehydrated media base to 1-liter of deionized water (E-Pure Barnstead, Thermo Fisher Scientific, Waltham, MA, USA), mixing thoroughly, and then autoclaving at 121°C for 20 minutes. Autoclave

performance was verified using *B. stearotheophilus* ProSpore vials. Media was then tempered to 55-60°C and hand poured (15-20 ml) into 100 mm x 15 mm petri plates in a biological safety cabinet (BSC Airstream Class 2, ESCO, Horsham, PA, USA).

After allowing the media to dry overnight (18-24 hours), TSA lots were bagged and placed in their respective storage locations, either room temperature (20-25°C) or refrigeration temperature (2-8°C). For the shelf life evaluation, enough media was made for each lot number to perform all analysis requirements at each time point. Three replications of each media lot number were tested. TSA plates for each replication of each lot number were made separately to ensure that they were truly independent replications.

General Testing Procedure and Shelf Life Parameters – Non-Selective Solid Media

Non-selective solid media lot numbers/replications were stored at room temperature (20-25°C) and refrigeration temperature (2-8°C) until reaching the predetermined sampling time points: 0, 3, 7, 14, 30, 60, and 98 days. Day “0” of shelf life testing was actually day “1” due to the overnight hold in the biological safety cabinet to allow the media to properly dry/harden. At each time point, prepared plates of non-selective solid media were retrieved from both room and refrigeration temperatures for all lot numbers and replications tested. All three replications were run simultaneously to reduce the amount of time necessary to complete analysis.

Plates were evaluated for quality factors of cracks in agar, drying or thinning of agar, presence of contamination, color change (lighter or darker), and pH. Plates under evaluation were selected at random for all quality checks and for determining growth. To

test for growth acceptability, randomly selected plates were inoculated with the control organisms using the ecometric streak scheme (template used for consistency) and incubated at 35-37°C for 20-28 hours. Samples of each lot/replication were tested in duplicate. After incubation, agar plates were given an absolute growth index score based on which streak scheme lines showed growth.

Non-Selective Solid Media (TSA) Quality Characteristics

Quality characteristics for non-selective solid media were evaluated at each time point throughout the shelf life study on randomly selected agar plates from each lot number and each replication for both storage temperatures – see Table 3.2. The quality parameters that were evaluated on a “Pass/Fail” basis included: cracks in agar, drying or thinning of agar, presence of contamination, and color change (lighter or darker from initial color). All quality parameters were evaluated visually and acceptance criteria for agar plates included:

1. Cracks in Agar: plates were checked visually for cracks by looking at the surface and bottom side of the agar. Only cracks that split the agar were considered for this parameter with the presence of any cracks giving a failing result and the absence giving a passing mark.
2. Drying or Thinning of Agar: plates were checked for drying and/or thinning of the agar visually. Pictures were taken of the plates after being prepared to determine the approximate depth of the agar within the petri plate. Plates were compared to these pictures to determine if drying/thinning had occurred. No drying provides a passing mark while any visually noticeable drying or thinning was considered a failing result.
3. Contamination: plates were evaluated visually for any microbiological contamination beginning to grow while the plates were in storage locations. No

contamination gives a passing mark and the presence of any contaminants regardless of size or quantity was considered a failing result.

4. Color Change: plates were evaluated visually to determine if the color of the agar plates have changed (lighter or darker). Pictures were taken of the plates after being prepared on the bench top under the same lighting conditions within the laboratory to determine the initial color of the agar within the petri plate. Plates were compared in the same location and lighting conditions to the pictures to determine if color changes had occurred. No notable color change was considered passing while any noticeable color change, either lighter or darker, was considered to be a failing result.

Non-Selective Solid Media (TSA) pH Testing

pH testing on non-selective solid media was performed by randomly selecting an agar plate at each time point for each lot number/replication and for each storage temperature. Agar plates were scored with a spatula 4-5 times, vertically and horizontally, to cut the agar into smaller pieces – see Figure 3.1. Agar pieces were then transferred to a vessel, smashed with a spatula multiple times to further decrease the size of the agar pieces, and deionized water was added to the vessel (3-5 ml), stirring to create a mixture. pH was determined for each mixture using a benchtop pH meter (PC700, Oakton, Vernon Hills, IL, USA) and pH probe (Orion 9106, Thermo Fisher Scientific, Waltham, MA, USA). The pH meter was calibrated before each use to verify the accuracy of the pH probe. Non-selective solid media was considered acceptable if pH range was within manufacturer's acceptance range of 7.3 ± 0.2 (TSA).

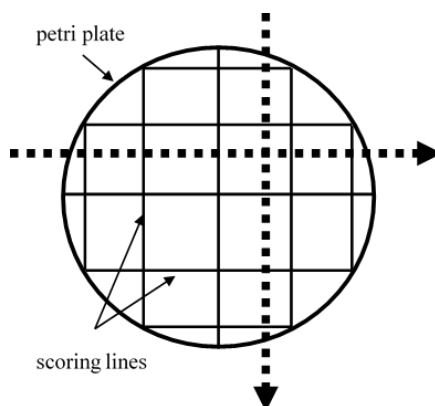


Figure 3.1: Non-Selective Solid Media pH Preparation. Figure shows an overhead view of a petri plate containing non-selective solid media. Figure shows the scoring pattern made to cut the agar into smaller pieces using a spatula. Larger arrows indicate the direction of the scoring patterns.

Non-Selective Solid Media (TSA) Growth Acceptability

Growth of non-selective solid media was evaluated at all time points throughout the shelf life study for each lot/replication and for each storage temperature utilizing the Absolute Growth Index (AGI) score determined from the ecometric method. The AGI score is a good way of applying a quantitative value to the media to determine if it is properly supporting the growth of target microorganisms. AGI consists of utilizing the ecometric streak plate scheme (Figure 3.2) and comparing the number of streak lines that show growth after incubation against a table of scores (Table 3.4).

To obtain an AGI score a 1 μ l loop is dipped into the inoculum and following the AGI steak pattern from Figure 3.2 the inoculum is transferred to the petri plate starting at A1 and streaking in sequence from A1, B1, C1, D1, A2, B2, C2... up to D5 until all lines have been inoculated lifting the loop in-between lines to create a dilution affect. The loop is inserted into the inoculum only once at the beginning of the streaking process. The number of streak lines on the plate that show growth after incubation correlates to the

AGI score seen in Table 3.4 (Basu et al. 2005; ASM 2012). Growth scores were then recorded so they could be compared at each time point to determine when the media began to fail. All media must have an AGI score ≥ 70 in order to be considered acceptable.

A template was used to trace the lines for the AGI streaking pattern onto the back side of the agar plates. This template (actual size) can be found in Figure 3.2. It is important that the streaking pattern be consistent from plate to plate so that the non-selective media plates receive the same amount of inoculum on each streak line. Inconsistency in the amount of inoculum from plate to plate could adversely affect the results. Table 3.5 contains the exact lengths that each streak line should be on the template to ensure consistency from sample to sample. Streak line patterns were traced onto the media plates immediately before inoculation procedures to ensure the plates had been properly stored for the full length of each shelf life time point.

Table 3.4: Absolute Growth Index Scores. Table displays absolute growth index scores which are determined from the ecometric method streak plate scheme. Score corresponds to the last line showing growth. Adapted from Basu et al 2005.

A1 = 5	B1 = 10	C1 = 15	D1 = 20
A2 = 25	B2 = 30	C2 = 35	D2 = 40
A3 = 45	B3 = 50	C3 = 55	D3 = 60
A4 = 65	B4 = 70	C4 = 75	D4 = 80
A5 = 85	B5 = 90	C5 = 95	D5 = 100

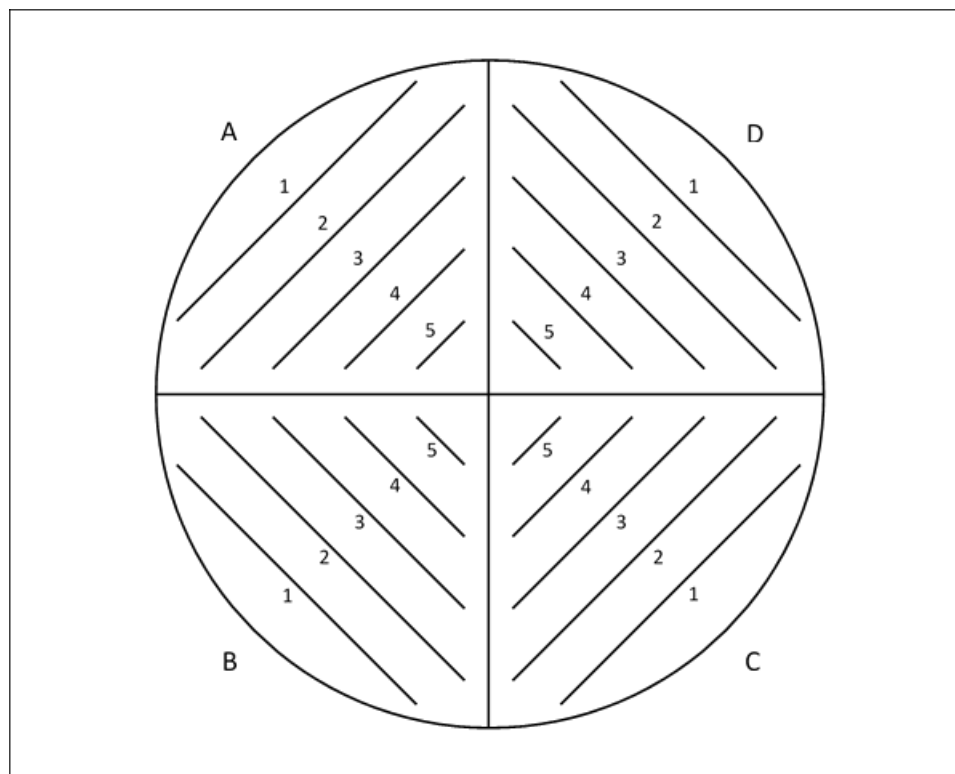


Figure 3.2: Ecometric Method Streak Plate Scheme Template. Template is actual size and may be utilized for tracing lines on a 100 mm x 15 mm petri-plate.

Table 3.5: Absolute Growth Index Template Dimensions. Table shows the Absolute Growth Index template streak pattern dimensions (line lengths) for a 100 mm x 15 mm petri plate (Line # applies to all quadrants A-D).

Line #	Length (inches)	Length (mm)
1	1.25	31.75
2	1.38	35.05
3	1.00	25.40
4	0.63	16.00
5	0.25	6.35
Diameter of Template	3.48	88.39

Non-Selective Liquid Method

Bacterial Cultures / Inoculum Preparation

Six designated cultures were used for analyzing non-selective liquid media, TSB and BPW, as part of the media qualification verification test procedure. An *Escherichia coli* strain, *E. coli* (Migula) Castellani and Chalmers ATCC 25922, was chosen for analyzing TSB. Five strains of *Salmonella* spp. were chosen for analyzing BPW as a cocktail: *Salmonella* Enteritidis PT 4 NVSL 94-13062, *Salmonella* Heidelberg 3347-1 Sheldon, *Salmonella* Agona 442967, *Salmonella* Senftenberg 447237, *Salmonella* Typhimurium ATCC 14028. Strains were acquired from the American Type Culture Collection and the University of Nebraska-Lincoln FPCLS culture collection and were verified for purity.

Strains were individually reactivated from -80°C freezer stocks (20% glycerol in tryptic soy broth) by aseptically transferring a loop full of frozen culture to a tryptic soy agar plate (TSA, Acumedia Neogen Corporation, Lansing, MI, USA), streaking for isolation, and incubating at 35-37°C (*E. coli*) and 41-43°C (*Salmonella* spp.) for 18-24 hours. Overnight cultures of each individual strain were then prepared by aseptically transferring 1 colony from the plate to a 9-ml tube of tryptic soy broth (TSB, Acumedia Neogen Corporation, Lansing, MI, USA) and incubating at 35-37°C (*E. coli*) and 41-43°C (*Salmonella* spp.) for 18-24 hours.

After incubation, a cocktail of *Salmonella* spp. was prepared by vortexing the overnight culture tubes for 5-10 seconds to ensure they were homogenous, and then transferring 1 ml of each of the five strains into a sterile test tube combining the strains. The cocktail was then vortexed for 5-10 seconds to create a homogenous mixture.

Inoculum was then prepared by transferring 900 µl of *E. coli* overnight culture or *Salmonella* spp. cocktail to a 5 ml tube of TSB to create a solution that is equivalent to a 0.5 McFarland's Standard (approximately 1.5×10^8 CFU/ml) and adjusting if necessary. Tubes (5-ml each) were then vortexed for 5-10 seconds to ensure inoculum was homogenous. Inoculum was made fresh each time plates were evaluated and used within 4 hours of preparation (inoculum prepared for each lot number and replication separately).

Preparation of Non-Selective Liquid Media

Tryptic Soy Broth from one manufacturer was selected for analysis with four lot numbers being evaluated and given generic names to protect the manufacturers identity – TSB-1, TSB-2, TSB-3, and TSB-4. All media was prepared per manufacturer's instructions by adding 30 grams of dehydrated media base to 1-liter of deionized water (E-Pure Barnstead, Thermo Fisher Scientific, Waltham, MA, USA), mixing thoroughly, transferring 5-ml aliquots to test tubes using a bottle pump, and then autoclaving at 121°C for 20 minutes. Autoclave performance was verified using *B. stearothersophilus* ProSpore vials.

Buffered Peptone Water (BPW) from two manufacturers (Acumedia, Cole Palmer) was selected for analysis with two lot numbers from one manufacturer and one lot number from the other manufacturer. Different lot numbers were given generic names to protect the manufacturers identities – BPW-1, BPW-2, and BPW-3. All media was prepared per manufacturer's instructions by adding 20 grams of dehydrated media base to 1-liter of deionized water (E-Pure Barnstead, Thermo Fisher Scientific, Waltham, MA,

USA), mixing thoroughly, transferring 5-ml aliquots to test tubes using a bottle pump, and then autoclaving at 121°C for 20 minutes. Autoclave performance was verified using *B. stearothermophilus* ProSpore vials.

Media tubes were then tempered to 20-25°C and placed in each shelf life storage location, either room temperature (20-25°C) or refrigeration temperature (2-8°C) and allowed to equilibrate overnight (18-24 hours) prior to testing. For the shelf life evaluation, enough media was made for each lot number to perform all analysis requirements at each time point. Three replications of each media lot number were evaluated. TSB and BPW tubes for each replication of each lot number were made separately to ensure that they were truly independent replications.

General Testing Procedure and Shelf Life Parameters – Non-Selective Liquid Media

Non-selective liquid media lot numbers/replications were stored at room temperature (20-25°C) and refrigeration temperature (2-8°C) until reaching the predetermined sampling time points: 0, 2, 4, 6, 8, 10, and 14 weeks (98 days). Day “0” of shelf life testing is actually day “1” due to overnight hold period allowing the media to properly equilibrate to the storage conditions. At each time point, prepared tubes of non-selective liquid media were retrieved from both room and refrigeration temperatures for all lot numbers and replications tested. All three replications were run simultaneously to reduce the amount of time necessary to complete analyses.

Tubes were evaluated for quality by checking for presence of contamination, color change (lighter or darker), pH, and volume loss by weight. Tubes under evaluation were selected at random for all quality checks and for determining growth. To test for

growth acceptability, randomly selected tubes were aseptically inoculated with the control organisms using sterile inoculation loops and incubated at 35-37°C (TSB) and 41-43°C (BPW) for 20-28 hours. Samples of each lot/replication were tested in duplicate. After incubation, media tubes were evaluated visually based on turbidity and then a portion of each sample was transferred to a microreader plate and processed to evaluate absorbance.

Quality Characteristics Non-Selective Liquid Media

Quality characteristics for non-selective liquid media were evaluated at each time point throughout the shelf life study on randomly selected media tubes from each lot number and each replication for both storage temperatures – see Table 3.2. The quality parameters that were evaluated on a “Pass/Fail” basis included; presence of contamination, and color change (lighter or darker from initial color). All quality parameters were evaluated visually so they could be easily utilized (if applicable) for laboratory media verification activities after the qualification process was concluded. Acceptance criteria for media in tubes included:

1. Contamination: media tubes were evaluated visually for any microbiological contamination while the tubes were in storage locations by holding the tubes up to a light source. No contamination or turbidity gives a passing mark and the presence of any contaminants or turbidity was considered a failing result.
2. Color Change: tubes were evaluated visually to determine if the color of the liquid media had changed (lighter or darker). Pictures were taken of the tubes on the bench top under the same lighting conditions after being prepared to determine the initial color of the media. Tubes at each time point were compared to these pictures under the same laboratory/lighting conditions to determine if color

changes had occurred. No noticeable color change was considered a passing result while any noticeable color change, either lighter or darker, was considered to be a failing result.

Volume Loss and pH of Non-Selective Liquid Media

Volume loss (by weight) testing on non-selective liquid media was performed by randomly selecting media tubes at each time point for each lot number/replication and for each storage temperature. A vessel was placed on the analytical balance (AX4202 Balance, OHAUS Corporation, Parsippany, NJ, USA), tared, and then the contents of the media tube were carefully transferred to the vessel with the weight being recorded. Weights were then compared to the values obtained at time “0” to determine evaporation or loss of weight over time in grams (acceptance criteria < 0.25 g loss). Vessels were then transferred to the pH recording area and pH was determined in each sample using a benchtop pH meter (PC700, Oakton, Vernon Hills, IL, USA) and pH probe (Orion 9106, Thermo Fisher Scientific, Waltham, MA, USA). The laboratory pH meter was calibrated before each use to verify the accuracy of the pH probe. Non-selective liquid media was considered acceptable if the pH value was within the acceptance range of 7.3 ± 0.2 (TSB) or 7.2 ± 0.2 (BPW).

Growth Non-Selective Liquid Media

Growth of non-selective liquid media was evaluated at each time point throughout the shelf life protocol for each lot/replication and for each storage temperature in two stages. After evaluating the quality characteristics, randomly selected media tubes were inoculated using a 1 μ l sterile loop by dipping the loop into the inoculum and then

aseptically transferring the cultures to the tubes, swirling gently to facilitate the release of the organisms, and then incubating at appropriate temperatures.

After incubation each tube was given a tube score utilizing a subjective visual system with three growth levels from 0-2 based on the turbidity. Tube scores were defined as: “0” for no turbidity or no growth observed; “1” for very light turbidity and some cloudiness corresponding to weak growth; and “2” for good turbidity heavy cloudiness corresponding to good growth (Figure 3.3). A sample must receive a “2” score to be considered acceptable.

Once growth score was recorded, 280 μ l of media from each tube was transferred to a 96 well microreader plate and tested at 600 nm to obtain absorbance values using a microplate reader (Bio-Rad Laboratories iMark™ Hercules, CA, USA). Initial absorbance values were taken after incubation for evaluation of time “0” to allow for comparisons of absorbance values at each time point. Growth turbidity scores and absorbance values were then recorded so they could be compared at each time point to determine when the media begins to fail and how the turbidity score correlates to the absorbance value. For evaluation purposes, tubes must have an absorbance at 600 nm \geq 0.500 to be considered a “2” score.

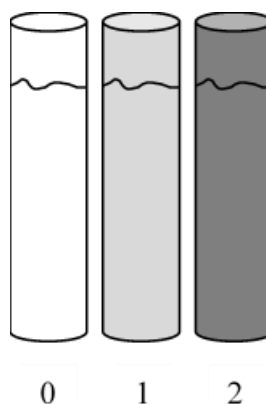


Figure 3.3: Non-Selective Liquid Media Tube Scores. Figure shows tube score visualization for determining growth acceptability in FPCLS. Tube turbidity scores are “0” “1” and “2” with “0” being no growth or clear, “1” light growth/turbidity, and “2” good or heavy growth/turbidity.

Selective Liquid Media Method

Bacterial Cultures and Inoculum Preparation

Designated cultures were used for analyzing selective liquid media, LRC and REC, as part of the media qualification verification test procedure. Five strains of *Listeria monocytogenes* were selected as the positive control for analyzing LRC as a cocktail; *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 49594, *L. monocytogenes* 104 ser. 4B (Scott A), *L. monocytogenes* 2 ser. 1/2A, and *L. monocytogenes* 110 ser. 1/2A. Two *Escherichia coli* strains, *E. coli* ATCC 25922 and *E. coli* O157:H7 ATCC 35150, were selected as the negative control organisms for analyzing LRC (negative control strains evaluated independently and not as a cocktail).

Five strains of *Escherichia coli* O157:H7 were selected as the positive control for analyzing REC as a cocktail; *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 ATCC 43894, *E. coli* O157:H7 USDA-FSIS 380-94, and *E. coli* O157:H7 E0019. *Staphylococcus aureus* Rosenbach ATCC 6538P, was selected as the

negative control for REC analysis. Strains were acquired from the American Type Culture Collection and the University of Nebraska-Lincoln FPCLS culture collection and were verified for purity.

Strains were individually reactivated from -80°C freezer stocks (20% glycerol in tryptic soy broth) by aseptically transferring a loop full of frozen culture to a tryptic soy agar plate (TSA, Acumedia Neogen Corporation, Lansing, MI, USA), streaking for isolation, and incubating at 35-37°C (*E. coli* spp. and *S. aureus*) and 29.5-30.5°C (*Listeria* spp.) for 18-24 hours. Overnight cultures of each individual strain were then prepared by aseptically transferring 1 colony from the plate to a 9-ml tube of tryptic soy broth (TSB, Acumedia Neogen Corporation, Lansing, MI, USA) and incubating at 35-37°C (*E. coli* spp. and *S. aureus*) and 29.5-30.5°C (*Listeria* spp.) for 18-24 hours.

After incubation, cocktails of both *Listeria* spp. and *E. coli* spp. were prepared by vortexing the overnight tubes for 5-10 seconds to ensure they were homogenous, and then transferring 1 ml of each of the five strains (by type) into a sterile test tube combining the strains. Each cocktail was then vortexed for 5-10 seconds to create a homogenous mixture. Inoculum was then prepared by transferring 900 µl of *E. coli* O157:H7 cocktail or *E. coli* controls, and 1 ml of *L. monocytogenes* cocktail and *S. aureus* controls, to 5 ml tubes of TSB to create solutions that are equivalent to a 0.5 McFarland's Standard (approximately 1.5×10^8 CFU/ml) and adjusting if necessary. Tubes (5-ml) were then vortexed for 5-10 seconds to ensure inoculum was homogenous. Inoculum was made fresh each time media was evaluated and used within 4 hours of preparation (inoculum prepared for each lot number and replication separately).

Preparation of Selective Liquid Media

RapidChek® *Listeria* media from Romer Labs (RapidChek, Romer Labs, Newark, DE, USA) was selected for analysis with three lot numbers being selected and given generic identities to avoid bias – LRC-1, LRC-2, and LRC-3. All media was prepared per manufacturer's instructions by adding 53 grams of dehydrated media base and 1 gram of *Listeria* RapidChek® Supplement to 1-liter of sterile deionized water in a biological safety cabinet (BSC Airstream Class 2, ESCO, Horsham, PA, USA), mixing thoroughly, and aseptically transferring 5-ml aliquots to test tubes using a serological pipette.

Reveal® 20-Hour for *E. coli* O157:H7 media from Neogen Corporation (Reveal, Neogen Corporation, Lansing, MI, USA) was selected for analysis with three lot numbers being selected and given generic identities to avoid bias – REC-1, REC-2, and REC-3. All media was prepared per manufacturer's instructions by adding 37 grams of dehydrated media base to 1-liter of sterile deionized water in a biological safety cabinet (BSC Airstream Class 2, ESCO, Horsham, PA, USA), mixing thoroughly, and aseptically transferring 5-ml aliquots to test tubes using a serological pipette.

Media tubes were then placed in each shelf life storage location, either room temperature (20-25°C) or refrigeration temperature (2-8°C) and allowed to equilibrate for ≤ 1 hour prior to testing time "0". For the shelf life evaluation, enough media was made for each lot number to perform all analysis requirements at each time point. Three replications of each media lot number were tested. LRC and REC tubes for each replication of each lot number were made separately to ensure that they were truly independent replications.

General Testing Procedure and Shelf Life Parameters – Selective Liquid Media

Selective liquid media lot numbers/replications were stored at room temperature (20-25°C) and refrigeration temperature (2-8°C) until reaching the predetermined sampling time points: 0, 6, 12, 24, 36, and 48 Hours. Hour “0” of shelf life testing is actually hour “1” due to preparation procedures and allowing the media to properly equilibrate to the storage conditions. At each time point, prepared tubes of selective liquid media were retrieved from both room and refrigeration temperatures for all lot numbers and replications tested. All three replications were run simultaneously to reduce the amount of time necessary to complete testing however, lot numbers were tested on different days to increase the robustness of the data.

Tubes were evaluated for quality by checking for presence of contamination in tubes or on non-selective agar, color change (lighter or darker), pH, and volume loss by weight. Tubes under evaluation were selected at random for all quality checks and for determining growth. To test for growth acceptability, randomly selected tubes were aseptically inoculated with the control organisms using sterile inoculation loops. Testing microorganisms included positive control cocktail, 50:50 (positive:negative control mix), negative control culture, and negative control (no organism, sterility) tubes.

All tubes were incubated at 29.5-30.5°C (LRC) and 35-37°C (REC) for 20-28 hours. Samples of each lot/replication were tested in duplicate. After incubation, each media tube was spread plated on selective and non-selective agars and incubated at 29.5-30.5°C (LRC) and 35-37°C (REC) for 20-28 hours. After final incubation, plates were evaluated for growth of control organisms on a Pass/Fail criterion taking into account the morphology of the control organisms.

Quality Characteristics Selective Liquid Media

Quality characteristics for selective liquid media were evaluated at each time point throughout the shelf life study on randomly selected media tubes from each lot number and each replication for both storage temperatures – see Table 3.2. The quality parameters that were evaluated on a “Pass/Fail” basis included; presence of contamination in tubes or on non-selective agar, and color change (lighter or darker from initial color). All quality parameters were evaluated visually and acceptance criteria for media in tubes included:

1. Contamination: media tubes were evaluated visually for any microbiological contamination beginning to grow while the tubes were in storage locations by holding the tubes up to a light source. Additionally, non-selective agar plates were used to spread plate the sterility sample tube and were also evaluated for any potential contaminants growing post incubation. No contamination, colonies, or turbidity gives a passing mark and the presence of any contaminants, colonies, or turbidity was considered a failing result.
2. Color Change: tubes were evaluated visually to determine if the color of the liquid media had changed becoming either lighter or darker in color. Pictures were taken of the tubes after being prepared to determine the initial color of the media. Tubes at each time point were compared to these pictures to determine if color changes had occurred. If no color change was noted then it was considered a passing result while any noticeable color change, either lighter or darker, was considered to be a failing result.

Volume Loss and pH of Selective Liquid Media

Volume loss (by weight) testing on selective liquid media was performed by randomly selecting media tubes at each time point for each lot number/replication and for each storage temperature. A vessel was placed on the analytical balance (AX4202

Balance, OHAUS Corporation, Parsippany, NJ, USA), tared, and then the contents of the media tube were carefully transferred to the vessel with the weight being recorded.

Weights were then compared to the values obtained at time “0” to determine evaporation or loss of weight over time in grams (acceptance criteria < 0.25 g loss). Vessels were then transferred to the pH recording area and pH was determined in each sample using a benchtop pH meter (PC700, Oakton, Vernon Hills, IL, USA) and pH probe (Orion 9106, Thermo Fisher Scientific, Waltham, MA, USA). The laboratory pH meter was calibrated before each use to verify the accuracy of the pH probe. The pH of selective liquids was compared to FPCLS historical laboratory data to confirm acceptability, as no acceptable pH parameters are provided by the manufacturers.

Growth Selective Liquid Media

Growth of selective liquid media was evaluated at each time point throughout the shelf life protocol for each lot/replication and for each storage temperature in two stages. After evaluating the quality characteristics, randomly selected media tubes were inoculated for growth determination of positive control culture, negative control culture, 50:50 (positive:negative control mixture), and negative control (media – no organism). First, positive control inoculum was aseptically transferred to the first tube by dipping a 1 µl loop into the positive control inoculum and then placing the loop into the tube and swirling gently to release the organisms.

Next, 100 µl of the negative control inoculum was transferred to a second tube using a pipettor and sterile filtered pipette tip. The 50:50 tube was created by transferring 200 µl each of both the positive and negative control cultures to a sterile test tube,

vortexing for 5-10 seconds, and then transferring 100 µl of the mixture to a third media tube. This step was done twice for the LRC media as there were two separate negative control cultures. The negative (media) control (no organism) tube was not inoculated but was incubated and plated to verify the sterility of the sample.

After incubation, the tubes were transferred to selective and non-selective agars to evaluate the growth of the control organisms. Procedures included:

1. Positive Control Culture – transfer 10 µl to selective media and spread plate. Selective agars included Oxford Agar (OX Agar Acumedia Neogen Corporation, Lansing, MI, USA) for *Listeria monocytogenes* and Sorbitol MacConkey Agar (SMAC Agar Acumedia Neogen Corporation, Lansing, MI, USA) for *Escherichia coli*.
2. Negative Control Culture – transfer 10 µl to non-selective media and spread plate. Non-selective agar utilized for analysis was TSA (TSA Acumedia Neogen Corporation, Lansing, MI, USA).
3. 50:50 Competition Positive and Negative Control Cultures – transfer 10 µl to selective media and spread plate. Selective agars included Oxford Agar (OX Agar Acumedia Neogen Corporation, Lansing, MI, USA) for *Listeria monocytogenes* and Sorbitol MacConkey Agar (SMAC Agar Acumedia Neogen Corporation, Lansing, MI, USA) for *Escherichia coli*.
4. Negative Control (No Organism, Sterility Sample) - transfer 10 µl to non-selective media and spread plate. Non-selective agar utilized for testing was TSA (TSA Acumedia Neogen Corporation, Lansing, MI, USA).

After spread plating the media tubes onto selective and non-selective agars, the plates were incubated at appropriate temperatures. Once incubation was completed the plates were evaluated for growth and were considered acceptable if: (1) > 10 CFU appeared on positive control culture plates; (2) > 10 CFU of the positive control culture

and < 10 CFU of the negative control culture appeared on the 50:50 plates; (3) < 10 CFU negative control culture formed on the negative control plates; and (4) no growth was observed on the negative (media) control plates. Additionally, the morphology of the organisms was taken into account including the size, shape, color, etc. to determine if the colonies being counted for acceptability were the correct organism. All results were recorded for further comparison.

Statistical Analysis

Statistical Analysis System (SAS) software version 9.4 (SAS Institute Inc., Cary, NC, USA), was utilized to analyze all media qualification verification data. Data for each parameter was evaluated by media lot (random block design) giving variance, residual variance estimates, and standard errors for the overall mean of each media type. Type III fixed effects were calculated for the differences based on Time, Temperature, and the interaction between them. In cases where interactions were significant, simple effects were evaluated looking at differences between temperatures at given time points. P-values < 0.05 were considered significant.

Least squares means (LSMEANS) for temperature*time interactions were analyzed using Tukey-Kramer to determine if they were significant from time zero. Simple effects were evaluated when interactions were significant. Analysis for Absolute Growth Index (growth scores) of tryptic soy agar were performed utilizing the beta distribution for scaled responses (divided by 100) since the data being analyzed were bound between 0 and 100.

Binary data (tube scores, color change, contamination, etc.), where possible, were analyzed using the binary distribution modeling the probability of the lowest response. LSMEANS were used to acquire estimates for the model scale. SAS code ILINK was utilized to display estimated means and standard errors of data LSMEANS (not the differences). Laplace (SAS) method was used to estimate the responses due to convergence issues with the data.

MEDIA QUALIFICATION VERIFICATION RESULTS

The following sections contain results obtained during media qualification verification procedures for non-selective solid (TSA), non-selective liquid (TSB, BPW), and selective liquid (LRC, REC) media types. Microbiological media were evaluated for cracks, dryness/thinning, color change, contamination, pH, volume loss (by weight), acceptable morphology (depending on media type), and each microbiological medias' ability to support acceptable levels of growth. During data analysis, overall mean results were reviewed to determine shelf life for each media type. The overall mean was the average of the media lot means and media lot mean was the average of each replication.

All procedures for evaluating quality and growth characteristics utilized for the shelf life testing of each media type were adapted from the ASM (2012) report, CLSI (2004) report, and Basu et al. (2005) publication. Each microbiological media type was evaluated for the purpose of meeting ISO requirements with the goal of obtaining ISO/IEC 17025 accreditation. In this context, all microbiological media must be “*verified to their performance*” and all purchased dehydrated media must be “*examined for suitability*” (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017).

Storage of Samples and Inoculum Adjustment

Shelf life tested solid and liquid microbiological media and stock cultures utilized in media qualification verification procedures were stored under temperature-controlled environment to aid in the consistency and accuracy of the qualification data.

Temperatures were taken daily to ensure that storage temperatures did not fluctuate too widely and were under control. Figure 3.F1 displays a representation of the temperatures taken for each storage location including: room temperature plates/tubes stored at 20-25°C in the laboratory storage cabinet; refrigerated plates/tubes stored at 2-8°C in the walk-in cooler; and frozen culture (control organisms) stocks stored at approximately -80°C in the ultra-low temperature freezer.

Microbiological cultures were adjusted to a 0.5 McFarland's Standard (1.5×10^8 CFU/ml or 8.18 LOG CFU/ml) equivalent prior to being utilized for media qualification verification testing purposes. Average inoculum levels for shelf life time points for TSA, TSB, BPW, LRC, and REC were ≥ 8.00 LOG CFU/ml (Figure 3.F6, Figure 3.F7, Figure 3.F8). All cultures were verified quantitatively using serial dilutions (10^{-10}) and plating methods on generic and selective agars.

Non-Selective Solid Agar – TSA

Quality characteristic acceptability score results for drying/thinning of TSA agar were recorded for shelf life time points 0, 3, 7, 14, 30, 60, and 98 days at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) for TSA 1, TSA 2, TSA 3, and TSA 4. Initially all lots received a score equal to 100 and gradually values reduced with failing results being achieved as early as day 7. More specifically, TSA 2 (RT), TSA

2 (2-8°C), TSA 3 (RT), TSA 4 (RT) were failing by day 7 with scores of 67 or below (Table 3.T3). Figure 3.F4 displays the overall mean acceptability scores during shelf life evaluation for drying/thinning of agar.

Acceptability score results for cracking of agar (Table 3.T3) and contamination (Table 3.T4) for lots TSA 1, TSA 2, TSA 3, and TSA 4 were all 100 during the evaluation period. The only exception was TSA 2 (RT) at 30 days which received a score of 67 (cracks seen on plate). Figures 3.F4 and 3.F5 display the overall mean acceptability scores for cracking of agar and contamination respectively. Acceptability score results for color change for all lots were 100 initially with only one time point (TSA RT at 98 days) showing failing results (score 0) by the end of the evaluation period (Table 3.T4). Figure 3.F5 also displays the overall mean acceptability scores for color change of agar.

Results for pH of TSA agar for shelf life time points 0, 3, 7, 14, 30, 60, and 98 days for TSA 1, TSA 2, TSA 3, and TSA 4 at room (RT, 20-25°C) and refrigeration (2-8°C) temperatures were 7.18, 7.19, 7.18, 7.24 and 7.19, 7.17, 7.21, 7.23 initially (day 0) and 7.05, 7.01, 7.03, 7.08 and 7.24, 7.21, 7.23, 7.25 (day 98) respectively. pH values obtained for media stored at room temperature reduced during storage while the pH values of media stored under refrigeration fluctuated slightly but remained at acceptable levels. For media stored at room temperature the last acceptable pH value (7.3 ± 0.2) was recorded by day 60 (Table 3.T1, Figure 3.F2).

Results for Absolute Growth Index (AGI) scores on TSA agar during shelf life time points 0, 3, 7, 14, 30, 60, and 98 days for TSA 1, TSA 2, TSA 3, and TSA 4 at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) were 99.2, 100.0, 100.0, 100.0 and 99.2, 99.2, 100.0, 100.0 initially (day 0) and 67.5, 71.7, 59.2, 65.8 and 95.8,

91.7, 87.5, 92.5 (day 98) respectively. AGI scores obtained for media at room temperature and under refrigeration reduced during storage, however RT lots had lower scores. AGI scores for TSA room temperature lots were outside the acceptable AGI score of ≥ 70 on day 98. Additionally, TSA 3 (RT) was outside adjusted FPCLS acceptable AGI value (≥ 80) at day 14 while TSA 1 (RT), TSA 2 (RT), TSA 4 (RT) were outside acceptable value on day 30. TSA 3 (2-8°C) was the only refrigerated sample failing on day 98 (Table 3.T2). Figure 3.F3 displays the overall mean AGI scores for growth acceptability.

For absolute growth index scores, the effects of temperature and time were significant ($p < 0.05$) while temperature*time interactions were not significant ($p < 0.05$). AGI scores reductions at room temperature were significant by 30 days while AGI scores at refrigeration temperature did not show any significant differences. Comparison of scores between room and refrigeration temperatures were significant after 30 days.

For pH of TSA, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The pH fluctuations at room temperature were significant at days 7 and 98, while pH at refrigerated temperatures was significant after day 14. Comparison between room and refrigeration temperatures were significant after 14 days.

For drying/thinning of TSA effects of time and temperature were significant ($p < 0.05$) with temperature*time interactions not being significant ($p > 0.05$). Both temperature drying/thinning scores were significant after day 7 except refrigeration temperature on day 30. For color change, the effect of temperature and temperature*time interactions were significant ($p < 0.05$) with time not being significant ($p > 0.05$).

However, when data was sliced by temperature no significant difference were seen and when sliced by days, p-values could not be calculated since all data points were the same prior to day 98 - significance is inferred at day 98 (RT color failed). Contamination and cracking of agar were not analyzed as all quality scores were identical (no differences) or not significant.

Non-Selective Liquid – TSB

Quality characteristic acceptability score results for contamination and color change of TSB were recorded for shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) for TSB 1, TSB 2, TSB 3, and TSB 4. Initially all lots received a score of 100 (Table 3.T8). Figure 3.F12 displays the overall mean acceptability scores during shelf life evaluation for contamination and color change of TSB.

Results for pH of TSB lots (1-4) at RT and under refrigeration were 7.19, 7.21, 7.21, 7.19 and 7.19, 7.19, 7.21, 7.18 initially (week 0), and 7.02, 7.04, 7.02, 7.01 and 7.20, 7.20, 7.19, 7.18 (week 14) respectively. The pH values obtained for media at room temperature trended down during storage while the pH values of media stored under refrigeration fluctuated slightly ending at acceptable levels. For media stored under refrigeration pH values fell outside the acceptable pH range (7.3 ± 0.2) at 2 and 6 weeks but recovered to normal values afterward. The pH values for TSB 1 (RT) and TSB 2 (RT) were outside the acceptable pH range on week 10 while all TSB room temperature lots were outside the acceptable pH range on week 14 (Table 3.T6). Figure 3.F10 displays the overall mean pH values for TSB.

Volume loss (by weight g) results were recorded for TSB shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) for TSB 1, TSB 2, TSB 3, and TSB 4. TSB volume loss results for room temperature and under refrigeration were 4.75, 4.75, 4.73, 4.77 and 4.74, 4.76, 4.73, 4.76 initially (week 0) and 3.03, 3.08, 3.04, 3.07 and 4.60, 4.60, 4.55, 4.59 (week 14) grams respectively. Both room temperature and under refrigeration volume loss values obtained during shelf life evaluation trended down during storage however RT lots showed increased volume loss. Volume loss values for all room temperature lots were approaching unacceptable levels at just 2 weeks whereas all media lots stored under refrigeration temperatures were more consistent throughout the shelf life dropping ≤ 0.18 g per lot at 14 weeks (Table 3.T5). Figure 3.F9 also displays the overall mean volume loss (by weight) values for TSB.

Results were recorded for absorbance at 600 nm for TSB shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) for TSB 1, TSB 2, TSB 3, and TSB 4. TSB absorbance readings for lots stored at room temperature and under refrigeration were 1.074, 1.105, 1.020, 1.086 and 1.064, 1.076, 0.993, 1.026 initially (week 0) and 1.396, 1.417, 1.419, 1.418 and 1.035, 1.058, 1.056, 1.043 (week 14) respectively. Absorbance values obtained for media stored at room temperature trended up during shelf life evaluation while absorbance values for media stored under refrigeration fluctuated but stayed fairly constant through week 14. No values were below the acceptable absorbance of ≥ 0.500 for either storage temperature.

Visual turbidity scores for all TSB lots at both room temperature and under refrigeration were initially “2” showing acceptable growth (Table 3.T7). Figure 3.F11 displays the overall mean absorbance (turbidity) values at 600 nm for TSB. Results for the TSB negative media control at 600 nm for room temperature and under refrigeration were all 0.000 initially (week 0) and -0.008, -0.008, -0.008, -0.018 and -0.007, -0.007, -0.008, 0.006 (week 14) respectively. All TSB lots concluded shelf life evaluation with negative absorbance values (less turbid). The only exception was TSB 4 (2-8°C) which had a final absorbance value of 0.006 at 14 weeks indicating it was more turbid (Table 3.T9). Figure 3.F13 also displays the overall mean negative control absorbance (turbidity) values at 600 nm for TSB.

For volume loss, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The volume loss at room temperature was significant after week 2, while losses at refrigeration temperature were significant after 6 weeks. Comparison between TSB volume losses at room and refrigeration temperatures were significant after 2 weeks.

For pH values, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The pH at room and refrigeration temperatures were significant after week 2. The only exception was pH at week 14 under refrigeration temperatures which was not significant compared to time “0”. Comparison between pH values during shelf life evaluation at room and refrigeration temperatures were significant after 0 weeks.

