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A Rapid Microbial ATP Bioluminescence Assay for Meat Carcasses

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SUMMARY

A new, rapid microbial ATP bioluminescence (R-mATP) assay has been developed in our laboratories that can be used to determine the levels of generic bacterial contamination on meat-animal carcasses. The R-mATP assay does in 5 min what standard culture methods for bacterial isolation take 36 h to accomplish. ATP is the energy molecule of all living cells, including bacteria. A sample with high amounts of microbial ATP indicates high levels of bacteria, which may be encountered when fecal contamination occurs on the carcass surface. The steps for the R-mATP assay are: (i) a sponge is used to sample the carcass surface; (ii) bacteria are removed from the sponge; (iii) nonbacterial ATP is removed from the sample; and (iv) the bacterial ATP is detected. The R-mATP assay was validated during several in-plant studies with over 1,000 beef, pork, and poultry carcasses, resulting in agreements between the R-mATP assay and standard culture methods of over 90%. Implementation of the assay in red-meat processing facilities demonstrated that the R-mATP assay can provide processors or meat inspectors with a rapid means to detect microbial contamination on carcasses and allow for improvements in the overall microbial quality of the carcasses before they leave the slaughter floor. Based on our laboratory and in-plant studies, the R-mATP assay is a rapid and near real-time means of estimating the microbial load of an animal carcass.

INTRODUCTION

Until recently, assessments of the microbiological quality of meats, meat-animal carcass surfaces, food-contact surfaces, foods, and equipment were done retrospectively by obtaining samples and performing standard plate counts requiring 24 to 48 h. Real-time or near real-time methods for detecting microorganisms are essential for implementation of a Hazard Analysis of Critical Control Point (HACCP) program in any food process.

ATP bioluminescence is an alternative to the standard plate count for estimating microbial loads. Adenosine triphosphate (ATP) is an energy molecule in all living cells, including insects, plants, animals, bacteria, molds, or yeast. ATP bioluminescence, as referred to in this paper, is the technique of measuring ATP based on light emission during a bioluminescent (light generated from a life form) reaction. The underlying premise of ATP bioluminescence testing is that the amount of ATP in a sample is proportional to the biomass. In the case of bacteria, there exists a strong correlation between cell number and

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Figure 1. The Luciferin/Luciferase bioluminescence reaction.

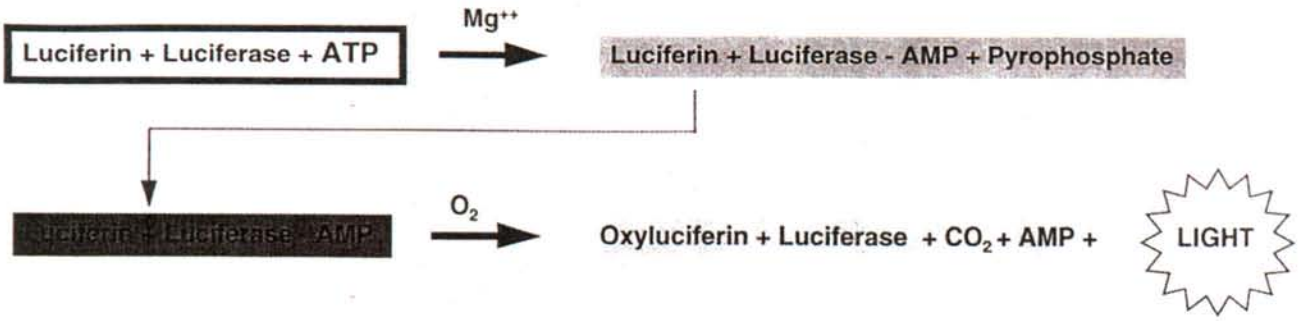


TABLE 1. Analysis using ATP bioluminescence

Sample type	Need for somatic ATP removal/inactivation	Analyte	End use
Hygiene monitoring or environmental surface monitoring	No	Total ATP	Sanitation of work and equipment surfaces
Meat animal carcass surfaces	Yes	Microbial ATP	Microbial load of product
Meats	Yes	Microbial ATP	Microbial load of product

ATP content. ATP can be measured using reagents extracted and purified from the abdomen of the firefly, *Photinus pyralis*. The very reaction that gives the glow worm its "glow" has been exploited for measuring ATP in a wide variety of samples *(15)*, including food and nonfoodstuffs. This technique is also known as ATP bioluminescence.

In the ATP bioluminescence reaction (Figure 1), luciferin is oxidized by the enzymatic reaction catalyzed in luciferase in the presence of magnesium and ATP. An end product of the reaction is energy in the form of yellow-green visible light ($\lambda \equiv 562$ nm). The emitted light, measurable with a luminometer, is directly proportional to the amount of ATP in the reaction mixture. Data can be reported as the actual amount of ATP calculated from standard curves. However, in practice, most workers report data directly from the luminometer as relative light units (RLU).

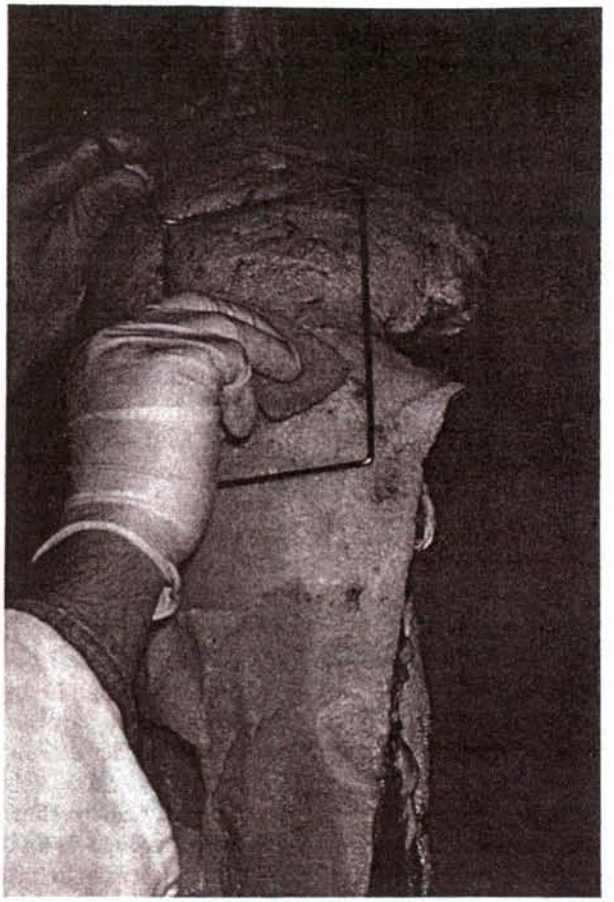
It is important to note that when measuring the ATP content of samples containing microorganisms, one is measuring an average of the ATP contents of the cells at that particular time. The ATP content of bacteria is in a state of constant flux and is also species dependent *(5, 7)*. When comparing ATP bioluminescence data to aerobic plate counts (APC), it is essential to remember that two different indicators of a microbial population are being compared: the APC is a growth-based assay; ATP bioluminescence, on the other hand, measures an existing metabolite.

