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Use of a Newly Developed Rapid Microbial ATP Bioluminescence Assay to Detect Microbial Contamination on Poultry Carcasses†‡

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A newly developed rapid microbial ATP bioluminescence test (R-mATP) was shown to be an adequate means to assay the microbial load of poultry carcasses. This assay utilizes differential extraction and filtration to separate somatic from microbial ATP in a very rapid timeframe. The assay requires approximately 5 min to complete; approximately 3.5 min to sample and 90 s analytical time. Correlation coefficient (*r*) between aerobic colony counts and R-mATP test results (*n*=329) was 0.82. Post-test probabilities to correctly classify carcasses with different levels of microbial contamination were as high as 98% for samples of ⩾3.5 log aerobic CFU per ml. Given the rapidity of this assay, the R-mATP holds potential for monitoring the microbial load of carcasses at poultry-processing critical control points. Other potential applications of this new version of the microbial ATP bioluminescence test are discussed. © 1996 by John Wiley & Sons, Ltd.

INTRODUCTION

In the case of meat animals and poultry, most microbial contamination is faecal or soilborne in origin (1) and, prior to washing or chilling, unusually

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‡ Mention of a trade name, proprietary product or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

high concentrations of bacterial contamination on a carcass are likely indicative of potential faecal contamination. Faeces and the intestinal tract are known to be sources of Gram-negative bacterial pathogens such as *Salmonella* spp., *Campylobacter jejuni* and *Escherichia coli* (2).

By definition, HACCP monitoring requires real-time or near real-time information for taking proactive measures to correct any process which is out of control (3). Meat animal and poultry processors concerned with product microbial safety have used traditional culture methods to monitor levels of generic aerobic mesophilic bacteria, coliforms and

faecal coliforms, and the incidence of specific patho-
genic bacteria. Such monitoring is not useful for
HACCP, since it usually requires at least 16–36 h to
obtain results; therefore, monitoring the microbial
load of meat animal or poultry carcasses during
processing requires a near real-time microbial test in
order for proactive measures to correct process
deviations resulting in microbial contamination to be
rectified.

Microbial ATP bioluminescence testing has been
shown to be a rapid and accurate means of measuring
the microbial load of certain foods and many non-
food samples (4, 5). We have recently developed a
new rapid microbial ATP bioluminescence test (R-
mATP) which can rapidly and accurately measure the
level of microbial contamination on beef and pork
carcasses (6). The primary advantage of the R-mATP
test is rapidity (total test time including sampling to
obtaining a result is approximately 5 min), which
allows plant personnel to assay the carcass microbial
load in real time, something that is not achievable in
the 36 h required to perform the standard aerobic
colony count.

The objective of this research was to evaluate the
efficacy of the R-mATP assay as a rapid monitor of
the poultry broiler carcass microbial load.

MATERIALS AND METHODS

Sampling

A total of 320 poultry broiler carcasses were obtained
from three different plants. Eighty poultry broiler
carcasses were selected from each of the following
same four sites or critical control points within each
plant: post-defeathering; post-evisceration; post-
wash; and post-chill. Carcasses were chosen from the
processing line to represent a variety of levels of
contamination ranging from visibly contaminated
with faeces to no visible faecal contamination. In the
plant, the entire exterior surface of each bird was
sponged using a Speci-Sponge (NASCO, Fort Atkin-
son, WI, USA) moistened in 25 mL of buffered
peptone water (Difco, Detroit, MI, USA) containing
0.5% (v/v) Tween 20 and 0.5% (w/v) glucose. The
solution was expressed from the sponge as it was
removed from the bag using a sterile glove. The
sponge was wiped firmly over the entire outside
surface of the bird carcass, turning the sponge at least
twice. The sponge was then placed into the bag
containing the residual sponge-moistening solution,

held at between 8 and 10°C and analysed within 2 h of
sampling. Sponges and all fluid contents were trans-
ferred to a filtered stomacher bag (Spiral Biotech,
Bethesda, MD, USA) and stomached in a LabBlender
400 stomacher (Tekmar, Cincinnati, OH, USA) for 2
min. The sample was withdrawn from the filtered
side, transferred to a conical centrifuge tube and
analysed.

Bacterial plate counts

Samples were serially diluted in buffered peptone
water (BBL, Cockeysville, MD, USA). For aerobic
colony counts, samples were either spiral plated
(Model D Spiral Plater: Spiral Systems Instruments,
Bethesda, MD, USA) or pour-plated with tryptic soy
agar (BBL, Becton and Dickinson, Cockeysville, MD,
USA) and incubated for 36 h at 35°C. Bacterial counts
were converted from colony forming units (CFU) per
mL to $\log_{10}$ CFU/mL.

Rapid microbial ATP assay (R-mATP)

The major challenge in using mATP as a means of
determining total microbial populations in food
samples is the separation of non-microbial ATP from
microbial ATP. The basis of the described R-mATP
assay is the use of a filtration device in which somatic
ATP is extracted and then removed within the same
device; extraction of bacterial ATP is followed by its
quantification.