For absorbance at 600 nm (turbidity/growth), the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The absorbance values at room

temperature were significant after week 6, while absorbance values at refrigeration temperatures were significant at weeks 6 and 10 only. Comparison between absorbance values during shelf life evaluation at room and refrigeration temperatures were significant after 8 weeks.

For absorbance at 600 nm for the negative control, the effects of time and temperature were significant ($p < 0.05$) while temperature*time interactions were not significant ($p > 0.05$). absorbances values were significant from weeks 4-6 and 10-14 but not significant on week 2 or 8 during shelf life evaluation. Contamination, color change, and visual turbidity tube scores (growth) were not analyzed statistically for TSB as all quality scores were identical (no differences).

Non-Selective Liquid – BPW

Quality characteristic acceptability score results for contamination and color change of BPW were recorded for shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) for BPW 1, BPW 2, and BPW 3. Initially all lots received a score equal to 100 and maintained this score throughout shelf life testing (Table 3.T10). Figure 3.F14 displays the overall mean acceptability scores for contamination and color change for BPW.

Results for pH of BPW lots (1-3) at room and refrigerated temperatures were 7.16, 7.16, 7.14 and 7.16, 7.16, 7.13 initially (week 0) and 7.14, 7.14, 7.11 and 7.21, 7.20, 7.18 (week 14) respectively. pH values for media stored at both room and refrigeration temperatures fluctuated up/down but stayed relatively consistent. However, pH values for samples stored under reirrigated conditions dropped at 2 and 6 weeks but recovered to

normal values afterward never going outside acceptable pH range of 7.2 ± 0.2 (Table 3.T13). Figure 3.F17 displays the overall mean pH values for BPW.

Results were obtained for volume loss (by weight g) for BPW shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks for samples stored at room temperature (RT, 20-25°C) and under refrigeration for BPW 1, BPW 2, and BPW 3. pH results for samples stored at room and under refrigeration were 4.77, 4.75, 4.74 and 4.74, 4.77, 4.75 initially (week 0) and 3.10, 3.15, 3.08 and 4.59, 4.64, 4.60 (week 14) grams respectively. Both room and refrigerated temperature volume loss values obtained during shelf life evaluation trended down. However, room temperature lots showed increased reductions in volume. Volume loss values for all samples stored at room temperature were approaching unacceptable levels (> 0.25 ml loss) at just 2 weeks, whereas samples stored under refrigerated conditions were more consistent throughout dropping ≤ 0.15 g per lot at 14 weeks (Table 3.T14). Figure 3.F18 displays the overall mean volume loss (by weight) values for BPW.

Results for absorbance scores were obtained during shelf life evaluation at 600 nm for BPW shelf life time points 0, 2, 4, 6, 8, 10, and 14 weeks at room temperature (RT, 20-25°C) and under refrigeration conditions for BPW 1, BPW 2, and BPW 3. Samples stored at room and refrigerated conditions had values of 0.554, 0.615, 0.645 and 0.549, 0.607, 0.620 initially (week 0) and 0.791, 0.979, 1.063 and 0.578, 0.729, 0.745 (week 14) respectively. Absorbance values for samples stored at both room and refrigeration temperatures trended up, however, room temperature samples increased by a larger margin. Some samples (BPW 1 (RT) and BPW 1 (2-8°C)) dropped below the acceptable absorbance of ≥ 0.500 during shelf life evaluation at week 6 but both

recovered to acceptable levels afterward. No other absorbance values recorded during shelf life evaluation were below the acceptance limit.

Visual turbidity scores for all BPW lots at both room temperature and under refrigerated conditions were “2” showing acceptable growth. Exceptions included BPW 1 at room and refrigeration temperatures at 6 weeks during shelf life evaluation giving visual scores of “1” (Table 3.T11). Figure 3.F15 displays the overall mean absorbance (turbidity) values at 600 nm for BPW. The results for the negative control at 600 nm at both room and refrigerated temperatures were all 0.000 initially (week 0) and -0.008, -0.014, -0.010 and -0.009, -0.019, -0.005 (week 14) respectively. All BPW lots ended with negative values indicating they were less turbid than initial readings (Table 3.T12). Figure 3.F16 displays the overall mean negative control absorbance (turbidity) values at 600 nm for BPW.

For volume loss, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The volume loss at room temperature was significant after week 2, while losses at refrigeration temperature were significant at 4 weeks (Adj $p = 0.0425$) and after 8 weeks. Comparison between room and refrigeration temperatures during shelf life evaluations were significant after 2 weeks.

For pH, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The pH at room and refrigeration temperatures were significant after week 2. Comparison between room and refrigeration temperatures during shelf life evaluations were significant at 2 weeks and after 6 weeks.

For absorbance (turbidity/growth), the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The sample absorbances at

room temperature were significant except week 6 (Adj $p = 0.4605$), while absorbances at refrigerated temperatures were significant from 8-10 weeks only. Comparison between room and refrigeration temperatures during shelf life evaluations were significant after 6 weeks.

For absorbance (negative control), only the effect of time was significant ($p < 0.05$) with temperature and temperature*time interactions not being significant ($p > 0.05$). The temperature absorbances were significant from week 2-6 and 10-14 but not significant on week 8. For visual tube score (turbidity), only the effect of time was significant ($p < 0.05$) with temperature and temperature*time interactions not being significant ($p > 0.05$). Visual scores were not significant but week 6 was significant compared to week 0. Contamination and color change were not analyzed for samples evaluated in this study as all quality scores were identical (no differences).

Selective Liquid – LRC

Quality characteristic acceptability score results for contamination, color change, bacterial morphology, and negative (media) control were recorded for LRC shelf life time points 0, 6, 12, 24, 36, 48 A, and 48 B hours at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions for LRC 1, LRC 2, and LRC 3. Samples stored at both temperatures had initial scores of 100 and stayed consistent throughout the shelf life evaluation (Table 3.T16, Table 3.T17). Figures 3.F20 and 3.F21 display the overall mean acceptability scores for morphology, negative (media) control, contamination, and color change during shelf life testing for LRC.

Results recorded for pH values for LRC lots (1-3) stored at room and refrigerated temperatures were 7.21, 7.24, 7.23 and 7.22, 7.25, 7.24 initially (hour 0) and 7.27, 7.26, 7.27 (48 A), 7.27, 7.27, 7.28 (48B) and 7.26, 7.25, 7.18 (48 A) 7.27, 7.25, 7.18 (48 B) (hour 48) respectively. pH results for samples stored at room and refrigeration temperatures fluctuated during shelf life evaluations but stayed relatively consistent. The only exception was the pH value of LRC 3 stored under refrigerated conditions which dropped at 36 hours to 7.18 and did not recover (about 0.1 lower than other lots) (Table 3.T18). Figure 3.F22 displays the overall mean pH values for LRC.

Results were recorded for volume loss (by weight g) of LRC samples prepared with a serological pipette for shelf life time points 0, 6, 12, 24, 36, 48 A, and 48 B hours at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions. Results for samples stored at room and refrigeration temperatures for LRC 1, LRC 2, and LRC 3 were 4.98, 4.94, 5.03 and 4.96, 5.07, 4.98 initially (hour 0) and 5.00, 5.00, 4.94 (48 A), 5.03, 5.00, 4.89 (48 B) and 5.06, 4.98, 4.97 (48 A), 5.03, 4.99, 5.03 (48 B) (hour 48) grams respectively. Samples stored at both temperatures that were serological prepared stayed consistent throughout the shelf life. Volume loss (by weight g) for LRC 1 samples prepared with a bottle pump showed results at room and refrigeration temperatures of 5.02 and 5.03 initially (hour 0) and 4.98 and 5.01 (hour 48) grams. Samples stored at both temperatures that were prepared using a bottle pump stayed consistent throughout the shelf life evaluation (Table 3.T15). Figure 3.F19 displays the overall mean volume loss (by weight) values for LRC.

Growth acceptability score results were obtained during shelf life evaluation for positive control, 50:50 positive control (competition plate), and 50:50 negative control

(competition plate) for LRC shelf life time points 0, 6, 12, 24, 36, and 48 hours at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions. Acceptability scores for samples stored at room and refrigeration temperatures for LRC 1, LRC 2, and LRC 3 were 100 initially and stayed consistent throughout shelf life testing (Table 3.T19, Table 3.T20). Figures 3.F23 and 3.F24 display the overall mean acceptability scores for positive control, 50:50 positive control (competition plate), and 50:50 negative control (competition plate) for LRC shelf life determination.

Results recorded for LRC lots (1-3) negative control *E. coli* ATCC 25922 for samples stored at both room and refrigeration temperatures were 100 initially (hour 0). Samples stored at both temperatures failed to control the growth of the negative control organism with LRC 1 (RT) and LRC 3 (RT) failing at 24 hours (LRC 1 (RT) recovered at 36 hours) and LRC 2 (RT) and LRC 3 (under refrigerated conditions) failing at 36 hours. Results recorded for LRC lots (1-3) negative control *E. coli* ATCC 35150 at room and refrigeration temperatures were 100 initially (hour 0) for LRC 1 (RT), LRC 2 (RT), and LRC 2 (2-8°C) but failing for all other lots. Results obtained for LRC negative control *E. coli* ATCC 35150 fluctuated at each time point without a pattern with only LRC 3 (RT and 2-8°C) failing at 6 hours, all lots failing at 24 hours, but only LRC 2 (RT) and LRC 3 (RT) failing at 48 hours (Table 3.T21). Figure 3.F23 displays the overall mean acceptability scores for negative controls *E. coli* ATCC 25922 and *E. coli* ATCC 35150 for LRC.

For volume loss (serological and pump), the effects of temperature, time, and temperature*time interactions were not significant ($p > 0.05$). For pH, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The pH

at room temperature was significant for hour 24 and 48 compared to time “0”, while pH at refrigerated temperatures was not significant. Comparison between room and refrigeration temperatures during shelf life evaluations were significant after 24 hours.

For negative control culture *E. coli* ATCC 25922, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The negative control scores at room temperature were significant after hour 24 while refrigeration temperature values were significant for hour 36 only. Comparison between room and refrigeration temperatures during shelf life evaluations were significant after 24 hours.

For negative control culture *E. coli* O157:H7 ATCC 35150, the effect of time was significant ($p < 0.05$) while temperature and temperature*time interactions were not significant ($p > 0.05$). Negative control culture *E. coli* O157:H7 ATCC 35150 scores were not significant at any time point compared to hour 0 (due to the erratic nature of the passing/failing of the control organism) since hour 0 failed. However, all time points (0-48) when compared to a passing value of 100 were significant for both temperatures except refrigerated temperature hour 12. Contamination, color change, positive control culture, 50:50 positive/negative control cultures (competition plate), negative (media) control, and morphology were not analyzed as all scores obtained during shelf life testing were identical (no differences).

Selective Liquid – REC

Quality characteristic acceptability score results were obtained throughout shelf life evaluations for contamination, color change, morphology, and negative (media) control for REC shelf life time points 0, 6, 12, 24, 36, 48 A, and 48 B hours at room

temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions. Samples stored at both temperatures for REC 1, REC 2, and REC 3 had scores of 100 initially and maintained these scores throughout shelf life testing (Table 3.T23, Table 3.T24). Figures 3.F26 and 3.F27 display the overall mean acceptability scores during shelf life evaluation for contamination, color change, morphology, and negative (media) control for REC.

Results recorded for pH values of REC lots (1-3) at both room and refrigerated temperatures were 6.88, 6.91, 6.93 and 6.88, 6.92, 6.95 initially (hour 0) and 6.90, 6.93, 6.93 (48 A), 6.89, 6.93, 6.94 (48B) and 6.90, 6.93, 6.94 (48 A), 6.88, 6.93, 6.94 (48 B) (hour 48) respectively. pH values for samples stored at both temperatures fluctuated slightly but stayed relatively consistent throughout the shelf life (Table 3.T25). Figure 3.F28 displays the overall mean pH values for REC.

Results were recorded for volume loss (by weight g) of REC prepared with a serological pipette for shelf life time points 0, 6, 12, 24, 36, 48 A, and 48 B hours at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions. Results for samples stored at room and refrigeration temperatures for REC 1, REC 2, and REC 3 were 4.70, 4.95, 4.97 and 4.78, 4.96, 5.00 initially (hour 0) and 4.83, 4.92, 4.90 (48 A), 4.86, 4.89, 4.91 (48 B) and 4.99, 5.01, 4.98 (48 A), 4.96, 4.92, 4.98 (48 B) (hour 48) grams respectively. Samples stored at both temperatures and prepared serologically had volume loss values that stayed consistent throughout the shelf life. Volume loss (by weight g) of LRC 1 prepared with a bottle pump had results at both room and refrigeration temperatures of 5.02 and 5.00 initially (hour 0) and 4.95 (48 A), 4.95 (48 B) and 5.00 (48 A), 4.99 (48 B) (hour 48) grams. Volume loss results for samples stored at both temperatures that were prepared using a bottle pump showed values that stayed

consistent throughout the shelf life evaluation (Table 3.T22). Figure 3.F25 displays the overall mean volume loss (by weight) values for REC throughout shelf life testing.

Growth acceptability score results were recorded for positive control, 50:50 positive control (competition plate), and 50:50 negative control (competition plate) for REC shelf life time points 0, 6, 12, 24, 36, 48 A, and 48 B hours at room temperature (RT, 20-25°C) and under refrigeration (2-8°C) conditions. Results for samples stored at both temperatures for REC 1, REC 2, and REC 3 were all 100 initially and stayed consistent throughout shelf life testing (Table 3.T26, Table 3.T27). Figures 3.F29 and 3.F30 display the overall mean acceptability scores for positive control, 50:50 positive control (competition plate), and 50:50 negative control (competition plate) for REC. Results for REC lots (1-3) negative control at both temperatures were 100 during the shelf life evaluation. The only exception was REC 3 (RT) failing at both 0 and 6 hours giving an acceptability score of 67 (only 1 replication failed). However, REC 3 (RT) passed for all other time points (Table 3.T26). Figure 3.F29 displays the overall mean acceptability scores for the negative control for REC throughout the shelf life evaluation.

For volume loss (serological) the effect of time was significant ($p < 0.05$) while temperature and temperature*time interactions were not significant ($p > 0.05$). The volume loss (by weight) was significant at hour 12 compared to time “0”. Comparison between room and refrigeration temperatures were not significant which may indicate that the media could potentially be stored at either temperature. For volume loss (bottle pump) the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The losses at room temperature were significant after hour 24, while losses at refrigerated temperatures were not significant. Comparison between room

and refrigeration temperatures during shelf life testing were significant after 36 hours. For pH, the effects of time and temperature*time interactions were significant ($p < 0.05$) while temperature was not significant ($p > 0.05$). pH was significant for room temperature at 24 and 48 hours but not significant at refrigerated temperatures.

For negative control culture *S. aureus* ATCC 6538P, the effects of temperature, time, and temperature*time interactions were significant ($p < 0.05$). The negative control acceptability scores at room temperature were significant at 0 and 6 hours while room temperature values were not significant. Comparison between room and refrigeration temperatures were significant at 0 and 6 hours. Contamination, color change, positive control culture, 50:50 positive/negative control cultures (competition plate), negative (media) control, and morphology were not analyzed as all scores were identical throughout shelf life testing (no differences).

DISCUSSION

Evaluating Microbiological Media

It is known that all microbiological media loses its ability to provide accurate results and perform as expected as the media's quality degrades over time. Studies have been done to show the failure rates of different types of media (Jones et al. 2002; CLSI 2004) which is a result of the degradation of the quality characteristics and/or growth capabilities of the media over time. Because media quality degrades, it becomes important to properly evaluate microbiological media that is utilized for testing purposes throughout its intended shelf life.

There are several publications and reports that have been issued on the evaluation of microbiological media and how to properly evaluate these reagents for use in research and in testing laboratories. One study evaluated the difference between BBL™ CHROMagar and Sorbitol MacConkey agar for detection of *E. coli* O157:H7 reporting the sensitivity, specificity, prevalence and accuracy of each media type but did not evaluate the media over the shelf life (Church et al. 2007) of the prepared media. Another study looked at several different types of media, including BPW, TSB, and REC (8-Hour) and their ability to support growth (doubling time at 3 and 6 hours) of *E. coli* O157:H7 for ground beef process analysis, but once again did not evaluate degradation of the media over its shelf life (Guerini et al. 2006).

Earlier publications also focused on media performance while not addressing stability over the shelf life of the prepared product. March and Ratnam (1986) evaluated Sorbitol MacConkey agar and showed it had a 100% sensitivity, 85% specificity, and 86% accuracy to detect *E. coli* O157:H7 in stool samples but they did not evaluate the media over time (March and Ratnam 1986). All of these publications describe how to evaluate media for their performance but they all fail to address stability of the media once it is prepared and stored.

Despite the majority of publications merely comparing media types, and the fact that most media qualification procedures are proprietary and not published, there are some documents/reports that are available to guide laboratories in performing proper microbiological media evaluation. These documents have been available for a long time with solid media evaluation being discussed in 1980 for classic and ecometric methods (Mossel et al. 1980), reviews of chemical and biological tests necessary to show the

quality of solid and liquid media types in 1985 (Curtis 1985), and a more in-depth evaluation of all media types including statistical evaluations in 1992 (Weenk 1992). However, for this study more recent documents were used where specific quality characteristics to evaluate microbiological media are described and growth parameters are determined, accounting for the degradation of the prepared media over its shelf life.

One of the documents that should be considered when designing a media qualification procedure is the report from the Clinical and Laboratory Standards Institute (CLSI) detailing quality characteristics to examine such as cracking of agar, color change, volume dispensed, dehydration (drying) of agar, contamination, and growth and providing some evaluation parameters such as organism to use and incubation requirements (Krisher 2001; CLSI 2004). However, a study by Cantarelli et al. showed that following the CLSI report (M22-A2/M22-A3) recommendations was not necessarily adequate for all media types and that the report was only adequate for media used in the recovery of fastidious pathogens and therefore other methods should be considered (Cantarelli et al. 2003).

Other reports and publications that are more useful, and the ones utilized by the FPCLS for determining all media qualification testing parameters, are the Guidelines for Assuring Quality of Microbiological Media from the Australian Society of Microbiology (ASM 2012) and the Quality Control of Culture Media in a Microbiology Laboratory (Basu et al. 2005). Basu et al. 2005 focuses on solid culture media and discusses what quality characteristics should be evaluated and how to perform those evaluations while the ASM 2012 report addresses all media types, provides detailed procedures on how to evaluate each media type, and addresses shelf life as a parameter.

Although there are no ISO requirements depicting what characteristics must be evaluated when qualifying in-house media, the FPCLS selected parameters that met its needs for performing media qualification verification. Parameters were then chosen based on the CLSI 2004 report, ASM 2012 report, and the Basu et al. 2005 publication. Some of the characteristics that have been recommended by CLSI and ASM include: drying and/or cracking, color changes, uneven filling or insufficient amount, growth, colony size and morphology, turbidity, volume, excessive moisture or dehydration, noticeable precipitants, biochemical responses, and contamination (CLSI 2004; ASM 2012). For solid media types Basu et al. 2005 recommended evaluating characteristics including; pH, excessive bubbles, evaluation of additives (blood for hemolysis), unequal filling of plates, cracking, growth, colony size and morphology, gel strength, and contamination (Basu et al. 2005).

In comparison, the FPCLS chose to evaluate ability to support growth (absolute growth index score, turbidity, etc.), cracking, drying/thinning, contamination, color change, morphology, pH, and volume loss pending on the media type being evaluated. The evaluation of these quality characteristics will provide the necessary information to effectively evaluate the quality of the prepared media while still keeping the process simple enough for any technician to perform.

Determining Shelf Life Parameters

ISO/IEC 17025 standards do not mention any guidelines for media qualification as it pertains to shelf life time points (ISO and IEC 2005; ISO and IEC 2017), while the AOAC guidelines simply state that, “*every batch of media...shall be examined to ensure*

it is suitable for use” (ISO and IEC 2005; AOAC 2015). Despite the lack of guidance, shelf life time points for each media type under evaluation were not merely selected at random. Certain types of media have predetermined shelf life requirements provided by the manufacturer.

For instance, Neogen Reveal[®] 20-Hour selective media for *E. coli* O157:H7 has a predetermined shelf life of only 6 hours (Neogen 2016), while the Romer RapidChek[®] *Listeria* spp. media has a predetermined shelf life of 3 hours at room temperatures or 24 hours at 4°C (Romer 2016a; Romer 2016b). However, media such as TSA, TSB, and BPW do not have specified shelf life requirements. The DIFCO and BBL Manual of Microbiological Culture Media states, “*the shelf life of some media... may be prolonged by refrigeration*” (BD 2009) and only provides shelf life information for very specific media types.

While manufacturer’s instructions and literature will suggest media should be used within several days of preparation, some studies have been done showing that various types of media are stable at refrigerated temperatures for up to 3 months (Ulisse et al. 2006) and potentially even a year under modified vacuum conditions (Choi and Rogers 2015). Due to the lack of established criterium it is necessary that each laboratory determine its own shelf life parameters and procedures for the shelf life of prepared media. Following these protocols would allow for verification that the media being utilized for testing purposes is acceptable and will not adversely affect testing results.

The ASM 2012 report addresses shelf life testing for prepared media to evaluate its stability over time (ASM 2012). Although this document provides examples of what a shelf life study should encompass, ASM continually stresses that each laboratory is

ultimately responsible for determining “*its own acceptance and rejection criteria*” and that each laboratory needs to determine the testing strategy that best fits their laboratory’s needs (ASM 2012). In general, the ASM 2012 shelf life protocol is vague and only recommends analysis on the different number of organism’s times the specified number of weeks that you prefer and only performing analysis at 2-8°C storage temperature (ASM 2012).

In contrast, the FPCLS designed its shelf life study to be much more robust for each media type by testing media samples in duplicate at each time point, including 6-7 time points per study, including negative media controls and negative culture controls (where appropriate), and utilizing two storage temperatures, room temperature (20-25°C) and refrigerated temperature (2-8°C). These testing conditions meet the requirements for commercial preparation of media when it should be subjected to unfavorable conditions during its shelf life (ASM 2012). Additionally, slight changes were made to the testing procedure for selective liquid media where spread plating was added to the final stage of each time point test. Originally the protocol by ASM (2012) suggested streak plating for confirmation of growth. However, it is believed that spread plating would provide more accurate results making the protocol more useful to the needs of the FPCLS and other laboratories.

Absolute Growth Index and Percent Relative Growth Index

Determining acceptable growth on non-selective solid media can be accomplished utilizing a semi-quantitative method that determines growth performance by applying a tabulated growth score to the observed growth in agar plates. This is known as the

ecometric method (Mossel et al. 1980; Weenk 1992; Basu et al. 2005; ASM 2012). This method involves streak plating a desired inoculum onto the solid agar following the ecometric streak plate pattern, growing the plate at appropriate temperatures, and then determining the last line displaying acceptable growth. This line is then associated to a tabulated value to indicate the absolute growth index (AGI) score, which is then compared to a standard or predetermined relative growth index (RGI) score and converted to % RGI by dividing the AGI of the sample by the AGI of the control or standard and then multiplying by 100 (Equation 1) (Basu et al. 2005; ASM 2012).

Following this method allows for growth measurement reflecting the performance of the evaluated medium as a percent value with the % RGI acceptance set at 70 % (Basu et al. 2005; ASM 2012). When evaluating selective solid media types, the AGI is the score from the non-selective media and the RGI is the score from the selective media. Selective media positive control organisms must have a % RGI > 50 % and negative control organisms must have a % RGI of < 25 % (Basu et al. 2005; ASM 2012). This is a good method of comparison for qualification methods, but not as effective for verification methods as a standard or baseline is needed for evaluating non-selective solid agar types.

Equation 1: Calculating Percent Relative Growth Index Scores

$$\text{RGI Score (\%)} = \frac{\text{Absolute Growth Index Sample Score}}{\text{Absolute Growth Index Control Score}} \times 100$$

Therefore, the FPCLS adapted this procedure to allow for the verification process over the shelf life of the prepared microbiological media. In the context of evaluating the ability to support growth, RGI scores will be utilized by the FPCLS for the laboratory

media qualification procedure (daily analysis – no shelf life). For this study where shelf life is evaluated, only the AGI scores were considered to determine the acceptable growth performance of the prepared media. Any AGI score ≥ 80 was considered acceptable.

By comparison, Basu et al. (2005) and ASM (2012) suggested an AGI score of ≥ 70 for acceptance. In this shelf life study the non-selective solid media scores were not converted to a percentage as a standard was not available. Moving forward with the everyday media qualification process the % RGI will be calculated using data from the shelf life study as the standard values.

Media Verification Results

Data associated with media qualification and verification procedures for ISO compliance is typically kept confidential and retained in the laboratory's records. This is to protect the establishment from its competitors gaining an advantage. Therefore, results are not available to compare the FPCLSs media qualification verification data against. Only the results obtained in this study will be discussed in the following paragraphs for each microbiological media evaluated during the media qualification verification testing procedures.

Tryptic Soy Agar Results

Tryptic soy agar (TSA) did not present any issues with cracking, even at room temperature, throughout the shelf life of the media with only one exception – Lot TSA 2 at 30 days. This was surprising as it was expected that the media would crack as it dried out. However, TSA had issues with drying/thinning of the agar at both room (20-25°C)

and refrigeration (2-8°C) temperatures. Issues arose as early as 7 days (significant at $p < 0.05$) with more noticeable issues for room temperature (media completely dried out) by day 14.

Refrigerated media began showing drying/thinning as early as 7 days but was fairly stable until day 60, displaying major drying/thinning issues only at day 98. This was surprising as it was expected that the media would maintain high quality if refrigerated according to the BD DIFCO manual (BD 2009). It was also noted that the pH of TSA room temperature samples significantly ($p < 0.05$) dropped over time starting at an average of 7.20 and ending around 7.04 which could be explained by the media drying out with the loss of water altering the pH value. Based on pH values failure was reached for room temperature media at day 98.

Finally, AGI scores were fairly consistent for both storage temperatures with only room temperature samples falling below the ≥ 70 requirement at 98 days. However, when the AGI score was adjusted to ≥ 80 , TSA 3 fell below this requirement at 14 days and all room temperature lots failed by 30 days. Even one lot under refrigeration (TSA 3) was potentially unacceptable at 98 days with the standard deviation exceeding the limit. AGI scores for media stored at room temperature began dropping at 7 days, dropped significantly ($p < 0.05$) at 30 days, and continued to deteriorate further over time. Based on these findings it is recommended that TSA quality should be evaluated based on a growth score ≥ 80 . Additionally, the shelf life of the agar should be based on the ability to support growth and drying/thinning, with shelf life of TSA set at 7 days for room temperature media and 60 days for media stored under refrigerated temperatures.

Tryptic Soy Broth Results

Tryptic soy broth (TSB) pH was found to decrease significantly ($p < 0.05$) over time for media stored at room temperature (25°C) samples starting at an average pH of 7.20 and ending at 7.02. This may have been due to the volume loss that was also observed in the media due to evaporation of the water from the broth and concentration of the nutrients. pH values dropped outside the acceptable range (7.3 ± 0.2) at 2 and 6 weeks for media stored under refrigerated conditions. The reason for this is unknown as the pH values recovered each time. However, pH fluctuations did not have an impact on the ability of the media to support growth at points where pH exceeded the acceptable range. Volume (by weight g) decreased over time for both room and refrigeration (2-8°C) samples significantly ($p < 0.05$). Room temperature TSB samples started at an average volume of 4.75 ml and ended at 3.06 ml showing significant losses at the beginning of week 2 (approaching losses of 0.25 ml). The loss of volume was not surprising since the FPCLS used standard test tubes with press on caps and not screw cap tubes.

Growth (absorbance at 600 nm) for both storage temperatures was acceptable (≥ 0.500) throughout the shelf life and corresponded to the visual score of “2” and it should be noted that absorbance values at both temperatures increased significantly ($p < 0.05$) by week 6. This may have been due to the volume loss concentrating the nutrients and reducing the dispersion area increasing the turbidity of the tubes. Based on these findings it is recommended that the TSB quality be evaluated based on visual scores for growth, pH (manufacturer requirement), and volume loss with the shelf life of TSB being set at 2 weeks for room temperature media and up to 13 weeks for media stored under refrigerated conditions.

Buffered Peptone Water Results

The pH of Buffered peptone water (BPW) was found to fluctuate over time for both room (20-25°C) and refrigeration (2-8°C) temperatures with significant ($p < 0.05$) differences beginning in week 2. This was surprising as it was expected that BPW would behave similarly to TSB with pH being reduced over time as volume was lost. Although pH of samples at room and refrigerated temperatures differed significantly over time, they did not fall outside the acceptable pH range (7.2 ± 0.2).

Volume (by weight) decreased over time for samples held at both room and refrigeration temperatures with losses being significant ($p < 0.05$) as early as 2 weeks for samples at room temperature and 4 weeks for refrigerated samples. Samples maintained at room temperature started the shelf life study at an average volume of 4.76 ml and ended at 3.11 ml (losses > 0.25 ml). Once again, the loss of volume was not surprising since the FPCLS used standard test tubes with press on caps and not screw cap tubes.

Ability to support growth (absorbance at 600 nm) was acceptable (≥ 0.500) for samples maintained at both storage temperatures throughout the evaluation period for shelf life. Additionally, acceptance of the samples as measured by absorbance corresponded to the visual scores except for Lot BPW 1 at 6 weeks. It was noted that the absorbance values for samples stored at room temperature increased significantly ($p < 0.05$) starting in week 2; while samples at refrigeration temperatures started to show increased values in week 8. This may have been due to the volume loss concentrating the nutrients and reducing the dispersion area increasing the turbidity of the tubes.

It is unknown why the results for refrigerated samples showed reduced absorbance values at 6 weeks. This may have been due to the microorganisms not being

transferred properly during inoculation or another unknown factor contributing to the result. Based on these findings it is recommended that the BPW quality should be evaluated based on visual scores for growth, pH (manufacturer requirement), and volume loss with the shelf life of BPW being set at 2 weeks for room temperature media and up to 13 weeks for refrigerated media.

Romer RapidChek[®] *Listeria* Results

Romer RapidChek[®] *Listeria* medium (LRC) did not have any quality issues related to bacterial morphology, negative (media) control, contamination, color change, or volume loss. This was not surprising as the shelf life study for this media was very short (only 48 hours) and therefore the media did not have enough time to physically change or experience enough time to evaporate (lose volume) by a significant amount. Growth parameters for positive control and 50:50 competition positive/negative culture controls were also as expected. The pH of the media appeared to be very stable but exhibited significant ($p < 0.05$) changes for samples stored at room temperature at 24 and 48 hours. Despite the significant changes in pH, there is no recommended acceptable pH range available for this medium and therefore determining if the fluctuations in pH are truly significant or not is difficult.

E. coli ATCC 25922 was one of the cultures chosen as a negative control. The ability of the media to prevent this bacterial strain from growing starting failing at 24 hours for samples stored at room temperature and 36 hours for refrigerated samples. The other negative control, *E. coli* O157:H7 ATCC 35150, which was expected to behave well as a negative control according to the exclusion list provided by AOCA (2017) was

associated with failing results at 0 hours and randomly led to failed results throughout the shelf life testing regardless of storage temperature. To date, there are no explanations as to why this organism was not inhibited by the Romer RapidChek[®] media, as according to the exclusion list it should have been inhibited. It is possible that the stock culture was contaminated with another organism that happens to not be inhibited, even though the culture had been verified for purity and identified as *E. coli* O157:H7 through other methods.

Based on these findings it is recommended that the LRC quality should be evaluated primarily based on its ability to support growth (positive, negative, and 50:50 competition cultures) with the shelf life of LRC being set at 3 hours for media stored at room temperature and up to 12 hours for refrigerated media. In comparison, Romer Labs recommends that LRC media be used within 3 hours if stored at room temperature (20-25°C) and within 24 hours if stored in the refrigerator (~4°C) (Romer 2016a; Romer 2016b). The shelf life of LRC has been reduced by 12 hours at the FPCLS for media stored at refrigeration temperatures because it was perceived as too unpredictable to be utilized beyond 12 hours while still providing acceptable results.

Neogen Reveal[®] 20-Hour for *E. coli* O157:H7 Results

The Neogen Reveal[®] 20-Hour for *E. coli* O157:H7 medium (REC) did not have any quality issues related to bacterial morphology, negative (media) control, contamination, or color change. This was not surprising as the shelf life study for this media was very short (only 48 hours) and therefore the media did not have enough time

to physically change, become contaminated, or experience other issues by a significant amount.

Volume loss (serological) appeared to be very stable but exhibited significant ($p < 0.05$) changes in samples stored at room and refrigerated temperatures at 12 hours. The differences observed at 12 hours were actually due to higher volumes at this time point which could be related to variations in filling volumes. Volume loss (bottle pump) also appeared to be very stable in samples stored at room and refrigerated temperatures. Significant ($p < 0.05$) changes were observed in samples stored at room temperature after 24 hours. pH values of media stored at both temperatures appeared to be very stable with significant ($p < 0.05$) changes starting at 24 and 48 hours. Despite the significant changes in values, there is no recommended acceptable pH range available for this medium and therefore determining if the fluctuations at 24 or 48 hours are truly significant or not is difficult.

Growth parameters for positive control, 50:50 competition positive/negative culture controls, and negative culture control were also as expected. However, there was one unexpected result for Lot REC 3 at 0 and 6 hours since one of its replications failed to inhibit the negative control when stored at room temperature. This event is possible as the REC media does not eradicate the negative control organism (*S. aureus* ATCC 6538P), but instead simply inhibits its growth. Since the pass-fail line is 10 CFU/ml on solid non-selective agar it is possible that *S. aureus* experienced some slight growth or the initial inoculation levels were high enough to allow for 10 CFU/ml in the final tube. It is also possible that the specific lot number was not produced correctly by the manufacturer. Regardless, this event was not perceived as a major failure of the ability of

the media to prevent growth of the negative control culture and was most likely an isolated event.

Based on these findings it is recommended that REC quality should be evaluated primarily based on its ability to support growth (positive, negative, and 50:50 competition cultures) with the shelf life of REC being set at 6 hours regardless of storage temperature. This is also the recommendation provided by the manufacturer, Neogen Corporation, which states that REC media be used within 6 hours of rehydration (Neogen 2016). However, we recommend a secondary shelf life be added as an option for usage of refrigerated REC media up to 48 hours under special circumstances, such as research activities, as it was shown a 48-hour shelf life meets requirements for quality and growth.

CONCLUSIONS

In conclusion this project has provided results showing that five types of media (non-selective solid – tryptic soy agar; non-selective liquid – tryptic soy broth and buffered peptone water; selective liquid – Romer RapidChek® *Listeria* and Neogen Reveal® 20-Hour for *E. coli* O157:H7) utilized by the FPCLS have passed media qualification verification procedures designed by the FPCLS and are suitable for use in performing analyses with client samples for ISO/IEC 17025 compliance (AOAC 2015). This project has also shown that each media type needs to be evaluated against different characteristics to determine its suitability for use. More specifically the parameters to be used should be: TSA – growth (≥ 80 score level), pH, and drying/thinning of the agar; TSB/BPW – growth (visual score), pH, and volume loss; LRC/REC – growth (positive, negative, and 50:50 competition cultures).

Additionally, media qualification verification results show that the shelf life of each media type should be: TSA – 7 days for media stored at room temperature and 60 days for refrigerated media; TSB/BPW – 2 weeks for media stored at room temperature and up to 13 weeks for refrigerated media; LRC – 3 hours for media stored at room temperature and up to 12 hours for refrigerated media; and REC – 6 hours regardless of storage temperature and up to 48 hours under special circumstances (research activities) at refrigeration temperatures.

Quality characteristics identified during this test procedure as necessary for determining acceptability of microbiological media will be applied as part of the FPCLS media verification program. These determined parameters will be utilized by the FPCLS as the basis for the acceptance criteria for the media verification procedures helping demonstrate that media utilized by the FPCLS for performing analyses under the scope of accreditation are acceptable and are of the highest quality.

Established shelf life designations for each media type will provide the FPCLS with media that is best suited for analysis without continuous evaluation. This will also ensure that there are less instances where the microbiological media adversely affects client results and will provide additional control and stability to laboratory procedures without having to continually evaluate the media. Media qualification verification procedures developed in this project meet and/or exceed the requirements for ensuring all microbiological media is suitable to be utilized in laboratory analyses for the purpose of obtaining ISO accreditation.

Data associated with media qualification and verification procedures for ISO compliance is confidential and not shared amongst laboratories. Therefore, there are not

many examples of processes or data like the ones described here that have been published for academic and industry establishments to follow to meet media qualification requirements when developing laboratory media verification processes. Therefore, to aid other academic and industry establishments, all media qualification verification procedures developed by the FPCLS as part of this project will be reviewed and made available to both industry and academia so they may serve as a guide to media qualification verification and be utilized by other laboratories attempting to develop media qualification programs, verify their media utilized for testing, increase the accuracy and reliability of their results, and/or prepare for obtaining ISO accreditation status.

In developing and conducting these media qualification verification procedures, the UNL-FPCLS has met the requirement for media verification within the ISO/IEC 17025 standard. This process provided the necessary evidence needed to demonstrate the FPCLS media qualification program is acceptable for meeting the ISO standards and becoming ISO compliant. The FPCLS is continuing to improve and progress in all areas within the laboratory and will pursue ISO/IEC 17025 accreditation when all requirements have been met.

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Media Qualification Verification Results Tables

Tables 3.T1: Tryptic Soy Agar pH Values. Table displays tryptic soy agar (TSA) pH values for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Days						
		0	3	7	14	30	60	98
TSA 1	20-25°C	7.18 ± 0.01	7.20 ± 0.02	7.16 ± 0.04	7.19 ± 0.01	7.22 ± 0.03	7.21 ± 0.02	7.05 ± 0.04 ¹
	2-8°C	7.19 ± 0.02	7.20 ± 0.02	7.14 ± 0.02	7.27 ± 0.01	7.28 ± 0.02	7.32 ± 0.01	7.24 ± 0.02
TSA 2	20-25°C	7.19 ± 0.03	7.16 ± 0.01	7.12 ± 0.01	7.19 ± 0.02	7.23 ± 0.02	7.19 ± 0.01	7.01 ± 0.04 ¹
	2-8°C	7.17 ± 0.01	7.21 ± 0.03	7.15 ± 0.03	7.25 ± 0.02	7.29 ± 0.02	7.30 ± 0.01	7.21 ± 0.01
TSA 3	20-25°C	7.18 ± 0.01	7.19 ± 0.02	7.16 ± 0.01	7.22 ± 0.03	7.22 ± 0.03	7.25 ± 0.03	7.03 ± 0.09 ¹
	2-8°C	7.21 ± 0.02	7.17 ± 0.02	7.17 ± 0.01	7.25 ± 0.03	7.30 ± 0.02	7.35 ± 0.02	7.23 ± 0.03
TSA 4	20-25°C	7.24 ± 0.02	7.18 ± 0.01	7.15 ± 0.01	7.25 ± 0.01	7.23 ± 0.04	7.23 ± 0.02	7.08 ± 0.03 ¹
	2-8°C	7.23 ± 0.02	7.23 ± 0.05	7.20 ± 0.02	7.25 ± 0.01	7.29 ± 0.02	7.33 ± 0.01	7.25 ± 0.03
Overall mean	20-25°C	7.20 ± 0.01	7.18 ± 0.01	7.15 ± 0.01*	7.21 ± 0.01	7.23 ± 0.01	7.22 ± 0.01	7.04 ± 0.01*
	2-8°C	7.20 ± 0.01	7.20 ± 0.01	7.17 ± 0.01	7.26 ± 0.01*	7.29 ± 0.01*	7.33 ± 0.01*	7.23 ± 0.01*

Expected pH of TSA is 7.3 ± 0.2 . Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All values are expressed in mean ± standard deviation. ¹pH values fall outside manufacturers acceptable range. Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

Table 3.T2: Tryptic Soy Agar Absolute Growth Index Scores. Table displays tryptic soy agar (TSA) absolute growth index (AGI) scores for media qualification verification testing at each shelf life time point. Growth determined using *E. coli* ATCC 25922.