A critical element of using an ATP bioluminescence assay is understanding that foods and processing-plant environmental samples contain large amounts of ATP that are from sources other than bacteria. While this additional source of ATP is not a consideration when doing hygiene or environmental testing, it is for the ATP bioluminescence testing of foods for microbial load (Table 1).

In microbiological tests based on ATP bioluminescence, the ATP that is from nonmicrobial sources is termed somatic ATP. As a guide, somatic cells contain about 100 to 1,000 times more ATP than do bacterial cells. For instance, the nonbacterial or somatic ATP contributed by cells from muscle in a ground beef sample has been calculated to be infinitely greater than the ATP contributed by bacteria on the ground beef *(11)*. In the case of environmental sampling in food plants (i.e., hygiene monitoring), ATP comes from a variety of sources (food, animal, and plant residues). Samples are tested for total ATP as an indicator of filth or potential breeding grounds for bacteria. In other words, any ATP in the sample, no matter its source, is taken as an indicator of filth or potential contamination. Other tests are designed to measure solely bacteria and must include means to either segregate the bacterial ATP from somatic ATP or destroy the somatic ATP so that all that is remaining in a sample is bacterial ATP. Since ATP from any source (plant, animal, bacterial) is chemically identical, separation of somatic from microbial ATP is a critical step when using ATP bioluminescence assays for measuring bacteria (Table 1). In fact, it was the development of such methods that opened the field of ATP bioluminescence testing for use with foodstuffs. Since it is not necessary to remove somatic ATP from environmental samples obtained for hygiene monitoring, this type of testing is simpler than that in instances in which only microbial ATP is of interest.

Figure 2. Sponge samples obtained from surfaces of meat animal carcasses.

A. A 150-cm² area of a skinned pork carcass is aseptically sampled in the bung area with a clean stainless-steel template and an ATP-free sponge premoistened in sponge solution.



A general procedure for separating somatic ATP from microbial ATP involves the following steps. First, nonmicrobial cells (animal cells such as those in milk or blood) are permeabilized by detergents that will not affect bacterial cell membranes. Secondly, the somatic ATP that is released into solution is destroyed using the enzyme ATPase (this step usually requires several minutes). Thirdly, the bacterial ATP is extracted from bacterial cells using reagents that can permeabilize bacteria. The remaining bacterial ATP is then measured using the ATP bioluminescence method (described earlier) and a luminometer.

The rapid microbial ATP (R-mATP) assay to be discussed, measures the microbial load of animal-carcass surfaces by separating somatic ATP from bacterial ATP during a differential chemical extraction, as described above, and an additional filtration step. This technique offers a very rapid means to segregate somatic from microbial ATP and the subsequent measurement of bacterial ATP for this type of sample. The rapidity of the R-mATP assay (total test time is 5 min including sampling) makes it a potentially useful tool for HACCP monitoring.

METHODS

ATP bioluminescence methods for meats

ATP bioluminescence has been used for microbial testing of a variety of meat products. Stannard and Wood *(16)* demonstrated that within 25 min, the ATP content of bacteria in raw beef, lamb, or pork homogenates could be determined following centrifugation, a cation-exchange resin treatment, filtration, and a bioluminescence assay. However, the procedures were only able to estimate ATP content from samples exhibiting ≥ 10^5 CFU/g. Bülte and Reuter *(3)* were able to correlate standard plate count assays and RLU values (r = 0.91) from beef samples inoculated with organisms at different growth phases after a 41-min procedure involving homogenization, centrifugation, and a bioluminescence assay. These procedures were only able to detect ATP from bacterial populations ≥ 5×10^6 CFU/g. For ground beef, a 1-h process involving homogenization, filtration, and incubation with a somatic releasing agent and ATPase, followed by a bioluminescence assay, yielded a correlation of 0.95 between APC and RLU values of samples *(8)*. In another study *(10)*, spoiled beef samples were homogenized, filtered through glass wool, subjected to a double-filtration technique to separate microbial cells from nonmicrobial cells, and assayed for microbial ATP. Despite correlations of (r) 0.96 between RLU and plate count data, the procedures took longer than 30 min and were not able to accurately detect ATP from samples with ≤ 5×10^4 CFU/g. Ward et al. *(17)* used an ATP bioluminescence test for fish (total time, 1 h) and obtained a correlation (r) of 0.97 with aerobic plate counts for samples ≥ 10^4 CFU/g. Vacuum-packaged, cooked, cured, meat products and ground beef were homogenized, somatic ATP extracted and hydrolyzed, and homogenates subjected to ATP bioluminescence assays within one hour *(9)*. The lower limit of sensitivity of this particular ATP bioluminescence test was approximately 10^4 CFU/g for ground meat and 10^5 CFU/g for cooked, cured meat products. More recently, Bautista et al. *(2)* demonstrated a correlation (r) of 0.85 for microbial number and RLU values obtained from chicken-carcass washings subjected to filtration, enzymatic treatment, and a microbial ATP bioluminescence test (total time, 15 min). The ATP content of bacteria taken from beef carcasses has also been demonstrated. Bautista et al. *(1)* treated excised beef carcass tissue with somatic cell extractant

Figure 2-B. The sponge is transferred to the WhirlPak™ bag and transported to the laboratory.