The steps of the R-mATP assay (6) are as follows.
50 μL of the carcass sponge sample was added to a
Filtravette™ (New Horizons Diagnostics, Columbia,
MD, USA) followed by 100 μL of a somatic ATP
extraction reagent, NRS™ (nucleotide releasing agent
for somatic cells: Lumac B.V., Perstorp Analytical,
The Netherlands). The fluid was then aspirated
through the Filtravette™ using a vacuum manifold
(New Horizons Diagnostics, Columbia, MD, USA)
and trap. Another 150 μL of NRS™ were added and
pulled through the device. At this stage, the Fil-
travette™ retained bacteria and other cellular debris
on the filter's surface, somatic cell ATP and free ATP
having been removed by the action of the somatic cell
extractor, NRS. The Filtravette™ , with its extracted
contents, was placed in the holding drawer of the
Profile™-1 Model 3550 Microluminometer (New
Horizons Diagnostics, Columbia, MD, USA). 30 μL
of the microbial ATP extractor, NRB™ (nucleotide ·
releasing agent for bacteria; Lumac B.V., Perstorp

Analytical, The Netherlands) were added, followed immediately by 30 μL of luciferin/luciferase reagent (New Horizons Diagnostics, Columbia, MD, USA) reconstituted to manufacturer's recommended volume with Lumit buffer (Lumac B.V., Perstorp Analytical, The Netherlands). The fluid was mixed by rapidly aspirating three times with the micropipettor, the drawer to the luminometer was closed and light emission was integrated over 10 s. The microbial ATP level was recorded as relative light units (RLU), taken directly from the luminometer's digital readout. Microbial ATP content is reported as the log_{10} relative light units normalized to 1 mL sample volumes (log RLU/mL). Each assay was performed in duplicate and the average RLU value calculated. All reagents were used at room temperature and were checked for contaminating ATP before use. The time required to perform the steps of the R-mATP after sampling is approximately 90 s.

Statistical analysis

Linear regression analysis was performed on scatter-plots of the mATP values (RLU) vs. the viable microbial counts using SAS (Cary, NC, USA) and InStat2 Version 2.0 statistical analysis package (GraphPad Software, San Diego, CA, USA). Likelihood ratios were calculated according to Sackett et al. (7).

RESULTS

The time required to sample a poultry carcass and perform the R-mATP test was under 5 min per sample.

The R-mATP test data obtained from poultry carcasses sampled from four sites in each of three different plants were plotted against the corresponding aerobic colony counts (Fig. 1). R-mATP results reflected the concomitant changes in aerobic colony count from carcasses sampled throughout the process. The aerobic colony count averages were 4.89, 5.01, 4.12, and 3.34 log_{10} CFU/mL from the post-defeathering, post-evisceration, post-wash and post-chill sampling sites, respectively. The R-mATP results were 3.83, 4.03, 2.94, and 1.59 log_{10} RLU/mL from the post-defeathering, post-evisceration, post-wash and post-chill sampling sites, respectively. Regression line parameters are presented in Table 1. The overall correlation from all three plants combined, $r=0.82$, was lower than that reported for beef ($r=0.91$) and pork ($r=0.89$) datasets (6). As indicated by comparable regression equations and $S_{y.x}$ values (6), the R-mATP test performed similarly in the case of poultry as in the response obtained from beef and pork sampling. Plotting the regression lines from the three different poultry plants indicated that the test response was similar between plants (Table 1). Graphical analysis of the regression lines between the three plants showed no difference in the assay response
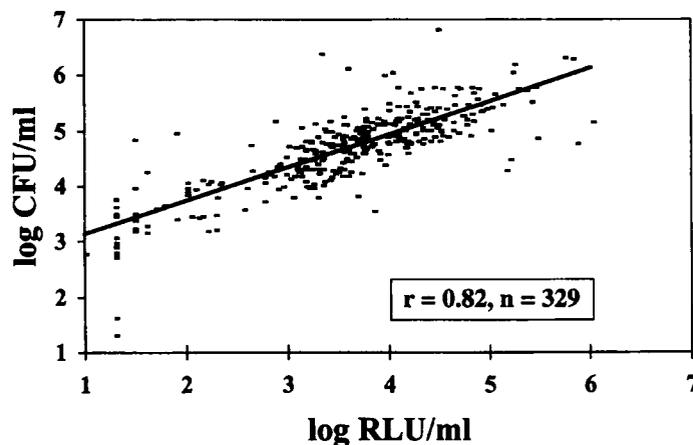

Figure 1. Scatterplot of R-mATP assay values (log RLU/mL) and 35°C aerobic colony counts (log CFU/mL) from poultry carcass in-plant sponge samples

Table 1. Regression parameters of linear regression plots from R-mATP assay data (RLU) and aerobic colony count (AER) culture method scatterplots from poultry in-plant data

Plant(s)	Regression equation[1]	n	r	$S_{y.x}^2$
1	AER = (0.64)RLU + 2.31	80	0.81	0.65
2	AER = (0.65)RLU + 2.41	40	0.80	0.58
3	AER = (0.51)RLU + 2.91	209	0.81	0.36
1, 2 and 3	AER = (0.60)RLU + 2.54	329	0.82	0.42

[1] RLU = $\log_{10}$ RLU/mL; AER = $\log_{10}$ aerobic CFU/mL.
[2] $S_{y.x}$ = standard deviation of residuals from regression line.

between plants.