Lot	Storage Temperature	Shelf Life Time Points - Days						
		0	3	7	14	30	60	98
TSA 1	20-25°C	99.2 ± 1.4	100.0 ± 0.0	98.3 ± 1.4	91.7 ± 3.8	80.8 ± 7.6 ²	78.3 ± 2.9 ²	67.5 ± 2.5 ¹²
	2-8°C	99.2 ± 1.4	98.3 ± 2.9	99.2 ± 1.4	100.0 ± 0.0	95.8 ± 5.2	95.0 ± 4.3	95.8 ± 5.2
TSA 2	20-25°C	100.0 ± 0.0	98.3 ± 2.9	93.3 ± 2.9	86.7 ± 2.9	86.7 ± 12.3 ²	85.0 ± 8.7 ²	71.7 ± 15.1 ¹²
	2-8°C	99.2 ± 1.4	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.5 ± 0.0	97.5 ± 2.5	91.7 ± 5.2
TSA 3	20-25°C	100.0 ± 0.0	100.0 ± 0.0	90.8 ± 5.2	82.5 ± 6.6 ²	85.8 ± 8.8 ²	81.7 ± 7.6 ²	59.2 ± 17.0 ¹²
	2-8°C	100.0 ± 0.0	97.5 ± 2.5	97.5 ± 2.5	99.2 ± 1.4	100.0 ± 0.0	99.2 ± 1.4	87.5 ± 8.7 ²
TSA 4	20-25°C	100.0 ± 0.0	99.2 ± 1.4	97.5 ± 2.5	90.0 ± 5.0	85.0 ± 8.7 ²	85.0 ± 5.0	65.8 ± 25.0 ¹²
	2-8°C	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	99.2 ± 1.4	98.3 ± 2.9	98.3 ± 1.4	92.5 ± 2.5
Overall mean	20-25°C	99.8 ± 0.0	99.4 ± 0.0	95.0 ± 0.0	87.7 ± 0.0	84.6 ± 0.0*	82.5 ± 0.0*	66.0 ± 0.0*
	2-8°C	99.6 ± 0.0	99.0 ± 0.0	99.2 ± 0.0	99.6 ± 0.0	97.9 ± 0.0	97.5 ± 0.0	91.9 ± 0.0

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Minimum acceptable AGI score is ≥ 70.0 . All values expressed in mean \pm standard deviation. ¹AGI scores fall outside acceptable range. ²AGI scores fall outside FPCLS adjusted acceptable range of ≥ 80.0 . Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

Table 3.T3: Tryptic Soy Agar Quality Acceptability Scores for Drying/Thinning and Cracking. Table displays tryptic soy agar (TSA) quality acceptability ratios for drying/thinning and cracking of agar for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Days								
Lot	Storage Temperature	0	3	7	14	30	60	98
Drying / Thinning of Agar								
TSA 1	20-25°C	100	100	100	0	0	0	0
	2-8°C	100	100	100	100	100	67	0
TSA 2	20-25°C	100	100	33	0	0	0	0
	2-8°C	100	100	67	100	100	100	33
TSA 3	20-25°C	100	100	67	0	0	0	33
	2-8°C	100	100	100	100	100	33	67
TSA 4	20-25°C	100	100	67	67	0	0	0
	2-8°C	100	100	100	67	100	100	67
Overall	20-25°C	100	100	67*	17*	0*	0*	8*
mean	2-8°C	100	100	92*	92*	100	75*	42*
Cracking of Agar								
TSA 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 2	20-25°C	100	100	100	100	67	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 4	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall	20-25°C	100	100	100	100	92	100	100
mean	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

Table 3.T4: Tryptic Soy Agar Quality Acceptability Scores for Contamination and Color Change. Table displays tryptic soy agar (TSA) quality acceptability ratios for contamination and color change (lighter or darker) for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Days								
Lot	Storage Temperature	0	3	7	14	30	60	98
Contamination								
TSA 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSA 4	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Color Change								
TSA 1	20-25°C	100	100	100	100	100	100	0
	2-8°C	100	100	100	100	100	100	100
TSA 2	20-25°C	100	100	100	100	100	100	0
	2-8°C	100	100	100	100	100	100	100
TSA 3	20-25°C	100	100	100	100	100	100	0
	2-8°C	100	100	100	100	100	100	100
TSA 4	20-25°C	100	100	100	100	100	100	0
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	0*
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

Table 3.T5: Tryptic Soy Broth Volume Loss (By Weight). Table displays tryptic soy broth (TSB) volume loss (by weight g) results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
TSB 1	20-25°C	4.75 ± 0.04	4.52 ± 0.01	4.24 ± 0.06	4.00 ± 0.01	3.84 ± 0.07	3.54 ± 0.06	3.03 ± 0.04
	2-8°C	4.74 ± 0.02	4.70 ± 0.01	4.75 ± 0.12	4.68 ± 0.03	4.70 ± 0.06	4.68 ± 0.08	4.60 ± 0.02
TSB 2	20-25°C	4.74 ± 0.04	4.52 ± 0.03	4.26 ± 0.05	4.02 ± 0.05	3.80 ± 0.05	3.56 ± 0.03	3.08 ± 0.06
	2-8°C	4.76 ± 0.01	4.73 ± 0.02	4.69 ± 0.02	4.65 ± 0.03	4.65 ± 0.02	4.62 ± 0.05	4.60 ± 0.03
TSB 3	20-25°C	4.73 ± 0.01	4.52 ± 0.05	4.26 ± 0.02	4.07 ± 0.06	3.84 ± 0.03	3.59 ± 0.03	3.04 ± 0.05
	2-8°C	4.73 ± 0.02	4.73 ± 0.03	4.69 ± 0.01	4.65 ± 0.02	4.66 ± 0.02	4.60 ± 0.03	4.55 ± 0.01
TSB 4	20-25°C	4.77 ± 0.04	4.54 ± 0.03	4.27 ± 0.02	4.02 ± 0.08	3.80 ± 0.03	3.60 ± 0.04	3.07 ± 0.11
	2-8°C	4.76 ± 0.04	4.74 ± 0.01	4.72 ± 0.03	4.69 ± 0.04	4.71 ± 0.03	4.63 ± 0.02	4.59 ± 0.03
Overall mean	20-25°C	4.75 ± 0.01	4.52 ± 0.01*	4.26 ± 0.01*	4.03 ± 0.01*	3.82 ± 0.01*	3.57 ± 0.01*	3.06 ± 0.01*
	2-8°C	4.75 ± 0.01	4.73 ± 0.01	4.71 ± 0.01	4.67 ± 0.01*	4.68 ± 0.01*	4.63 ± 0.01*	4.58 ± 0.01*

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Values represent total volume. All values expressed in mean ± standard deviation. Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T6: Tryptic Soy Broth pH Values. Table displays tryptic soy broth (TSB) pH results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
TSB 1	20-25°C	7.19 ± 0.01	7.18 ± 0.00	7.21 ± 0.01	7.17 ± 0.01	7.15 ± 0.01	7.09 ± 0.01 ¹	7.02 ± 0.02 ¹
	2-8°C	7.19 ± 0.00	7.07 ± 0.01 ¹	7.25 ± 0.01	7.07 ± 0.01 ¹	7.22 ± 0.02	7.22 ± 0.01	7.20 ± 0.00
TSB 2	20-25°C	7.21 ± 0.02	7.18 ± 0.01	7.22 ± 0.01	7.18 ± 0.00	7.15 ± 0.01	7.12 ± 0.01	7.04 ± 0.01 ¹
	2-8°C	7.19 ± 0.01	7.08 ± 0.01 ¹	7.26 ± 0.00	7.08 ± 0.01 ¹	7.23 ± 0.01	7.24 ± 0.01	7.20 ± 0.00
TSB 3	20-25°C	7.21 ± 0.01	7.17 ± 0.01	7.22 ± 0.00	7.18 ± 0.00	7.16 ± 0.01	7.12 ± 0.00	7.02 ± 0.01 ¹
	2-8°C	7.21 ± 0.00	7.08 ± 0.01 ¹	7.25 ± 0.01	7.09 ± 0.01 ¹	7.23 ± 0.02	7.24 ± 0.00	7.19 ± 0.00
TSB 4	20-25°C	7.19 ± 0.01	7.15 ± 0.01	7.21 ± 0.01	7.16 ± 0.02	7.15 ± 0.00	7.09 ± 0.01 ¹	7.01 ± 0.01 ¹
	2-8°C	7.18 ± 0.00	7.07 ± 0.01 ¹	7.25 ± 0.01	7.08 ± 0.00 ¹	7.23 ± 0.01	7.22 ± 0.01	7.18 ± 0.00
Overall mean	20-25°C	7.20 ± 0.00	7.17 ± 0.00*	7.21 ± 0.00*	7.17 ± 0.00*	7.15 ± 0.00*	7.11 ± 0.00*	7.02 ± 0.00*
	2-8°C	7.19 ± 0.00	7.07 ± 0.00*	7.25 ± 0.00*	7.08 ± 0.00*	7.22 ± 0.00*	7.23 ± 0.00*	7.19 ± 0.00

Expected pH of TSB is 7.3 ± 0.2. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All values are expressed in mean ± standard deviation. ¹pH values fall outside manufacturers acceptable range. Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T7: Tryptic Soy Broth Visual and Absorbance Turbidity Scores. Table displays tryptic soy broth (TSB) visual scores and absorbance results for turbidity (growth of *E. coli* ATCC 25922) for media qualification verification testing at each shelf life time point.

		Shelf Life Time Points - Weeks						
Lot	Storage Temperature	0	2	4	6	8	10	14
Turbidity Absorbance Values								
TSB 1	20-25°C	1.074 ± 0.047	1.140 ± 0.051	1.195 ± 0.125	1.148 ± 0.088	1.245 ± 0.037	1.222 ± 0.134	1.396 ± 0.082
	2-8°C	1.064 ± 0.008	1.095 ± 0.045	1.122 ± 0.084	1.104 ± 0.094	1.036 ± 0.008	1.097 ± 0.100	1.035 ± 0.041
TSB 2	20-25°C	1.105 ± 0.009	1.104 ± 0.036	1.061 ± 0.073	1.203 ± 0.078	1.314 ± 0.034	1.233 ± 0.142	1.417 ± 0.022
	2-8°C	1.076 ± 0.028	1.047 ± 0.095	1.107 ± 0.060	1.120 ± 0.072	1.094 ± 0.011	1.153 ± 0.138	1.058 ± 0.064
TSB 3	20-25°C	1.020 ± 0.032	1.075 ± 0.005	1.104 ± 0.029	1.182 ± 0.069	1.279 ± 0.007	1.173 ± 0.108	1.419 ± 0.009
	2-8°C	0.993 ± 0.014	1.026 ± 0.023	1.061 ± 0.075	1.123 ± 0.066	1.052 ± 0.026	1.101 ± 0.088	1.056 ± 0.029
TSB 4	20-25°C	1.086 ± 0.025	1.110 ± 0.038	1.114 ± 0.081	1.192 ± 0.064	1.253 ± 0.016	1.212 ± 0.114	1.418 ± 0.035
	2-8°C	1.026 ± 0.025	1.066 ± 0.032	1.063 ± 0.052	1.179 ± 0.086	1.094 ± 0.007	1.127 ± 0.076	1.043 ± 0.024
Overall mean	20-25°C	1.071 ± 0.019	1.107 ± 0.019	1.118 ± 0.019	1.181 ± 0.019*	1.273 ± 0.019*	1.210 ± 0.019*	1.412 ± 0.019*
	2-8°C	1.040 ± 0.019	1.058 ± 0.019	1.088 ± 0.019	1.132 ± 0.019*	1.069 ± 0.019	1.120 ± 0.019*	1.048 ± 0.019
Visual Scores								
TSB 1	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
TSB 2	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
TSB 3	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
TSB 4	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
Overall mean	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Acceptable absorbance scores for turbidity are ≥ 0.500. All absorbance values were taken at 600 nm and are expressed in mean ± standard deviation, all visual scores based on scoring system (0 no growth, 1 weak growth, 2 good growth). Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T8: Tryptic Soy Broth Quality Acceptability Scores for Contamination and Color Change. Table displays tryptic soy broth (TSB) quality acceptability ratios for contamination and color change (lighter or darker) for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Weeks								
Lot	Storage Temperature	0	2	4	6	8	10	14
Contamination								
TSB 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 4	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Color Change								
TSB 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
TSB 4	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean TSB values were not significantly different then Week 0 ($p < 0.05$).

Table 3.T9: Tryptic Soy Broth Negative Control Absorbance Scores. Table displays tryptic soy broth (TSB) negative (media) control absorbance results for turbidity for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
TSB 1	20-25°C	0.000 ± 0.000	-0.005 ± 0.002	-0.006 ± 0.002	-0.007 ± 0.002	-0.006 ± 0.001	-0.007 ± 0.002	-0.008 ± 0.002
	2-8°C	0.000 ± 0.000	-0.002 ± 0.002	-0.005 ± 0.002	-0.006 ± 0.003	-0.001 ± 0.002	-0.005 ± 0.003	-0.007 ± 0.001
TSB 2	20-25°C	0.000 ± 0.000	-0.007 ± 0.001	-0.007 ± 0.003	-0.008 ± 0.005	-0.004 ± 0.001	-0.007 ± 0.004	-0.008 ± 0.003
	2-8°C	0.000 ± 0.000	-0.005 ± 0.002	-0.007 ± 0.004	-0.007 ± 0.003	-0.002 ± 0.003	-0.008 ± 0.003	-0.007 ± 0.003
TSB 3	20-25°C	0.000 ± 0.000	-0.006 ± 0.001	-0.007 ± 0.004	-0.007 ± 0.003	-0.006 ± 0.001	-0.008 ± 0.003	-0.008 ± 0.002
	2-8°C	0.000 ± 0.000	-0.003 ± 0.002	-0.005 ± 0.004	-0.005 ± 0.005	-0.001 ± 0.002	-0.007 ± 0.006	-0.008 ± 0.004
TSB 4	20-25°C	0.000 ± 0.000	-0.013 ± 0.012	-0.016 ± 0.012	-0.020 ± 0.010	-0.019 ± 0.012	-0.021 ± 0.011	-0.018 ± 0.014
	2-8°C	0.000 ± 0.000	0.007 ± 0.005	0.004 ± 0.002	0.006 ± 0.002	0.005 ± 0.003	0.002 ± 0.003	0.006 ± 0.005
Overall mean	20-25°C	0.000 ± 0.002	-0.008 ± 0.002	-0.009 ± 0.002*	-0.011 ± 0.002*	-0.009 ± 0.002	-0.011 ± 0.002*	-0.011 ± 0.002*
	2-8°C	0.000 ± 0.002	-0.001 ± 0.002	-0.003 ± 0.002*	-0.003 ± 0.002*	0.000 ± 0.002	-0.005 ± 0.002*	-0.004 ± 0.002*

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All absorbance values were taken at 600 nm and are expressed in mean ± standard deviation. Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T10: Buffered Peptone Water Quality Acceptability Scores for Contamination and Color Change. Table displays buffered peptone water (BPW) quality acceptability ratios for contamination and color change (lighter or darker) for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Weeks								
Lot	Storage Temperature	0	2	4	6	8	10	14
Contamination								
BPW 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
BPW 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
BPW 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Color Change								
BPW 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
BPW 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
BPW 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean BPW values were not significantly different then Week 0 ($p < 0.05$).

Table 3.T11: Buffered Peptone Water Visual and Absorbance Turbidity Scores. Table displays buffered peptone water (BPW) visual scores and absorbance results for turbidity (growth of *Salmonella* spp. cocktail) for media qualification verification testing at each shelf life time point.

		Shelf Life Time Points - Weeks						
Lot	Storage Temperature	0	2	4	6	8	10	14
Turbidity Absorbance Values								
BPW 1	20-25°C	0.554 ± 0.012	0.637 ± 0.019	0.680 ± 0.033	0.506 ± 0.173 ¹	0.748 ± 0.024	0.706 ± 0.054	0.791 ± 0.043
	2-8°C	0.549 ± 0.008	0.654 ± 0.018	0.665 ± 0.045	0.498 ± 0.181 ¹	0.646 ± 0.015	0.674 ± 0.051	0.578 ± 0.085
BPW 2	20-25°C	0.615 ± 0.014	0.760 ± 0.018	0.747 ± 0.047	0.713 ± 0.088	0.890 ± 0.028	0.837 ± 0.127	0.979 ± 0.058
	2-8°C	0.607 ± 0.036	0.680 ± 0.009	0.705 ± 0.035	0.609 ± 0.023	0.684 ± 0.018	0.752 ± 0.109	0.729 ± 0.029
BPW 3	20-25°C	0.645 ± 0.014	0.787 ± 0.005	0.764 ± 0.035	0.780 ± 0.099	0.927 ± 0.016	0.884 ± 0.134	1.063 ± 0.029
	2-8°C	0.620 ± 0.022	0.718 ± 0.017	0.660 ± 0.049	0.630 ± 0.027	0.733 ± 0.015	0.800 ± 0.132	0.745 ± 0.033
Overall mean	20-25°C	0.605 ± 0.046	0.728 ± 0.046*	0.730 ± 0.046*	0.666 ± 0.046	0.855 ± 0.046*	0.809 ± 0.046*	0.944 ± 0.046*
	2-8°C	0.592 ± 0.046	0.684 ± 0.046	0.677 ± 0.046	0.579 ± 0.046	0.688 ± 0.046*	0.742 ± 0.046*	0.684 ± 0.046
Visual Scores								
BPW 1	20-25°C	2	2	2	1 ²	2	2	2
	2-8°C	2	2	2	1 ²	2	2	2
BPW 2	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
BPW 3	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2
Overall mean	20-25°C	2	2	2	2	2	2	2
	2-8°C	2	2	2	2	2	2	2

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Acceptable absorbance scores for turbidity are ≥ 0.500. All absorbance values were taken at 600 nm and are expressed in mean ± standard deviation, all visual scores based on scoring system (0 no growth, 1 weak growth, 2 good growth). ¹Absorbance values fall below acceptable range. ²Weak growth seen for BPW replication 1 only resulting in average score below 2. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T12: Buffered Peptone Water Negative Control Absorbance Scores. Table displays buffered peptone water (BPW) negative control (media) absorbance results for turbidity for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
BPW 1	20-25°C	0.000 ± 0.000	-0.004 ± 0.004	-0.009 ± 0.003	-0.004 ± 0.004	-0.002 ± 0.005	-0.009 ± 0.004	-0.008 ± 0.004
	2-8°C	0.000 ± 0.000	-0.006 ± 0.003	-0.008 ± 0.003	-0.004 ± 0.003	-0.001 ± 0.005	-0.009 ± 0.004	-0.009 ± 0.003
BPW 2	20-25°C	0.000 ± 0.000	-0.012 ± 0.007	-0.016 ± 0.008	-0.012 ± 0.013	-0.004 ± 0.003	-0.016 ± 0.007	-0.014 ± 0.010
	2-8°C	0.000 ± 0.000	-0.017 ± 0.004	-0.020 ± 0.005	-0.012 ± 0.007	-0.005 ± 0.003	-0.021 ± 0.004	-0.019 ± 0.005
BPW 3	20-25°C	0.000 ± 0.000	-0.008 ± 0.004	-0.012 ± 0.004	-0.010 ± 0.005	-0.002 ± 0.004	-0.012 ± 0.005	-0.010 ± 0.005
	2-8°C	0.000 ± 0.000	-0.002 ± 0.004	-0.007 ± 0.002	-0.002 ± 0.004	-0.001 ± 0.003	-0.008 ± 0.003	-0.005 ± 0.003
Overall mean	20-25°C	0.000 ± 0.003	-0.008 ± 0.003*	-0.012 ± 0.003*	-0.009 ± 0.003*	-0.002 ± 0.003	-0.012 ± 0.003*	-0.011 ± 0.003*
	2-8°C	0.000 ± 0.003	-0.008 ± 0.003*	-0.012 ± 0.003*	-0.006 ± 0.003*	-0.002 ± 0.003	-0.013 ± 0.003*	-0.011 ± 0.003*

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All absorbance values were taken at 600 nm and are expressed in mean ± standard deviation. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T13: Buffered Peptone Water pH Values. Table displays buffered peptone water (BPW) pH results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
BPW 1	20-25°C	7.16 ± 0.01	7.20 ± 0.01	7.27 ± 0.01	7.21 ± 0.01	7.24 ± 0.01	7.21 ± 0.01	7.14 ± 0.01
	2-8°C	7.16 ± 0.01	7.11 ± 0.01	7.27 ± 0.01	7.11 ± 0.01	7.25 ± 0.02	7.25 ± 0.00	7.21 ± 0.01
BPW 2	20-25°C	7.16 ± 0.02	7.19 ± 0.02	7.26 ± 0.02	7.22 ± 0.02	7.23 ± 0.02	7.20 ± 0.02	7.14 ± 0.02
	2-8°C	7.16 ± 0.02	7.11 ± 0.01	7.26 ± 0.02	7.11 ± 0.01	7.24 ± 0.02	7.24 ± 0.02	7.20 ± 0.02
BPW 3	20-25°C	7.14 ± 0.01	7.17 ± 0.01	7.24 ± 0.01	7.20 ± 0.01	7.20 ± 0.01	7.18 ± 0.01	7.11 ± 0.01
	2-8°C	7.13 ± 0.00	7.09 ± 0.01	7.24 ± 0.01	7.10 ± 0.01	7.22 ± 0.01	7.22 ± 0.01	7.18 ± 0.01
Overall mean	20-25°C	7.15 ± 0.01	7.19 ± 0.01*	7.26 ± 0.01*	7.21 ± 0.01*	7.22 ± 0.01*	7.19 ± 0.01*	7.13 ± 0.01*
	2-8°C	7.15 ± 0.01	7.10 ± 0.01*	7.26 ± 0.01*	7.10 ± 0.01*	7.24 ± 0.01*	7.24 ± 0.01*	7.20 ± 0.01*

Expected pH of BPW is 7.2 ± 0.2 . Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All values are expressed in mean ± standard deviation. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T14: Buffered Peptone Water Volume Loss (By Weight). Table displays buffered peptone water (BPW) volume loss (by weight g) results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Weeks						
		0	2	4	6	8	10	14
BPW 1	20-25°C	4.77 ± 0.03	4.57 ± 0.03	4.29 ± 0.03	4.02 ± 0.01	3.80 ± 0.11	3.53 ± 0.01	3.10 ± 0.10
	2-8°C	4.74 ± 0.02	4.73 ± 0.03	4.69 ± 0.02	4.71 ± 0.03	4.67 ± 0.02	4.69 ± 0.02	4.59 ± 0.03
BPW 2	20-25°C	4.75 ± 0.03	4.57 ± 0.03	4.31 ± 0.04	4.09 ± 0.09	3.88 ± 0.03	3.59 ± 0.03	3.15 ± 0.06
	2-8°C	4.77 ± 0.04	4.73 ± 0.03	4.70 ± 0.02	4.73 ± 0.02	4.70 ± 0.01	4.69 ± 0.01	4.64 ± 0.02
BPW 3	20-25°C	4.74 ± 0.01	4.54 ± 0.03	4.29 ± 0.03	4.01 ± 0.06	3.79 ± 0.05	3.58 ± 0.04	3.08 ± 0.11
	2-8°C	4.75 ± 0.02	4.75 ± 0.02	4.70 ± 0.03	4.70 ± 0.02	4.68 ± 0.02	4.67 ± 0.02	4.60 ± 0.03
Overall mean	20-25°C	4.76 ± 0.02	4.56 ± 0.02*	4.30 ± 0.02*	4.04 ± 0.02*	3.82 ± 0.02*	3.57 ± 0.02*	3.11 ± 0.02*
	2-8°C	4.75 ± 0.02	4.74 ± 0.02	4.70 ± 0.02*	4.71 ± 0.02	4.68 ± 0.02*	4.68 ± 0.02*	4.61 ± 0.02*

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Values represent total volume. All values expressed in mean ± standard deviation. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

Table 3.T15: Romer RapidChek® Listeria Media Volume Loss (By Weight). Romer RapidChek® *Listeria* media (LRC) volume loss (by weight g) results for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Serological Pipette								
LRC 1	20-25°C	4.98 ± 0.04	4.99 ± 0.07	5.01 ± 0.05	4.95 ± 0.04	4.99 ± 0.05	5.00 ± 0.09	5.03 ± 0.09
	2-8°C	4.96 ± 0.04	4.94 ± 0.08	4.98 ± 0.02	4.98 ± 0.07	4.89 ± 0.06	5.06 ± 0.20	5.03 ± 0.10
LRC 2	20-25°C	4.94 ± 0.04	5.06 ± 0.10	4.99 ± 0.07	5.00 ± 0.10	4.94 ± 0.11	5.00 ± 0.09	5.00 ± 0.05
	2-8°C	5.07 ± 0.08	4.97 ± 0.03	5.03 ± 0.09	4.98 ± 0.04	5.05 ± 0.14	4.98 ± 0.04	4.99 ± 0.06
LRC 3	20-25°C	5.03 ± 0.14	4.97 ± 0.06	4.96 ± 0.06	4.98 ± 0.14	4.92 ± 0.09	4.94 ± 0.02	4.89 ± 0.05
	2-8°C	4.98 ± 0.03	4.97 ± 0.06	4.97 ± 0.05	5.01 ± 0.06	4.97 ± 0.04	4.97 ± 0.07	5.03 ± 0.07
Overall mean	20-25°C	4.98 ± 0.02	5.01 ± 0.02	4.99 ± 0.02	4.97 ± 0.02	4.95 ± 0.02	4.98 ± 0.02	4.97 ± 0.02
	2-8°C	5.00 ± 0.02	4.96 ± 0.02	4.99 ± 0.02	4.99 ± 0.02	4.97 ± 0.02	5.00 ± 0.02	5.01 ± 0.02
Bottle Pump ¹								
LRC	20-25°C	5.02 ± 0.01	5.02 ± 0.01	5.04 ± 0.01	5.02 ± 0.01	5.01 ± 0.01	4.98 ± 0.01	
	2-8°C	5.03 ± 0.01	5.02 ± 0.01	5.03 ± 0.01	5.02 ± 0.01	5.02 ± 0.01	5.01 ± 0.01	

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). ¹Volume loss retested using bottle pump due to inconsistency of filling tubes with serological pipette. Values represent total volume. All values expressed in mean ± standard deviation. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T16: Romer RapidChek® *Listeria* Media Quality Acceptability Scores for Morphology and Negative (Media) Control.
Table displays Romer RapidChek® *Listeria* media (LRC) quality acceptability ratios for morphology and negative (media) control for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours							
Lot	Storage Temperature	0	6	12	24	36	48
Morphology ¹							
LRC 1	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Negative Control (Media)							
LRC 1	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100

¹Acceptable morphology determined for *L. monocytogenes* cocktail. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T17: Romer RapidChek® Listeria Media Quality Acceptability Scores for Contamination and Color Change. Table displays Romer RapidChek® *Listeria* Media (LRC) quality acceptability ratios for contamination and color change (lighter or darker) for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Contamination								
LRC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Color Change								
LRC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T18: Romer RapidChek® Listeria Media pH Values. Table displays Romer RapidChek® *Listeria* media (LRC) pH results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Hours						
		0	6	12	24	36	48 A	48 B
LRC 1	20-25°C	7.21 ± 0.02	7.23 ± 0.02	7.22 ± 0.01	7.24 ± 0.01	7.25 ± 0.00	7.27 ± 0.01	7.27 ± 0.01
	2-8°C	7.22 ± 0.02	7.22 ± 0.02	7.20 ± 0.01	7.23 ± 0.02	7.23 ± 0.00	7.26 ± 0.00	7.27 ± 0.00
LRC 2	20-25°C	7.24 ± 0.01	7.25 ± 0.01	7.24 ± 0.01	7.26 ± 0.01	7.24 ± 0.01	7.26 ± 0.01	7.27 ± 0.01
	2-8°C	7.25 ± 0.00	7.26 ± 0.00	7.23 ± 0.00	7.26 ± 0.01	7.23 ± 0.01	7.25 ± 0.01	7.25 ± 0.01
LRC 3	20-25°C	7.23 ± 0.02	7.24 ± 0.01	7.21 ± 0.02	7.27 ± 0.01	7.26 ± 0.00	7.27 ± 0.01	7.28 ± 0.01
	2-8°C	7.24 ± 0.02	7.23 ± 0.02	7.19 ± 0.04	7.21 ± 0.04	7.18 ± 0.05	7.18 ± 0.07	7.18 ± 0.06
Overall mean	20-25°C	7.23 ± 0.01	7.24 ± 0.01	7.22 ± 0.01	7.26 ± 0.01*	7.25 ± 0.01	7.27 ± 0.01*	7.27 ± 0.01*
	2-8°C	7.24 ± 0.01	7.24 ± 0.01	7.21 ± 0.01	7.23 ± 0.01	7.21 ± 0.01	7.23 ± 0.01	7.23 ± 0.01

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). There is no manufacturer recommended pH value for LRC. All values expressed in mean ± standard deviation. Overall mean LRC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

Table 3.T19: Romer RapidChek® Listeria Media Positive Control Scores. Table displays Romer RapidChek® *Listeria* Media (LRC) positive control (*Listeria* spp. cocktail) growth acceptability ratios for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Hours					
		0	6	12	24	36	48
LRC 1	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T20: Romer RapidChek® Listeria Media 50:50 Positive/Negative Control Scores. Table displays Romer RapidChek® Listeria Media (LRC) 50:50 competition plate positive/negative control (*Listeria* spp. cocktail / *E. coli* ATCC 25922 and *E. coli* O157:H7 ATCC 35150) growth acceptability ratios for media qualification verification testing at each shelf life time point.

		Shelf Life Time Points - Hours					
Lot	Storage Temperature	0	6	12	24	36	48
50:50 Positive Control							
LRC 1	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
50:50 Negative Control							
LRC 1	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T21: Romer RapidChek® Listeria Media Negative Control Scores. Table displays Romer RapidChek® *Listeria* Media (LRC) negative control (*E. coli* ATCC 25922 and *E. coli* O157:H7 ATCC 35150) growth acceptability ratios for media qualification verification testing at each shelf life time point.

		Shelf Life Time Points - Hours					
Lot	Storage Temperature	0	6	12	24	36	48
Negative Control <i>E. coli</i> ATCC 25922							
LRC 1	20-25°C	100	100	100	75	100	100
	2-8°C	100	100	100	100	100	100
LRC 2	20-25°C	100	100	100	100	50	100
	2-8°C	100	100	100	100	100	100
LRC 3	20-25°C	100	100	100	75	50	50
	2-8°C	100	100	100	100	75	100
Overall mean	20-25°C	100	100	100	83*	67*	83*
	2-8°C	100	100	100	100	92*	100
Negative Control <i>E. coli</i> O157:H7 ATCC 35150							
LRC 1	20-25°C	100	100	100	0	0	100
	2-8°C	0	100	100	0	100	100
LRC 2	20-25°C	100	100	75	25	25	75
	2-8°C	100	100	100	75	75	100
LRC 3	20-25°C	50	75	50	25	75	75
	2-8°C	50	25	100	50	50	100
Overall mean	20-25°C	83*	92*	75*	17*	33*	83*
	2-8°C	50*	75*	100	42*	75*	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean LRC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

Table 3.T22: Neogen Reveal® 20-Hour for E. coli O157:H7 Media Volume Loss (By Weight). Neogen Reveal® 20-Hour media (REC) volume loss (by weight g) results for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Serological Pipette								
REC 1	20-25°C	4.70 ± 0.01	4.93 ± 0.07	5.03 ± 0.07	4.92 ± 0.05	4.94 ± 0.13	4.83 ± 0.05	4.86 ± 0.05
	2-8°C	4.78 ± 0.08	4.90 ± 0.04	5.07 ± 0.09	4.94 ± 0.12	4.97 ± 0.03	4.99 ± 0.03	4.96 ± 0.00
REC 2	20-25°C	4.95 ± 0.07	4.93 ± 0.05	4.87 ± 0.04	4.94 ± 0.16	4.95 ± 0.05	4.92 ± 0.01	4.89 ± 0.06
	2-8°C	4.96 ± 0.11	4.98 ± 0.06	4.98 ± 0.11	4.93 ± 0.02	4.91 ± 0.09	5.01 ± 0.08	4.92 ± 0.06
REC 3	20-25°C	4.97 ± 0.07	4.99 ± 0.07	5.01 ± 0.10	4.95 ± 0.06	4.93 ± 0.10	4.90 ± 0.10	4.91 ± 0.03
	2-8°C	5.00 ± 0.03	4.92 ± 0.05	4.94 ± 0.01	4.98 ± 0.03	4.97 ± 0.02	4.98 ± 0.02	4.98 ± 0.03
Overall mean	20-25°C	4.87 ± 0.03	4.95 ± 0.03	4.97 ± 0.03*	4.94 ± 0.03	4.94 ± 0.03	4.89 ± 0.03	4.89 ± 0.03
	2-8°C	4.91 ± 0.03	4.93 ± 0.03	5.00 ± 0.03*	4.95 ± 0.03	4.95 ± 0.03	4.99 ± 0.03	4.95 ± 0.03
Bottle Pump ¹								
REC1	20-25°C	5.02 ± 0.01	4.99 ± 0.01	4.99 ± 0.01	4.98 ± 0.01*	4.96 ± 0.01*	4.95 ± 0.01*	4.95 ± 0.01*
	2-8°C	5.00 ± 0.01	4.99 ± 0.01	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01	4.99 ± 0.01

¹Volume loss retested using bottle pump due to inconsistency of filling tubes with serological pipette. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All values expressed in mean ± standard deviation. Overall mean REC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

Table 3.T23: Neogen Reveal® 20-Hour for E. coli O157:H7 Media Quality Acceptability Scores for Morphology and Negative (Media) Control. Table displays Neogen Reveal® 20-Hour media (REC) quality acceptability ratios for morphology and negative (media) control for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Morphology ¹								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Negative Control (Media)								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

¹Acceptable morphology determined for *Escherichia coli* O157:H7 cocktail. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T24: Neogen Reveal® 20-Hour for E. coli O157:H7 Media Quality Acceptability Scores for Contamination and Color Change. Table displays Neogen Reveal® 20-Hour media (REC) quality acceptability ratios for contamination and color change (lighter or darker) for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Contamination								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Color Change								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

Table 3.T25: Neogen Reveal® 20-Hour for E. coli O157:H7 Media pH Values. Table displays Neogen Reveal® 20-Hour media (REC) pH results for media qualification verification testing at each shelf life time point.

Lot	Storage Temperature	Shelf Life Time Points - Hours						
		0	6	12	24	36	48 A	48 B
REC 1	20-25°C	6.88 ± 0.01	6.88 ± 0.01	6.87 ± 0.01	6.90 ± 0.01	6.89 ± 0.01	6.90 ± 0.01	6.89 ± 0.01
	2-8°C	6.88 ± 0.03	6.88 ± 0.01	6.86 ± 0.01	6.88 ± 0.01	6.88 ± 0.01	6.90 ± 0.01	6.88 ± 0.02
REC 2	20-25°C	6.91 ± 0.01	6.92 ± 0.01	6.91 ± 0.00	6.91 ± 0.01	6.90 ± 0.01	6.93 ± 0.01	6.93 ± 0.01
	2-8°C	6.92 ± 0.01	6.93 ± 0.01	6.92 ± 0.01	6.90 ± 0.01	6.90 ± 0.01	6.93 ± 0.02	6.93 ± 0.01
REC 3	20-25°C	6.93 ± 0.00	6.94 ± 0.01	6.93 ± 0.01	6.95 ± 0.01	6.94 ± 0.01	6.93 ± 0.01	6.94 ± 0.01
	2-8°C	6.95 ± 0.00	6.94 ± 0.00	6.94 ± 0.01	6.94 ± 0.01	6.93 ± 0.00	6.94 ± 0.01	6.94 ± 0.01
Overall mean	20-25°C	6.90 ± 0.02	6.91 ± 0.02	6.90 ± 0.02	6.92 ± 0.02*	6.91 ± 0.02	6.92 ± 0.02*	6.92 ± 0.02*
	2-8°C	6.91 ± 0.02	6.91 ± 0.02	6.91 ± 0.02	6.91 ± 0.02	6.90 ± 0.02	6.92 ± 0.02	6.92 ± 0.02

There is no manufacturer recommended pH value for REC. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All values expressed in mean ± standard deviation. Overall mean REC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

Table 3.T26: Neogen Reveal® 20-Hour for E. coli O157:H7 Media Positive and Negative Control Scores. Table displays Neogen Reveal® 20-Hour media (REC) positive control (*E. coli* O157:H7 cocktail) and negative control (*S. aureus* ATCC 6538P) growth acceptability ratios for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
Positive Control								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Negative Control								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	67	67	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	89*	89*	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean REC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

Table 3.T27: Neogen Reveal® 20-Hour for E. coli O157:H7 Media 50:50 Positive/Negative Control Scores. Table displays Neogen Reveal® 20-Hour media (REC) 50:50 competition plate positive/negative control (*E. coli* O157:H7 cocktail / *S. aureus* ATCC 6538P) growth acceptability ratios for media qualification verification testing at each shelf life time point.

Shelf Life Time Points - Hours								
Lot	Storage Temperature	0	6	12	24	36	48 A	48 B
50:50 Positive Control								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
50:50 Negative Control								
REC 1	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 2	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
REC 3	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100
Overall mean	20-25°C	100	100	100	100	100	100	100
	2-8°C	100	100	100	100	100	100	100

Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable. Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

Media Qualification Verification Results Figures

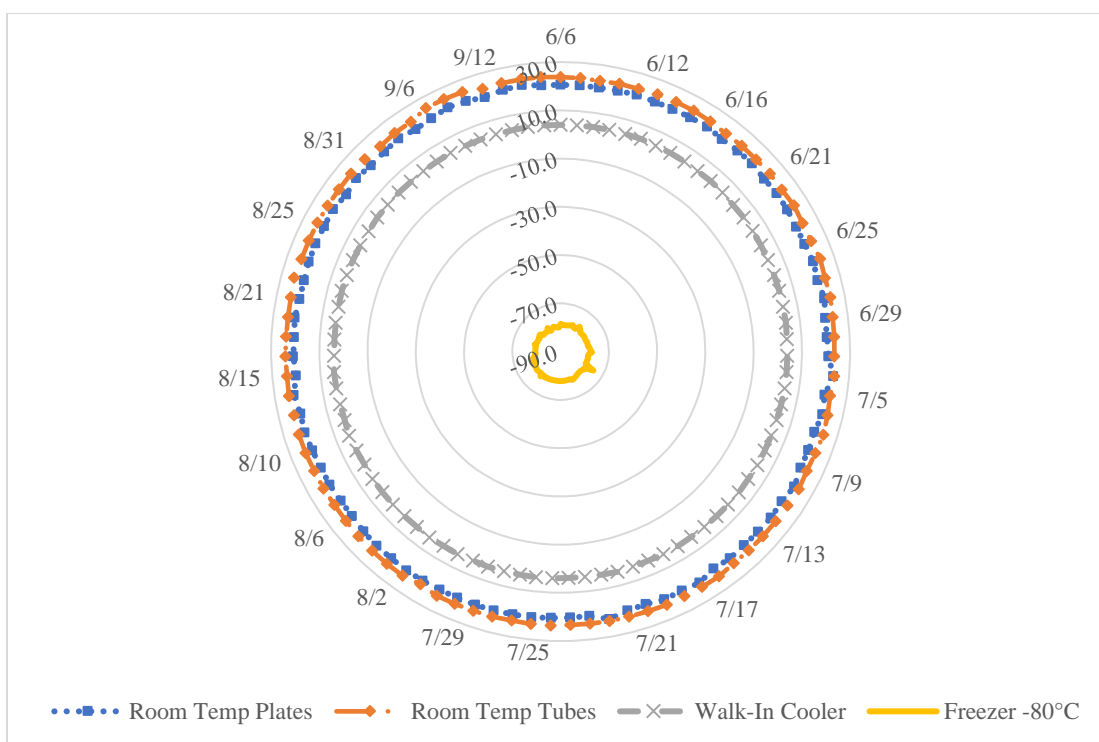


Figure 3.F1: Sample and Stock Culture Storage Temperature Control Chart. Media qualification verification procedure temperature control of storage areas for shelf life testing of solid and liquid media and stock cultures. Figure shows the stability of the storage environments utilized for shelf life testing. Room temperature plates/tubes stored at 20-25°C, refrigerated plates/tubes stored at 2-8°C, and frozen culture stocks stored at approximately -80°C.