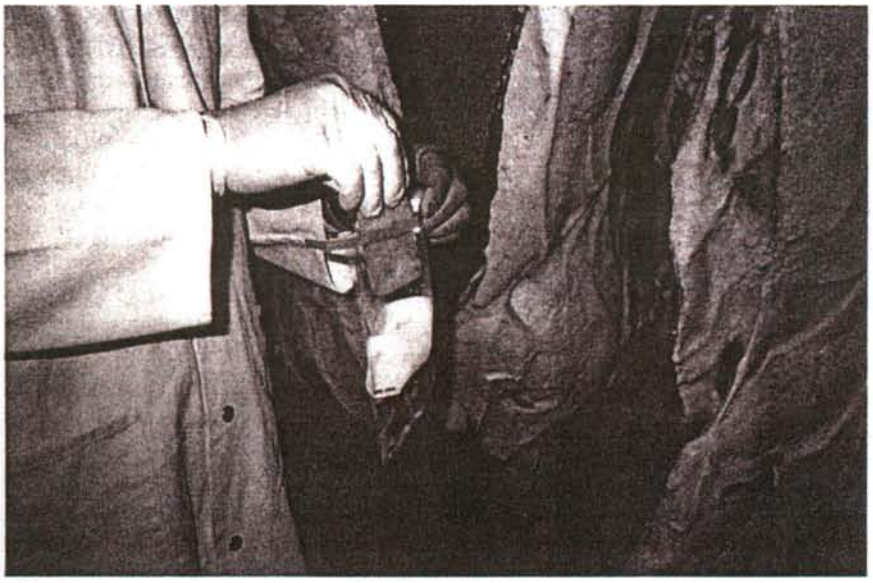
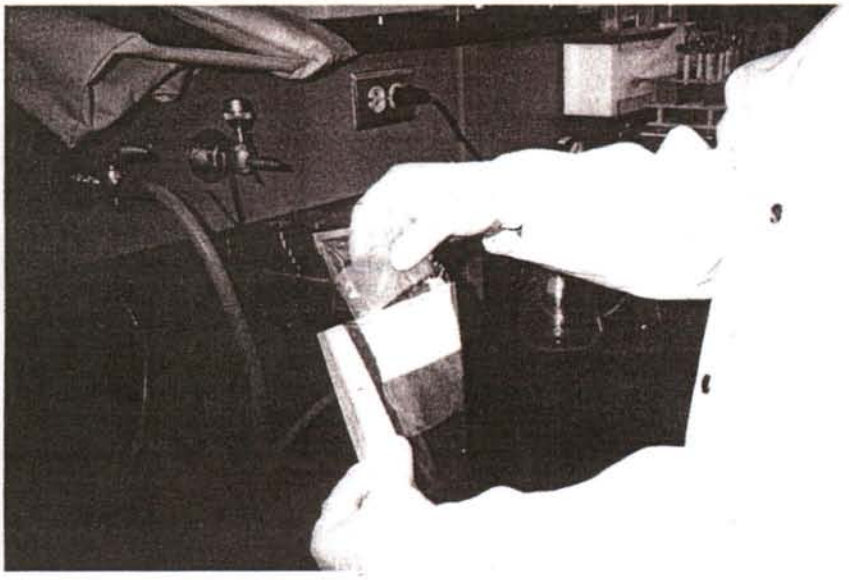


Figure 2-C. The sponge and all fluid is transferred aseptically to a Sterefil™ stomacher bag.



containing lipase, removed bacteria by filtration, and determined ATP content. Bacteria at 4×10^4 CFU/cm^2 were detected in 15 min using this methodology *(6)*. Siragusa and Cutter *(12)* used an ATP bioluminescence assay to determine the microbial load of fecally contaminated beef-carcass tissue. Microbial ATP was selectively distinguished from nonmicrobial ATP by the assay procedure and resulted in correlations (r) of > 0.90 between RLU values and aerobic plate count data *(12)*. As indicated by these examples, microbial testing of meat, meat products, and animal carcasses can be accomplished in hours or minutes using ATP bioluminescence tests versus days when using traditional culture methods. The remaining challenges to improving the ATP bioluminescence test are to decrease the level of sensitivity and to reduce assay time in order for these assays to be used on a real-time basis under normal in-plant processing conditions. In the next section, we will describe a newly developed microbial ATP test (R-mATP) that requires approximately 90 s of analytical time and can measure microbial ATP in carcass samples with high levels of somatic ATP.

Steps of the R-mATP assay

One of the major sources of microbial contamination on animal carcasses is feces, which are also a reservoir for enteric pathogens such as *E. coli* O157:H7. Since feces contain high numbers (10^{11} to 10^{14} CFU/g) of bacteria, fecal contamination during the slaughter process results in high levels of bacteria on the carcass surface. Although carcasses that exhibit low bacterial counts may not be pathogen free, reducing fecal contamination should improve the microbial safety. Thus, detection of high numbers of bacteria on a carcass can be used as a potential indicator of fecal contamination *(13)*.

The R-mATP assay is the result of several laboratory and in-plant studies that encompassed methods for carcass sampling (swabbing, sponging, excision), concentration of microbial cells (centrifugation, filtration), types and amounts of reagents, luminometers, and statistical analyses. The R-mATP assay utilizes a sponge sampling procedure and a filtration device in combination with a microbial ATP bioluminescence assay to detect levels of bacteria on meat-animal carcasses.

Sampling

An area is delineated on the surface of pork or beef carcasses with a clean (dipped in 80°C water for 15 s), stainless-steel template (Figure 2A), or the area is measured to specific dimensions with a ruler and outlined with a sterile cotton swab dipped in edible ink. Samples are taken using an ATP-free sponge (NASCO, Fort Atkinson, WI) packaged in a Whirlpak™ bag. The sponge method of sampling is chosen since it contributes very few carcass tissue components that may interfere with the ATP bioluminescence assay; sponging also

Figure 2-D. The sponge and bag are placed into the Tekmar™ stomacher and homogenized for 2 min.

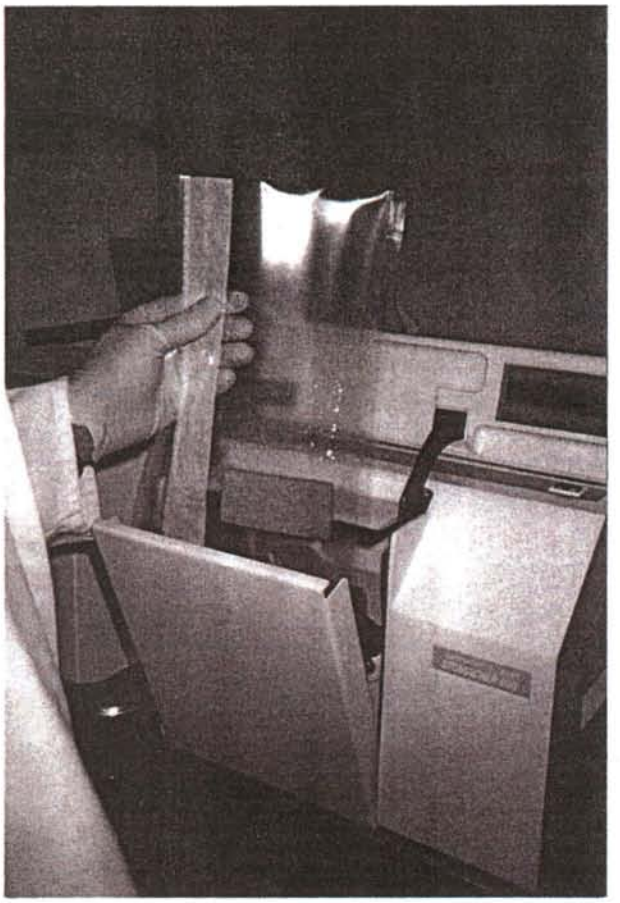
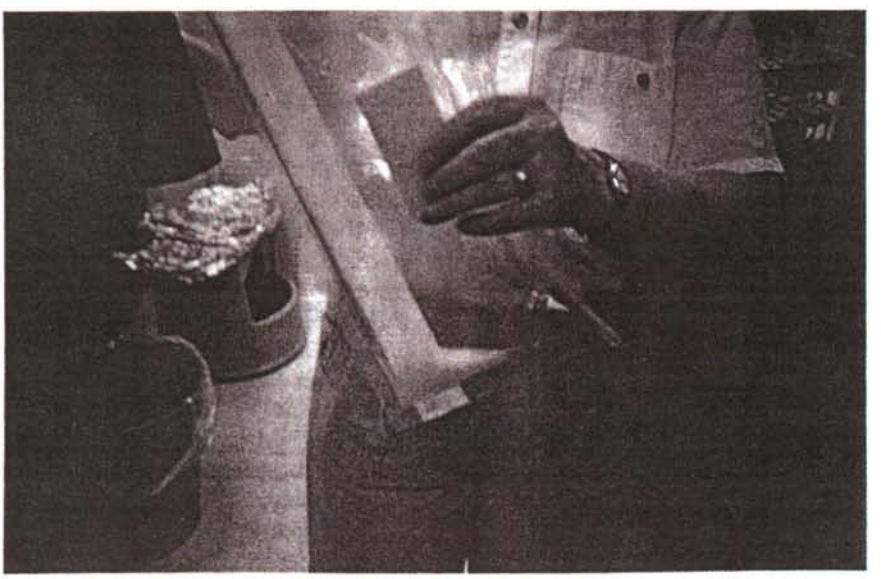