Likelihood ratios were used to calculate post-test probability that a specific R-mATP test result would indicate a given aerobic microbial count level for a poultry carcass sponge sample within the collected dataset. Table 2 lists the post-test probabilities of a sample containing a specified aerobic bacterial population for different R-mATP test results. Post-test probabilities that the R-mATP test result correctly indicated the aerobic colony counts were more than 92% for samples which were within the range of the greater majority of samples composing this dataset (≥ 3.5–4.5 $\log_{10}$ CFU/mL).

DISCUSSION

A number of factors affect the ATP content of bacterial cells and these were discussed previously (4). The nature of poultry processing is such that carcasses are left with the skin on the carcass, and then are subjected to a number of treatments that have the potential to greatly affect the per cell ATP content. These treatments include scalding, spray washing

Table 2. Post-test probabilities (calculated from likelihood ratios) of the R-mATP test result predicting aerobic microbial population of poultry sponge samples

R-mATP result[1]	Aerobic Count[2]	Post-test probability (%)
≥ 2.5	≥ 3.5	98
≥ 3.5	≥ 4.0	97
≥ 4.0	≥ 4.5	92
≥ 5.0	≥ 5.0	75

[1] $\log_{10}$ RLU/mL.
[2] $\log_{10}$ CFU/mL.

with chlorinated water and chilling in chlorinated chill tanks (4–5°C) for up to approximately 45 min, all of which are treatments not encountered in beef or pork processing.

Likelihood ratios are used to evaluate clinical diagnostic tests for post-test probabilities of both a positive and negative result occuring for the condition in question. The likelihood ratio is a means of evaluating the predictive power of a diagnostic test, and was chosen for the current study since this statistic is not affected by prevalence or sample distribution within a target population, and can be used to evaluate different levels of response of a test for a specified condition (7). For the current application, the likelihood ratio is defined as the odds that a given test result is expected in a sample with the target level microbial load. This information would provide the in-plant user with a means of interpreting the R-mATP test results obtained for monitoring the microbial load of poultry carcasses. The R-mATP is not meant to be a replacement for the aerobic colony count method, neither is it necessarily indicative of the microbial load of carcasses at all different processing plants, although the between-plant response was similar (Table 1).

Bautista *et al.* (8) have used a different microbial ATP bioluminescence test, requiring approximately 15 min to perform, to validate the use of microbial ATP bioluminescence to monitor microbial load of poultry scald and chill tank water. The same group used the standard poultry rinse sampling method (9) with a different microbial ATP bioluminescence test to monitor poultry carcasses (10). In that study, from a database of 149 carcass rinses, the overall correlation (r) was 0.85 and the reported agreement between standard aerobic colony counts and the 15 min microbial ATP bioluminescence test was classed 'very good' by descriptive kappa statistic (10). The method of Bautista (10) differed from the R-mATP procedure in that it utilized enzymatic degradation of somatic ATP as a means of eliminating the somatic ATP content from samples, lipase treatment and a dual filtration step (10). As in the R-mATP technique, the method of Bautista *et al.* (10) relies on a coarse filtration to enhance the response of the microbial ATP bioluminescence test. The R-mATP procedure uses a coarse glass fibre filter incorporated into the stomacher bag to accomplish a similar filtration.

Since the R-mATP test requires less than 90 s to perform (excluding sampling), R-mATP test results could provide the user with a rapid means of assessing the microbiological status of a poultry-processing operation. For use as a HACCP monitor, R-mATP test

data would have to be collected over time at the specific critical control points of concern to each individual processor. Such data could be useful as an indicator of unusually high levels of microbial contamination, such as that contributed by excessive faecal contamination or a breakdown in the usual processing treatments designed to clean carcasses and reduce microbial loads, as they are processed.

The R-mATP test described has the potential for use as a microbial load monitor of the poultry conversion process at critical control points such as those described in this report. In its current form, the R-mATP test is not a substitute for the standard culture method aerobic colony count, since the R-mATP test could possibly detect any ATP contributed not only by aerobic but also by anaerobic bacteria. However, the R-mATP test offers microbial load data in a rapid time-frame, allowing processors to take proactive measures in the event of process deviations.

As far as other potential uses of the R-mATP test, liquid samples that are easily filtered with microbial loads of greater than approximately 10^3 CFU of bacteria per mL are ideally suited for microbial load testing using this assay. Among the advantages to using the R-mATP is the removal of substances (colour contributing substances, high salt content, etc.) which might interfere with or inhibit the luciferin–luciferase reaction in standard ATP bioluminescence tests. Applications to starter culture testing for fermented foods and beverages, industrial fermentations and antimicrobial efficacy testing are well suited for this type of assay due to its versatility. The rapidity of the R-mATP assay renders it ideal, not only for food plant HACCP monitoring but also for the study of bioenergetic or metabolic inhibition assays that require rigid sample timing. These possibilities remain to be documented utilising the R-mATP protocol.

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