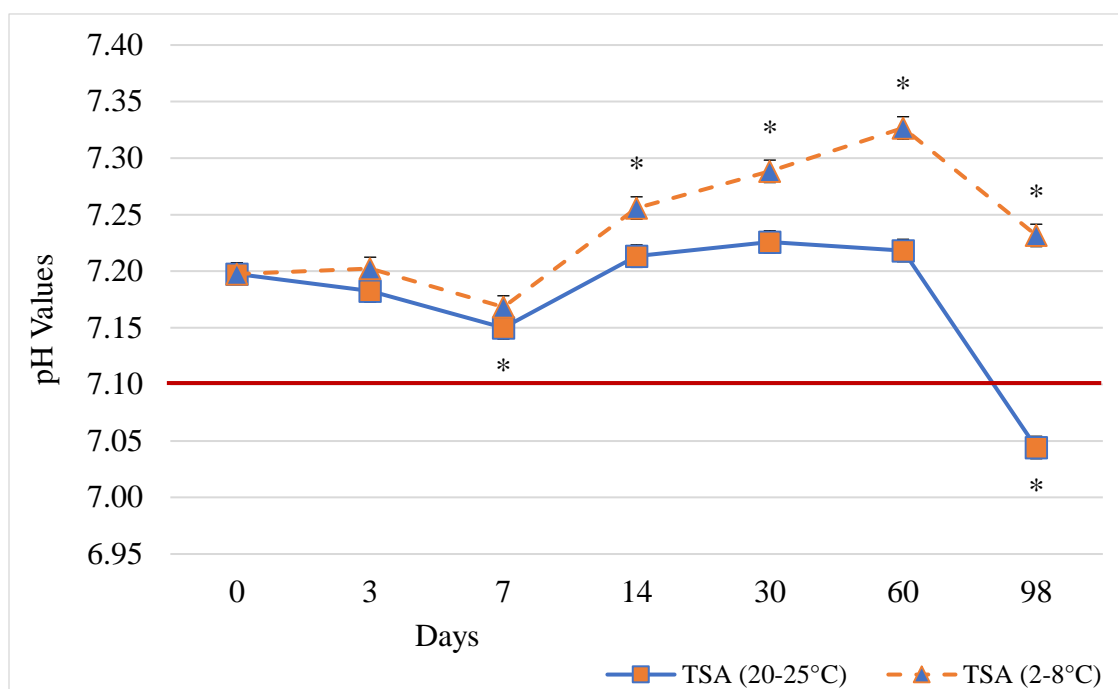


Figure 3.F2: Tryptic Soy Agar Overall Mean pH Values. Figure displays tryptic soy agar (TSA) pH values for media qualification verification testing at each shelf life time point. Expected pH of TSA is 7.3 ± 0.2 (limits indicated by red lines). Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

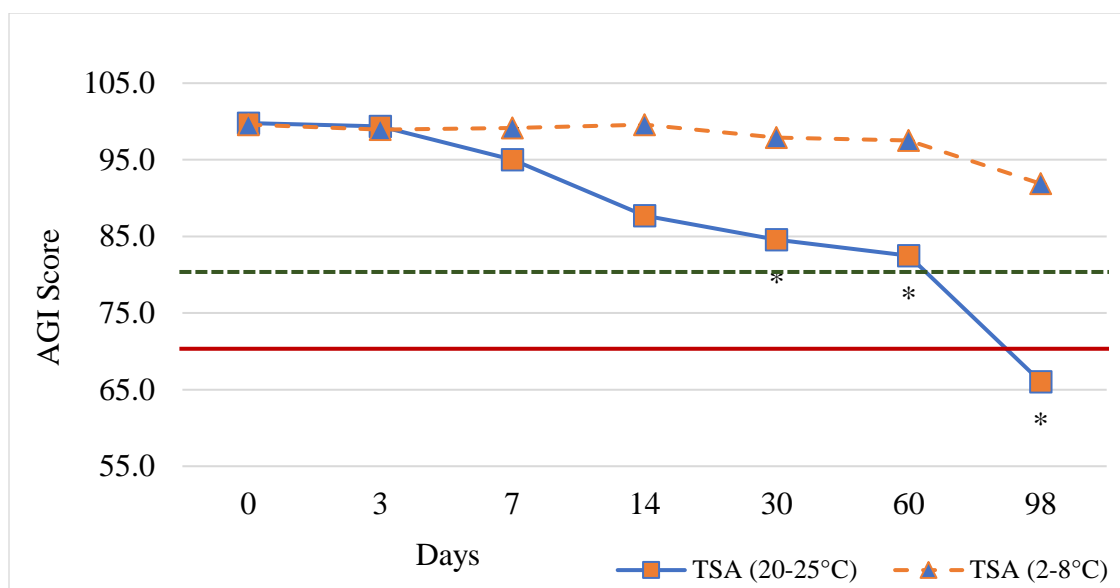


Figure 3.F3: Tryptic Soy Agar Overall Mean Absolute Growth Index Scores. Figure displays tryptic soy agar (TSA) absolute growth index (AGI) scores for media qualification verification testing at each shelf life time point. Growth determined using *E. coli* ATCC 25922. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Minimum acceptable AGI score is ≥ 70.0 (solid red line) with FPCLS acceptability set at ≥ 80.0 (dashed green line). Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

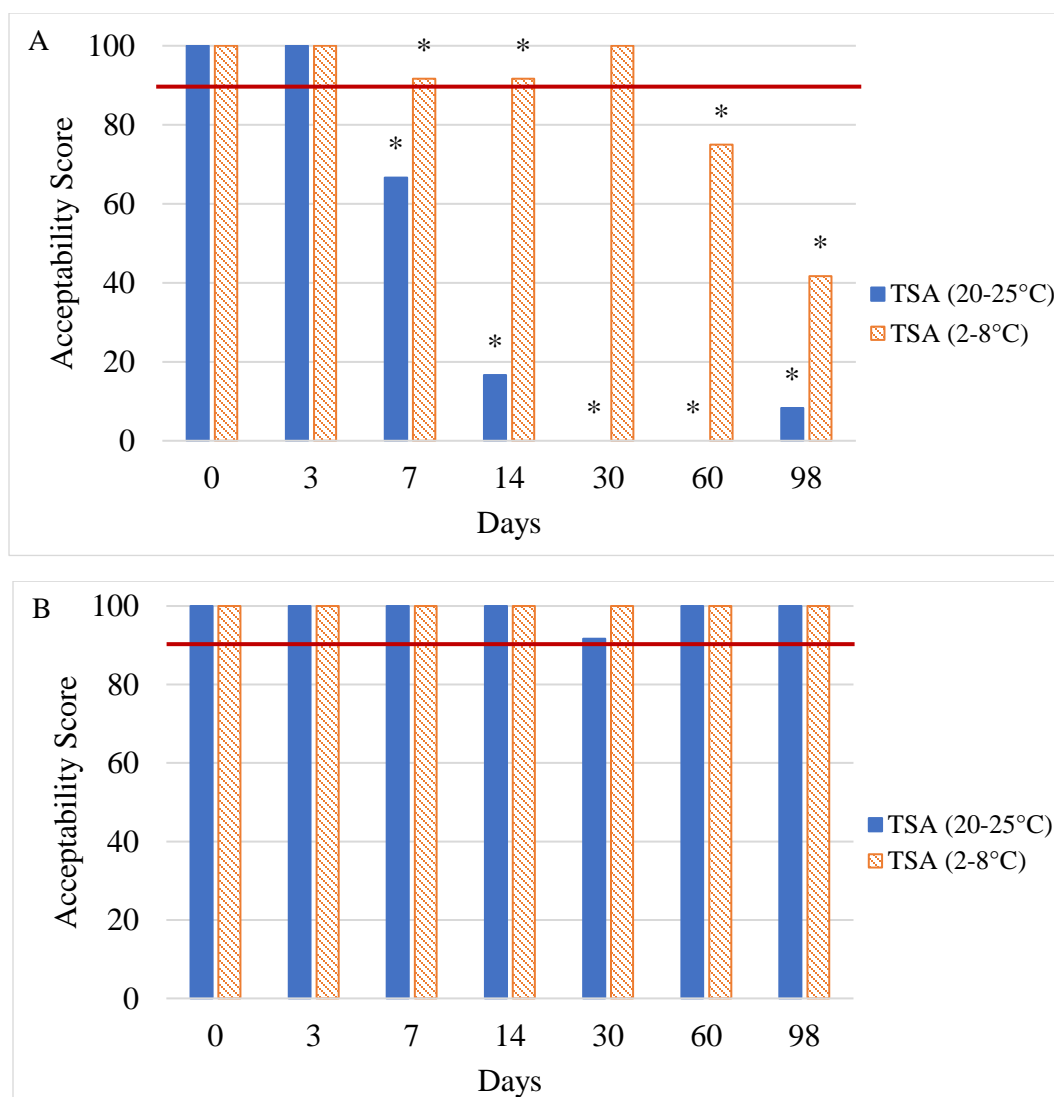


Figure 3.F4: Tryptic Soy Agar Overall Mean Quality Acceptability Scores for Drying/Thinning and Cracking. Figure displays tryptic soy agar (TSA) quality acceptability ratios for drying/thinning (A) and cracking (B) of agar for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

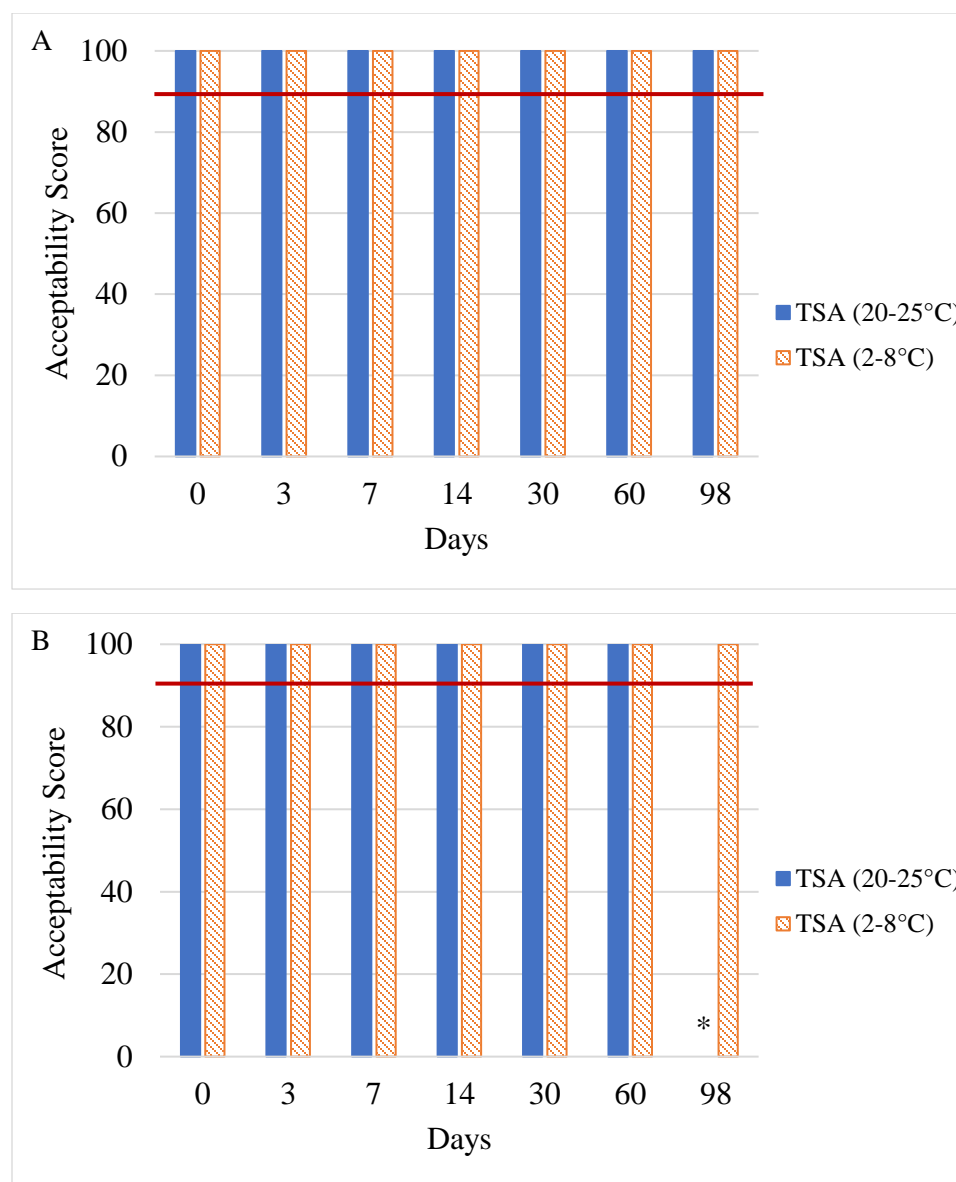


Figure 3.F5: Tryptic Soy Agar Overall Mean Quality Acceptability Scores for Contamination and Color Change. Table displays tryptic soy agar (TSA) quality acceptability ratios for contamination (A) and color change – lighter or darker (B), for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean TSA values marked with an * are significantly different then Day 0 ($p < 0.05$).

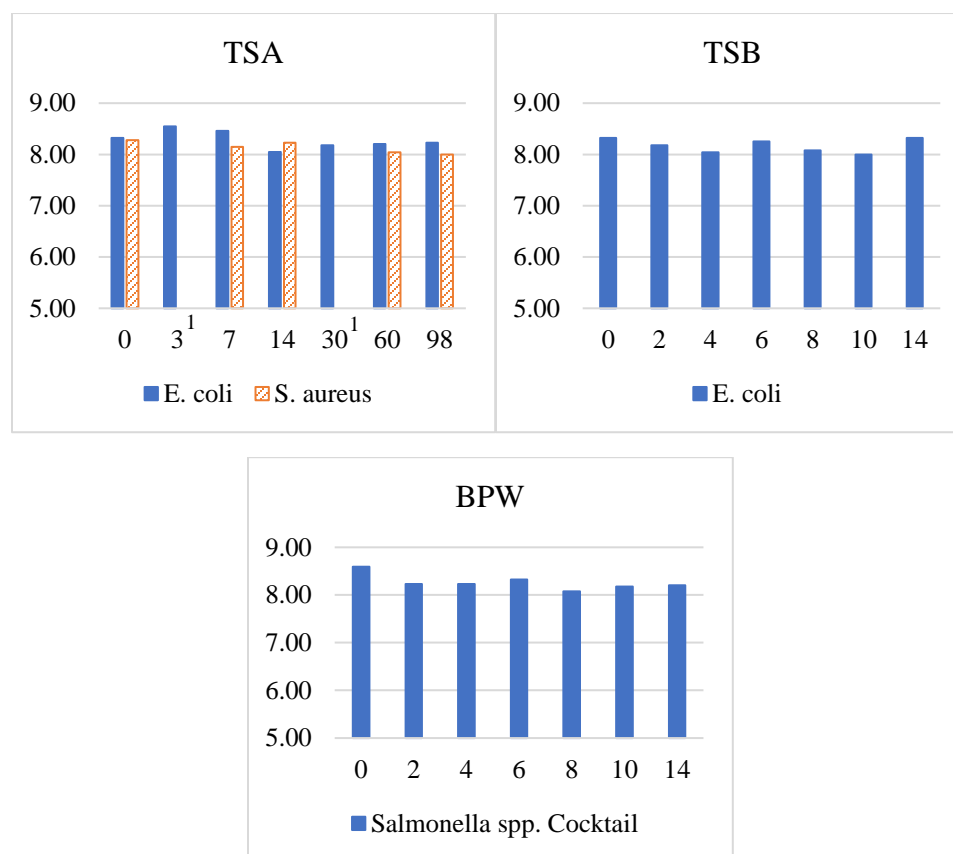


Figure 3.F6: Inoculum Level McFarland's Standard Adjustment Verification – TSA, TSB, BPW. Figure displays the inoculum levels achieved for each organism after adjusting to 0.5 McFarland's Standard (1.5×10^8 CFU/ml) for media qualification verification testing purposes. Values displayed as LOG CFU/ml across time points in days (TSA), and weeks (TSB, BPW). *E. coli* ATCC 25922 on/in TSA and TSB, *S. aureus* ATCC 6538P on TSA, and *Salmonella* spp. cocktail in BPW. Figures are as follows: Tryptic Soy Agar (TSA) top left; Tryptic Soy Broth (TSB) top right; and Buffered Peptone Water (BPW) bottom. ¹*S. aureus* was not tested at 3 and 30 days.

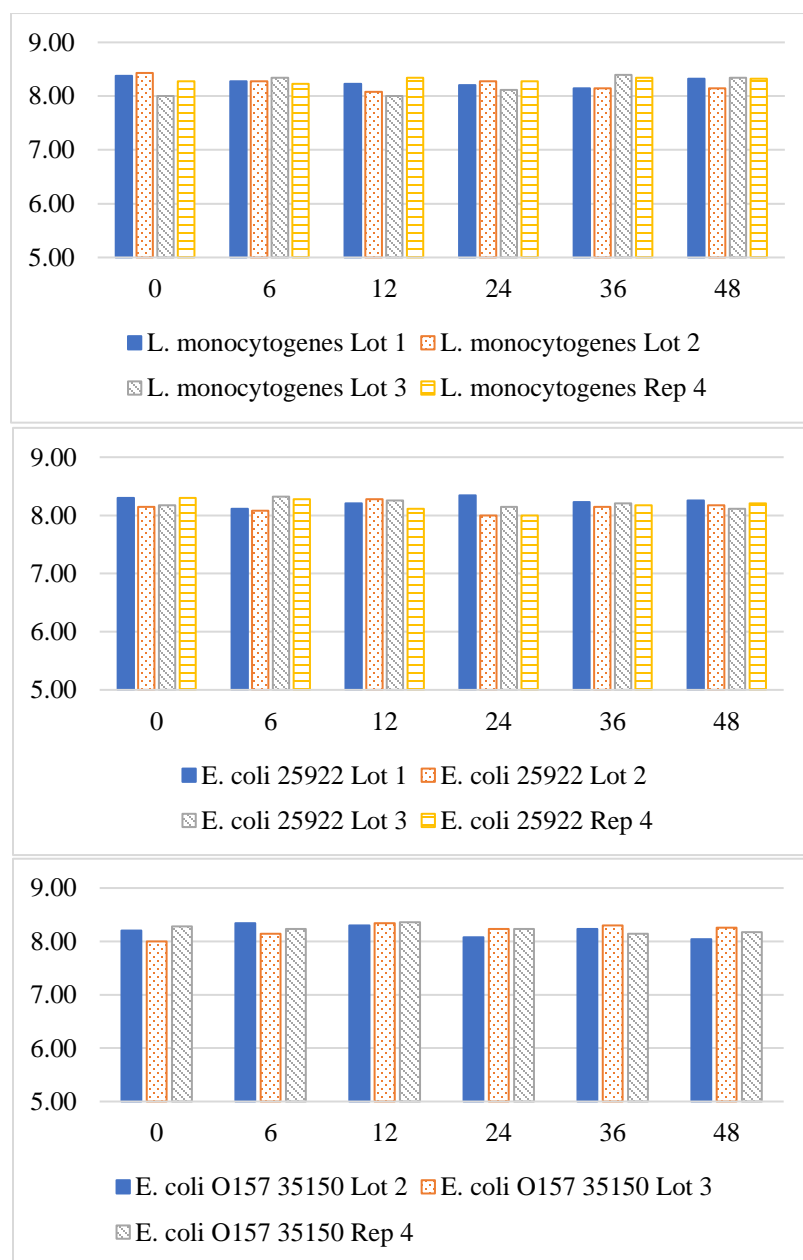


Figure 3.F7: Inoculum Level McFarland's Standard Adjustment Verification – LRC. Figure displays the inoculum levels achieved for each organism after adjusting to 0.5 McFarland's Standard (1.5×10^8 CFU/ml) for media qualification verification testing purposes. Values displayed as LOG CFU/ml across time points in hours. Figures are as follows: Romer RapidChek® *Listeria* (LRC) *L. monocytogenes* cocktail top; *E. coli* ATCC 25922 middle; *E. coli* O157:H7 ATCC 35150 bottom.

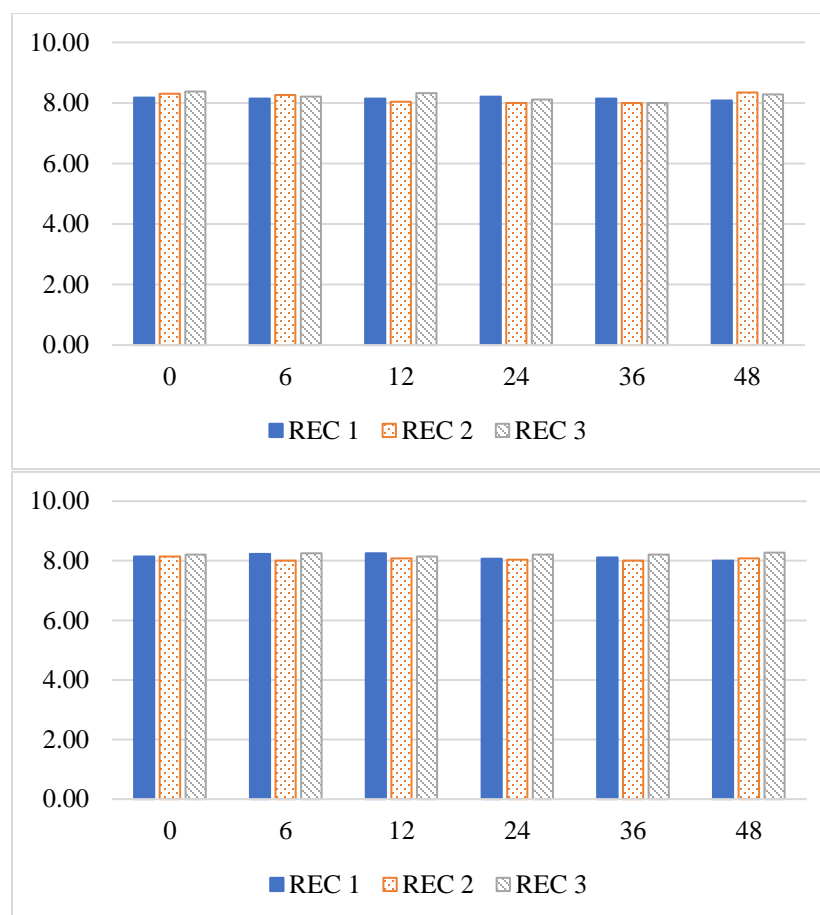


Figure 3.F8: Inoculum Level McFarland's Standard Adjustment Verification – REC. Figure displays the inoculum levels achieved for each organism after adjusting to 0.5 McFarland's Standard (1.5×10^8 CFU/ml) for media qualification verification testing purposes. Values displayed as LOG CFU/ml across time points in hours. Figures are as follows: Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) media *E. coli* O157:H7 cocktail (top) and *S. aureus* ATCC 6538P (bottom).

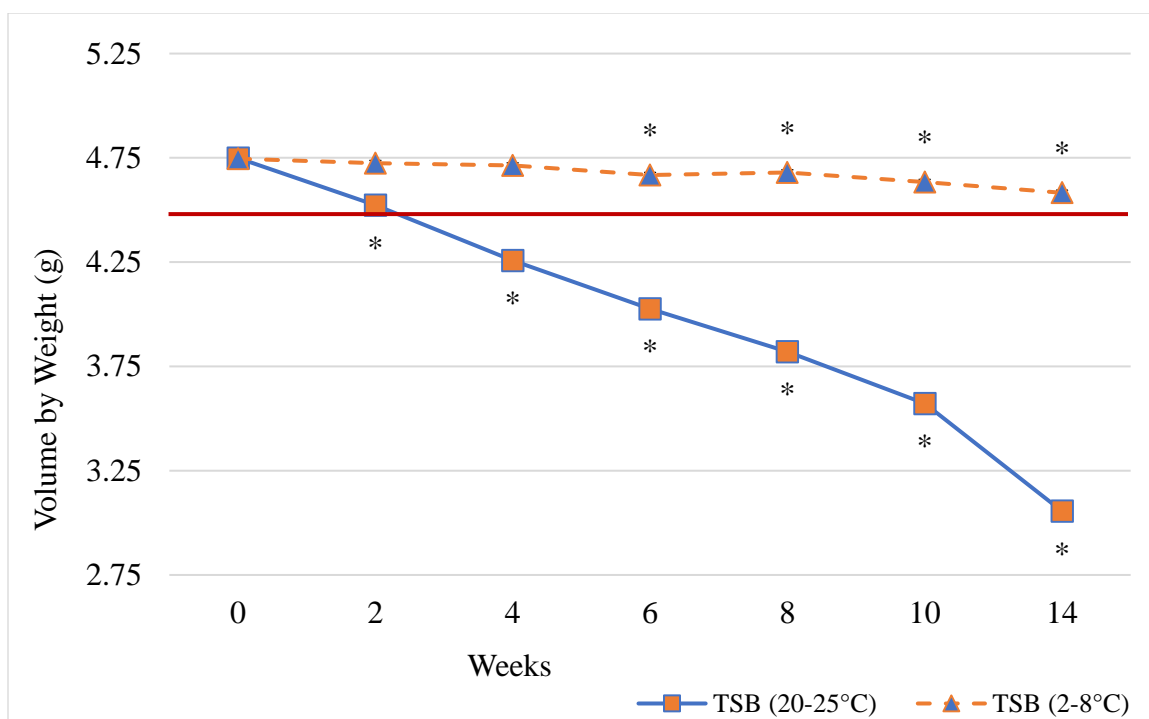


Figure 3.F9: Tryptic Soy Broth Overall Mean Volume Loss (By Weight). Figure displays tryptic soy broth (TSB) volume loss (by weight g) results for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 4.50 (loss of 0.25) is considered unacceptable (solid red line). Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

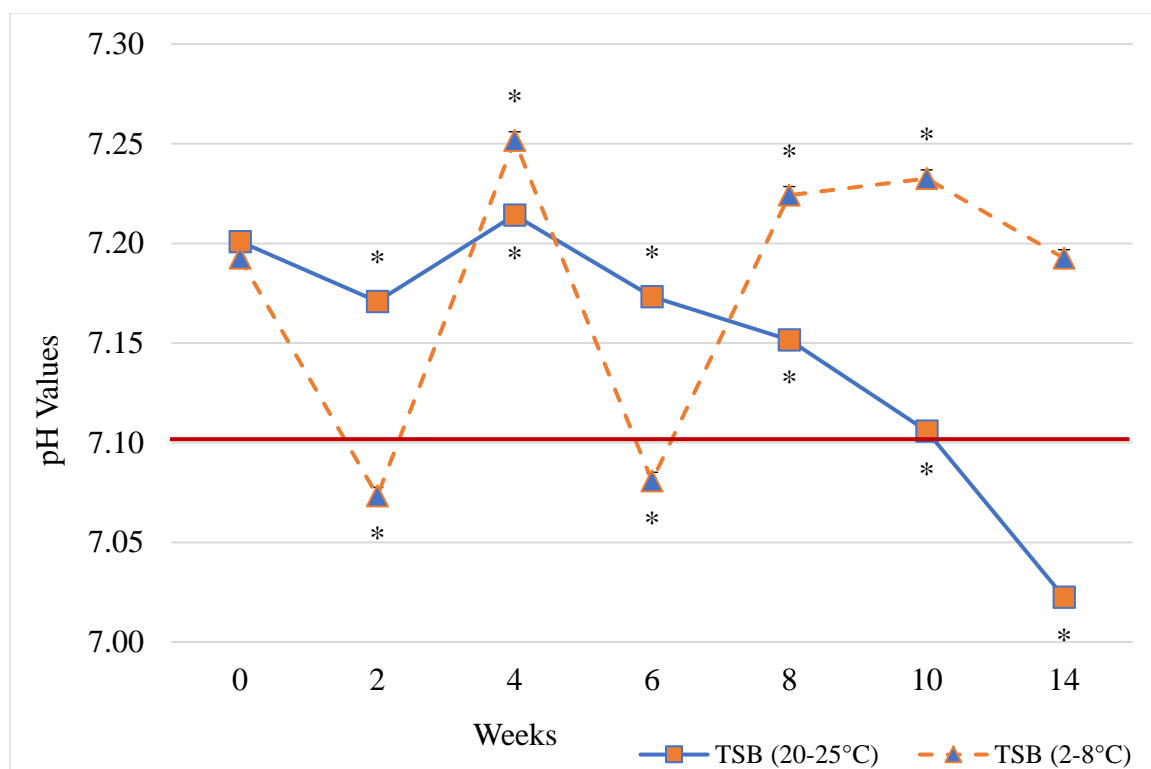


Figure 3.F10: Tryptic Soy Broth Overall Mean pH Values. Figure displays tryptic soy broth (TSB) pH results for media qualification verification testing at each shelf life time point. Expected pH of TSB is 7.3 ± 0.2 (limits indicated by red lines). Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Overall mean TSB values marked with an * are significantly different than Week 0 ($p < 0.05$).

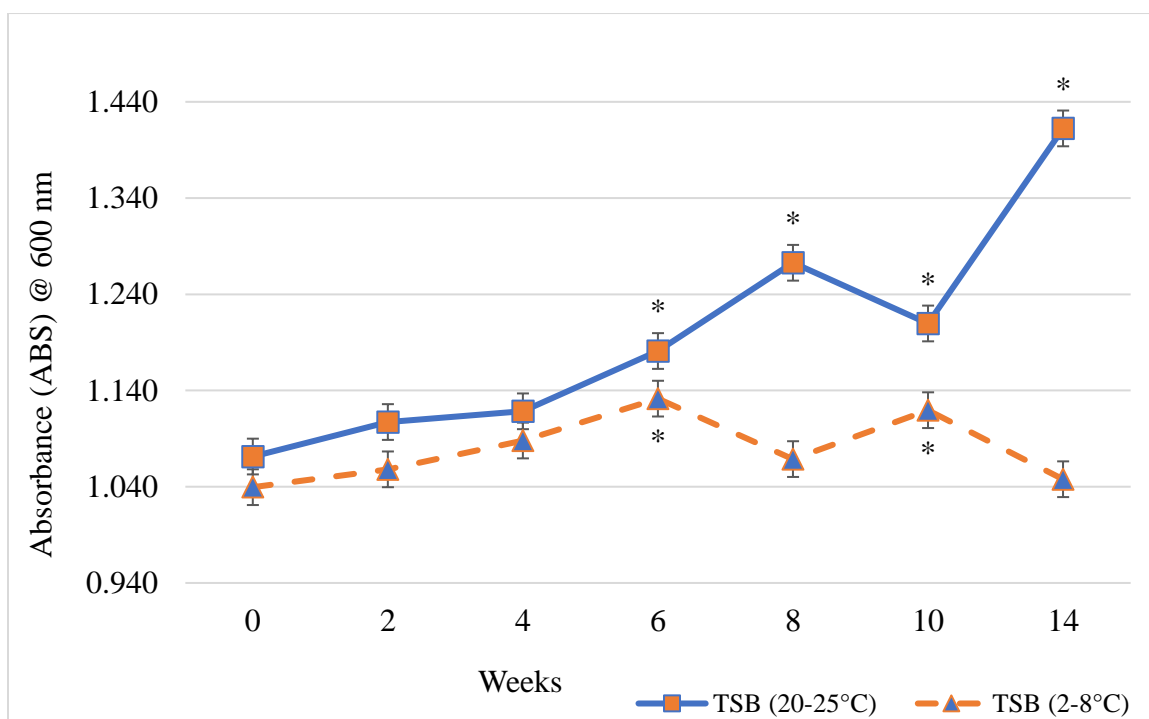


Figure 3.F11: Tryptic Soy Broth Overall Mean Absorbance Scores. Figure displays tryptic soy broth (TSB) absorbance results for turbidity (growth of *E. coli* ATCC 25922) for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Acceptable absorbance scores for turbidity are ≥ 0.500 . All absorbance values were taken at 600 nm. Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

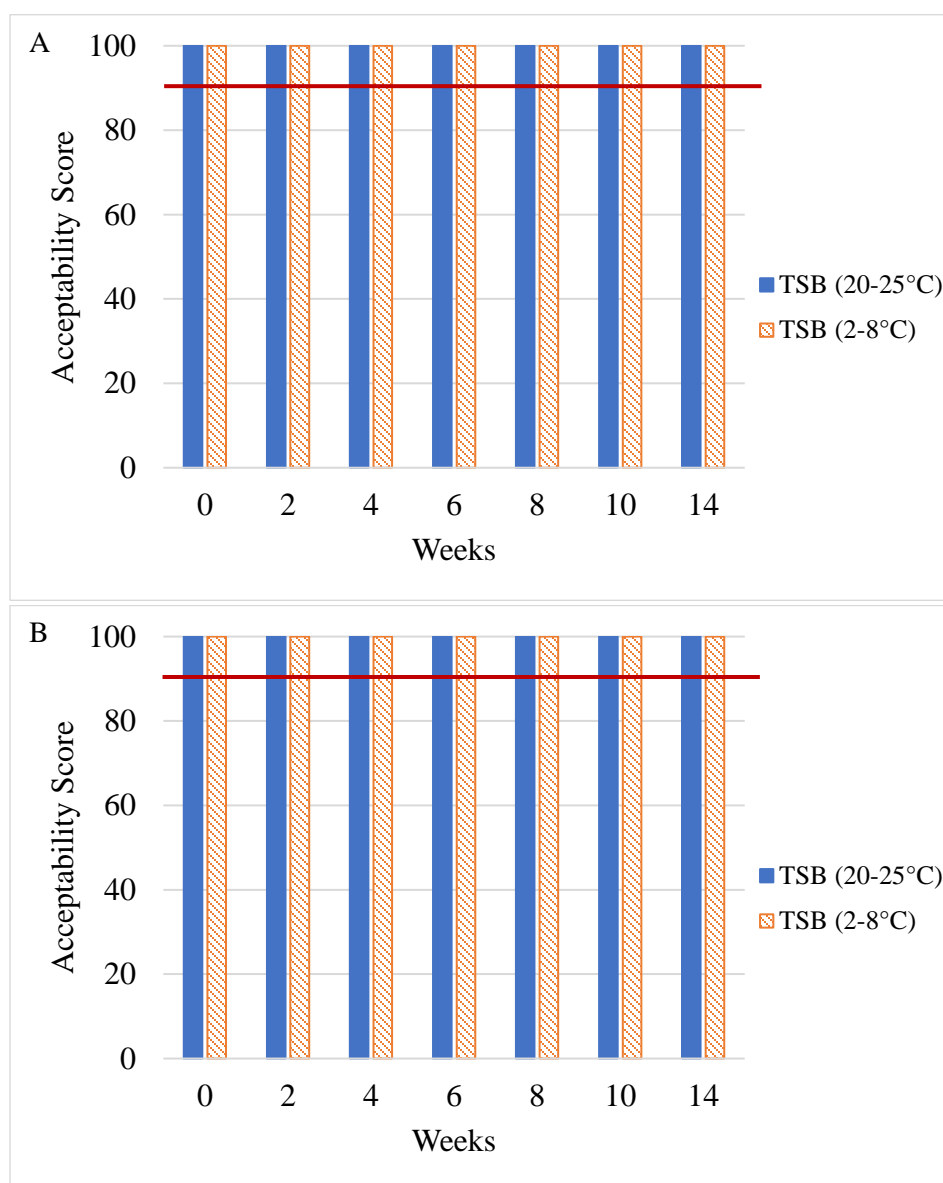


Figure 3.F12: Tryptic Soy Broth Overall Mean Quality Acceptability Scores for Contamination and Color Change. Figure displays tryptic soy broth (TSB) quality acceptability ratios for contamination (A) and color change, lighter or darker (B), for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean TSB values were not significantly different then Week 0 ($p < 0.05$).

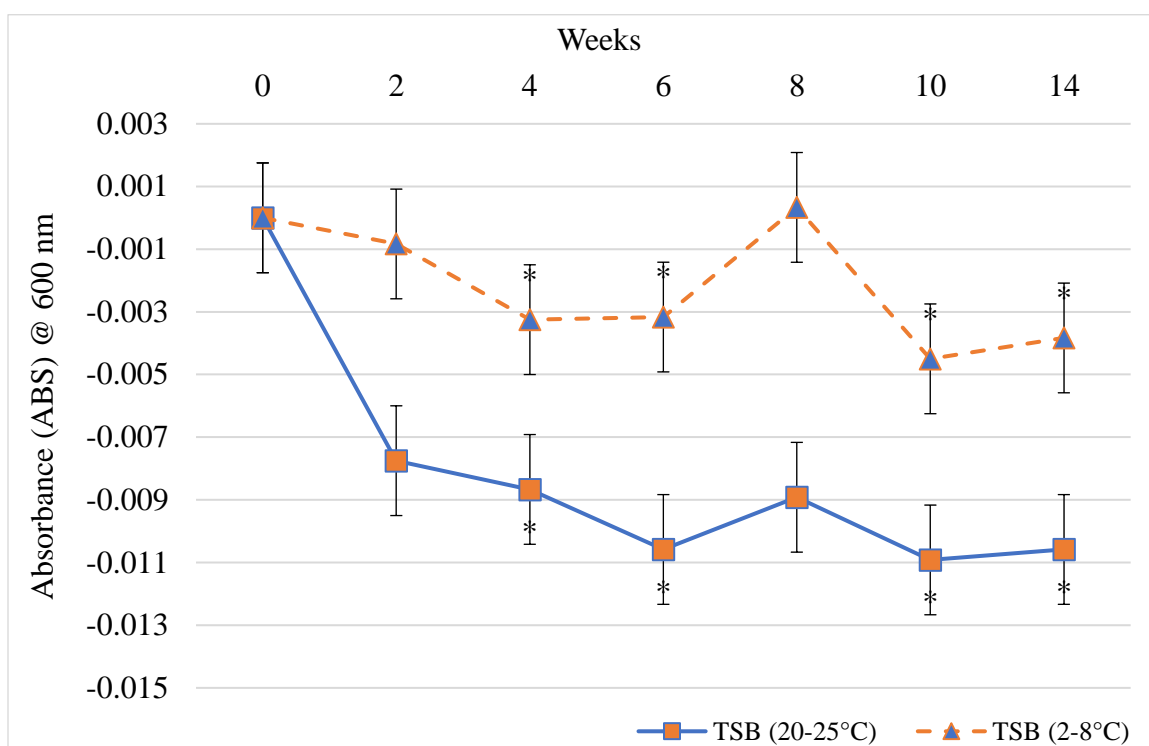


Figure 3.F13: Tryptic Soy Broth Overall Mean Negative Control Absorbance Scores. Figure displays tryptic soy broth (TSB) negative (media) control absorbance results for turbidity for media qualification verification testing at each shelf life time point. Shelf life time points are 0, 2, 4, 6, 8, 10, and 14 weeks. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All absorbance values were recorded at 600 nm. Overall mean TSB values marked with an * are significantly different then Week 0 ($p < 0.05$).

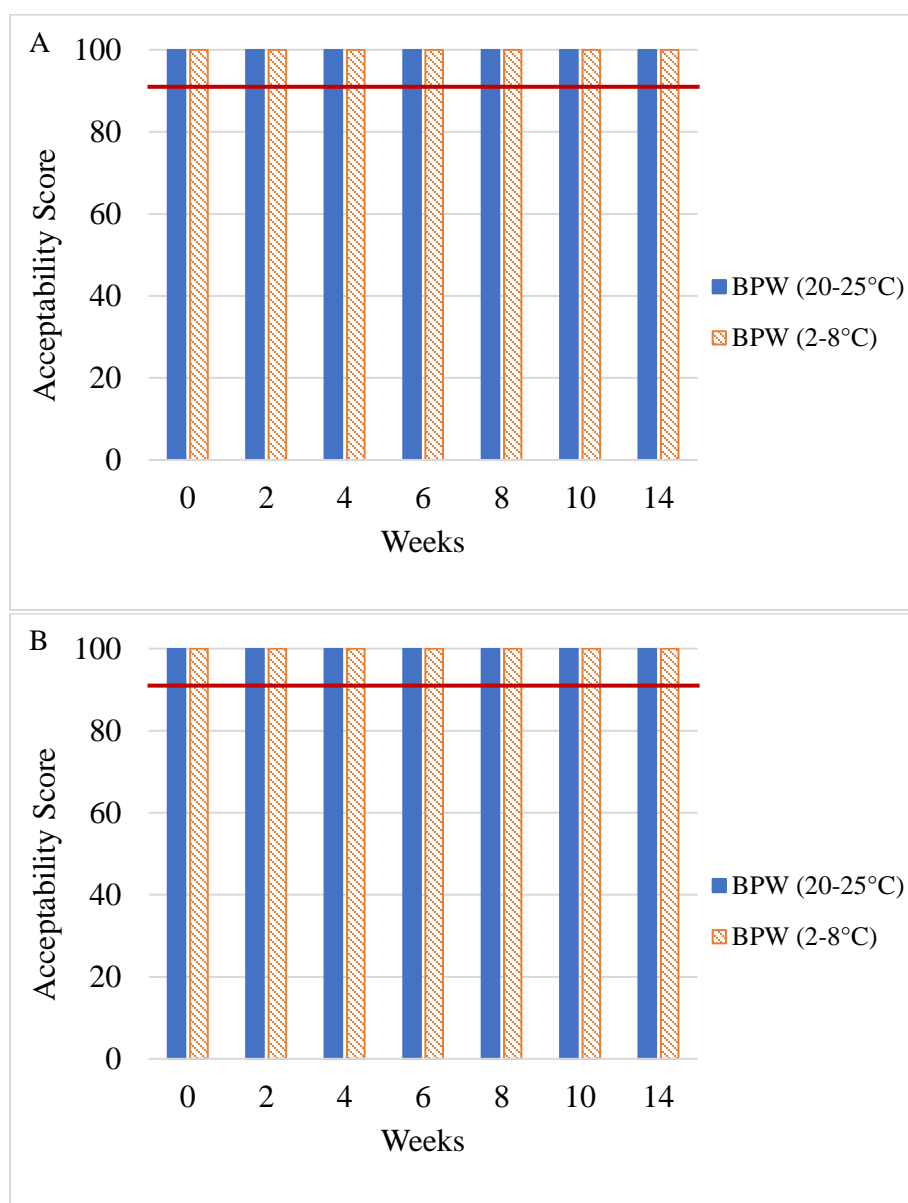


Figure 3.F14: Buffered Peptone Water Overall Mean Quality Acceptability Scores for Contamination and Color Change. Figure displays buffered peptone water (BPW) quality acceptability ratios for contamination (A) and color change, lighter or darker (B), for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean BPW values were not significantly different than Week 0 ($p < 0.05$).