Figure 2-E. The liquid is expressed from the sponge and transferred via a sterile pipette into a sterile tube.



is nondestructive, rapid, and reproducible *(4, 13)*. The sponge is moistened with 25 ml of a sterile sponge solution composed of 0.085% (wt/vol) sodium chloride and 0.05% (vol/vol) Tween 20, adjusted to pH 7.8. After donning a sterile glove, the worker expresses the solution by hand from the sponge while removing it from the Whirlpak™ bag. The sponge is wiped firmly over the sample area 10 times in both vertical and horizontal directions (Figure 2A). The sponge is then placed into the bag containing the residual sponge solution and held at 8 to 10°C for < 2 h until analyses are performed (Figure 2B) *(13)*.

For sampling chickens, the entire exterior carcass surface is sponged with an ATP-free sponge (NASCO, Fort Atkinson, WI) moistened in 25 ml of a sterile sponge solution composed of buffered peptone water containing 0.5% Tween 20 (vol/vol) and 0.5% glucose (wt/vol). After donning a sterile glove, the worker expresses the solution from the sponge while removing it from the Whirlpak™ bag. The sponge is wiped firmly over the entire outside surface of the bird carcass, turning the sponge at least two times *(14)*. The sponge is placed into the Whirlpak™ bag containing the residual sponge solution and held between 8 and 10°C for < 2 h until analyses are performed *(14)*.

Following sampling and transport to a laboratory, all sponges and all fluid contents are transferred to a filtered stomacher bag (Figure 2C; Spiral Biotech, Bethesda, MD) and stomached in a LabBlender 400 stomacher (Figure 2D; Tekmar, Cincinnati, OH) for 2 min. Approximately 15 ml of each of the stomached samples are withdrawn from the filtered side of the stomacher bag with a sterile pipette (Figure 2E), transferred to a sterile tube, and the resulting sample analyzed with the rapid microbial ATP assay *(13, 14)*.

Rapid microbial ATP bioluminescence (R-mATP) assay

As stated in the introduction, separating somatic ATP from microbial ATP is the central challenge in microbial ATP testing of foods. The R-mATP assay uses a filtration device (Filtravette™, New Horizons Diagnos-

Figure 3. The R-mATP assay.

A. Items needed to perform the R-mATP assay: A, stack of paper towels; B, Filtravette™; C, vacuum manifold; D, luminometer; E, reagents; F, pipettor; G, waste vessel; H, sterile pipettor tips; I, forceps (Siragusa et al., 1995).

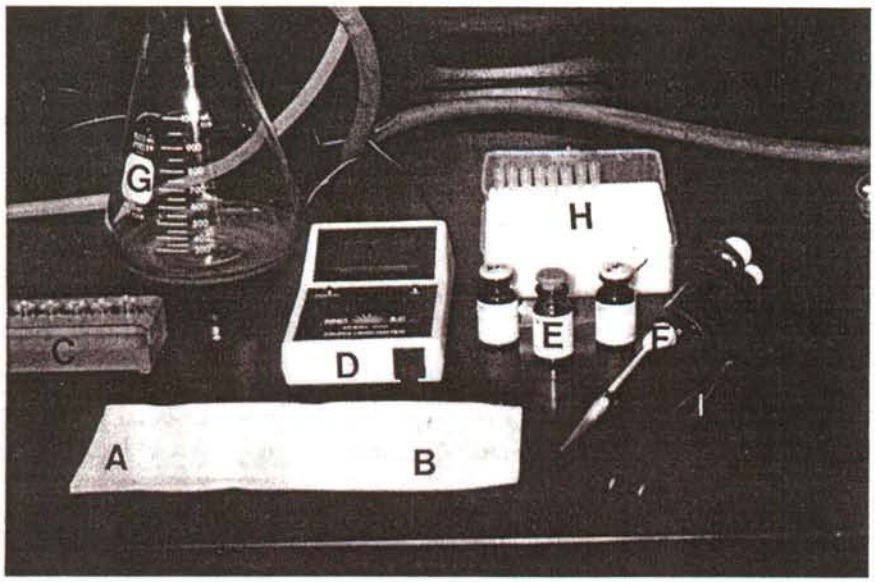
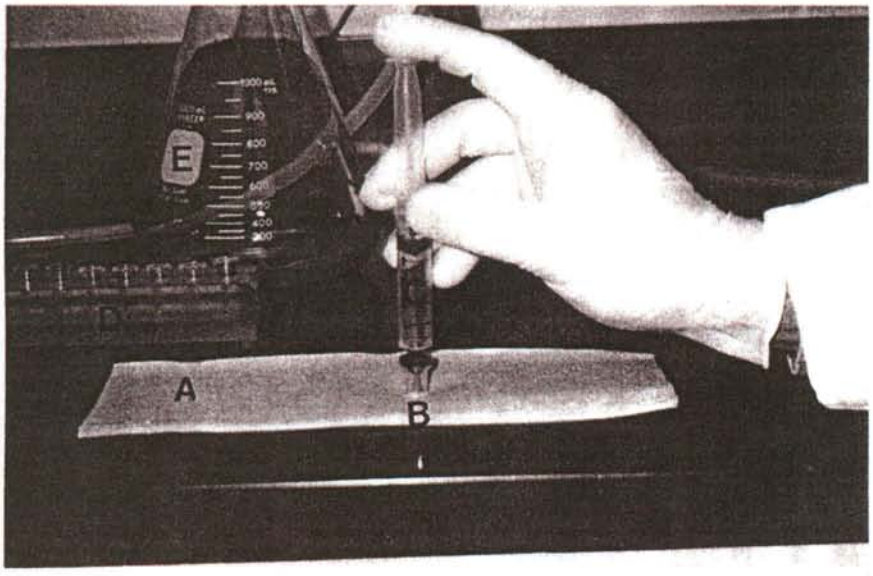


Figure 3-B. For single samples, 50 µl of sample is transferred to a Filtravette™, followed by the addition of 100 µl of somatic cell extracting reagent. A positive-pressure device (C) is used to push the liquid through the Filtravette™ (B) onto a stack of paper towels (A). If performing multiple samples, the vacuum manifold (D) is used, and the filtrate is captured in the waste vessel (E). Another 150 µl of somatic releasing agent is added to each Filtravette™ and the fluid from each filter is pushed or suctioned again. At this stage, the Filtravette™ retains bacteria and other cellular debris on the filter's surface. Somatic cell ATP and free ATP are extracted by the action of the somatic cell extracting reagent and removed from the Filtravette™ by suction or positive pressure.



tics, Columbia, MD) in which somatic ATP is extracted, and within the same device bacterial ATP is extracted and measured using the firefly luciferin/luciferase *(13)*. Briefly, 50 µl of sample is transferred to a Filtravette™, followed by the addition of 100 µl of somatic ATP extraction reagent (NRS™, nucleotide-releasing agent for somatic cells; Lumac B. V., Perstorp Analytical, The Netherlands. If multiple samples are being assayed, the fluid from each Filtravette™ is suctioned using a vacuum manifold (Figure 3B; New Horizons Diagnostics), capturing the filtrate in a waste vessel. If individual samples are used, a positive pressure device (Figure 3B; New Horizons Diagnostics) is used to push the liquid through the Filtravette™ onto a stack of paper towels. After initial removal of the liquid, another 150 µl of NRS™ is added to the Filtravette™ and the liquid is pulled through with the vacuum manifold or pushed through with the positive pressure device. At this stage, the Filtravette™ retains bacteria and other cellular debris on the Filtravette™ surface. Somatic cell ATP and free ATP are extracted by the action of the somatic cell extracting reagent (NRS™) and removed from the Filtravette™ by suction or positive pressure. The Filtravette™ is then placed into the holding drawer of the microluminometer (Figure 3C; Model 3550, New Horizons Diagnostics and ILC Dover, Inc., Frederica, DE) and 50 µl of the microbial ATP extractant, NRB™ (nucleotide releasing agent for bacterial cells; Lumac B. V., Netherlands) is added (Figure 3D). Immediately after the addition of the NRB™, 50 µl of the luciferin/luciferase reagent (New Horizons Diagnostics) reconstituted in Lumit buffer (Lumac, B. V., Perstorp Analytical, Netherlands) is added. The fluid in the Filtravette™ is mixed rapidly three times by aspirating the fluid up and down in the same micropipettor tip used to deliver the luciferin/luciferase reagent. The drawer to the luminometer is closed and light emission is measured by integration over 10 s. Microbial ATP is reported as relative light units (RLU) taken directly from the luminometer's digital readout (Figure 3E). All re-

Figure 3-C. The Filtravette™ is then placed into the holding drawer of the microluminometer with a pair of forceps.

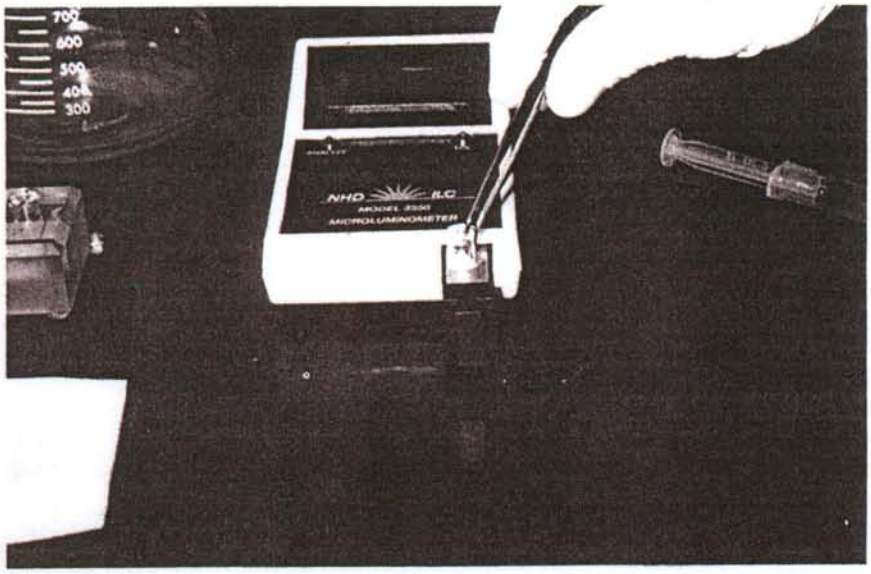
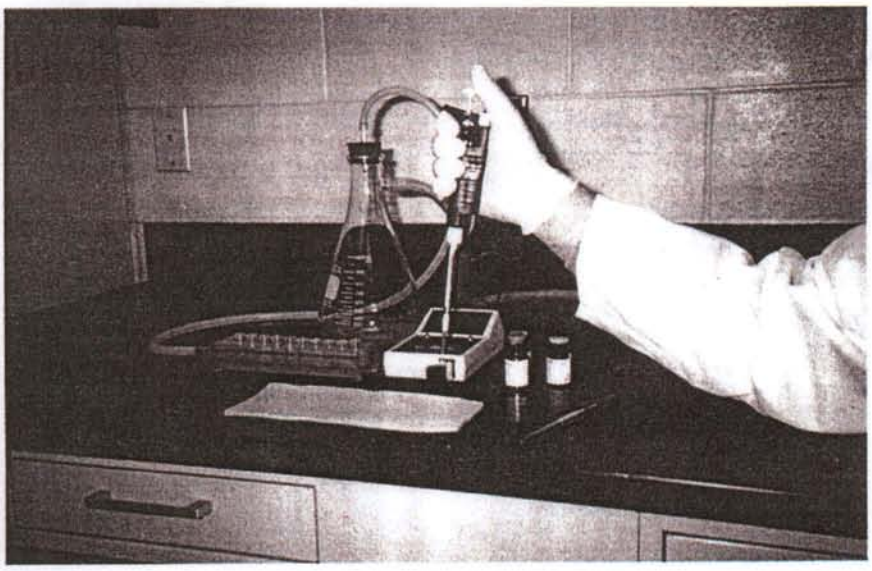


Figure 3-D. After placement of the Filtravette™ in the luminometer, 50 µl of the microbial ATP extractant is added. Immediately after the addition of the microbial ATP extractant, 50 µl of the luciferin/luciferase reagent is added and mixed rapidly three times.



agents are used at room temperature and are checked for contaminating ATP before use. Special handling is required of tips, reagents, Filtravette™, and luminometer to prevent ATP contamination throughout the process. The time required to perform the R-mATP assay is 1.5 min (90 s). When combined with sampling, the entire test can be performed in under 5 min per sample *(13, 14)*.