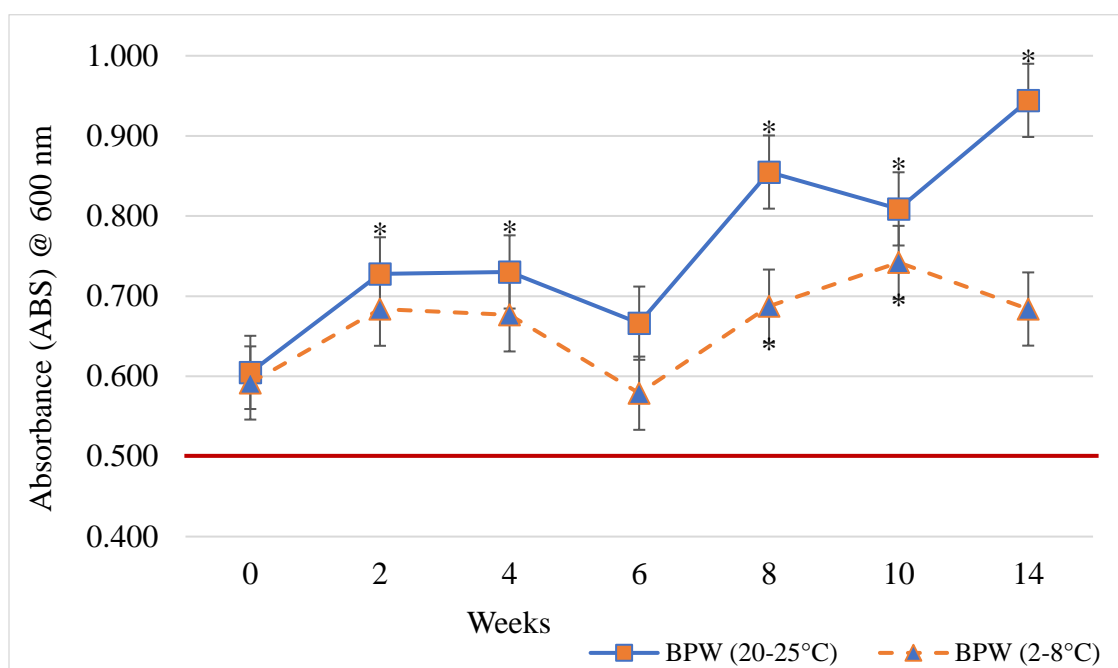


Figure 3.F15: Buffered Peptone Water Overall Mean Absorbance Scores. Figure displays buffered peptone water (BPW) absorbance results for turbidity (growth of *Salmonella* spp. cocktail) for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Acceptable absorbance scores for turbidity are ≥ 0.500 (solid red line). All absorbance values were recorded at 600 nm. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

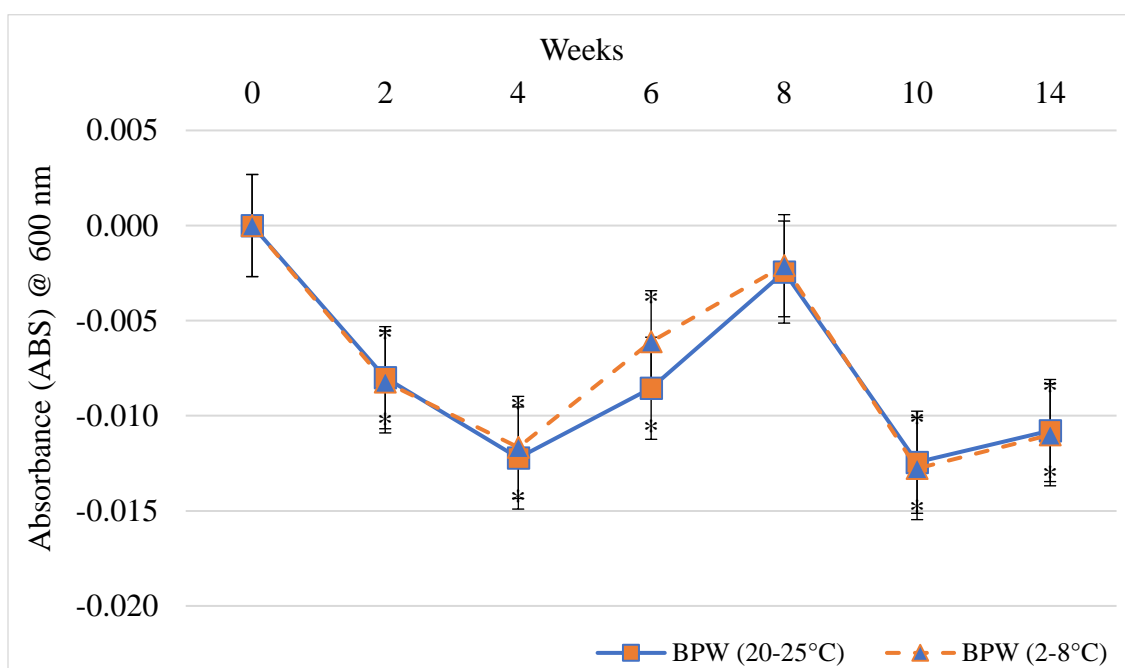


Figure 3.F16: Buffered Peptone Water Negative Control Overall Mean Absorbance Scores. Figure displays buffered peptone water (BPW) negative (media) control absorbance results for turbidity for media qualification verification testing at each shelf life time point. Shelf life time points are 0, 2, 4, 6, 8, 10, and 14 weeks. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). All absorbance values were recorded at 600 nm. Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

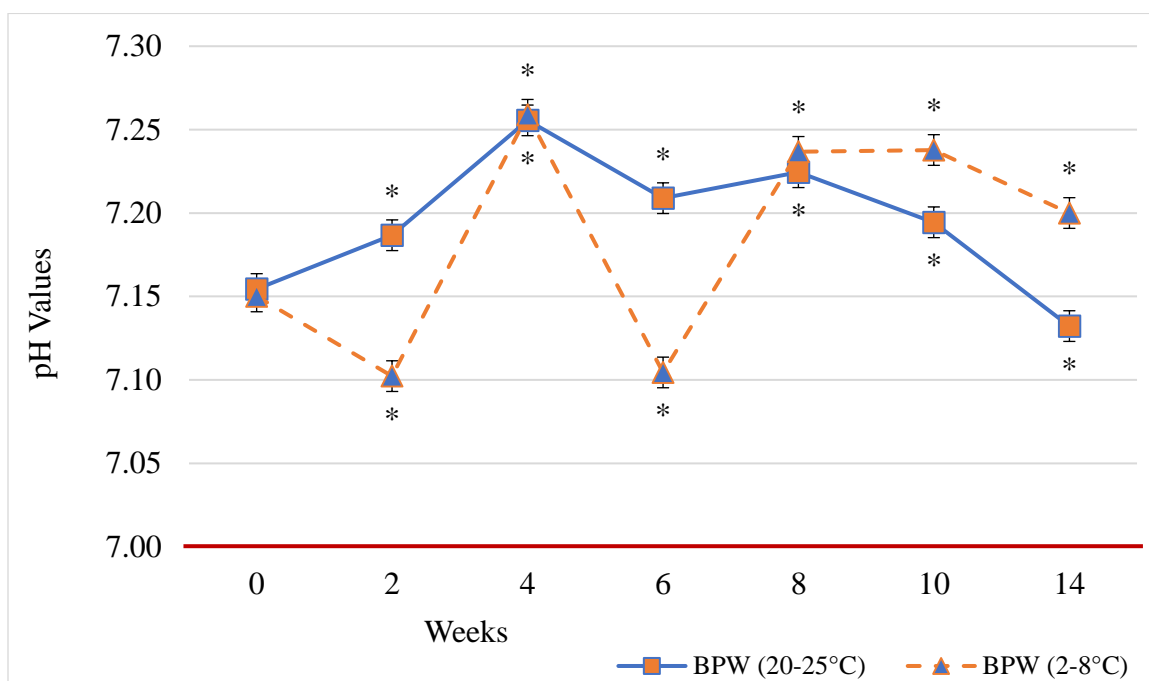


Figure 3.F17: Buffered Peptone Water Overall Mean pH Values. Figure displays buffered peptone water (BPW) pH results for media qualification verification testing at each shelf life time point. Expected pH of BPW is 7.2 ± 0.2 (limits indicated by red lines). Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Overall mean BPW values marked with an * are significantly different than Week 0 ($p < 0.05$).

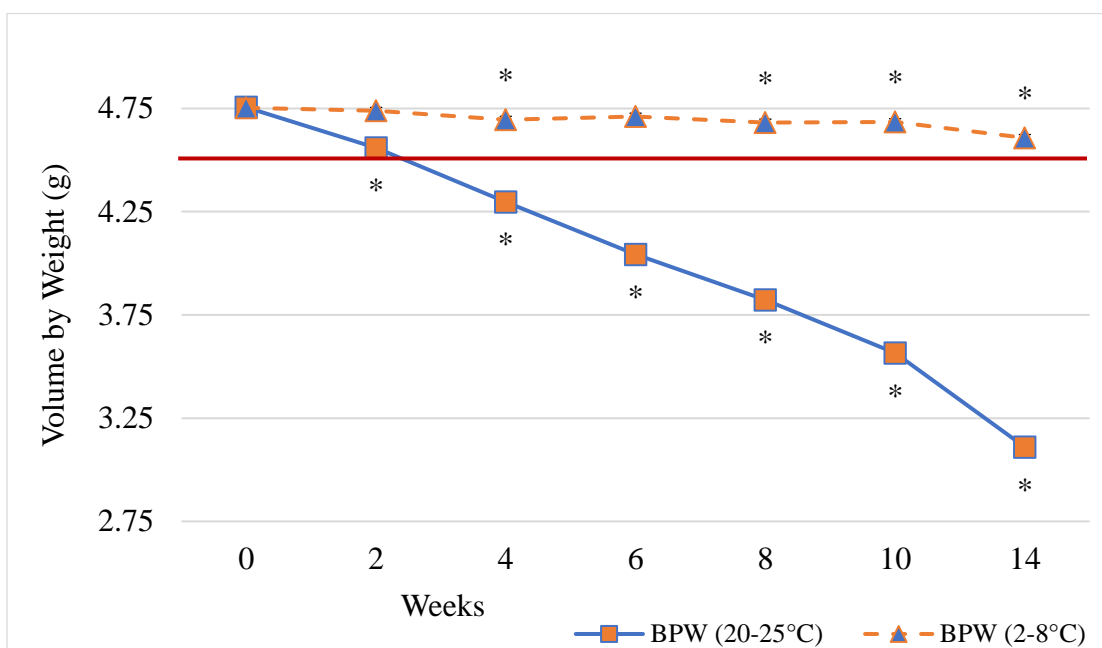


Figure 3.F18: Buffered Peptone Water Overall Mean Volume Loss (By Weight).

Figure displays buffered peptone water (BPW) volume loss (by weight g) results for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 4.50 (loss of 0.25) is considered unacceptable (solid red line). Overall mean BPW values marked with an * are significantly different then Week 0 ($p < 0.05$).

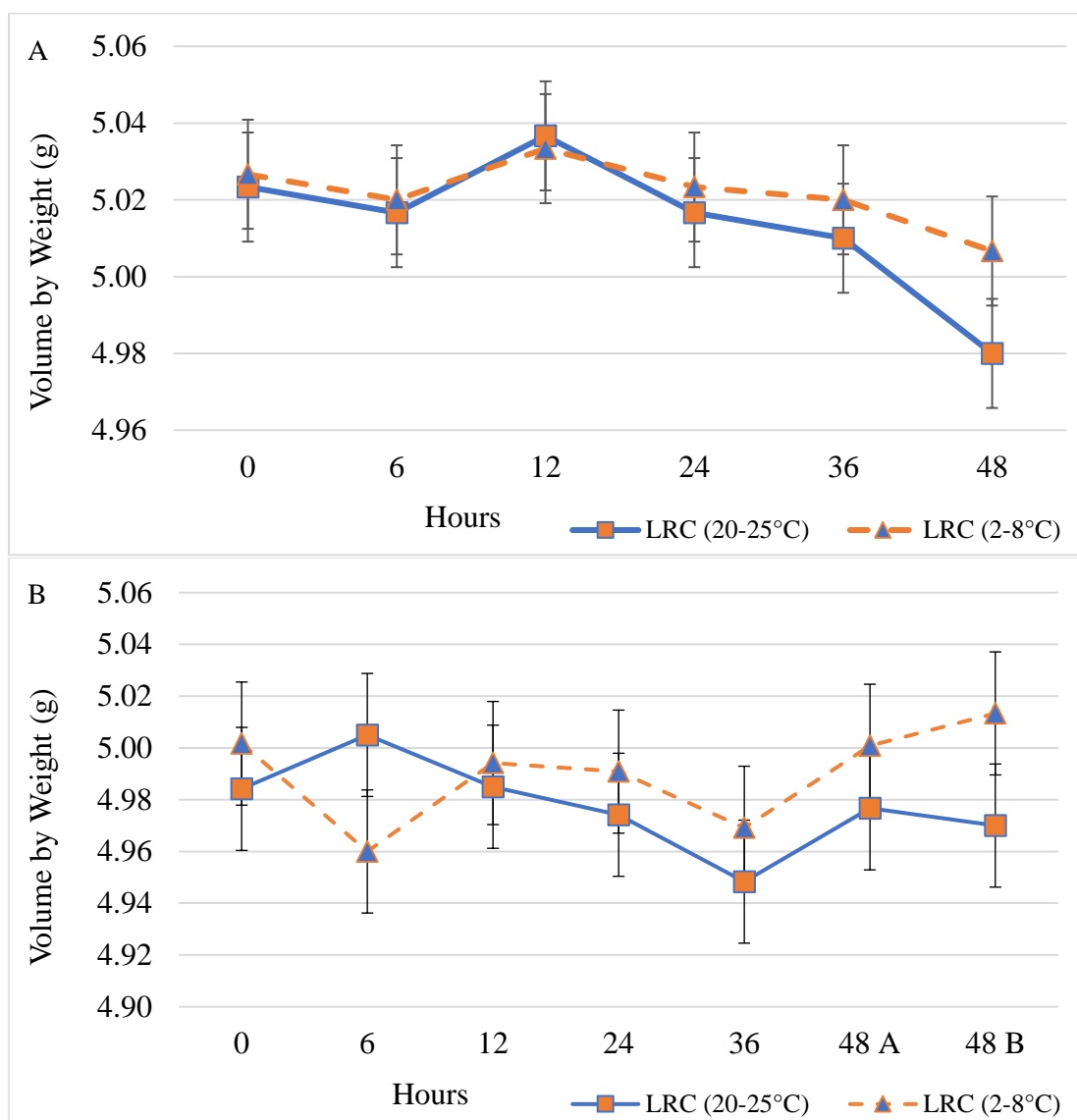


Figure 3.F19: Romer RapidChek® *Listeria* Overall Mean Volume Loss (By Weight). Figure displays Romer RapidChek® *Listeria* (LRC) volume loss (by weight g) results for media qualification verification testing at each shelf life time point. Media tubes prepared using a bottle pump (A) and serological pipette (B). Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 4.75 (loss of 0.25) is considered unacceptable. Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

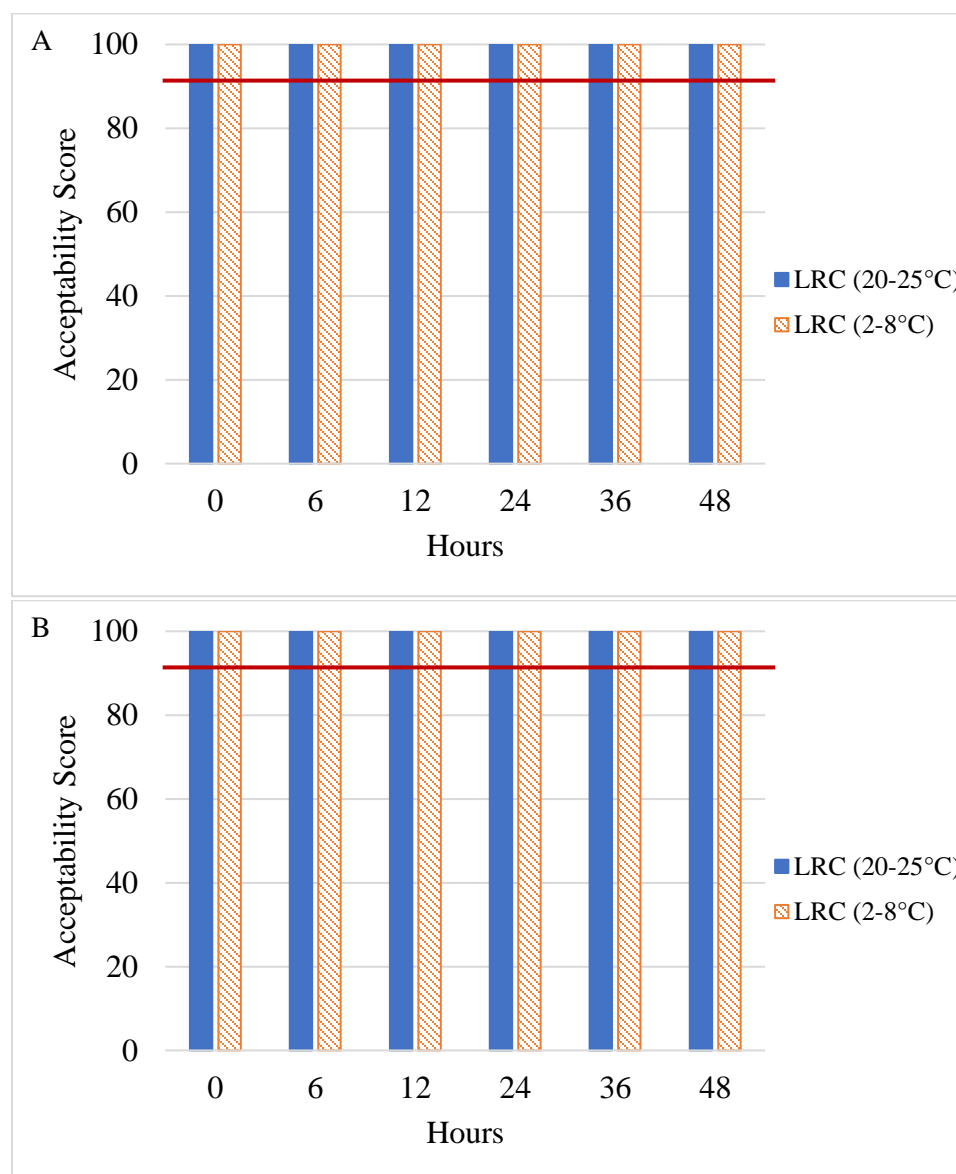


Figure 3.F20: Romer RapidChek® *Listeria* Media Overall Mean Quality

Acceptability Scores for Morphology and Negative (Media) Control. Figure displays Romer RapidChek® *Listeria* media (LRC) quality acceptability ratios for morphology (A) and negative (media) control (B) for media qualification verification testing at each shelf life time point. Acceptable morphology determined for *L. monocytogenes* cocktail. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

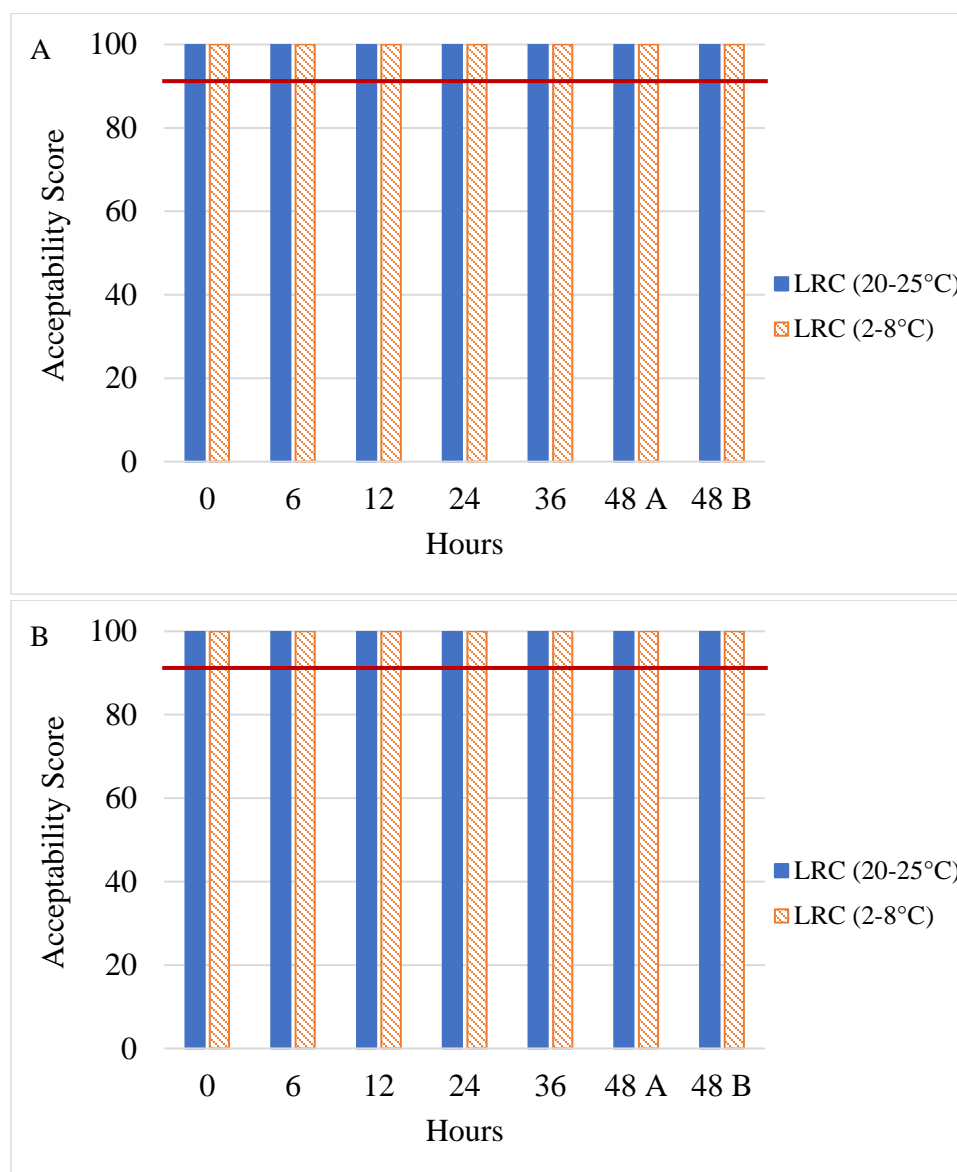


Figure 3.F21: Romer RapidChek® *Listeria* Media Overall Mean Quality Acceptability Scores for Contamination and Color Change. Figure displays Romer RapidChek® *Listeria* Media (LRC) quality acceptability ratios for contamination (A) and color change, lighter or darker (B), for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean LRC values were not significantly different than Hour 0 ($p < 0.05$).

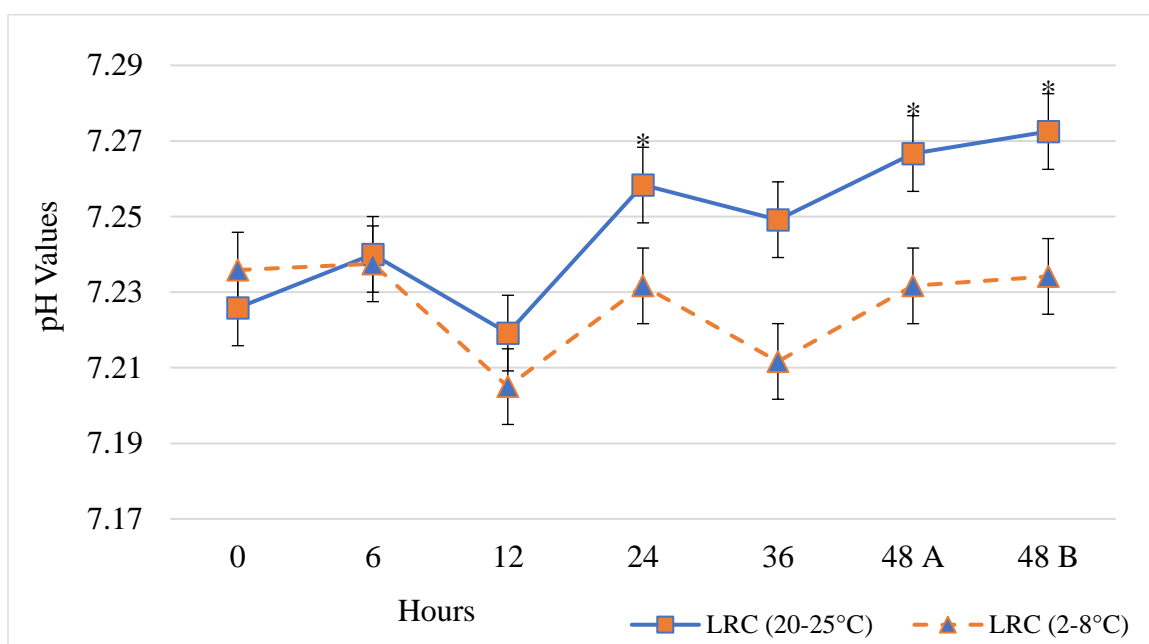


Figure 3.F22: Romer RapidChek® *Listeria* Media Overall Mean pH Values. Table displays Romer RapidChek® *Listeria* media (LRC) pH results for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). There is no recommended manufacturer pH range for Romer RapidChek® *Listeria* media. Overall mean LRC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

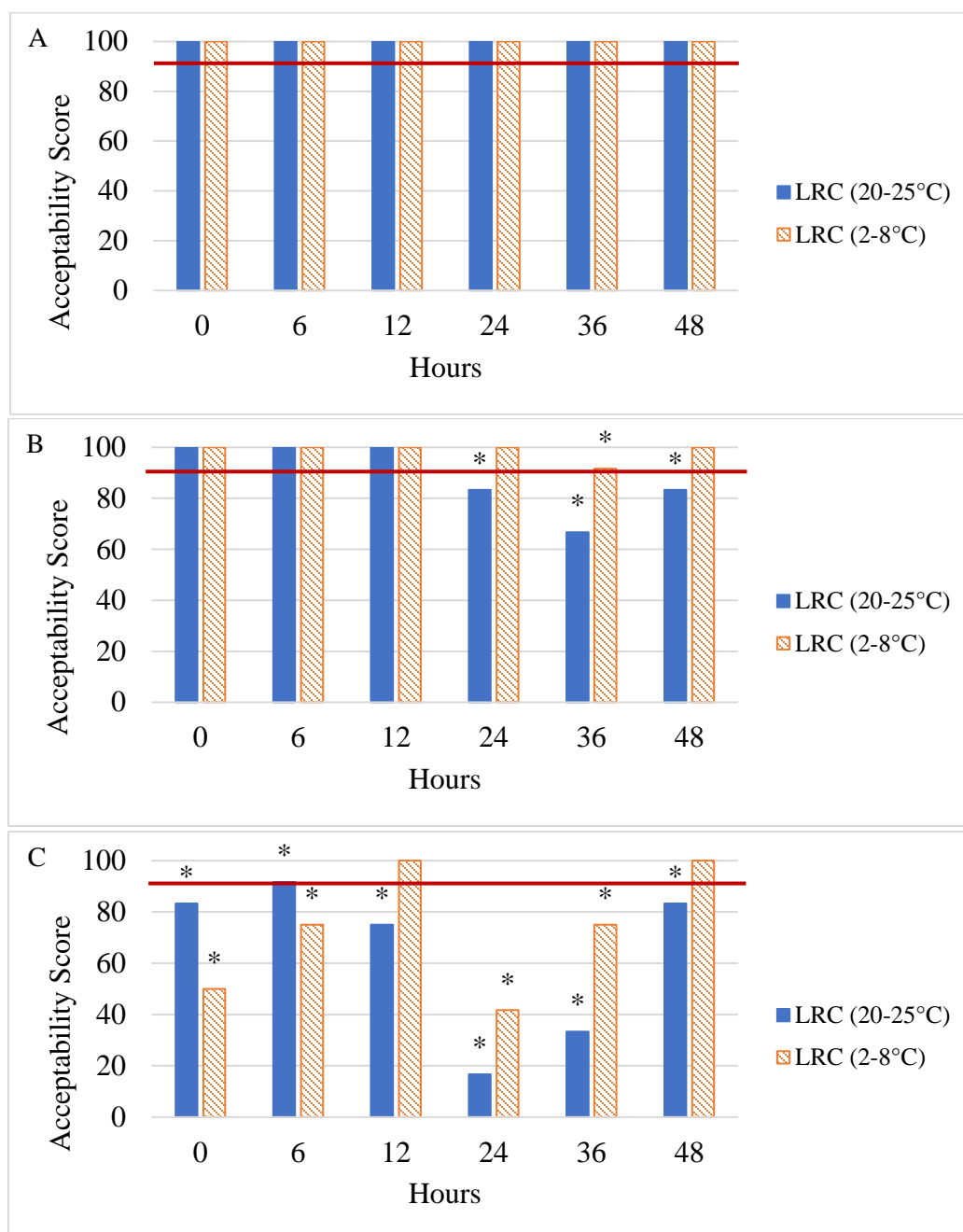


Figure 3.F23: Romer RapidChek® *Listeria* Media Overall Mean Positive and Negative Control Scores. Table displays Romer RapidChek® *Listeria* Media (LRC) positive control *Listeria* spp. cocktail (A), negative controls *E. coli* ATCC 25922 (B), and *E. coli* O157:H7 ATCC 35150 (C) growth acceptability ratios for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean LRC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

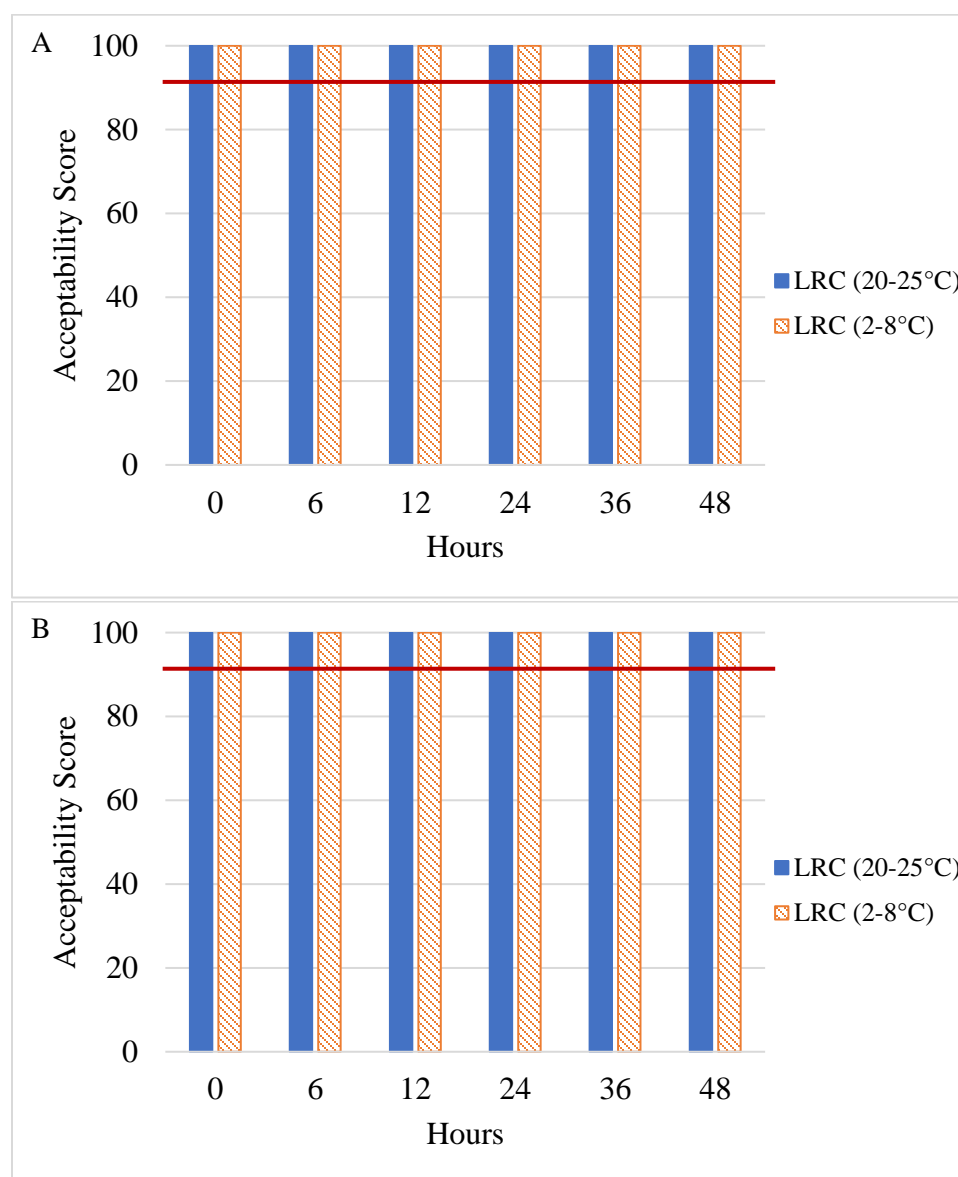


Figure 3.F24: Romer RapidChek® *Listeria* Media Overall Mean 50:50 Positive and Negative Control Scores. Figure displays Romer RapidChek® *Listeria* Media (LRC) 50:50 competition plate positive control *Listeria* spp. cocktail (A) and negative controls *E. coli* ATCC 25922 and *E. coli* O157:H7 ATCC 35150 (B) growth acceptability ratios for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean LRC values were not significantly different then Hour 0 ($p < 0.05$).

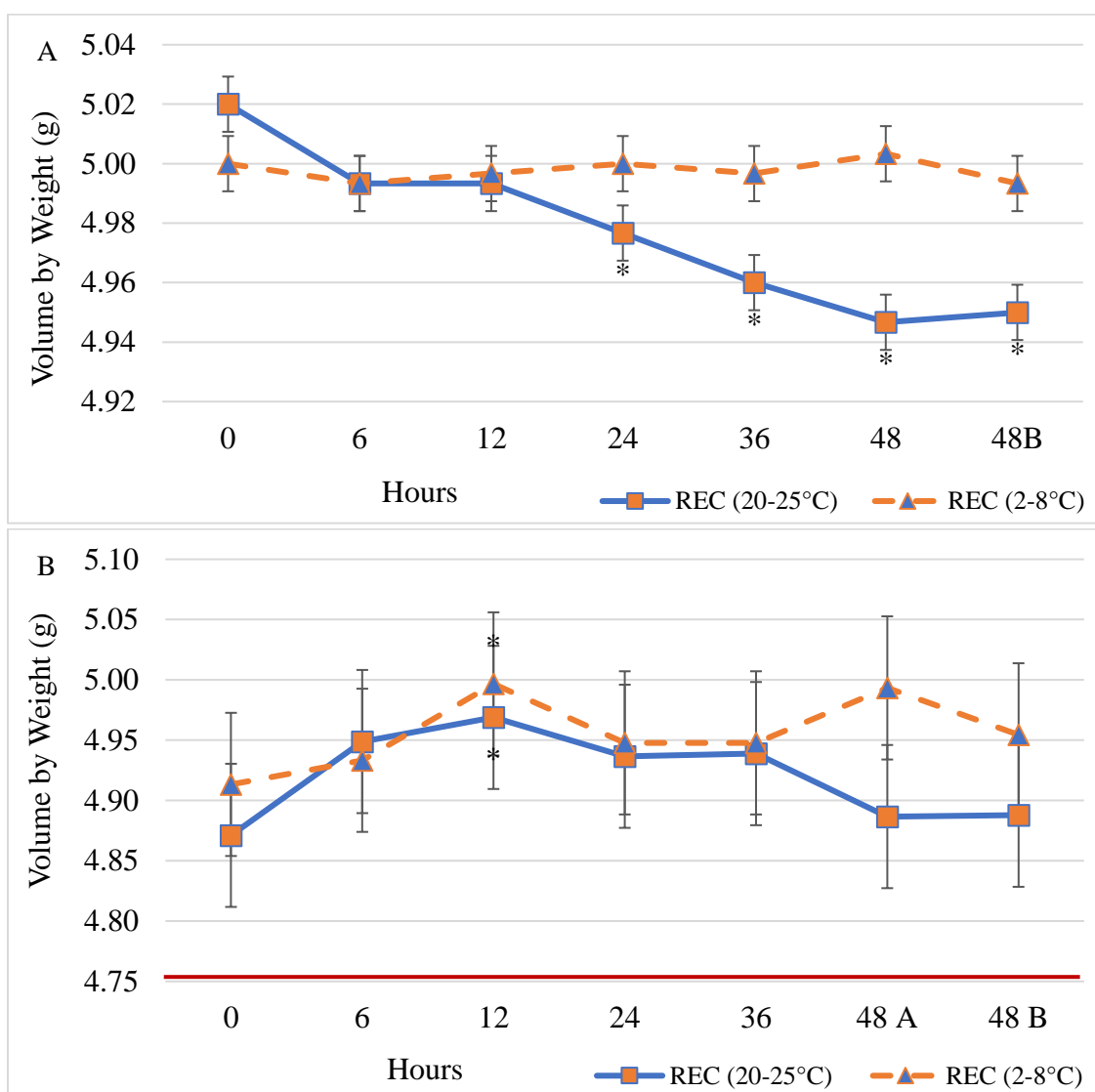


Figure 3.F25: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Overall Mean Volume Loss (By Weight). Figure displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) volume loss (by weight g) results for media qualification verification testing at each shelf life time point. Media tubes prepared using a bottle pump (A) and serological pipette (B). Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 4.75 (loss of 0.25) is considered unacceptable (solid red line). Overall mean REC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

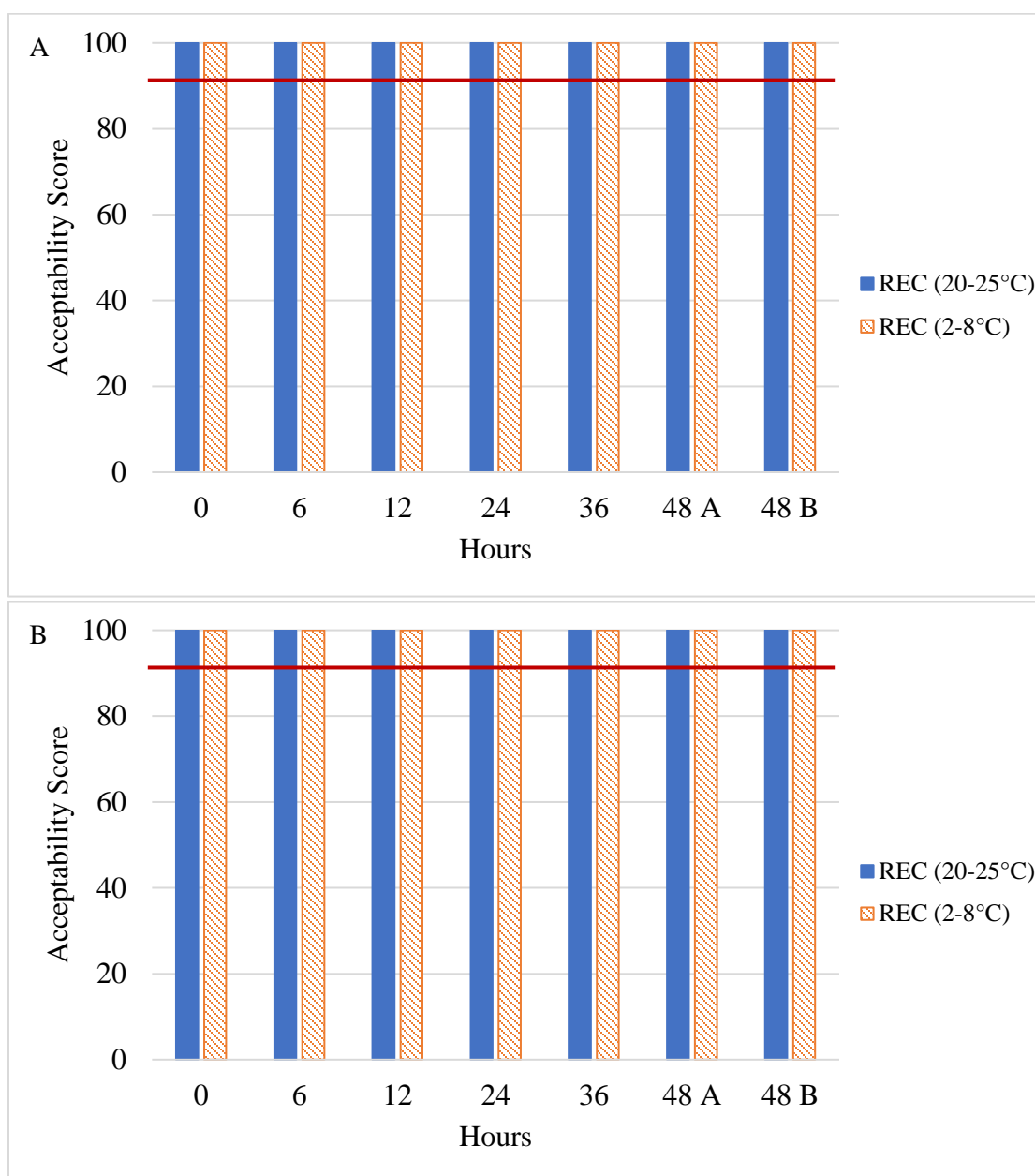


Figure 3.F26: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Media Overall Mean Quality Acceptability Scores for Morphology and Negative (Media) Control. Figure displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) quality acceptability ratios for morphology (A) and negative (media) control (B) for media qualification verification testing at each shelf life time point. Acceptable morphology determined for *Escherichia coli* cocktail. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

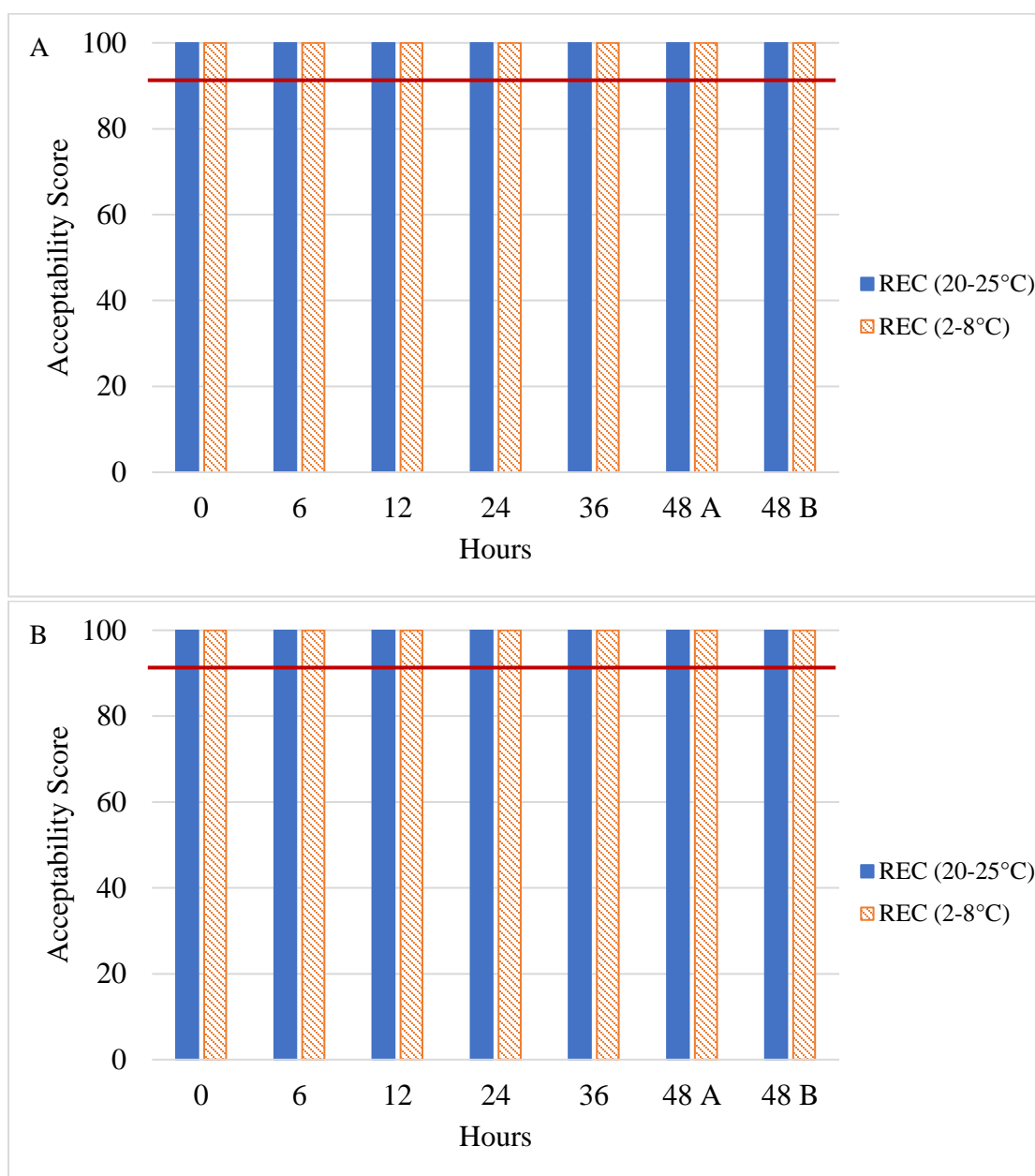


Figure 3.F27: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Media Overall Mean Quality Acceptability Scores for Contamination and Color Change. Figure displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) quality acceptability ratios for contamination (A) and color change, lighter or darker (B), for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

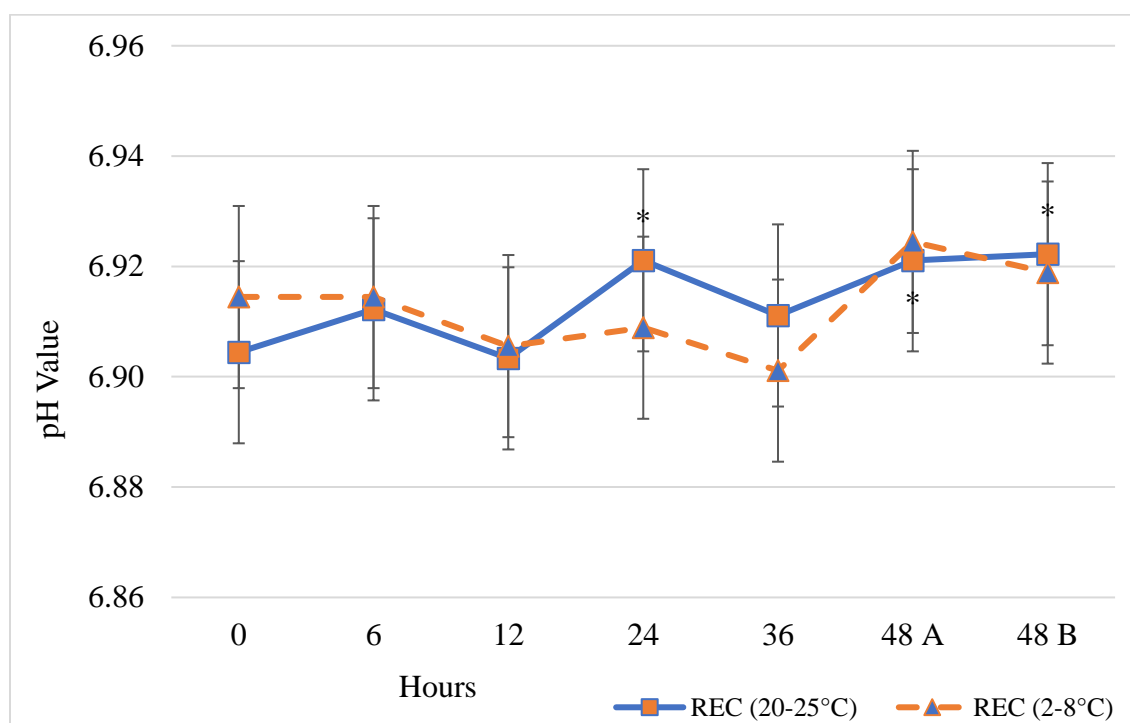


Figure 3.F28: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Media Overall Mean pH Values. Table displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) pH results for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). There is no recommended manufacturer pH range for Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) media. Overall mean REC values marked with an * are significantly different then Hour 0 ($p < 0.05$).