Conversion charts

After a RLU reading is obtained from the luminometer and recorded, R-mATP assay conversion charts can be used to determine the approximate log APC/cm² for beef carcasses. On the chart corresponding to the size of the area sampled (100, 150, or 500 cm², Figure 4, A to D), locate the value taken directly from the luminometer on the horizontal (RLU) axis. At the location of the horizontal RLU axis value, use a straight edge to find the abscissa ($\log_{10}$ APC/cm²) from the printed line.

EXAMPLES

In-plant studies

Three hundred sixty-five beef-carcass samples were obtained from two different commercial processing plants. One plant produced graded beef (heifers and steers) and the other produced carcasses for deboning and grinding from cows and bulls. Beef samples were taken immediately before and after the final spray wash, but before chilling. Randomly chosen carcasses were sampled mainly from the brisket area using a 500 cm² stainless-steel template or marked areas as described previously. For in-plant studies involving pork, 320 pork-carcass samples were obtained from three large swine processors. Using the procedures described and a 100 cm² template, all pork samples were taken from skinned carcasses. Samples obtained for the in-plant poultry studies were taken from broiler carcasses in three different plants. Poultry carcasses were randomly selected from the following sites: postdefeathering, postevisceration, postwash, and postchill. In all instances, beef, pork, and poultry carcasses were randomly selected from the processing line to represent a variety of possible contamination levels, ranging from visibly contaminated with feces to no visible fecal contamination *(13, 14)*.

Aerobic plate count (APC) and R-mATP assay data were obtained from each sample. Sponge samples cultured for APC were serially diluted in buffered peptone water (BBL, Cockeysville, MD), plated in duplicate with a Model D Spiral Plater (Spiral Biotech, Bethesda, MD) on tryptic soy agar (BBL), and the plates were incubated 48 h at 35°C. Bacterial counts were converted from colony-forming units (CFU) per ml to log CFU/cm² on the basis of the area sampled. Plots of R-mATP and APC data obtained from the beef and pork

Figure 3-E. The drawer to the luminometer is closed and light emission is measured by integration over 10 s. Microbial ATP is reported as relative light units (RLU) taken directly from the luminometer's digital readout (RLU = 437).

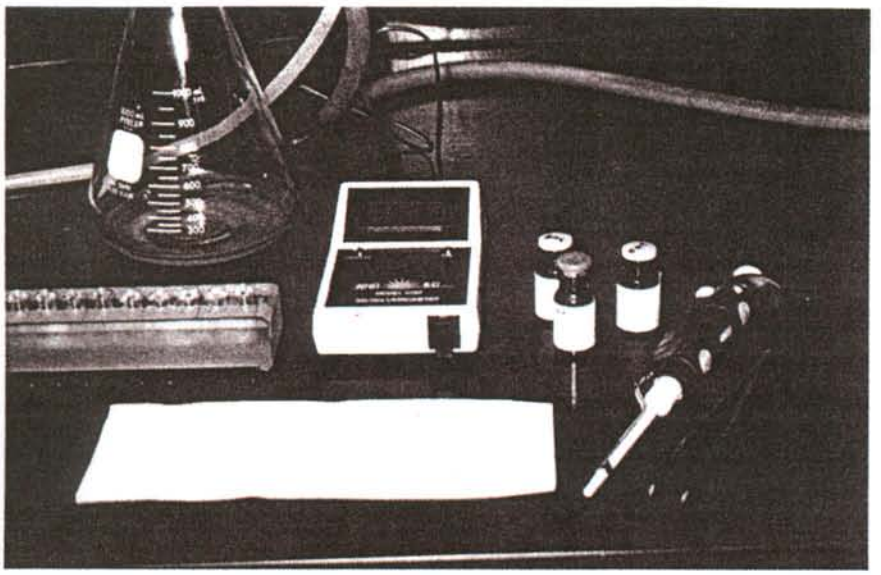


Figure 4. Conversion charts for beef.

A. Instructions for R-mATP assay conversion charts.

1. Select the chart that corresponds to the size of the area sampled on each carcass (100 cm^2, 150 cm^2, or 500 cm^2).

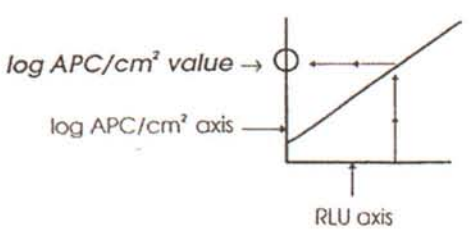
2. Once an RLU reading from the luminometer is determined, locate this value on the horizontal RLU axis of the chart.

3. At the location of the horizontal RLU axis value, use a straight edge and follow a straight, vertical line up to the dark line moving across the chart.

4. Once at the position where the RLU value intersects with the dark line, use a straight edge and follow a straight, horizontal line left to the log APC/cm^2 y-axis.

5. At the log APC/cm^2 y-axis, locate the log APC/cm^2 value and record.

EXAMPLE



samples are shown in Figures 5 and 6, respectively. The lower limit of assay sensitivity for samples from beef carcasses was determined to be 2 log APC/cm^2, with a correlation (r) between APC and R-mATP of 0.91 (Figure 5) *(13)*. For samples taken from pork carcasses, the lower limit of sensitivity was determined to be 3.2 log APC/cm^2 and the correlation (r) between APC and R-mATP was 0.93 (Figure 6) *(13)*. Data obtained from poultry carcasses sampled from the four sites averaged 4.89, 5.01, 4.12, and 3.34 log APC/ml while R-mATP data were 3.83, 4.03, 2.04, and 1.59 log RLU/ml from the post defeathering, postevisceration, postwash, and postchill sampling sites, respectively (Figure 7). The overall correlation (r) of APC and R-mATP samples taken from the three poultry plants was 0.82. On the basis of the data obtained from all three in-plant studies, the R-mATP assay provides a reliable measure of generic microbial levels on beef, pork, and poultry carcasses.

R-mATP assay as HACCP monitor

Two red-meat processing facilities have successfully used the R-mATP assay for more than a year as a HACCP monitor. These plants use the R-mATP assay to monitor carcasses to determine the daily microbial profile of their process on a near real-time basis. Plant A is a 2,400 head per day fed-beef slaughter facility. Plant B is a 3,000 head per day lamb slaughter facility. During initial implementation of the R-mATP assay in both plants, the Quality Control (QC) personnel obtained samples from several carcasses a day, following the final spray wash, over the course of several months. From these samples, plate count data and RLU values were determined in-house and these data were used to constitute the baseline readings for each plant.

In both plants, the R-mATP assay has been utilized to successfully identify several deviations of critical processes. All deviations were rectified immediately with resulting reductions in R-mATP values and aerobic plate counts. As an example, Plant A no-

Figure 4-B. This chart is used for samples obtained from 100-cm² areas.

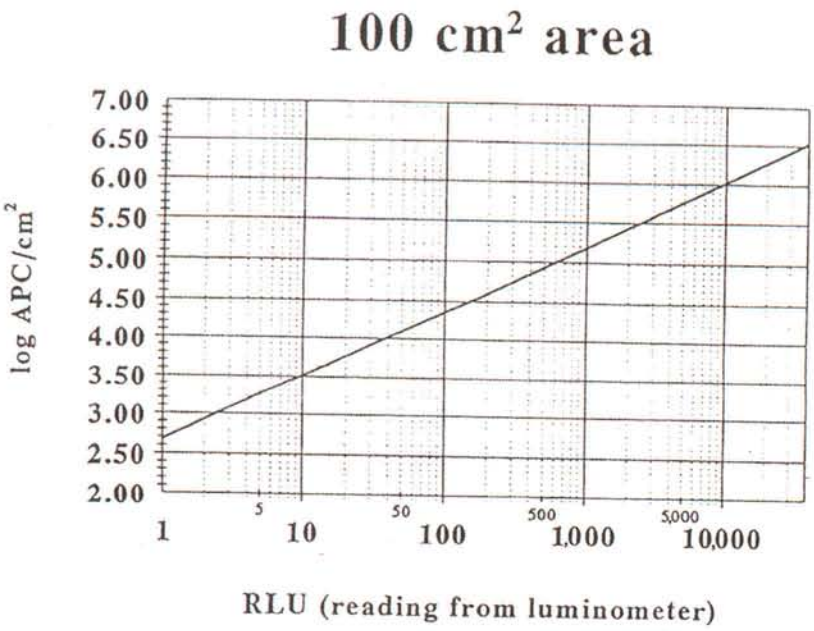
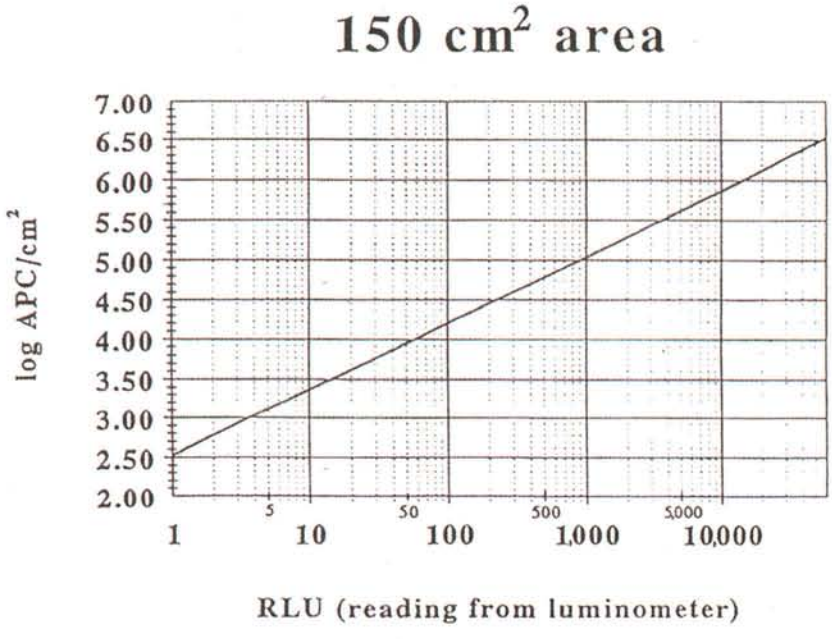


Figure 4-C. This chart is used for samples obtained from 150-cm² areas.



ticed an increase in RLU values from samples taken from beef carcasses as they exited the final wash. The QC personnel immediately began investigating possible deviations and it was determined that the tank supplying chlorine to the water used for spray washing was empty. Once the tank was refilled and operational, additional samples were taken from the carcasses and the R-mATP assay performed. RLU values taken from these samples had returned to acceptable baseline levels.

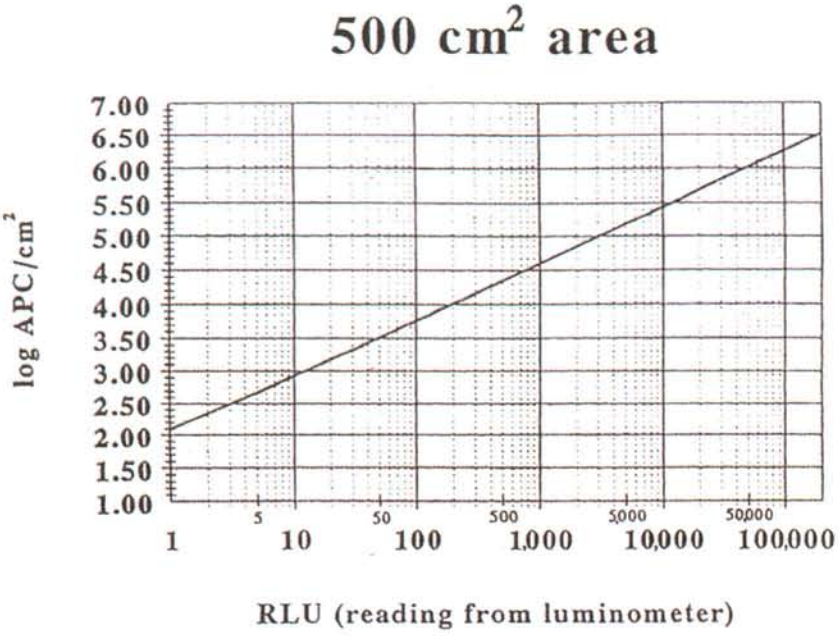
In another instance, Plant B experienced an increase in RLU values on carcasses following the final spray wash which prompted QC personnel to investigate potential problems in the process. It was determined that injectors for delivering an organic acid to spray-washing cabinets were clogged. Once the situation was rectified and spray washes were restored, QC personnel sampled carcasses, performed the R-mATP assay, and obtained RLU values that were reflective of their earlier baseline values.