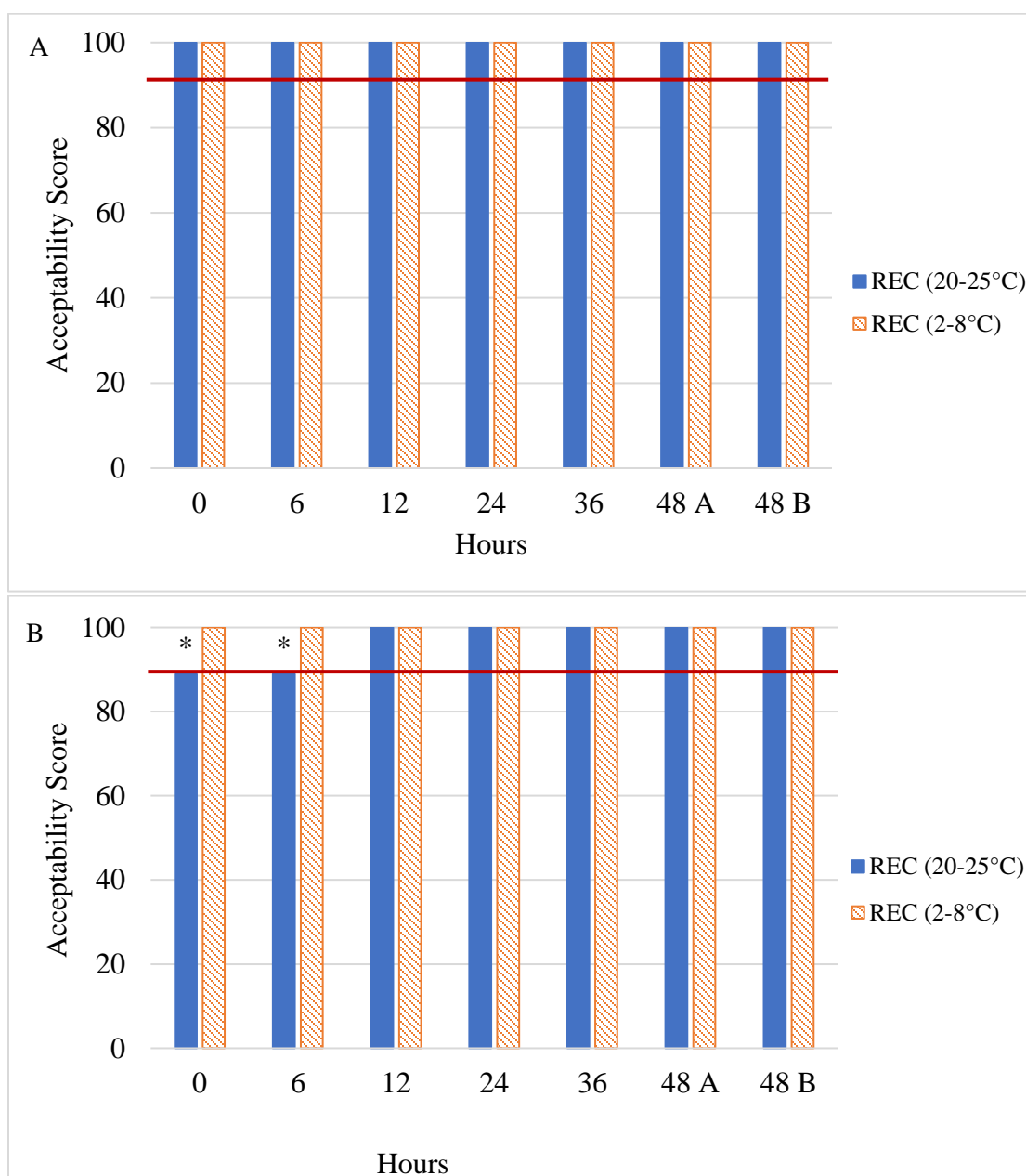


Figure 3.F29: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Media Overall Mean Positive and Negative Control Scores. Table displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) positive control, *E. coli* O157:H7 cocktail (A) and negative control *S. aureus* ATCC 6538P (B) growth acceptability ratios for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean REC values marked with an * were significantly different ($p < 0.05$).

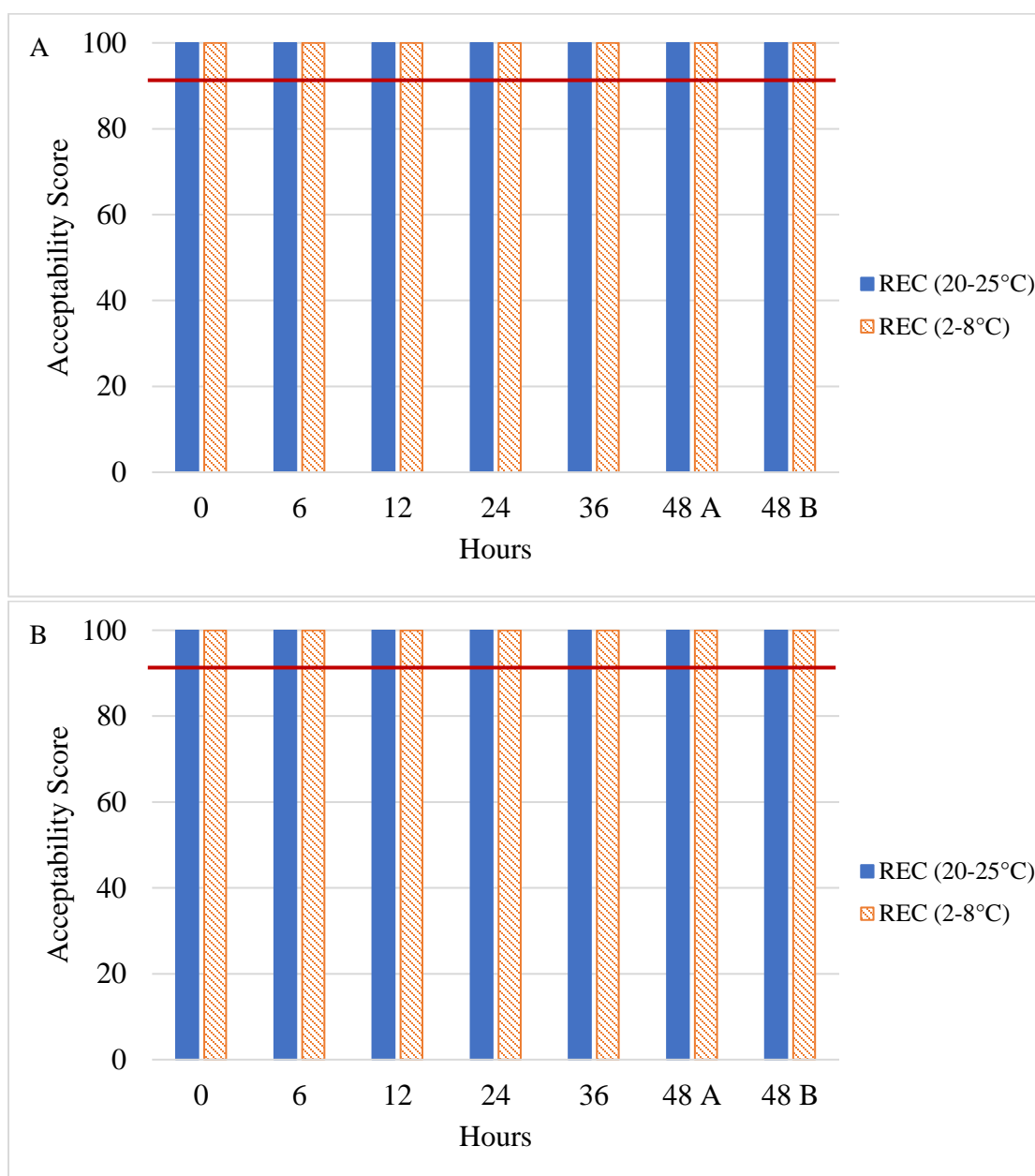


Figure 3.F30: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Media Overall Mean 50:50 Positive and Negative Control Scores. Figure displays Neogen Reveal® 20-Hour for *E. coli* O157:H7 (REC) media 50:50 competition plate positive control *E. coli* O157:H7 cocktail (A) and negative control *S. aureus* ATCC 6538P (B) growth acceptability ratios for media qualification verification testing at each shelf life time point. Storage temperatures were room temperature (20-25°C) and refrigeration temperature (2-8°C). Any score < 90 is considered unacceptable (solid red line). Overall mean REC values were not significantly different then Hour 0 ($p < 0.05$).

Bibliography

A2LA. 2001. *A2LA Food Microbiology Program Requirements*. "AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing." The American Association for Laboratory Accreditation. <https://www.a2la.org/>.

AOAC. 2015. *AOAC International: Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals*. AOAC International.

AOAC. 2017. *AOAC Performance Tested # 020401: RapidChek(R) Listeria - Romer Labs Technology Inc.* AOAC International Standards Development. AOAC Performance Tested. Rockville, Maryland.

Argyri, Anthoula A., Olga S. Papadopoulou, Aspasia Nisiotou, Chrysoula C. Tassou, and Nikos Chorianopoulos. 2018. "Effect of High Pressure Processing on the Survival of Salmonella Enteritidis and Shelf-Life of Chicken Fillets." *Food Microbiology*, 70 (Supplement C): 55-64. doi:10.1016/j.fm.2017.08.019.

ASM. 2012. *Guidelines for Assuring Quality of Medical Microbiology Culture Media*, edited by M. Arghyros, G. Douglass, M. Locher, P. Mugg, D. C. Myatt, T. Olma, A. Scholtes and I. Wilkinson. 2nd ed. Australian Society for Microbiology, Inc.

Basu, S., A. Pal, and P. K. Desai. 2005. "Quality Control of Culture Media in a Microbiology Laboratory." *Indian Journal of Medical Microbiology*, 23 (3): 159-163. doi:10.4103/0255-0857.16586. <http://www.bioline.org.br/abstract?id=mb05047>.

BD. 2009. *Difco and BBL Manual: Manual of Microbiological Culture Media*, edited by Mary Jo Zimbro, David Power, Sharon Miller, George Wilson and Julie Johnson. 2nd ed. Sparks, Maryland: Becton Dickinson and Company.

Cantarelli, Vlademir Vicente, Everton Inamine, Teresa Cristina Zenobin Brodt, Carina Secchi, Fabiana de Souza Pereira, Maria Cristina Amaro, Alice Aurora Batalha, and Simone Dornellas Ligiero. 2003. "Quality Control for Microbiological Culture Media: Is it enough to Follow the NCCLS M22-A2 Procedures?" *Brazilian Journal of Microbiology*, 34 (1): 8-10. doi:10.1590/S1517-83822003000500003.

Choi, Young and James Rogers. 2015. "Shelf-Life Assessment of Agar Plates Vacuum-Sealed in Nylon/Polyethylene Packaging Flushed with Nitrogen." *Journal of Microbiology & Experimentation*, 3. doi:10.15406/jmen.2015.03.00075.

Church, D. L., D. Emshey, H. Semeniuk, T. Lloyd, and J. D. Pitout. 2007. "Evaluation of BBL CHROMagar O157 Versus Sorbitol-MacConkey Medium for Routine Detection of

Escherichia coli O157 in a Centralized Regional Clinical Microbiology Laboratory." *Journal of Clinical Microbiology*, 45 (9): 3098-3100. doi:10.1128/JCM.00426-07.

CLSI. 2004. *Quality Control for Commercially Prepared Microbiological Culture Media - Approved Standard M22-A3 Third Edition*, 19. Vol. 24. 3rd ed. The National Committee for Clinical Laboratory Standards. Pennsylvania, USA, 0273-3099.

Curtis, G. D. W. 1985. "A Review of Methods for Quality Control of Culture Media." *International Journal of Food Microbiology*, 2 (1): 13-20. doi:10.1016/0168-1605(85)90051-0.

Erickson, Marilyn C. and Yen-Con Hung. 1997. *Quality in Frozen Food*. Chapman & Hali, Springer Science Business Media, B.V.

FDA. 2017. *Bacteriological Analytical Manual Chapter 10: Detection of Listeria Monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria Monocytogenes in Foods*. edited by Anthony Hitchins, Karen Jinneman and Yi Chen. US Food and Drug Administration. Silver Spring, MD.

Grochau, Inês, Carlos Ferreira, Jane Ferreira, and Carla ten Caten. 2010. "Implementation of a Quality Management System in University Test Laboratories: A Brief Review and New Proposals." *Accreditation and Quality Assurance*, 15 (12): 681-689. doi:10.1007/s00769-010-0713-6.

Guerini, Michael N., Terrance M. Arthur, Steven D. Shackelford, and Mohammad Koohmaraie. 2006. "Evaluation of *Escherichia Coli* O157:H7 Growth Media for use in Test-and-Hold Procedures for Ground Beef Processing." *Journal of Food Protection*, 69 (5): 1007-1011.

ISO. 2014. *ISO 11133:2014 Microbiology of Food, Animal Feed and Water — Preparation, Production, Storage and Performance Testing of Culture Media*. International Organization for Standardization. Geneva, Switzerland.

ISO. "ISO: Popular Standards." International Organization for Standardization, accessed 4/12/2018. 2018. <https://www.iso.org/>.

ISO. "ISO: The Main Benefits of ISO Standards." International Organization for Standardization, accessed 4/12/2018. 2018.

ISO and IEC. 2005. *ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories*. 2nd ed. Geneva, Switzerland: International Organization for Standardization.

ISO and IEC. 2017. *ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories*. 3rd ed. International Organization for Standardization. Geneva, Switzerland.

Jones, R. N., K. Krisher, D. Bird, and CAPMRC. 2002. *Assessment of Quality Assurance Failure Rates for Commonly used Diagnostic Media: Results of a College of American Pathologist Questionnaire in 2001*. The Jones Group and CAP Microbiology Resource Committee Poster. North Liberty, Iowa.

Krisher, Karen. 2001. "Survey Results: The use of the NCCLS Standard, M22-A2, and "Quality Assurance for Commercially Prepared Microbiological Culture Media" by Clinical Laboratories." *Clinical Microbiology Newsletter*, 23 (8): 59-63. doi:10.1016/S0196-4399(01)89016-4.

March, S. B. and S. Ratnam. 1986. "Sorbitol-MacConkey Medium for Detection of *Escherichia coli* O157:H7 Associated with Hemorrhagic Colitis." *Journal of Clinical Microbiology*, 23 (5): 869-872. <http://jcm.asm.org/content/23/5/869.abstract>.

Mossel, D. A. A., F. Van Rossem, M. Koopmans, M. Hendriks, M. Verouden, and I. Eelderink. 1980. "Quality Control of Solid Culture Media: A Comparison of the Classic and the so-Called Ecometric Techniques." *Journal of Applied Bacteriology*, 49: 439-454.

Neogen. 2017. *Neogen Acumedia Tryptic Soy Agar 7100*. Neogen Corporation. Lansing, MI.

Neogen. 2016. *Neogen Reveal for E. coli O157:H7 Instruction Pamphlet*, "Reveal 20-Hour *E. coli* O157:H7 AOAC No.2000.14." Neogen Corporation. Lansing, MI. www.neogen.com.

Parn. "Quality Control of Culture Media In A Microbiology Laboratory." Pakistan Antimicrobial Resistance Network, accessed May 3, 2018.

Perry, J. D. and A. M. Freydière. 2007. "The Application of Chromogenic Media in Clinical Microbiology." *Journal of Applied Microbiology*, 103 (6): 2046-2055. doi:10.1111/j.1365-2672.2007.03442.x.

Possé, Björn, Lieven De Zutter, Marc Heyndrickx, and Lieve Herman. 2008. "Novel Differential and Confirmation Plating Media for Shiga Toxin-Producing *Escherichia coli* Serotypes O26, O103, O111, O145 and Sorbitol-Positive and -Negative O157." *FEMS Microbiology Letters*, 282 (1): 124-131. doi:10.1111/j.1574-6968.2008.01121.x.

Romer. 2016a. *Romer Labs Listeria Quick Guide - RapidChek Listeria System*. Romer Labs Technology Inc. Newark, DE.

Romer. 2016b. *Romer Labs RapidChek Listeria Species Test System Package Insert*. Romer Labs Technology Inc. Newark, DE.

Romero, J., I. Manero, and J. Laso. 2007. "Validation of Geosmin and 2-Methyl-i-Borneol Analysis by CLSA-GC-FID Method to Obtain ISO-17025 Accreditation." *Journal of Chromatographic Science*, 45 (7): 439-446. doi:10.1093/chromsci/45.7.439.

Sandle, Tim. 2016. *Pharmaceutical Microbiology: Essentials for Quality Assurance and Quality Control. Chapter 5 Microbiological Culture Media*. Biomedicine. 1st ed. Cambridge, UK. Woodhead Publishing Limited and Imprint of Elsevier.

Sutton, Scott. "Quality Control of Microbiological Culture Media." PMF Newsletter, accessed May 3, 2018. www.microbiol.org/resources/monographwhite-papers/quality-control-of-microbiological-culture-media/.

Ulisse, S., A. Peccio, G. Orsini, and B. Di Emidio. 2006. "A Study of the Shelf-Life of Critical Culture Media." *Veterinaria Italiana*, 42 (3): 237-247. <http://www.ncbi.nlm.nih.gov/pubmed/20429062>.

Weenk, G. H. 1992. "Microbiological Assessment of Culture Media: Comparison and Statistical Evaluation of Methods." *International Journal of Food Microbiology*, 17 (2): 159-181. doi:10.1016/0168-1605(92)90113-H.

CHAPTER 4

VERIFICATION OF TEST METHODS FOR DETERMINING THE PRESENCE OF PATHOGENIC MICROORGANISMS IN FOOD PRODUCTS IN MEETING ISO/IEC 17025 REQUIREMENTS

“Method Verification Procedure for Small and Academic Labs”

INTRODUCTION

Overview of Method Verification and Validation

International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC) standards exist to help guide laboratories in performing higher quality testing producing results that can be trusted worldwide (Romero et al. 2007; ISO 2018b). ISO has developed a standard that directly affects the food testing industry known as ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017; ISO 2018b).

Preparing a laboratory to obtain ISO/IEC 17025 accreditation is an extremely challenging task that takes months or even years to accomplish and is especially challenging for academic laboratories that are focused on research and teaching activities (Grochau et al. 2010). This preparation process involves the implementation of a quality management system, development of various support programs, and the creation of standard operating procedures and paperwork for all processes that take place within the laboratory (ISO and IEC 2005; AOAC 2015).

However, one of the more burdensome areas of preparing for accreditation that might be underestimated is the verification of test methods that are part of the laboratory's scope of accreditation. Proper method verification is essential to the success of any laboratory attempting ISO accreditation as it provides evidence the laboratory is capable of performing testing methods, achieving expected results (AOAC 2015), and showing its qualifications and competency (Taverniers et al. 2004). When performing method verification, it is important to remember that you are not validating the method,

but instead are simply verifying that your laboratory is capable of performing the procedure as it was intended.

Validation is defined as “*confirmation by examination...objective evidence that the particular requirements...are fulfilled*” (ISO and IEC 2005; Araujo 2009; AAFCO 2014). It can be further defined as “*the process of establishing the performance characteristics and limitations of a method, and of identifying the influences that may change these characteristics*” (Rambla-Alegre et al. 2012). When performing method validation, you are establishing the performance characteristics of the method, comparing that method to other reference methods to show its equivalence, and then performing statistical evaluations such as selectivity, specificity, precision, and detection limits on the data to show how well it performs against those reference methods (A2LA 2001; Feinberg and Laurentie 2006; AAFCO 2014; Kurbanoglu et al. 2018).

Method verification is when the laboratory demonstrates the ability of its technicians in performing a previously validated method that is fit for the intended purpose by adequately meeting all of the analytical requirements of the method (AAFCO 2014; AOAC 2015). Method verification has been defined as “*confirmation by examination and provisions of evidence that specified requirements have been met*” (A2LA 2001) and “*process by which a laboratory confirms by examination and provides objective evidence that the particular requirements for specific uses are fulfilled*” (FDA 2014; FDA 2015).

Similar to method validation, microbiological method verification would involve inoculating samples with known organisms and evaluating those samples using the approved validated method. However, while verifying a method, the laboratory must

show that the inoculated organism can be recovered or suppressed by the method only evaluating the sensitivity (A2LA 2001) and not determining what the actual limits of the test are.

In more simple terms, for a microbiological testing method, the laboratory is proving they can perform the method by showing the method detects the organisms it claims to detect. In order to verify any method, the laboratory must verify all parts of the method, including support programs and processes that could influence the results (NATA 2013), are being followed exactly as described and without deviation. The laboratory must design their verification study to make sure all outside factors that could affect the results have been accounted for to guarantee the results generated during analysis are valid and can be trusted (Araujo 2009; Rambla-Alegre et al. 2012).

Method Verification Guidelines

Laboratories attempting to obtain ISO/IEC 17025 accreditation are required to perform verification on all methods they intend to become accredited against (AOAC 2015). Each establishment has a scope of accreditation unique to its testing facility that includes the methods the laboratory performs. According to the ISO/IEC 17025 standard a valid/verified method “*must meet specifications*” that revolve around the intended use of the method and any of the methods that fall within the scope of accreditation “*shall have been validated*” or verified prior to attempting to obtain certification (A2LA 2001; ISO and IEC 2005; AOAC 2015). Additionally, any methods that have been developed by the laboratory for a specific client may be approved for use as an in-scope method if

the method is considered “*appropriate for the intended use...and validated*” or verified (ISO and IEC 2005; AOAC 2015).

Although there are several guidelines for the validation of laboratory methods, such as AOAC International Methods Committee guidelines for validation of microbiological methods for food and environmental surfaces Appendix J and guidelines for the validation and verification of quantitative and qualitative test methods from the National Association of Testing Authorities (NATA) (AOAC 2012; AOAC 2013; NATA 2013; FDA 2014; FDA 2015), there are currently very few available guidelines on how to conduct method verification (NATA 2013; FDA 2014; FDA 2015).

Because of this, ISO has released, or is in the process of releasing, two standards to help guide laboratories in their preparation for accreditation as it pertains to method validation and verification. ISO 16140-2:2016 standard focuses on method validation; while ISO/DIS 16140-3, which is currently under development, will focus on verification of reference methods implemented in single laboratories (ISO 2018a). Utilizing these ISO guidelines will help to standardize the verification process for all laboratories attempting to obtain ISO/IEC 17025 accreditation by providing guidance as to the testing parameters that must be met for proper method verification.

OBJECTIVES OF PROJECT

The Food Processing Center Laboratory Services (FPCLS) located at the University of Nebraska-Lincoln (UNL) Food Processing Center (FPC) set a goal to meet all of the requirements set forth by ISO for obtaining accreditation to ISO/IEC 17025 standards. This included the verification of all test methods part of the FCPLSs scope of

accreditation for obtaining ISO accreditation status. The experiences that the FPCLS had and the processes that they followed may be used as a guide for other small and academic laboratories who wish to improve their processes or prepare for obtaining ISO accreditation.

Test method verification procedures from this project will provide the FPCLS, and other laboratories, with a guide for the verification of standard methods and will demonstrate that test methods utilized by the laboratory that are part of the scope of accreditation are effective and fit for the needs of the FPCLS. Additionally, these method verification procedures will demonstrate the competency of the FPCLSs staff in being able to perform those methods to an acceptable level consequently meeting the requirements for ISO accreditation.

The specific goal of this project is to develop and implement the verification procedures for all test methods utilized by the FPCLS that will be part of the scope of ISO/IEC 17025 accreditation. The FPCLS will focus on three pathogen screening methods for the method verification process: Romer RapidChek[®] *Listeria* for the detection of *Listeria* spp., Neogen Reveal[®] 20-Hour for the detection of *E. coli* O157:H7, and BioMérieux VIDAS[®] UP *Salmonella* SPT for the detection of *Salmonella* spp.

Establishing method verification procedures and verifying all FPCLSs in-scope methods will further position the FPCLS to become ISO/IEC 17025 compliant and accomplish one of the many requirements necessary to obtain ISO/IEC 17025 accreditation. To achieve the specific goal aforementioned, two primary objectives were carried out in this project.

Objective 1. Develop and carry out a procedure for the verification of test methods part of the FPCLS testing laboratory scope of accreditation that would allow for the

FPCLS to demonstrate competency in each method. This method was designed to accommodate the capabilities of the FCPLS while still meeting the requirements set forth in the ISO/IEC 17025 standard (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017). A study was designed to test the sensitivity of each test method against various food matrices utilizing recognized quality assurance microorganisms in accordance with manufacturers and internationally approved/recognized procedures for each method. Three test procedures were selected for method verification testing (Romer RapidChek® *Listeria* for the detection of *Listeria* spp., Neogen Reveal® 20-Hour for the detection of *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp.) to ensure the FPCLS is capable of performing approved recognized methods as they were designed and capable of obtaining accurate results that can be trusted, reproducible, and are reliable within the laboratory.

Objective 2. Challenge the ability of each test method (kit) being evaluated as part of the method verification process to detect the microorganism selected for analysis at low levels approximately 1.0×10^3 CFU/ml (expected kit(s) threshold is 10^6 CFU/ml). A study was conducted on all three methods selected for method verification for low inoculum level threshold evaluation. Testing showed the ability of the FPCLS to properly inoculate samples while also providing evidence that the limit of detection must be achieved for the methods (kits) to work correctly with the enrichment step of each method being absolutely critical to each methods ability to provide a valid result. Testing provided further evidence of the FPCLSs competency in performing method verification and following approved recognized methods to meet the method verification requirements for ISO/IEC 17025 accreditation.

Note: Method verification procedures are vital in providing evidence that the FPCLS is capable of performing approved methods and is competent for meeting ISO/IEC requirements for method verification. Method verification is unique to each laboratory as each laboratory must select methods that meet its needs and cover the testing that they wish to include in their scope of accreditation. However, method verification procedures conducted for this project may be used as a guide to other laboratories and academic institutions for their own method verification processes.

MATERIALS AND METHODS

As a part of certifying the FPCLS testing facility against ISO/IEC 17025 requirements many programs must be implemented, documentation created, and training of personnel performed. But one of the most important parts of the accreditation process is the verification of methods that are part of the laboratory's scope of accreditation. These in-scope procedures include rapid methods for the detection of *Escherichia coli* O157:H7, *Listeria* spp., and *Salmonella* spp. Performing these verification studies can be very time consuming and expensive, therefore ensuring that they have been designed correctly is an absolute necessity. The following sections detail the procedures and the experiences that the FPCLS had in developing and conducting verification procedures for the methods (kits) it determined would be part of the scope of accreditation for ISO compliance.

Approved Methods

Approved, internationally recognized, validated methods should be used as the primary test methods by laboratories attempting ISO accreditation. It is stated in the international standard ISO/IEC 17025 that, "*Methods published in international, regional, or national standards shall preferably be used*" (ISO and IEC 2005). All FPCLS methods part of the scope of accreditation for ISO compliance utilized by the laboratory are approved AOAC methods. These methods include; AOAC-RI # 020401 (Romer RapidChek® *Listeria* for the detection of *Listeria* spp.), AOAC # 2000.14 (Neogen Reveal® 20-Hour for the detection of *E. coli* O157:H7), and AOAC # 2013.01 (BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp.) (AOAC

2002; AOAC 2009; AOAC 2016a; AOAC 2017; USDA 2017). Verifying only approved validated methods will further enhance how the FPCLS is viewed by the accreditation body and by FPCLS clients providing those clients insurance that the laboratory is capable of analyzing samples according to international standards (AOAC 2015) and able to produce quality reliable results while meeting the requirements for ISO accreditation.

Rapid Lateral Flow Method Devices – How They Work

Romer RapidChek[®] *Listeria* for the detection of *Listeria* spp. test system was selected by the FPCLS as the primary method for evaluating *Listeria* spp. in food and environmental samples as part of the scope of accreditation for ISO compliance. This lateral flow immunoassay utilizes a double antibody sandwich setup providing presumptive evidence of the presence of *Listeria* spp. in various food products and from environmental surfaces.

The lateral flow device, which can be seen in Figure 4.1, consists of a test line containing *Listeria* specific antibodies and a reagent pad seeded prior to the test line containing a second *Listeria* antibody labeled with colloidal gold. As fluid moves up the sample device via capillary action through the reagent pad the colloidal gold antibody binds *Listeria* and together they continue to move towards the test line. As the liquid containing the bound *Listeria* passes over the test line immobilized *Listeria* antibody binds the *Listeria*-antibody complex creating a sandwich between the antibody and the bound *Listeria*. This leads to a reaction that is visualized as a red line on the test device.

It's important to note that these complexes are not formed in the absence of *Listeria* and therefore no red line would be seen. Further up the test device is a control

line that captures excess gold reagent resulting in a red line indicating that the device was working correctly and that the fluid from the sample was flowing through the device as expected (Romer 2016a; Romer 2016b).

Results are interpreted as:

1. Positive Result (*Listeria* Present) = 2 lines present on device with red lines appearing in the “Control” and “Test” zones
2. Negative Result (*Listeria* Absent) = Red line appears in the “Control” zone but not in the “Test” zone
3. If no red line appears in the “Control” zone, regardless if there is a red line in the “Test” zone then the test results are “Invalid” and the test must be repeated

(Romer 2016a; Romer 2016b)

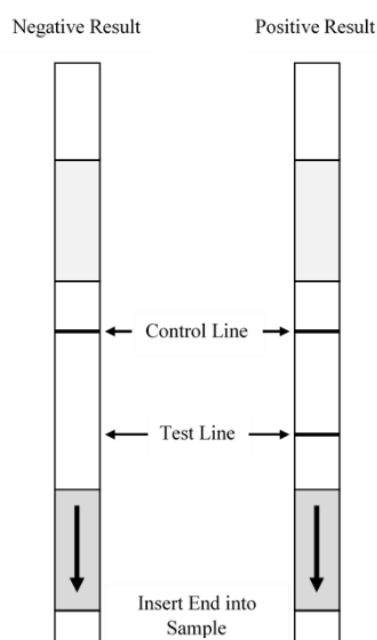


Figure 4.1: Romer RapidChek® *Listeria* Test Device. Visual depiction of Romer Labs RapidChek® *Listeria* test system devices displaying a presumptive positive and negative result. Adapted from Romer RapidChek® *Listeria* Species Test System Pamphlet (Romer 2016a; Romer 2016b).

Neogen Reveal[®] 20-Hour for the detection of *E. coli* O157:H7 test system was selected by the FPCLS as the primary method for evaluating *E. coli* O157:H7 in food samples as part of the scope of accreditation for ISO compliance. This lateral flow immunoassay utilizes an antibody sandwich model providing presumptive evidence of the presence of *E. coli* O157:H7 in various food products.

The lateral flow device, which can be seen in Figure 4.2, has a sample port where the enrichment broth is injected followed by a reagent zone containing *E. coli* O157:H7 specific antibodies attached to colloidal gold particles and gold conjugate attached to a proprietary antigen. As fluid moves through the reagent zone antigens from *E. coli* O157:H7 bind to the antibodies forming an antibody complex. The fluid then continues up the device via capillary action towards the test line (zone). As the liquid containing the *E. coli* O157:H7-antibody complex passes over the test line immobilized *E. coli* O157:H7 antibody binds the *E. coli* O157:H7-antibody complex giving a reaction that is visualized as a red line on the test device.

It's important to note that these complexes are not formed in the absence of *E. coli* O157:H7 and therefore no red line would be seen. Further down the test device is a control line (zone) that captures the proprietary gold-antigen also being visualized as a red line indicating that the device was working correctly and that the fluid from the sample was flowing through the device as expected (Neogen 2016).

Results are interpreted as:

1. Positive Result (*E. coli* O157:H7 Present) = 2 lines present on device with red lines appearing in the “Control” and “Test” zones

2. Negative Result (*E. coli* O157:H7 Absent) = Red line appears in the “Control” zone but not in the “Test” zone
3. If no red line appears in the “Control” zone, regardless if there is a red line in the “Test” zone then the test results are “Invalid” and the test must be repeated

(Neogen 2016)

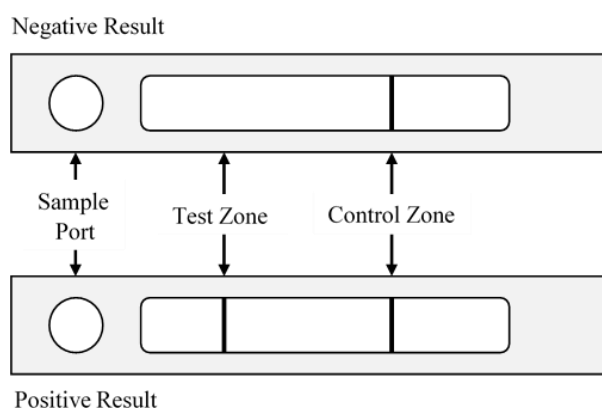


Figure 4.2: Neogen Reveal® 20-Hour for *E. coli* O157:H7 Test Device. Visual depiction of Neogen Reveal® 20-Hour for *E. coli* O157:H7 test system devices displaying a presumptive positive and negative result. Adapted from Neogen Reveal® for *E. coli* O157:H7 Test System Pamphlet (Neogen 2016).

BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp.

BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp.

test system was selected by the FPCLS as the primary method for evaluating *Salmonella* spp. in food and environmental samples as part of the scope of accreditation for ISO compliance. This automated enzyme linked fluorescent assay (ELFA) utilizes generic enrichment media (buffered peptone water) in combination with a proprietary *Salmonella* supplement to support the growth of *Salmonella* spp. while inhibiting the growth of other organisms. A test strip, seen in Figure 4.3, is utilized to hold the initial sample and

contains all transfer wells for the assay with the sample being transferred between wells using a Solid Phase Receptacle (SPR) within a VIDAS® or miniVIDAS® instrument (miniVIDAS, BioMérieux Industry, Hazelwood, MO, USA).

Salmonella spp. target receptors bind to capture proteins inside the SPR as the sample fluid is drawn up and transferred to each well in the test strip. Proteins bound to alkaline phosphatase inside wells further down the strip bind the *Salmonella* spp. target receptors which are attached to the proteins on the SPR as the fluid is pipetted from well to well. Within the final well of the test strip, the SPR is washed with a substrate (4-methyl-umbelliferyl phosphate), which is then catalyzed by the enzyme conjugate causing the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone) which can then be measured at 450 nm (BioMérieux 2013; BioMérieux 2017b; BioMérieux 2017a). Results are interpreted as:

1. Positive Result (*Salmonella* spp. Present) = test value threshold (TV) ≥ 0.25
2. Negative Result (*Salmonella* spp. Absent) = test value threshold (TV) < 0.25
3. Test Value = (Sample RFV* / Standard RFV*)

*RFV = Relative Fluorescence Value

Example: Sample RFV must be 993 or greater if standard RFV is 3971 in order to be a positive result. $TV = 993 / 3971 = 0.250063 = \text{Positive}$. Whereas $992 / 3971 = 0.249811$ and therefore would be a Negative result (BioMérieux 2013).

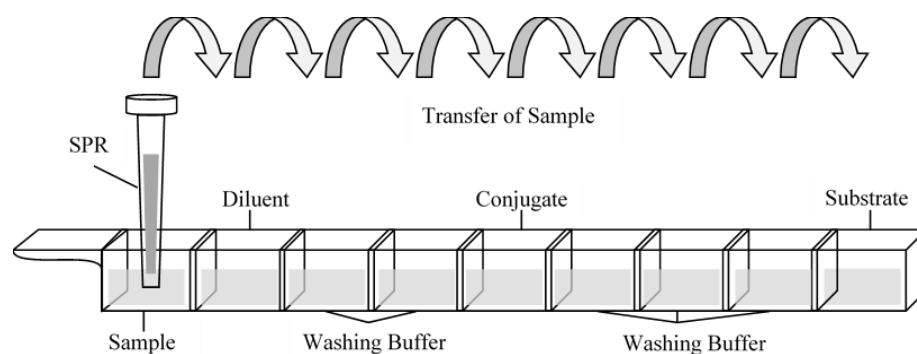


Figure 4.3: BioMérieux VIDAS® UP *Salmonella* SPT Test Device. Visual depiction of BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp. test system strip. Figure shows the Solid Phase Receptacle (SPR) with capture proteins attached inside. Sample is placed in first well and then transferred to each subsequent well to attach and wash targets for *Salmonella* ending up with a fluorescent substrate that can be measured by the miniVIDAS® at 450 nm giving a pass/fail result based on the test value threshold. Adapted from (BioMérieux 2017a; BioMérieux 2017b).

Determining Sample Size

One important aspect of setting up any verification study is determining the number of samples necessary to not only perform the study but also that will provide the desired information. Unfortunately, there are no set regulations as to the number of samples required for verifying qualitative test kit methods. Many of the resources that are available simply state that “*it depends*” on the test being verified and that the “*laboratory is responsible*” for determining the appropriate number of samples to produce statistically valid results and to meet the goals of the verification process (Thompson et al. 2002; AOAC 2006; NATA 2013). The major theme throughout all of the available resources is that qualitative validations require less samples than quantitative validations and the sample size for qualitative methods may be simply determined by the resources available to the laboratory and/or by the objectives the study is trying to achieve (Statistical Solutions 2017).

However, some of the documents available attempt to provide at least some input into the number of samples necessary, but there is no consensus. One of the recommendations suggested that at least three replicates of each sample type for each condition should be tested depending on the desired confidence limit. For these three replicates example was based on a 95% confidence interval (LOD50) (AOAC 2006). Another recommendation was to utilize a predetermined table provided by AOAC that predicts the sample number needed based on the desired probability of detection for a 95% confidence interval (AOAC 2016b) but, this would mainly apply for quantitative studies. Based on this table at least 3 samples per matrix would be needed to obtain a 50% correlation coefficient which would be equivalent to the LOD50 testing recommended for single laboratory verifications/validations (AOAC 2006; AOAC 2016b).

Another AOAC document also recommended, when using multiple matrices, that at least 3 samples be performed for each matrix (AOAC 2012). Based on these recommendations it would be in the best interest of the FPCLS to test a minimum of three samples from each matrix. Therefore, in order to meet these recommendations, the FPCLS method verification studies for each test kit would need to include, at minimum, three matrices, three replications, and five microorganisms for a total of at least 45 samples. Additionally, negative controls would need to be included for each test method in order to achieve the recommended 50% correlation coefficient.

Most recommended sample amounts are designated for validation studies. In order to provide establishments with a better resource, ISO is in the process of generating a new standard, ISO/DIS 16140-3, with guidelines for minimum number of samples

(recommended 10 per matrix) to be used in verification of methods for qualitative procedures (sensitivity testing) (IAFP 2018). Despite these recommendations, it is currently still up to the laboratory to determine how many samples to ultimately test.

Based on all of the recommendations, the FPCLS chose to test 12 samples from each matrix of interest (four samples each from three different suppliers) along with three negative controls per matrix (one from each supplier) and three non-inoculated sample controls (one from each supplier) for a total of 36 samples for each test method verification. In addition to these samples one sample from each matrix/supplier (six samples total) were tested for background flora and eight samples (one from each matrix/supplier plus two extra for negative control organisms) were tested for low level device (media enrichment threshold) testing. If the FPCLS wanted to increase the precision of the verification test methods, the number of samples could be increased but this would not necessarily increase the accuracy (Trullols et al. 2004; NATA 2013; Penn State University 2017).

Calculating Sensitivity/Specificity for Qualitative Methods

Sensitivity can be described as the probability that any sample that has been inoculated (presumed positive) will give a positive result upon testing of the sample giving the true rate at which a positive result will occur (Trullols et al. 2004; NATA 2013; Penn State University 2017). Sensitivity can be calculated according to Table 4.1 where the true number of positive results is divided by the true number of positive results plus the number of false negative results (number of presumed positive results that

provide a negative response) multiplied by 100 – sensitivity = $[A/(A+C) \times 100]$ (NATA 2013; Penn State University 2017). Refer to Table 4.1 for explanation of terms.

Specificity is the opposite of sensitivity. Specificity can be described as the probability that any sample that is not inoculated (presumed negative) will give a negative result upon testing of the sample giving the true rate at which a negative result will occur (Trullols et al. 2004; NATA 2013; Penn State University 2017). Specificity can be calculated according to Table 4.1 where the true number of negative results is divided by the true number of negative results plus the number of false positive results (number of presumed negative results that provide a positive response) multiplied by 100 – specificity = $[D/(D+B) \times 100]$ (NATA 2013; Penn State University 2017). Refer to Table 4.1 for explanation of terms.

Since neither sensitivity or specificity is affected by the population within a study (Penn State University 2017) these formulas do not change for binary methods such as qualitative methods with positive/negative results. However, only sensitivity will be required by ISO going forward (IAFP 2018; ISO 2018a) for qualitative method verification as the new standard will require that percent sensitivity be 100% and therefore the other statistical factors such as specificity, positive predictive values, negative predictive values, and positive/negative ratios are no longer relevant.

Table 4.1: Qualitative Data Statistical Calculations Table. Calculations for sensitivity, specificity, positive predictive and negative predictive values for qualitative studies. Adapted from PSU.edu STAT 507 section 10.3 Sensitivity, Specificity, Positive Predictive Values and Negative Predictive Values and NATA 2013 pg. 12. (NATA 2013; Penn State University 2017).

Testing Results	Obtained Test Values			
		Inoculated Samples (#)	Non-Inoculated Samples (#)	Total (#)
	Positive Result (#)	A (True Positive)	B (False Positive)	T _{Test Positive}
	Negative Result (#)	C (False Negative)	D (True Negative)	T _{Test Negative}
		Total Number of Samples =		T _{Total}

Bacterial Cultures and Inoculum Preparation

Designated cultures were used for verifying the performance of each method: Romer RapidChek® *Listeria* for the detection of *Listeria* spp., Neogen Reveal® 20-Hour for the detection of *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT for the detection of *Salmonella* spp. Two strains were selected for evaluating each method: *L. monocytogenes* ATCC 19111 (positive control) and *E. coli* ATCC 25922 (negative control) for Romer RapidChek®, *E. coli* O157:H7 ATCC 43888 (positive control) and *S. aureus* Rosenbach ATCC 6538P (negative control) for Neogen Reveal®, and *Salmonella* Typhimurium ATCC 14028 (positive control) and *E. coli* ATCC 25922 (negative control) for BioMérieux VIDAS® UP SPT analysis. Strains were acquired from the American Type Culture Collection and were verified for purity.

Strains were individually reactivated from -80°C freezer stocks (20% glycerol in tryptic soy broth) by aseptically transferring a loop full of frozen culture to a tryptic soy

agar plate (TSA, Acumedia Neogen Corporation, Lansing, MI, USA), streaking for isolation, and incubating at 35-37°C (*E. coli* spp. and *S. aureus*) and 29.5-30.5°C (*Listeria* spp.) for 18-24 hours. Overnight cultures of each individual strain were then prepared by aseptically transferring 1 colony from the plate to a 9-ml tube of tryptic soy broth (TSB, Acumedia Neogen Corporation, Lansing, MI, USA) and incubating at 35-37°C (*E. coli* spp. and *S. aureus*) and 29.5-30.5°C (*Listeria* spp.) for 18-24 hours. After incubation overnight culture tubes were vortexed for 5-10 seconds to ensure inoculum was homogenous, and then serially diluted (1/10) in Butterfields Stock Solution to adjust controls to approximately 1.0×10^5 CFU/ml. Inoculum was made fresh for each procedure and used within 1 hour of preparation. Additionally, inoculum was prepared for each matrix separately.

Romer RapidChek® *Listeria* System Sample Preparation

Two separate matrices, ricotta cheese and oven roasted turkey breast deli slices, sold from three local grocery chains (HyVee, Super Saver, Walmart) were selected for the evaluation of the Romer RapidChek® *Listeria* system. Samples were prepared by weighing 25 grams of matrix into a sterile dish, transferring the samples to a biological safety cabinet, and then dividing the samples into testing categories for background flora, non-inoculated, inoculation level check, positive, and negative control samples. Matrices were then inoculated (if applicable) in the biological safety cabinet with 250 µl of previously prepared inoculum to get a final contamination level of approximately 1.0×10^3 CFU/g in the samples (BSC Airstream Class 2, ESCO, Horsham, PA, USA).

Ricotta cheese was inoculated by pressing the cheese flat inside a sample bag and then evenly distributing the inoculum over the surface of the product followed by hand blending the inoculum into the cheese by squeezing the exterior of the sample bag. Deli slices were inoculated by evenly distributing dots of inoculum onto the surface of the deli slices (see Figure 4.4 for examples of dotting procedure). Samples were allowed to dry for a minimum of 45 minutes before placing them into a sample bag to minimize the amount of inoculum potentially transferred to the sample bag walls. Sample bags were then placed in holding bins and kept at refrigeration temperatures (2-8°C) for 18-24 hours to facilitate attachment of the inoculum (organisms) to each food matrix.

Romer RapidChek® Sample Testing Procedures

Samples under evaluation for background flora were mixed with Butterfields Dilution Water to achieve a decimal dilution by weight on an analytical balance (AX4202 Balance, OHAUS Corporation, Parsippany, NJ, USA). Samples and dilution water were stomached for 30 seconds on normal speed, serially diluted, and plated on tryptic soy agar (TSA, Acumedia Neogen Corporation, Lansing, MI, USA), Oxford *Listeria* Agar (OX Agar Acumedia Neogen Corporation, Lansing, MI, USA), and Sorbitol MacConkey Agar (SMAC Agar Acumedia Neogen Corporation, Lansing, MI, USA). Plates were incubated at $30 \pm 2^\circ\text{C}$ for 40-48 hours.

Inoculated samples were allowed 24-hours for pathogen attachment. After the 24-hour attachment period, sample bags were removed from the refrigerator, allowed to equilibrate to room temperature, aseptically diluted to achieve a decimal dilution with RapidChek® *Listeria* media from Romer Labs (RapidChek®, Romer Labs, Newark, DE,

USA). Dilution was done by weight on an analytical balance. Samples were then stomached for 30 seconds on normal speed, and (non-inoculated, positive, and negative control samples) were placed into the incubator at $30 \pm 2^{\circ}\text{C}$ for 40-48 hours for enrichment.

Low level device samples, after the 24-hour attachment period, were not enriched but tested using the device testing protocol. These samples were mixed with RapidChek[®] *Listeria* media to achieve a decimal dilution, then stomached for 30 seconds. Further serial dilutions were prepared and plated on TSA, OX, and SMAC, and incubated at $30 \pm 2^{\circ}\text{C}$ for 40-48 hours to determine pre-enrichment inoculation levels. The RapidChek[®] *Listeria* media was prepared in bulk following manufacturer's instructions. Sample weights, volumes, incubation parameters, and device testing procedures were conducted according to AOAC-RI # 020401 and manufacturers testing guidelines (Romer 2016b; AOAC 2017).

Romer RapidChek[®] Test Device Protocol

Enriched samples were removed from the incubator and allowed to equilibrate to room temperature. An aliquot of 400 μl of enrichment broth was transferred from each sample bag to a sterile 1.5 ml microcentrifuge tube, placed into a heating block (Thermomixer R, Eppendorf, Hauppauge, NY, USA), and heated at $95-100^{\circ}\text{C}$ for 5 minutes (5-7 minutes accounting for load/unload times). Tubes were removed from the heating block, cooled for 10 minutes (achieving $25-30^{\circ}\text{C}$), and then tested using Romer RapidChek[®] *Listeria* lateral flow test strips for the presence/absence of *Listeria* spp. Device test strips placed into microcentrifuge tubes and allowed to absorb enrichment

media for 10 minutes. After the testing time is completed results are recorded as positive (control and test lines visible), negative (only control line visible), or invalid (no control line visible) – Table 4.2 (Romer 2016b; AOAC 2017).

Neogen Reveal® Sample Preparation

Two separate matrices, ground beef (73/27 and 80/20) and spinach (bagged), sold from three local grocery chains (HyVee, Super Saver, Walmart) were selected for the evaluation of the Neogen Reveal® 20-Hour system. Samples were prepared by weighing 375 grams (ground beef) or 25 grams (spinach and ground beef background flora) of matrix into a sterile vessel (dish or bag), transferring the samples to a biological safety cabinet, and then dividing the samples into testing categories for background flora, non-inoculated, inoculation level check, positive, and negative control samples. Matrices were then inoculated (if applicable) in the biological safety cabinet with 3-ml (ground beef) or 250- μ l (spinach) of previously prepared inoculum to get a final contamination level of approximately 1.0×10^3 CFU/g in the samples (BSC Airstream Class 2, ESCO, Horsham, PA, USA).

Ground beef was inoculated by pressing the meat flat inside a sample bag and then evenly distributing the inoculum over the surface followed by hand blending the inoculum into the ground beef by squeezing the exterior of the sample bag. Spinach was inoculated by evenly distributing dots of inoculum onto the surface of the leaves (see Figure 4.4 for examples of dotting procedure). Samples were allowed to dry for a minimum of 45 minutes before placing them into a sample bag to minimize the amount of inoculum potentially transferred to the sample bag walls. Sample bags were then

placed in holding bins and kept at refrigeration temperatures (2-8°C) for 18-24 hours to facilitate attachment of the inoculum to each food matrix.

Neogen Reveal[®] Sample Testing Procedures

Samples under evaluation for background flora samples were mixed with Butterfields Dilution Water to achieve a decimal dilution by weight on an analytical balance (AX4202 Balance, OHAUS Corporation, Parsippany, NJ, USA). Samples and dilution water were hand blended (ground beef) until evenly distributed or stomached (spinach) for 2 minutes on normal speed, serially diluted, and plated on tryptic soy agar (TSA, Acumedia Neogen Corporation, Lansing, MI, USA) and Sorbitol MacConkey Agar (SMAC Agar Acumedia Neogen Corporation, Lansing, MI, USA). Plates were incubator at $36 \pm 1^\circ\text{C}$ for 18-24 hours.

Inoculated samples were allowed 24-hours for pathogen attachment. After the 24-hour attachment period, sample bags were removed from the refrigerator, allowed to equilibrate to room temperature, aseptically mixed with 1125-ml (ground beef) or diluted to achieve a decimal dilution (spinach) with Neogen Reveal[®] 20-Hour media from Neogen Corporation (Reveal[®], Neogen Corporation, Lansing, MI, USA) by weight on an analytical balance. Samples were then hand blended (ground beef) until evenly distributed or stomached (spinach) for 2 minutes on normal speed, and (non-inoculated, positive, and negative control) were placed into the incubator at $36 \pm 1^\circ\text{C}$ for 21 ± 1 hours for enrichment.

Low level device samples, after the 24-hour attachment period, were not enriched, but tested using the device testing protocol. These samples were mixed with Neogen

Reveal[®] media appropriately, and then blended or stomached appropriately. Further dilutions were prepared, plated on TSA and SMAC, and incubated at $36 \pm 1^{\circ}\text{C}$ for 18-24 hours to determine pre-enrichment inoculation levels. Neogen Reveal[®] media was prepared according to manufacturer's instructions but was prepared in each sample bag individually to mirror the process followed for analyzing client samples at the FPCLS. Sample weights, volumes, incubation parameters, and device testing procedures were followed according to AOAC # 2000.14 and manufacturers testing guidelines (AOAC 2002; AOAC 2009; Neogen 2016).

Neogen Reveal[®] Test Device Protocol

Enriched samples were removed from the incubator while Reveal[®] test strips were removed from the refrigerator and allowed to equilibrate to room temperature (20-25°C). Using a pipettor, 120 µl of the enrichment broth was transferred from each sample bag directly into the sample port on a Neogen Reveal[®] 20-Hour *E. coli* O157:H7 lateral flow test strip to determine the presence/absence of *E. coli* O157:H7. Device test strips were allowed to develop for 15 minutes. After testing time results were recorded as positive (control and test lines visible), negative (only control line visible), or invalid (no control line visible) – Table 4.2 (AOAC 2002; AOAC 2009; Neogen 2016).



Figure 4.4: Spotting and Blending Inoculation Methods. Figure displays spinach leaves (left) in a sterile dish inoculated using the spotting inoculation method. Dots of desired inoculum (equaling 250 μ l combined) were transferred to the leaves surface using a pipettor and then allowed to dry in a biological safety cabinet. This method was also used for cucumber, oven roasted turkey breast deli slices, and rotisserie seasoned chicken breast deli slices. Ricotta cheese and ground beef samples were inoculated by spreading the inoculum evenly over the surface of the product and then hand blending the inoculum into the product by massaging the exterior of the sample bag (right).

VIDAS[®] UP *Salmonella* SPT Sample Preparation

Two separate matrices, cucumber (bulk) and rotisserie seasoned chicken breast deli slices, sold from three local grocery chains (HyVee, Super Saver, Walmart) were selected for the evaluation of the VIDAS[®] UP *Salmonella* SPT system. Samples were prepared by weighing 25 grams of matrix into a sterile dish, transferring the samples to a biological safety cabinet, and then dividing the samples into testing categories for background flora, non-inoculated, inoculation level check, positive, and negative control samples. Matrices were then inoculated (if applicable) in a biological safety cabinet with 250 μ l of previously prepared inoculum to get a final contamination level of approximately 1.0×10^3 CFU/g in the samples (BSC Airstream Class 2, ESCO, Horsham, PA, USA).

Cucumber and chicken deli slice samples were inoculated by evenly distributing dots of inoculum onto the surface of the samples (see Figure 4.4 for example of dotting procedure). Samples were allowed to dry for a minimum of 45 minutes before placing them into a sample bag to minimize the amount of inoculum potentially transferred to the sample bag walls. Sample bags were then placed in holding bins and kept at refrigeration temperatures (2-8°C) for 18-24 hours to facilitate attachment of the inoculum to each food matrix.

VIDAS® UP *Salmonella* SPT Sample Testing Procedures

Samples under evaluation for background flora samples were mixed with Butterfields Dilution Water to achieve a decimal dilution by weight on an analytical balance (AX4202 Balance, OHAUS Corporation, Parsippany, NJ, USA). Samples and dilution water were stomached for 2 minutes on normal speed, serially diluted, and plated on tryptic soy agar (TSA, Acumedia Neogen Corporation, Lansing, MI, USA) and XLT4 agar (XLT4 Agar, Acumedia Neogen Corporation, Lansing, MI, USA). Plates were placed into the incubator at $42 \pm 1^\circ\text{C}$ for 18-24 hours.

Inoculated samples were allowed 24-hours for pathogen attachment. After the 24-hour attachment period, sample bags were removed from the refrigerator, allowed to equilibrate to room temperature, and aseptically diluted to achieve a decimal dilution with Buffered Peptone Water (Acumedia Neogen Corporation, Lansing, MI, USA) by weight on an analytical balance. Samples were then stomached for 2 minutes on normal speed. Then, 1-ml of BioMérieux *Salmonella* Supplement was added aseptically, samples bags were hand blended to distribute supplement, and (non-inoculated, positive, and

negative control) then placed into the incubator at $42 \pm 1^\circ\text{C}$ for 18-24 hours for enrichment.

Low level device samples, after the 24-hour attachment period, were not enriched but tested using the device testing protocol. These samples were mixed with Buffered peptone water to achieve a decimal dilution, then stomached for 2 minutes. Further dilutions were prepared, plated on TSA and XLT4 agars, and incubated at $42 \pm 1^\circ\text{C}$ for 18-24 hours to determine pre-enrichment inoculation levels. Buffered peptone water was prepared in bulk following manufacturer's instructions. Sample weights, volumes, incubation parameters, and device testing procedures were followed according to AOAC # 2013.01 and manufacturers testing guidelines (AOAC 2016a; BioMérieux 2013; BioMérieux 2017b).

VIDAS[®] UP *Salmonella* SPT Test Device Protocol

Enriched samples were removed from the incubator while VIDAS[®] UP *Salmonella* SPT test strips and SPRs were removed from the refrigerator and allowed to equilibrate to room temperature. Test strips were placed into a strip holder and the SPRs were put into the VIDAS[®] instrument (miniVIDAS[®], BioMérieux Industry, Hazelwood, MO, USA). An aliquot of 500 μl of enrichment broth was then transferred from each sample bag directly to VIDAS[®] UP *Salmonella* SPT test strips, heated for 5 ± 1 minute at $131 \pm 5^\circ\text{C}$ on a heating block (Heat and Go, Techne (Cole-Parmer), Beacon Road, Stone, Staffordshire, UK), cooled for 10 minutes to 25-30 $^\circ\text{C}$ (room temperature), and then ran on the miniVIDAS[®] instrument for the presence/absence of *Salmonella* spp. The device provides a printout with final results defined as positive ($\text{TV} \geq 0.25$) or negative ($\text{TV} <$

0.25) (AOAC 2016a; BioMérieux 2013; BioMérieux 2017b). Table 4.2 shows a summary of all methods verified, types of samples evaluated, the expected results, and the test device responses for each method.

Statistical Analysis of Data

Statistical analysis could not be performed on the data generated during this method verification since it was qualitative in nature and all of the results obtained for sensitivity were identical – all passing (100%). Percent sensitivity was determined by dividing the true number of positive results by the true number of positive results plus the number of false negative results (number of presumed positive results that provide a negative response) and then multiplying the obtained value by 100. The following formula was used:

$$\% \text{ Sensitivity} = \frac{\# \text{ True Positives}}{(\# \text{ True Positives} + \# \text{ False Negatives})} \times 100$$

Although no statistical analysis could be performed, the results indicated that the procedure used for method verification was adequate as it met the requirements from ISO that all qualitative test methods provide 100% sensitivity, with no false negative or false positive results (IAFP 2018). Therefore, the methods and procedures used by FPCLS meet this guideline/requirement and all data obtained from this project is suitable to be used for method verification and ISO accreditation.

Table 4.2: Verification Method Test Device Responses and Expected Results.

Verification Method test kit expected results for inoculated positive/negative control and non-inoculated products and test device responses for FPCLS method verification testing (BioMérieux 2013; Neogen 2016; Romer 2016b).

Method	Sample Type	Expected Result	Test Device Response ¹
Neogen Reveal® 20-Hour <i>E. coli</i> O157:H7	Positive Control	Positive	Control Line – present Test Line – present
	Negative Control	Negative	Control Line – present Test Line – absent
	Non-Inoculated	Negative	Control Line – absent Test Line – present/absent
Romer RapidChek® <i>Listeria</i>	Positive Control	Positive	Control Line – present Test Line – present
	Negative Control	Negative	Control Line – present Test Line – absent
	Non-Inoculated	Negative	Control Line – absent Test Line – present/absent
BioMérieux VIDAS® UP <i>Salmonella</i> SPT	Positive Control	Positive	TV ≥ 0.25
	Negative Control	Negative	TV < 0.25
	Non-Inoculated	Negative	TV < 0.25

¹Neogen Reveal® 20-Hour *E. coli* O157:H7 and Romer RapidChek® *Listeria* lateral flow device testing responses: Positive = 2 lines present on device with lines appearing in the “Control” and “Test” zones; Negative = line appears in the “Control” zone but not in the “Test” zone; Invalid = no line appears in “Control” zone, regardless of line in “Test” zone then result “Invalid” and test must be repeated. BioMérieux VIDAS® UP *Salmonella* SPT device test value threshold (TV).

METHOD VERIFICATION RESULTS

The following sections contain results obtained during method verification procedures for Romer RapidChek® *Listeria* for the detection of *Listeria* spp., Neogen Reveal® 20-Hour for the detection of *E. coli* O157:H7, and BioMérieux VIDAS® UP

Salmonella SPT for the detection of *Salmonella* spp. Each method was evaluated for sensitivity (qualitative responses) utilizing positive and negative control cultures and tested for background flora, pre/post enrichment inoculum levels, low level device analysis, and verification of each method following manufacturer and AOAC guidelines using positive and negative spiked samples and non-inoculated sample controls. We evaluated each method for the purpose of meeting ISO requirements for obtaining ISO/IEC 17025 accreditation that all approved methods part of the scope of accreditation must be verified and fit for their intended purpose (ISO and IEC 2005; AOAC 2015; ISO and IEC 2017).

Negative / Positive Control Acceptability Pretesting Verification

Positive and negative controls for each method were tested (overnight cultures of organisms in tryptic soy broth between 3.4×10^8 and 1.14×10^9 CFU/ml) against each method device to verify that they were appropriate for testing prior to performing actual method verification procedures. Control culture results were: Romer RapidChek® *L. monocytogenes* ATCC 19111 (positive control) at 1.11×10^9 CFU/ml – Positive/Pass, *E. coli* ATCC 25922 (negative control) at 1.14×10^9 CFU/ml – Negative/Pass; Neogen Reveal® 20-Hour *E. coli* O157:H7 ATCC 43888 (positive control) at 5.20×10^8 CFU/ml – Positive/Pass, *S. aureus* ATCC 6538P (negative control) at 7.30×10^8 CFU/ml – Negative/Pass; BioMérieux VIDAS® UP *Salmonella* SPT *Salmonella* Typhimurium ATCC 14028 (positive control) at 3.40×10^8 CFU/ml, TV 1.76 – Positive/Pass, *E. coli* ATCC 25922 (negative control) at 4.60×10^8 CFU/ml, TV 0.07 – Negative/Pass. See Table 4.T1.

Romer RapidChek® *Listeria* Method Results

Background flora for ricotta cheese on Tryptic Soy Agar (TSA), Oxford *Listeria* Selective Agar (OX), and Sorbitol MacConkey Agar (SMAC) for HyVee (HV), Super Saver (SS), and Walmart (WM) products were 0.00, 3.07, 2.79 and 0.00, 0.00, 1.00 and 0.00, 0.00, 0.00 LOG CFU/g respectively. Background flora for oven roasted turkey breast deli slices on TSA, OX, and SMAC for HV, SS, and WM products were 0.00, 2.82, 0.00 and 0.00, 1.00, 0.00 and 0.00, 0.00, and 0.00 LOG CFU/g respectively (Table 4.T2, Figure 4.F1). Bacterial load averages for non-inoculated ricotta cheese and oven roasted turkey breast deli slice products on TSA pre and post enrichment were 1.95, 0.94 (pre) and 7.15, 7.93 (post) LOG CFU/g respectively (Figure 4.F4).

Negative control, *E. coli* ATCC 25922, inoculum levels pre and post enrichment for ricotta cheese and oven roasted turkey breast deli slice products were 4.26, 4.23 (pre) and 0.00, 0.00 (post) LOG CFU/g respectively (Figure 4.F3). Positive control, *L. monocytogenes* ATCC 19111, inoculum levels pre and post enrichment for ricotta cheese on TSA and OX, for HV, SS, and WM products were 4.32, 4.01, 4.16 and 4.06, 3.92, 4.12 (pre) and 9.77, 9.74, 9.86 and 9.74, 9.67, 9.80 (post) LOG CFU/g respectively (Figure 4.F2). Positive control, *L. monocytogenes* ATCC 19111, inoculum levels pre and post enrichment for oven roasted turkey breast deli slices on TSA and OX, for HV, SS, and WM products were 4.22, 4.36, 4.19 and 4.17, 4.20, 4.18 (pre) and 9.73, 9.71, 9.92 and 9.74, 9.72, 9.85 (post) LOG CFU/g respectively (Figure 4.F2).

Low level device testing results for percent sensitivity of all products tested (ricotta cheese and oven roasted turkey breast deli slices), for all distributors (HV, SS, WM), and for both positive control (*L. monocytogenes* ATCC 19111) and negative

control (*E. coli* ATCC 25922) inoculated samples, were 100%. Ricotta cheese positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS, WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively. Oven roasted turkey breast deli slices positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS, WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively (Table 4.T2, Table 4.T3).

Method verification sample testing by Romer RapidChek[®] *Listeria* system for percent sensitivity of positive (*L. monocytogenes* ATCC 19111) and negative control (*E. coli* ATCC 25922) inoculated samples and non-inoculated control samples were 100% for all products tested, ricotta cheese and oven roasted turkey breast deli slices, and for all distributors (HV, SS, WM). Ricotta cheese positive, negative, and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3). Oven roasted turkey breast deli slices positive, negative, and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3).

Neogen Reveal[®] 20-Hour *E. coli* O157:H7 Method Results

Background flora for spinach on Tryptic Soy Agar (TSA) and Sorbitol MacConkey Agar (SMAC) for HyVee (HV), Super Saver (SS), and Walmart (WM)

products were 6.05, 4.79, 5.49 and 5.97, 4.80, 5.43 LOG CFU/g respectively.

Background flora for ground beef on TSA and SMAC for HV, SS, and WM products were 5.00, 5.04, 3.16 and 3.30, 3.32, 1.60 LOG CFU/g respectively (Table 4.T2, Figure 4.F1). Bacterial load averages for non-inoculated spinach and ground beef products on TSA pre and post enrichment were 5.45, 4.40 (pre) and 9.77, 9.76 (post) LOG CFU/g respectively (Figure 4.F4).

Negative control, *S. aureus* ATCC 6538P, inoculum levels pre and post enrichment for spinach and ground beef products were 3.78, 4.00 (pre) and 4.45, 5.48 (post) LOG CFU/g respectively (Figure 4.F3). Positive control, *E. coli* O157:H7 ATCC 43888, inoculum levels pre and post enrichment for spinach on TSA and SMAC, for HV, SS, and WM products were 4.15, 4.01, 4.35 and 4.08, 3.97, 4.30 (pre) and 9.74, 9.57, 9.43 and 9.32, 9.33, 9.07 (post) LOG CFU/g respectively (Figure 4.F2). Positive control, *E. coli* O157:H7 ATCC 43888, inoculum levels pre and post enrichment for ground beef on TSA and SMAC, for HV, SS, and WM products were 3.99, 3.34, 3.32 and 3.84, 3.36, 3.16 (pre) and 9.95, 9.82, 9.78 and 9.77, 9.22, 9.30 (post) LOG CFU/g respectively (Figure 4.F2).

Low level device testing results for percent sensitivity of all products tested (spinach and ground beef) for all distributors (HV, SS, WM), and for both positive control (*E. coli* O157:H7 ATCC 43888) and negative control (*S. aureus* ATCC 6538P) inoculated samples, were 100%. Spinach positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS, WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively. Ground beef positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS,

WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively (Table 4.T2, Table 4.T3).

Method verification sample testing by Neogen Reveal® 20-Hour *E. coli* O157:H7 system for percent sensitivity of positive (*E. coli* O157:H7 ATCC 43888) and negative control (*S. aureus* ATCC 6538P) inoculated samples and non-inoculated control samples were 100% for all products tested, spinach and ground beef, and for all distributors (HV, SS, WM). Spinach positive, negative, and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3). Ground beef positive, negative and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3).

BioMérieux VIDAS® UP *Salmonella* SPT Method Results

Background flora for cucumber on Tryptic Soy Agar (TSA) and XLT4 for HyVee (HV), Super Saver (SS), and Walmart (WM) products were 3.92, 2.26, 2.20 and 0.00, 0.00, 0.00 LOG CFU/g respectively. Background flora for rotisserie seasoned chicken breast deli slices on TSA and XLT4 for HV, SS, and WM products were 2.15, 0.00, 1.70 and 0.00, 0.00, 0.00 LOG CFU/g respectively (Table 4.T2, Figure 4.F1). Bacterial load averages for non-inoculated cucumber and rotisserie seasoned chicken breast deli slice

products on TSA pre and post enrichment were 2.79, 1.28 (pre) and 5.66, 0.83 (post) LOG CFU/g respectively (Figure 4.F4).

Negative control, *E. coli* ATCC 25922, inoculum levels pre and post enrichment for cucumber and rotisserie seasoned chicken breast deli slice products were 4.31, 4.26 (pre) and 9.89, 9.71 (post) LOG CFU/g respectively (Figure 4.F3). Positive control, *Salmonella* Typhimurium ATCC 14028, inoculum levels pre and post enrichment for cucumber on TSA and XLT4, for HV, SS, and WM products were 3.97, 3.80, 4.08 and 3.94, 3.58, 3.97 (pre) and 10.04, 9.98, 9.90 and 9.92, 9.72, 9.58 (post) LOG CFU/g respectively (Figure 4.F2). Positive control, *Salmonella* Typhimurium ATCC 14028, inoculum levels pre and post enrichment for rotisserie seasoned chicken breast deli slices on TSA and XLT4, for HV, SS, and WM products were 3.95, 3.90, 3.90 and 3.89, 3.61, 3.79 (pre) and 9.95, 9.62, 9.92 and 9.69, 9.18, 9.66 (post) LOG CFU/g respectively (Figure 4.F2).

Low level device testing results for percent sensitivity of all products tested (cucumber and rotisserie seasoned chicken breast deli slices) for all distributors (HV, SS, WM), and for both positive control (*Salmonella* Typhimurium ATCC 14028) and negative control (*E. coli* ATCC 25922) inoculated samples, were 100%. Cucumber positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS, WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively. Rotisserie seasoned chicken breast deli slices positive and negative control samples were 3 positive, 0 negative, 0 false positive/negative (1 each HV, SS, WM) and 0 positive, 1 negative, 0 false positive/negative (HV) respectively (Table 4.T2, Table 4.T3).

Method verification sample testing by BioMérieux VIDAS® UP *Salmonella* SPT system for percent sensitivity of positive (*Salmonella* Typhimurium ATCC 14028) and negative control (*E. coli* ATCC 25922) inoculated samples and non-inoculated control samples were 100% for all products tested, cucumber and rotisserie seasoned chicken breast deli slices, and for all distributors (HV, SS, WM). Cucumber positive, negative, and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3).

Rotisserie seasoned chicken breast deli slices positive, negative and non-inoculated control samples were 12 positive, 0 negative, 0 false positive/negative (4 each HV, SS, WM), 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM), and 0 positive, 3 negative, 0 false positive/negative (1 each HV, SS, WM) respectively (Table 4.T2, Table 4.T3). Test threshold values (TV) for verifying positive and negative outputs on the VIDAS® were obtained for cucumber and rotisserie seasoned chicken breast deli slices, and for all distributors (HV, SS, WM). TV for positive and negative control cultures as well as non-inoculated samples for low level analysis and method verification testing can be seen in tables 4.T4 and 4.T5.

DISCUSSION

Method Verification

Method verification is an involved process that is very time consuming and requires a lot of resources to complete. However, it must be performed by each laboratory

attempting to obtain ISO/IEC 17025 accreditation as it is required in the standard that each method “*must meet specifications*” that revolve around the intended use of the method and that any of the methods that fall within the scope of accreditation “*shall have been validated*” or verified prior to attempting to obtain certification (A2LA 2001; ISO and IEC 2005; AOAC 2015).

Since the majority of the laboratories that are ISO/IEC 17025 accredited are in industry and not academia (A2LA 2018), their method verification data is not available to the public and is not published or shared as a guide for others to follow. The UNL FPCLS has decided that all method verification activities conducted as part of the ISO/IEC 17025 accreditation process for the FPCLS testing laboratory will be published or made available as a guide for other laboratories or academic institutions to utilize as a guide for improvement or as a template for obtaining ISO accreditation within their laboratories.

Qualitative Sample Size Determination and Sensitivity Testing

As part of preparing for method verification the sample size and tests to perform must be determined. Method validation/verification procedures exist that help guide in determining the sample size and tests to perform for quantitative methods, but there is very little information available for qualitative testing (NATA 2013; FDA 2015).

Determining sample size for qualitative methods is not directly provided in the guidance documents and it has been stated that the “*laboratory is responsible*” for determining the appropriate number of samples to produce statistically valid results and to meet the goals of the verification process (Thompson et al. 2002; AOAC 2006; NATA 2013), and the sample size for qualitative methods may be simply determined by the resources available

to the laboratory and/or objectives the study is trying to achieve (Statistical Solutions 2017).

However, ISO 16140-3 which is under development will provide a recommended minimum number of samples for qualitative methods of 10 samples per matrix (IAFP 2018). FPCLS method verification procedures were designed to be compliant with and/or exceed ISO 16140-3 testing 12 samples per matrix (positive controls), negative controls, and evaluating 2 matrices per method. Additionally, ISO 16140-3 only requires that sensitivity be evaluated when performing method verification on qualitative methods (IAFP 2018) therefore FPCLS method verification procedures only accounted for sensitivity and no other statistical parameters such as selectivity, precision, accuracy, and detection limits.

Media Inhibition

It was found that the Romer RapidChek® *Listeria* media inhibited growth of the negative control organism *E. coli* ATCC 25922 during analysis with average inoculation levels pre-enrichment of 4.25 LOG CFU/g and post enrichment average levels of 0.00 LOG CFU/g (Figure 4.F3). This is due to the inhibitory and selective nature of the proprietary *Listeria* media and/or *Listeria* Supplement part of the Romer RapidChek® *Listeria* testing system (Romer 2016b; AOAC 2017). Neogen Reveal® 20-Hour media also inhibited the growth of the negative control organism *S. aureus* ATCC 6538P during analysis with average inoculation levels pre-enrichment of 3.89 LOG CFU/g and post enrichment average levels of 4.97 LOG CFU/g (Figure 4.F3).

Although the Neogen Reveal[®] 20-Hour media inhibited the growth of the *S. aureus* ATCC 6538P it did not eradicate the control organism as with the Romer RapidChek[®] *Listeria* media. This is due to the partially selective nature of the proprietary Neogen Reveal[®] 20-Hour media utilized as part of the Neogen Reveal[®] 20-Hour for *E. coli* O157:H7 testing system (AOAC 2002; AOAC 2009; Neogen 2016).

It was also noted that buffered peptone water containing proprietary BioMérieux *Salmonella* Supplement (BioMérieux 2013; BioMérieux 2017b) did not inhibit the negative control organism *E. coli* ATCC 25922 during analysis with average inoculation levels pre-enrichment of 4.29 LOG CFU/g and post enrichment average levels of 9.8 LOG CFU/g (Figure 4.F3). Despite the high levels of negative control post enrichment, the results for all negative controls were still negative (passing) on the VIDAS[®] showing the specificity of the method.

Control Organism Selection

Organisms available to the FPCLS were selected for both positive and negative controls received from the American Type Culture Collection. The FPCLS selected *L. monocytogenes* ATCC 19111 (serotype 1/2A) as the positive control and *E. coli* ATCC 25922 as the negative control for Romer RapidChek[®] analysis. Although neither of these organisms are on the inclusion or exclusion lists provided by Romer Labs they are closely related with Romer Labs including *L. monocytogenes* ATCC 51774 and *L. monocytogenes* USDA 472 (both 1/2A strains) on the inclusion list, and *E. coli* O157:H7 ATCC 35150 on the exclusion list (AOAC 2017).

The FPCLS selected *E. coli* O157:H7 ATCC 43888 as the positive control and *S. aureus* ATCC 6538P as the negative control for Neogen Reveal® 20-Hour analysis. There are no inclusion or exclusion lists available for Neogen Reveal® 20-Hour therefore the FPCLS selected the control organisms that they felt best fit the method and provided a chance for success based on historical data. The FPCLS selected *Salmonella* Typhimurium ATCC 14028 as the positive control and *E. coli* ATCC 25922 as the negative control for BioMérieux VIDAS® UP *Salmonella* SPT analysis. Although neither of these organisms ATCC designations are on the inclusion or exclusion lists provided by BioMérieux, the species are on each list with *Salmonella* Typhimurium on the inclusion list, and *Escherichia coli* on the exclusion list (BioMérieux 2017b).

Sample Matrix Selection

Sample matrices for method verification were selected based on two parameters, matrices tested by the manufacturers of each method kit and if samples had been tested previously for clients. The FPCLS selected ricotta cheese and oven roasted turkey breast deli slices for performing Romer RapidChek® *Listeria* analysis. Romer Labs tested both ricotta cheese and deli turkey during their method validation of the Romer RapidChek® *Listeria* test system (AOAC 2017).

The FPCLS selected spinach and ground beef for performing Neogen Reveal® 20-Hour analysis. AOAC method for Neogen Reveal® 20-Hour for *E. coli* O157:H7 recommends the method for lettuce (similar to spinach) and ground beef (AOAC 2002; AOAC 2009; Neogen 2016). Spinach is a product that has been tested by the FPCLS, is

very similar to lettuce, and is easily obtained therefore it was selected for analysis by Neogen Reveal[®] 20-Hour for *E. coli* O157:H7.

The FPCLS selected cucumber and rotisserie seasoned chicken breast deli slices for performing BioMérieux VIDAS[®] UP *Salmonella* SPT analysis. BioMérieux recommends the VIDAS[®] UP *Salmonella* SPT system for a variety of products with the official AOAC method mentioning cheeses, deli roast beef, chicken carcass rinsate, ice cream, fish, ground turkey, almonds, etc. (AOAC 2016a; BioMérieux 2017b). Cucumbers and rotisserie seasoned chicken breast deli slices are not on their list of products but they are easily accessible, have been involved in outbreaks (FDA 2018; USDA 2018), and are similar enough to other products on the list that the FPCLS felt they would be good matrices for analyzing the BioMérieux VIDAS[®] UP *Salmonella* SPT test system.

Method Verification Sensitivity Results

Romer Labs tested several matrices for percent sensitivity as part of the method validation for RapidChek[®] *Listeria* acquiring the following results: roast beef 100%, ricotta cheese 100%, deli turkey 93%, hot dogs 100%, pepperoni 100%, smoked fish 100%, cooked shrimp 100%, potato salad 94%, whole milk 100%, and ice cream 100% (AOAC 2017) using the Romer RapidChek[®] *Listeria* system (lateral flow device). In comparison, the FPCLS results obtained from method verification procedures for percent sensitivity were: ricotta cheese 100% and oven roasted turkey breast deli slices 100%. This shows the capabilities of the FPCLS to perform Romer RapidChek[®] *Listeria* system testing and achieve an expected result.

Neogen Corporation tested several products for percent sensitivity as part of the method validation and AOAC official method approval for Neogen Reveal[®] 20-Hour for *E. coli* O157:H7 acquiring the following results: apple cider 89.5-97.1%, lettuce rinse 96.5-100%, raw beef cubes 100%, and raw ground beef 100% based on interlaboratory comparisons (AOAC 2002) and retest results with the method update for raw beef cubes 100% and raw ground beef 100% (AOAC 2009). In comparison, the FPCLS results obtained from method verification procedures for percent sensitivity were: spinach 100% and ground beef 100%. This shows the capabilities of the FPCLS to perform Neogen Reveal[®] 20-Hour for *E. coli* O157:H7 system testing and achieve an expected result.

BioMérieux tested several products for percent sensitivity as part of the method validation and AOAC official method approval for BioMérieux VIDAS[®] UP *Salmonella* SPT acquiring the following results: raw ground beef (25g) 100%, raw ground beef (375g) 100%, deli roast beef 100%, chicken carcass rinsate 100%, vanilla ice cream 100%, bagged lettuce 90-100%, peanut butter 100%, cooked shrimp 100%, raw cod 100%, liquid/powdered eggs 100%, ground black pepper 100%, almonds 100%, dog food 100%, and others based on internal and independent laboratory comparisons (AOAC 2016a; BioMérieux 2017b). In comparison, the FPCLS results obtained from method verification procedures for percent sensitivity were: cucumber 100% and rotisserie seasoned chicken breast deli slices 100%. This shows the capabilities of the FPCLS to perform BioMérieux VIDAS[®] UP *Salmonella* SPT system testing and achieve an expected result.

CONCLUSIONS

In conclusion, this project has provided evidence that the FPCLS is capable and competent to perform three approved laboratory methods (Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour for *E. coli* O157:H7, BioMérieux VIDAS® UP *Salmonella* SPT) as they were intended in order to meet ISO/IEC 17025 requirements for accreditation (AOAC 2015). This project has also shown that enrichment is extremely important in obtaining acceptable results and to the success of each method chosen for method verification by the FPCLS. Low inoculum level device testing data suggests that if the enrichment stage of any of the selected methods fails, the test would give a false negative result despite the presence of the target organism which would lead to poor performance, lack of control of the test system, and misrepresentation of the true results.

Method verification procedures and processes developed in this project already meet the requirements that will be released under ISO 16140-3 for acceptable method verification of approved methods for the purpose of obtaining ISO accreditation. There are not many examples that have been published for academic and industry establishments to follow to meet method verification requirements therefore all method verification procedures developed by the FPCLS as part of this project will be reviewed and made available to both industry and academia so they may serve as a guide to method verification and be utilized by any laboratory attempting to verify their own methods, improve the performance of their laboratory, increase the accuracy and reliability of their results, and/or prepare for obtaining ISO accreditation.

In developing and conducting these method verification procedures, the UNL-FPCLS has met the requirement for method verification within the ISO/IEC 17025

standard and are one step closer to becoming ISO compliant setting the FPCLS apart from other small and academic laboratories while raising the bar for quality experimentation, service to industry, and laboratory excellence. The FPCLS is now on track to meet all ISO requirements for accreditation and is ready to pursue ISO/IEC 17025 accreditation when the time is right for the laboratory.

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Method Verification Procedures Tables Section

Table 4.T1: Positive and Negative Control Pre-Testing Results. Table displays results verifying positive and negative controls selected for method verification procedures are suitable for testing.

Test Method	Organism	ATCC #	Inoculum Level ¹	Device Response	Test Result
RapidChek®	<i>L. monocytogenes</i>	19111	1.11 x 10 ⁹	Positive	Pass
	<i>E. coli</i>	25922	1.14 x 10 ⁹	Negative	Pass
Reveal® 20-Hour	<i>E. coli</i> O157:H7	43888	5.20 x 10 ⁸	Positive	Pass
	<i>S. aureus</i>	6538P	7.30 x 10 ⁸	Negative	Pass
VIDAS® UP SPT	<i>Salmonella</i> Typhimurium	14028	3.40 x 10 ⁸	Positive	Pass
	<i>E. coli</i>	25922	4.60 x 10 ⁸	Negative	Pass

¹Inoculum levels were achieved by growing the cultures in tryptic soy broth for 18-24 hours at appropriate temperatures. All organisms gave passing results with positive control cultures having positive responses (present) and negative control cultures having negative responses for each test method respectively. All inoculum levels above theoretical threshold for devices to work of 1.0 x 10⁶ CFU/ml.

Table 4.T2: Method Verification Sample Matrices. Table displays sample matrices selected for performing method verification procedures at the UNL-FPCLS.

Testing Device	Matrix	Brand	Store Location
Romer Labs RapidChek® <i>Listeria</i>	Ricotta Cheese	HyVee Best Choice Great Value	HyVee Super Saver Walmart
	Oven Roasted Turkey Breast Deli Slices	HyVee Buddig Great Value	HyVee Super Saver Walmart
Neogen Reveal® 20-Hour <i>E. coli</i> O157:H7	Ground Beef (73/27 and 80/20)	73/27 TSD Sales	HyVee
		80/20 TSD Sales 73/27 TSD Sales	Super Saver Walmart
	Spinach	Popeye Super Foods Fresh Express Classic	HyVee Super Saver Walmart
BioMérieux VIDAS® UP <i>Salmonella</i> SPT	Cucumber ¹	N/A	HyVee
		N/A	Super Saver
		N/A	Walmart
	Rotisserie Seasoned Chicken Breast Deli Slices	HyVee Oscar Meyer Great Value	HyVee Super Saver Walmart

¹Cucumbers did not have a brand association that was available – pulled fresh from the produce section.

Table 4.T3: Method Verification Sensitivity Results. Table displays sensitivity results for all method verification testing procedures – Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT.

Testing Device	Matrix	Test Performed	# of Samples	# Positive	# Negative	% Sensitivity
Romer Labs RapidChek® <i>Listeria</i>	Ricotta Cheese	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100
	Oven Roasted Turkey Breast Deli Slices	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100
Neogen Reveal® 20-Hour <i>E. coli</i> O157:H7	Ground Beef (73/27 and 80/20)	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100
	Spinach	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100
BioMérieux VIDAS® UP <i>Salmonella</i> SPT	Cucumber	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100
	Rotisserie Seasoned Chicken Breast Deli Slices	Low Level (PC)	3	3	0	100
		Low Level (NC)	1	0	1	100
		Positive Control Culture	12	12	0	100
		Negative Control Culture	3	0	3	100
		Non-Inoculated	3	0	3	100

Table 4.T4: VIDAS® UP *Salmonella* SPT Low Level and Method Verification Test Value Threshold Results. Table displays the test value threshold (TV) for low level testing of cucumbers and rotisserie seasoned chicken breast deli slices and the TV for cucumber samples evaluated during VIDAS® UP *Salmonella* SPT method verification procedures.

Test Performed	Sample ID ¹	TV	Device Response	Test Result ²
Low Level ² (Inoculum approx. 1.0 x 10 ³ – 1.0 x 10 ⁴)	HVCUSAL1	0.08	Negative	Fail
	SSCUSAL2	0.08	Negative	Fail
	WMCUSAL3	0.08	Negative	Fail
	HVCHSAL4	0.08	Negative	Fail
	SSCHSAL5	0.08	Negative	Fail
	WMCHSAL6	0.07	Negative	Fail
	HVCUEC7	0.07	Negative	Pass
	HVCHEC8	0.07	Negative	Pass
Method Verification	HVCUPOS1	1.87	Positive	Pass
	HVCUPOS2	1.86	Positive	Pass
	HVCUPOS3	1.85	Positive	Pass
	HVCUPOS4	1.82	Positive	Pass
	HVCUNEG5	0.08	Negative	Pass
	HVCUNON6	0.08	Negative	Pass
	SSCUPOS7	1.78	Positive	Pass
	SSCUPOS8	1.76	Positive	Pass
	SSCUPOS9	2.21	Positive	Pass
	SSCUPOS10	2.20	Positive	Pass
	SSCUNEG11	0.08	Negative	Pass
	SSCUNON12	0.07	Negative	Pass
	WMCUPOS13	2.17	Positive	Pass
	WMCUPOS14	2.13	Positive	Pass
	WMCUPOS15	2.11	Positive	Pass
	WMCUPOS16	2.12	Positive	Pass
	WMCUNEG17	0.08	Negative	Pass
	WMCUNON18	0.07	Negative	Pass

¹Sample IDs: HyVee (HV), Super Saver (SS), Walmart (WM), Cucumber (CU), Rotisserie Seasoned Chicken Breast Deli Slices (CH), *Salmonella* Typhimurium ATCC 14028 (SAL), *E. coli* ATCC 25922 (EC), Non-Inoculated (NON), Negative Control Culture (NEG), Positive Control Culture (POS). ²Results for low level device testing failed to produce an acceptable result at low levels since the inoculum on the matrix was below the necessary threshold for the device to work.

Table 4.T5: VIDAS® UP *Salmonella* SPT Method Verification Test Value Threshold Results. Table displays the test value threshold (TV) for rotisserie seasoned chicken breast deli slices evaluated during VIDAS® UP *Salmonella* SPT method verification procedures.

Test Performed	Sample ID ¹	TV	Device Response	Test Result
Method Verification	HVCHPOS19	1.85	Positive	Pass
	HVCHPOS20	1.80	Positive	Pass
	HVCHPOS21	1.82	Positive	Pass
	HVCHPOS22	1.79	Positive	Pass
	HVCHNEG23	0.07	Negative	Pass
	HVCHNON24	0.07	Negative	Pass
	SSCHPOS25	1.84	Positive	Pass
	SSCHPOS26	1.81	Positive	Pass
	SSCHPOS27	2.25	Positive	Pass
	SSCHPOS28	2.23	Positive	Pass
	SSCHNEG29	0.07	Negative	Pass
	SSCHNON30	0.07	Negative	Pass
	WMCHPOS31	2.20	Positive	Pass
	WMCHPOS32	2.12	Positive	Pass
	WMCHPOS33	2.15	Positive	Pass
	WMCHPOS34	2.15	Positive	Pass
	WMCHNEG35	0.08	Negative	Pass
	WMCHNON36	0.07	Negative	Pass

¹Sample IDs: HyVee (HV), Super Saver (SS), Walmart (WM), Rotisserie Seasoned Chicken Breast Deli Slices (CH), *Salmonella* Typhimurium ATCC 14028 (SAL), *E. coli* ATCC 25922 (EC), Non-Inoculated (NON), Negative Control Culture (NEG), Positive Control Culture (POS).

Method Verification Procedures Results Figures

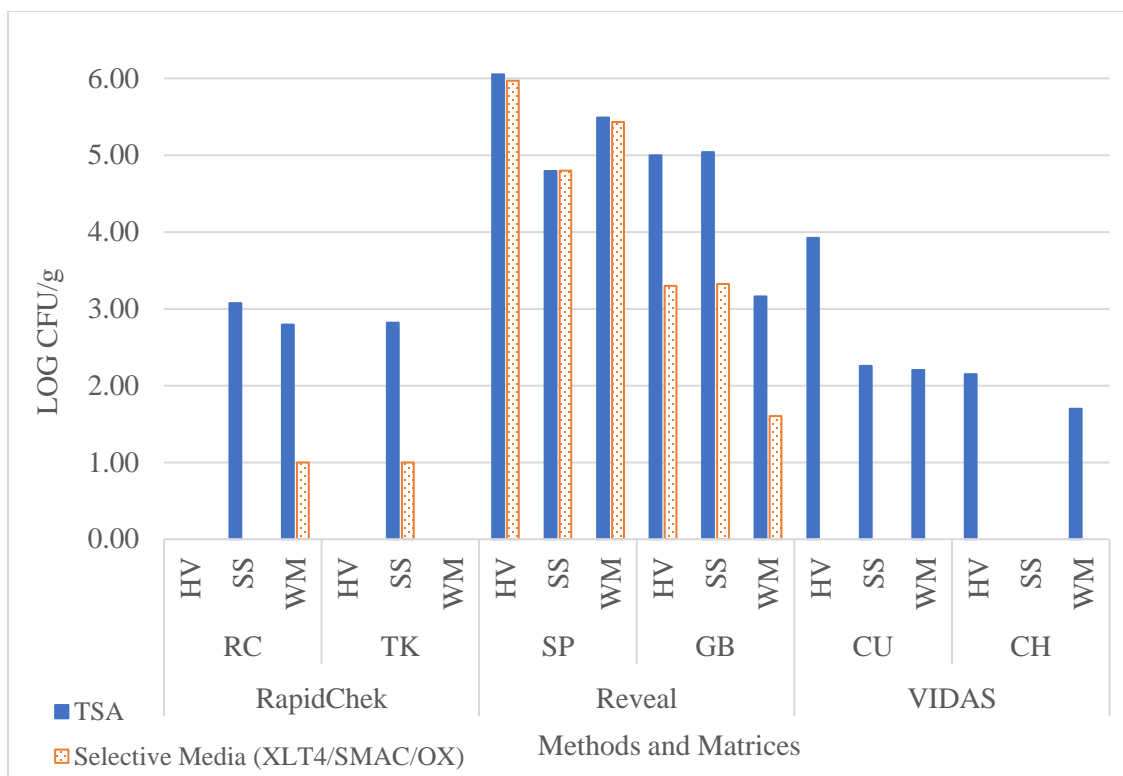


Figure 4.F1: Method Verification Background Flora Results. Background flora of products selected for method verification testing for Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT. Sample IDs: HyVee (HV), Super Saver (SS), Walmart (WM), Ricotta Cheese (RC), Oven Roasted Turkey Breast Deli Slices (TK), Spinach (SP), Ground Beef (GB), Cucumber (CU), Rotisserie Seasoned Chicken Breast Deli Slices (CH). Media: Tryptic Soy Agar (TSA) general purpose media, Sorbitol MacConkey Agar (SMAC) for coliforms and *E. coli* spp., Oxford *Listeria* Agar Base (OX) for *Listeria* spp., and XLT4 for *Salmonella* spp.

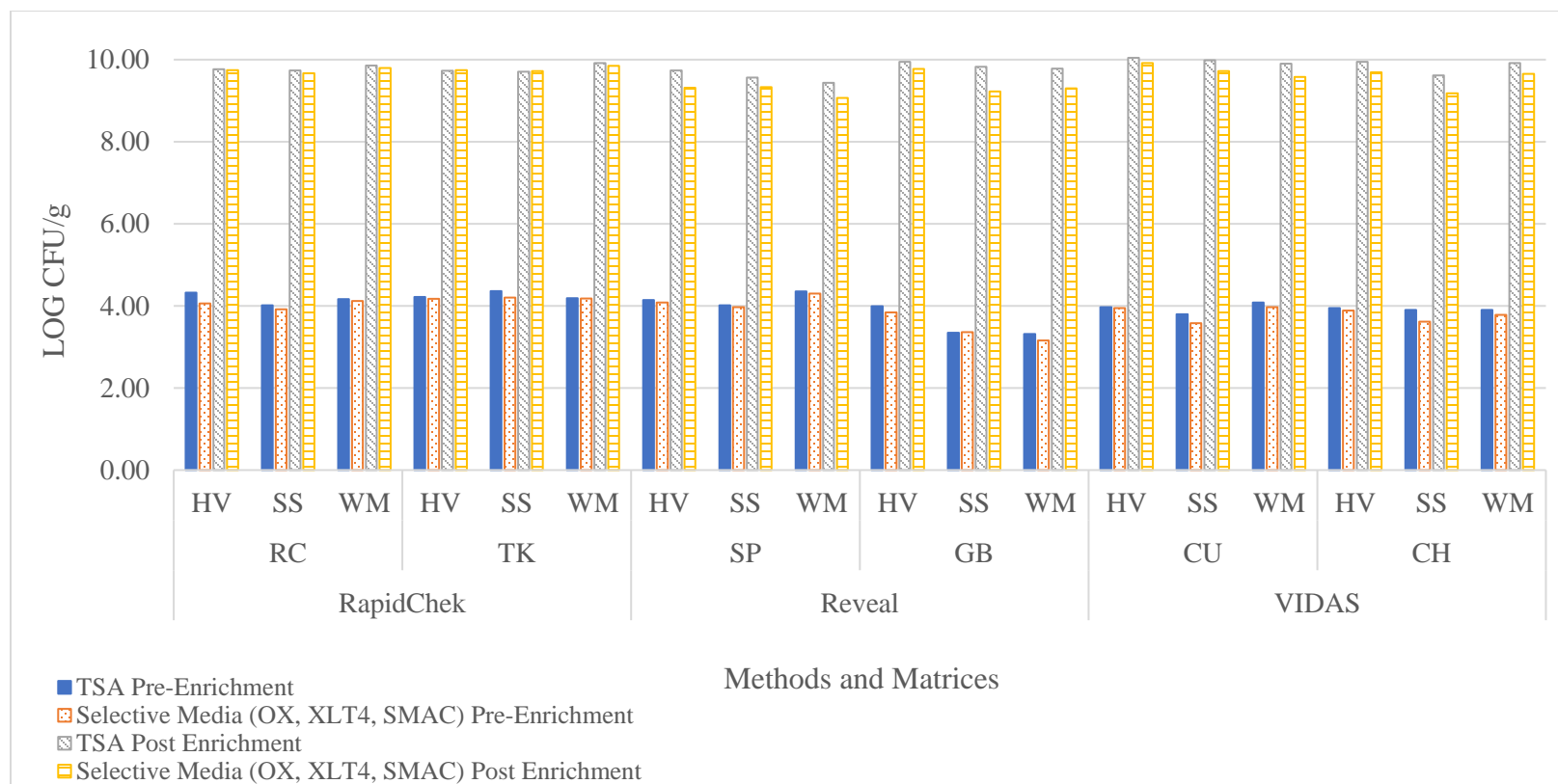


Figure 4.F2: Method Verification Pre and Post Enrichment Positive Control Culture Inoculation Levels. Positive Control Culture inoculation levels pre and post enrichment for products selected for method verification testing for Romer RapidChek[®] *Listeria*, Neogen Reveal[®] 20-Hour *E. coli* O157:H7, and BioMérieux VIDAS[®] UP *Salmonella* SPT. Post enrichment results are averages. Sample IDs: HyVee (HV), Super Saver (SS), Walmart (WM), Ricotta Cheese (RC), Oven Roasted Turkey Breast Deli Slices (TK), Spinach (SP), Ground Beef (GB), Cucumber (CU), Rotisserie Seasoned Chicken Breast Deli Slices (CH). Media: Tryptic Soy Agar (TSA) general purpose media, Sorbitol MacConkey Agar (SMAC) for coliforms and *E. coli* spp., Oxford *Listeria* Agar Base (OX) for *Listeria* spp., and XLT4 for *Salmonella* spp.

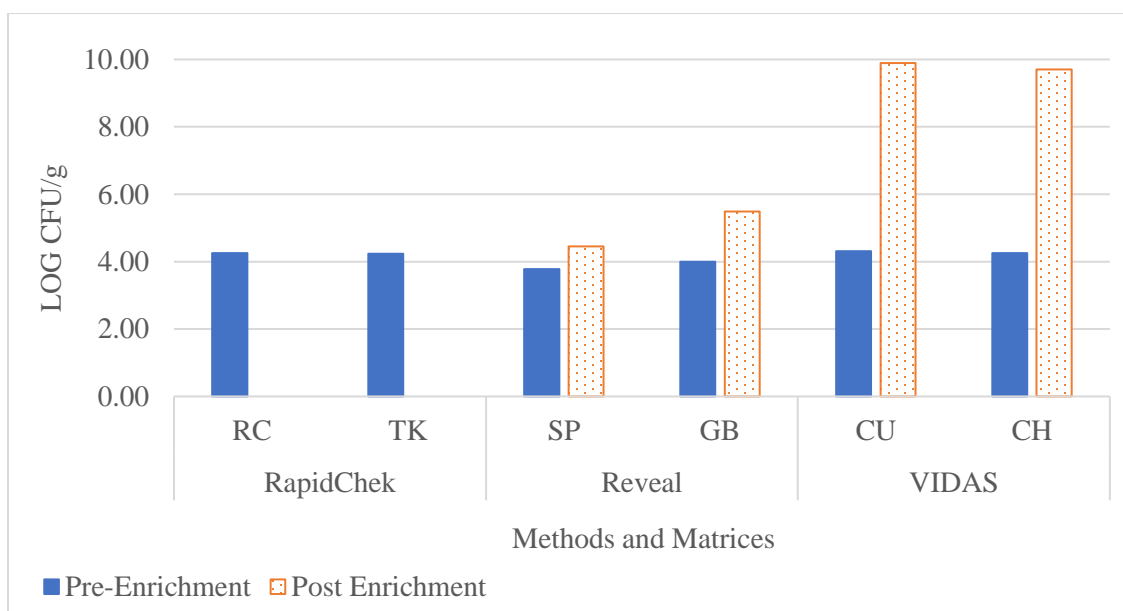


Figure 4.F3: Method Verification Negative Control Culture Inoculation Levels.

Negative Control Culture inoculation levels pre and post enrichment for products selected for method verification testing for Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT. Post enrichment results are averages. Results shown are on tryptic soy agar. Sample IDs: Ricotta Cheese (RC), Oven Roasted Turkey Breast Deli Slices (TK), Spinach (SP), Ground Beef (GB), Cucumber (CU), Rotisserie Seasoned Chicken Breast Deli Slices (CH).

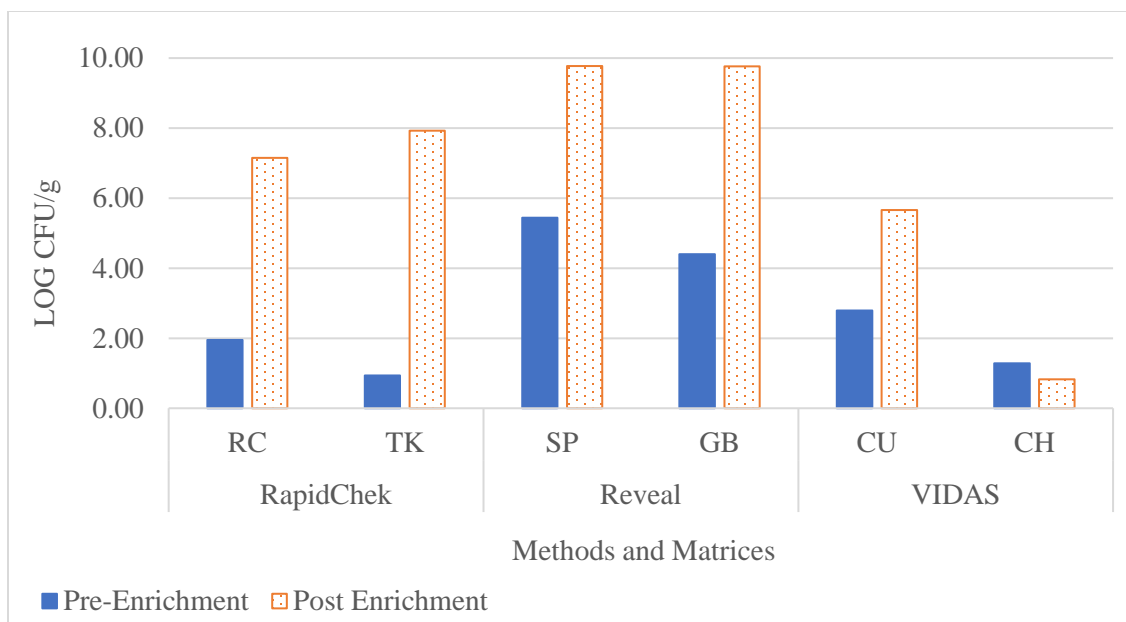


Figure 4.F4: Method Verification Pre and Post Enrichment Non-Inoculated Control Bacterial Levels. Non-Inoculated Control bacterial load levels pre and post enrichment for products selected for method verification testing for Romer RapidChek® *Listeria*, Neogen Reveal® 20-Hour *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT. Post enrichment results are averages. Results shown are on tryptic soy agar. Sample IDs: Ricotta Cheese (RC), Oven Roasted Turkey Breast Deli Slices (TK), Spinach (SP), Ground Beef (GB), Cucumber (CU), Rotisserie Seasoned Chicken Breast Deli Slices (CH).

Bibliography

- A2LA. 2001. *A2LA Food Microbiology Program Requirements*. "AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing," The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- A2LA. 2018. *A2LA: Currently Accredited Organizations*, The American Association for Laboratory Accreditation. <https://www.a2la.org/>.
- AAFCO. 2014. *ISO 17025 Accreditation Quality Management Systems Panel Discussion*. edited by Kristi McCallum Association of American Feed Control Officials, Colorado Department of Agriculture. Colorado.
- AOAC. 2006. *AOAC International Presidential Task Force on the Best Practices in Microbiological Methodology*. AOAC International Standards Development. Gaithersburg, MD.
- AOAC. 2015. *AOAC International: Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals*. AOAC International.
- AOAC. 2002. *AOAC Official Method 2000.14 Reveal for E. coli O157:H7 Test in Selected Foods and Environmental Swabs*. AOAC International Standards Development.
- AOAC. 2009. *AOAC Official Method 2000.14 Reveal for E. coli O157:H7 Test in Selected Foods and Environmental Swabs 20-Hour Method. 2*. Vol. 92. AOAC International Standards Development. Journal of AOAC International.
- AOAC. 2016a. *AOAC Official Method 2013.01 Salmonella in a Variety of Foods VIDAS UP Salmonella (SPT) Method*. AOAC Standards Development.
- AOAC. 2017. *AOAC Performance Tested # 020401: RapidChek Listeria - Romer Labs Technology Inc*. AOAC International Standards Development, AOAC Performance Tested. Rockville, Maryland.
- AOAC. 2016b. *Appendix F Guidelines for Standard Method Performance Requirements*. AOAC International Standards Development. aoac.org.
- AOAC. 2012. *Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*. AOAC International Standards Development.
- AOAC. 2013. *Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*. AOAC International Standards Development. aoac.org.

Araujo, Pedro. 2009. "Key Aspects of Analytical Method Validation and Linearity Evaluation." *Journal of Chromatography, B*, 877 (23): 2224-2234.
doi:10.1016/j.jchromb.2008.09.030.

BioMérieux. 2013. *VIDAS UP Salmonella (SPT) REF 30 707 Instructions*, REF 30 707 9300912 F-EN. BioMérieux. Hazelwood, MO, USA.

BioMérieux. 2017a. *VIDAS: Constantly Evolving with You*. BioMérieux. Hazelwood, MO, USA.

BioMérieux. 2017b. *VIDAS UP Salmonella (SPT) Ultra Performance Summary*. BioMérieux, Web. Hazelwood, MO, USA.

FDA. Food and Drug Administration, "Recalls, Market Withdrawals, and Safety Alerts." last modified Apr 12 2018, accessed 4/12/2018. 2018. <https://www.fda.gov/>.

FDA. 2015. *Guidelines for the Validation of Analytical Methods for the Detection of Pathogens in Foods and Feeds*. 2nd ed. Department of Health and Human Services, Public Health Services, United States Food and Drug Administration Office of Foods and Veterinary Medicine.

FDA. 2014. *ORA Laboratory Procedure Food and Drug Administration: Methods, Method Verification and Validation*, ORA-LAB.5.4.5. ORA-LAB.5.4.5, 1.71-19. Food and Drug Administration. http://www.fda.gov/ora/science_ref/lm/default.htm.

Feinberg, Max and Michel Laurentie. 2006. "A Global Approach to Method Validation and Measurement Uncertainty." *Accreditation and Quality Assurance*, 11 (1): 3-9.
doi:10.1007/s00769-005-0081-9.

Grochau, Inês, Carlos Ferreira, Jane Ferreira, and Carla ten Caten. 2010. "Implementation of a Quality Management System in University Test Laboratories: A Brief Review and New Proposals." *Accreditation and Quality Assurance*, 15 (12): 681-689.
doi:10.1007/s00769-010-0713-6.

IAFP. 2018. *Validation, Verification Or just Semantics?* Edited by Michael Brodsky and DeAnn Benesh. International Association for Food Protection.
<https://www.foodprotection.org/>.

ISO. "ISO: Popular Standards." International Organization for Standardization, accessed 4/12/2018. 2018. <https://www.iso.org/>.

ISO. "ISO: The Main Benefits of ISO Standards." International Organization for Standardization, accessed 4/12/2018. 2018.

ISO and IEC. 2005. *ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories*. 2nd ed. Geneva, Switzerland: International Organization for Standardization.

ISO and IEC. 2017. *ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories*. 3rd ed. International Organization for Standardization. Geneva, Switzerland.

Kurbanoglu, Sevinc, Bengi Uslu, and Sibel A. Ozkan. 2018. "Chapter 4 - Validation of Analytical Methods for the Assessment of Hazards in Food." In *Food Safety and Preservation*. edited by Alexandru Mihai Grumezescu and Alina Maria Holban, 59-90. Ankara, Turkey. Academic Press, Elsevier Inc.

NATA. 2013. *Guidelines for the Validation and Verification of Quantitative and Qualitative Test Methods*. National Association of Testing Authorities. Commonwealth of Australia. Australia.

Neogen. 2016. *Neogen Reveal for E. coli O157:H7 Instruction Pamphlet*. Reveal 20-Hour *E. coli* O157:H7 AOAC No.2000.14. Neogen Corporation. Lansing, MI. www.neogen.com.

Penn State University. "10.3 Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value STATS 507." accessed 10/25/2017. 2017.

Rambla-Alegre, Maria, Josep Esteve-Romero, and Samuel Carda-Broch. 2012. "Is it really Necessary to Validate an Analytical Method or Not? that is the Question." *Journal of Chromatography A*, 1232: 101-109. doi:10.1016/j.chroma.2011.10.050.

Romer. 2016a. *Romer Labs Listeria Quick Guide - RapidChek Listeria System*. Romer Labs Technology Inc. Newark, DE.

Romer. 2016b. *Romer Labs RapidChek Listeria Species Test System Package Insert*. Romer Labs Technology Inc. Newark, DE.

Romero, J., I. Manero, and J. Laso. 2007. "Validation of Geosmin and 2-Methyl-i-Borneol Analysis by CLSA-GC-FID Method to Obtain ISO-17025 Accreditation." *Journal of Chromatographic Science*, 45 (7): 439-446. doi:10.1093/chromsci/45.7.439.

Statistical Solutions. "Qualitative Sample Size.", accessed Oct 25 2017. 2017. <http://www.statisticssolutions.com/qualitative-sample-size/>.

Taverniers, Isabel, Marc De Loose, and Erik Van Bockstaele. 2004. "Trends in Quality in the Analytical Laboratory II Analytical Method Validation and Quality Assurance." *TrAC Trends in Analytical Chemistry*, 23 (8): 535-552. doi:10.1016/j.trac.2004.04.001.

Thompson, Michael, Stephen L. R. Ellison, and Roger Wood. 2002. "Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis (IUPAC Technical Report)." *Pure and Applied Chemistry*, 74 (5): 835-855. doi:10.1351/pac200274050835.

Trullols, Esther, Itziar Ruisánchez, and F. Xavier Rius. 2004. "Validation of Qualitative Analytical Methods." *Trends in Analytical Chemistry*, 23 (2): 137-145. doi:10.1016/S0165-9936(04)00201-8.

USDA, FSIS. 2017. *Foodborne Pathogen Test Kits Validated by Independent Organizations*. United States Department of Agriculture Food Safety Inspection Services.

USDA, FSIS. "Recalls and Public Health Alerts: Current Recalls and Alerts." United States Department of Agriculture Food Safety and Inspection Service, last modified Feb 14 2018. accessed Apr 12, 2018. <https://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/current-recalls-and-alerts>.

CHAPTER 5

SUMMARY, THE FUTURE, AND CLOSING REMARKS

SUMMARY OF RESULTS

Throughout this thesis the implementation of ISO practices in small and academic laboratories as it relates to accreditation requirements set forth in ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories have been documented and studied. The following paragraphs are a summary of the ISO/IEC management and technical requirements developed and implemented, results from methods developed for control of various laboratory processes, and conclusions stated within the results sections for each chapter. All information shared in this section can be found in greater detail throughout the other chapters of this dissertation.

Implementation of a quality management system (QMS) and an all-encompassing management system is extremely time consuming and involves the development of many documents, policies, processes, and procedures. It takes the dedication of not only laboratory staff but also management as this process can take several years to complete with development of the QMS and implementation of all support programs taking the FPCLS 1 ½ years to accomplish. Management requirements for ISO accreditation that were addressed included: confidentiality policies; development of the QMS; service to the customer and complaints; document control; corrective and preventive action; internal audits; and management reviews. Technical requirements that were addressed for ISO accreditation included: personnel policies and procedures; sampling; handling test and calibration items; ensuring the quality of test results and reporting of results to clients; and the implementation of several support programs such as environmental monitoring, temperature monitoring, equipment maintenance and calibration, proficiency testing, and training programs.

In all, the FPCLS developed over 63 standard operating procedures, 103 forms for various laboratory operations and test methods, 19 manuals and lists to guide and support laboratory functions, and 6 support programs to make sure the management system works as intended. Despite the size of the FPCLS, each of these programs, policies, and procedures were necessary to prepare in becoming ISO/IEC 17025 compliant with their implementation leading to improvements in all laboratory processes with many benefits that have already been realized.

As part of the preparation for ISO accreditation, a media qualification verification procedure was developed to evaluate the suitability of various types of microbiological media utilized by the FPCLS as part of in scope methods looking for the presence of foodborne pathogens. Media qualification is very important to the success of any laboratory as microbiological media is part of almost all laboratory test procedures and can greatly affect the results if the media is not suitable for use. As part of this project the FPCLS evaluated three categories of media and five media types that directly affect analyses part of the scope of accreditation for ISO/IEC 17025.

These media categories and types included: non-selective solid media – tryptic soy agar (TSA); non-selective liquid media – tryptic soy broth (TSB) and buffered peptone water (BPW); and selective liquid media – Romer RapidChek[®] *Listeria* media (LRC) and Neogen Reveal[®] 20-Hour *E. coli* O157:H7 media (REC). Each media type was evaluated at several time points throughout its shelf life for growth acceptability (AGI scores, turbidity) and various quality parameters such as cracks in agar, drying or thinning of agar, presence of contamination, color change (lighter or darker from initial color), pH, and volume loss (by weight) depending on the media type.

It was found that most microbiological media were more stable, as expected, at refrigeration temperatures with TSA being fairly stable throughout its shelf life of 98 days at refrigeration temperatures with only a few failing marks for growth and drying/thinning but approaching unacceptable marks at just 7 days at room temperature. We also found that the pH of TSB dropped at room temperature but not refrigeration temperatures over the shelf life of 14 weeks, and that the pH of BPW was not as affected by storage temperature or time and but still showed statistically significant changes.

Volume loss at room temperature for both TSB and BPW were significant ($p < 0.05$) over time affecting the growth of the control organisms while volume loss was less of a factor for LRC or REC (despite being significant for REC) media types as the shelf life is too short to see a dramatic effect. In addition, we found that REC media is very stable over its shelf life only failing for negative control growth twice throughout all testing, while LRC was unstable by 24 hours for one of the negative controls and failing sporadically, even at time “0” hours, for the other negative control regardless of storage temperature.

Based on the results from this study we were able to determine the acceptable growth and quality parameters necessary to distinguish the difference between acceptable and non-acceptable microbiological media for use in the FPCLS. We also utilized the media qualification verification procedure as a template for developing a media qualification standard operating procedure for use in evaluating purchased dehydrated media on an everyday basis. These media qualification findings and procedures may be utilized by other laboratories looking to improve their processes or aid them in qualifying

their microbiological media for acceptability in use of procedures for obtaining ISO/IEC 17025 accreditation.

After developing a quality management system and implementing an encompassing management system to guide and direct the laboratory, it became necessary to verify the methods that were selected as part of the scope of accreditation for ISO/IEC 17025 compliance. Method verification is an extremely important part of the preparation process for obtaining ISO/IEC 17025 accreditation. As part of this project the FPCLS developed verification methods for and verified three rapid qualitative methods (Romer RapidChek® *Listeria*, Neogen Reveal® for *E. coli* O157:H7, and BioMérieux VIDAS® UP *Salmonella* SPT) for the detection of foodborne pathogens as part of the scope of accreditation for ISO compliance.

It was determined that the FPCLS is capable of performing approved methods (AOAC) as they were intended and are competent in obtaining expected results. All three rapid qualitative methods tested gave results of 100% sensitivity for all food matrices evaluated meeting the requirements for qualitative method verification within the ISO standards. Method verification procedures and processes developed in this project already meet the requirements that will be released under ISO 16140-3 for acceptable qualitative method verification of approved methods for the purpose of obtaining ISO accreditation. Furthermore, the procedures developed as part of this method verification process may be used as a guide to assist other laboratories who are attempting to verify qualitative methods for improvement or for obtaining ISO accreditation status.

This project shows that academic laboratories are capable of implementing quality management systems and verifying media control procedures and foodborne pathogen

detection methods to become ISO compliant and pursue ISO/IEC 17025 accreditation. Furthermore, this project has provided the FPCLS the opportunity to grow and progress while becoming a suitable option for food industry establishments looking for assistance and guidance with their food safety concerns and needs. Other academic and professional laboratories may use the documents and procedures developed by the FPCLS as a guide for improvement and utilize the method verification procedures as a template for pursuing ISO accreditation status. Finally, the FPCLS is now in a position to pursue ISO/IEC 17025 accreditation if they desire to do so.

FUTURE WORK AND IMPROVEMENTS

Adulterated food products potentially leading to foodborne illnesses continue to be a major concern for the food industry and for consumers worldwide. Outbreaks associated with foodborne pathogens are occurring at an alarming rate and are causing not only illnesses leading to millions of dollars in medical expenses and loss of food supply, but also have recently been leading to more and more deaths in many countries from the United States of American to South Africa. Because of this, it is important to continue to properly monitor the food supply prior to it being released into commerce through sample analyses conducted by accredited laboratories. In this project we evaluated the requirements necessary for a laboratory to become accredited to ISO/IEC 17025 standards in order to accurately and competently test food products for pathogenic microorganisms and aid the food industry in lowering the risk of foodborne pathogen related recalls and outbreaks while also providing safer food products to consumers.

Based on the evaluations of ISO practices conducted in this dissertation there are several more experiments and alternations that should be considered to further prepare the FPCLS for ISO/IEC 17025 accreditation and guide other laboratories in improving their processes to meet ISO accreditation requirements. During the initial stages of this project we developed and implemented many management requirements to design a unique quality management system for the FPCLS. It is essential to reevaluate the entire system now that it has been implemented in order to determine areas that could be improved upon to better meet or exceed ISO accreditation requirements. One example where improvement could be made would be to reevaluate the Quality Manual to verify that it meets all of the requirements within the new standards that are being released and contains all of the necessary information needed for the laboratory to be successful.

Other areas that should be reevaluated within the management system include implementing more robust environmental monitoring and laboratory maintenance (cleaning) programs. Although the current programs are adequate and meet the minimum requirements for ISO accreditation, they should be expanded and enhanced. The environmental monitoring program should include more precise sampling locations that are controlled and randomly selected. Currently the environmental monitoring sites are not on a predetermined list and are chosen by the technician performing the procedure and not a random generator. By adding all monitoring sites to a list and allowing the list to randomly select the sites for evaluation, the environmental monitoring program becomes less subjective and more controlled.

Along with environmental monitoring, laboratory maintenance (cleaning) programs should be improved to include additional cleaning requirements and weekly

checkups. Currently the system is in place but is not always being followed as weekly maintenance (cleaning) is not tracked ensuring standard cleaning functions are being performed. The program needs to be reevaluated to determine how to truly implement the cleaning portion of the program and make it effective while holding the laboratory accountable.

Finally, methods need to be developed and experiments need to be performed within the FPCLS to expand the scope of accreditation, specifically for quantitative procedures. As part of this dissertation we only developed methods for the media qualification program and performed method verification for three rapid qualitative methods; Romer RapidChek[®] *Listeria*, Neogen Reveal[®] for *E. coli* O157:H7, and BioMérieux VIDAS[®] UP *Salmonella* SPT. Quantitative procedures are vital to foodborne pathogen testing and the expansion and success of the laboratory will be dependent on being able to add these types of procedures to the scope of accreditation.

Quantitative procedures that should be considered include; aerobic plate count (TSA and Petrifilm), yeast and mold count (DRBC and Petrifilm), *Enterobacteriaceae* counts (Petrifilm), coliform and *Escherichia coli* count (Petrifilm), lactic acid bacteria count (MRS or other agars), *Listeria* spp. count (OX and Petrifilm), *Salmonella* count (XLT4, XLD), pH, water activity, and BAX[®] PCR. Adding these types of tests to the scope of accreditation will help the FPCLS provide better service to the food industry while the methods developed for their evaluation will serve as guides for other laboratories to follow for improvement or for attempting ISO/IEC 17025 accreditation within their laboratory's.

CLOSING REMARKS

In closing, it has been my privilege to work in the Food Processing Center Laboratory Services and help develop and implement a fully functional quality management system that will be the backbone for the FPCLSs testing laboratory in obtaining ISO/IEC 17025 accreditation. Additionally, it has been exciting to learn about ISO guidelines related to testing facilities, all of the requirements in order to create a management system and determine the best solutions for laboratory issues related to ISO compliance. It was also gratifying to be involved in ensuring that all laboratory processes, procedures, and methods were ISO compliant in order to show the competency of the laboratory and aid the FPCLS in providing better higher quality service to the food industry.

Developing methods and guides that will aid not only the FPCLS but also other laboratories in providing testing services that facilitate safer food products reaching consumers is essential for the growth and progress of the food industry. Continued understanding of food safety guidelines and the addition of more accredited food testing facilities that are competent to perform analyses on food products for the presence of foodborne pathogens is not only necessary but is vital for the future of the food industry and the health of the human population.

If we continue to grow and progress as an industry in understanding the regulations and guidelines necessary to produce safer higher quality products for commerce and continue to improve laboratory testing capabilities for ensuring the food supply is safe, we will ultimately find those solutions we need to make our food products safer for everyone worldwide. If we do not continue to grow and progress in the food

industry, especially in laboratory testing facilities, then outbreaks related to foodborne pathogens will continue to be a major food safety concern causing death and disease to mankind regardless of geographical location.

I am very grateful that I was given the opportunity to help develop methods and procedures that will aid the food testing industry in improving laboratory processes and procedures for the detection of foodborne pathogens in food products. I look forward to continuing with process and program development as well as research to help make food safer for all consumers around the world so that they might have peace of mind when consuming the foods that they enjoy!