Plants A and B also have cited several examples of elevated RLU values following the addition of new, inexperienced employees to their processing lines. In these cases, retraining of personnel resulted in reduced RLU values. Because both plants A and B have been using the R-mATP for a year or more, these plants also have observed elevated RLU values and concurrent microbial increases related to seasonal effects.

One other incident occurred during our in-plant pork studies. Over the course of several days, we obtained both RLU and plate count data from a pork-processing plant (5,000 head per day). During one day of sampling, RLU (and concomitant plate count values) were increased on carcasses immediately after the debunging process. Upon closer inspection by plant personnel, it was determined that the debunger was not operating properly. After the debunger was repaired and samples were taken, RLU and plate count values were found to be reduced to initial baseline levels.

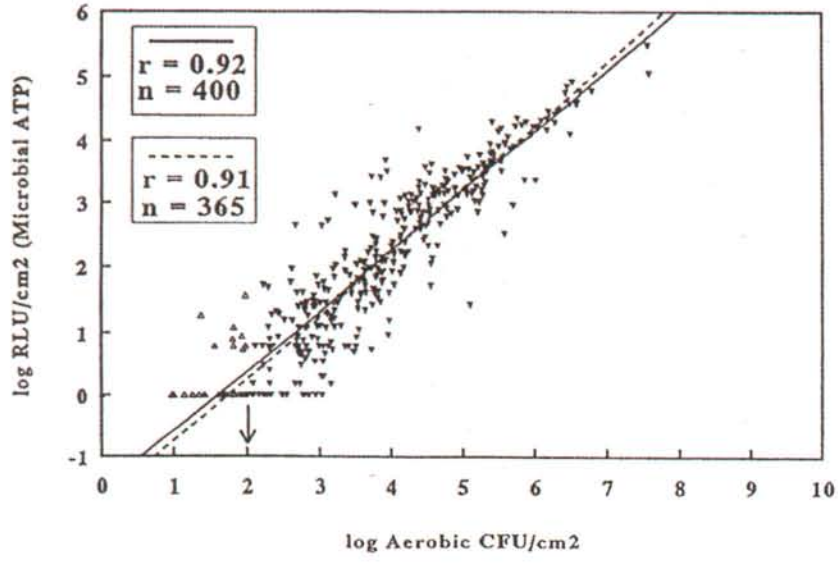
In all of these situations, the QC personnel identified a process deviation almost as soon as it was occurring, as opposed to after 24 to 48 h, the time at which aerobic plate count data would be available. Rectifying these situations probably resulted in the extension of shelf life and improvements to the microbial safety of products from these plants, but it certainly lowered the level of microbial contamination on these carcasses. As demonstrated in these actual examples, using the R-mATP assay provides the means to take proactive measures to maintain low microbial contamination on carcasses and improves the overall microbial quality of the carcasses.

Figure 4-D. This chart is used for samples obtained from 500-cm² areas.



Note: The conversion charts were derived from data obtained during the in-plant studies *(13)*. These charts may not be applicable to all plants.

Figure 5. Scatterplot of R-mATP assay values (log RLU/ml) and aerobic plate counts (log CFU/cm²) from beef- carcass in-plant samples. The solid line is the regression including all data points. The dashed regression line is calculated from data points above the lower limit of assay sensitivity (2 log CFU/cm²; indicated by the arrow). Data points below the threshold are open symbols and those above the threshold are solid symbols. (Reprinted with permission from *Journal of Food Protection* 58:770-775.)



Status and economics of R-mATP assay

The R-mATP assay has been performed by trained plant personnel, veterinarians, laboratory personnel, and USDA meat inspectors. In all instances, the R-mATP assay was easy to perform and could give results in less than 5 min.

Without considering the initial startup costs for stomacher, pipettors, luminometer, and vacuum manifold, the R-mATP assay can be performed for approximately $3.00/sample. This cost includes reagents (NRS™, NRB™, buffer, and luciferin/ luciferase) and disposable supplies (sponge, Whirl-Pak™ bag, pipette, tips, and Filtra-vette™).

CONCLUSIONS

Based on our laboratory and in-plant studies, the R-mATP assay is a rapid and near real-time means of estimating the microbial load of an animal carcass. The R-mATP assay should be of considerable interest to persons who are implementing a HACCP program in animal-processing plants. This assay can be used by meat animal processors or inspectors as a way to monitor their process and take proactive measures rather than retrospective action.

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Figure 6. Scatterplot of R-mATP assay values (log RLU/ml) and aerobic plate counts (log CFU/cm²) from pork-carcass in-plant samples. The solid line is the regression including all data points. The dashed regression line is calculated from data points above the lower limit of assay sensitivity (3.2 log CFU/cm²; indicated by the arrow). Data points below the threshold are open symbols and those above the threshold are solid symbols. (Reprinted with permission from *Journal of Food Protection* 58:770-775.)

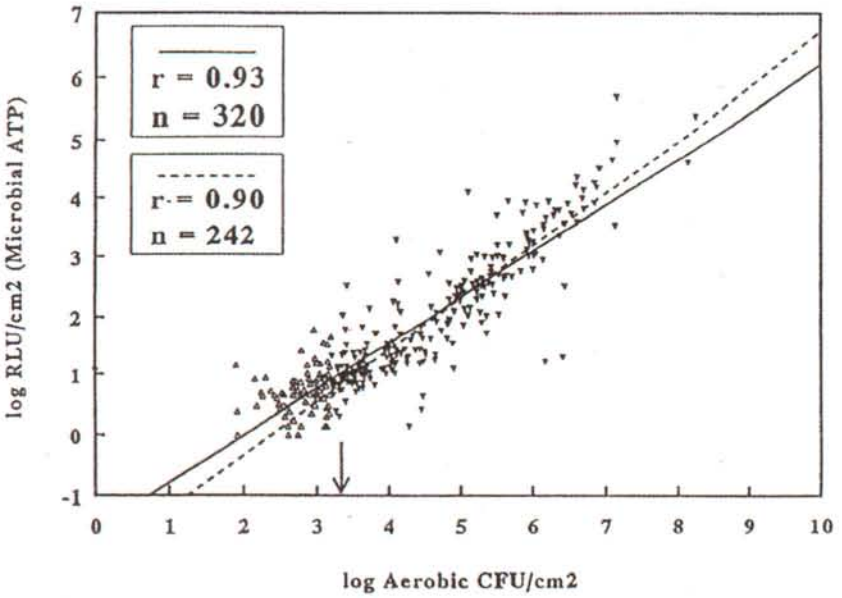
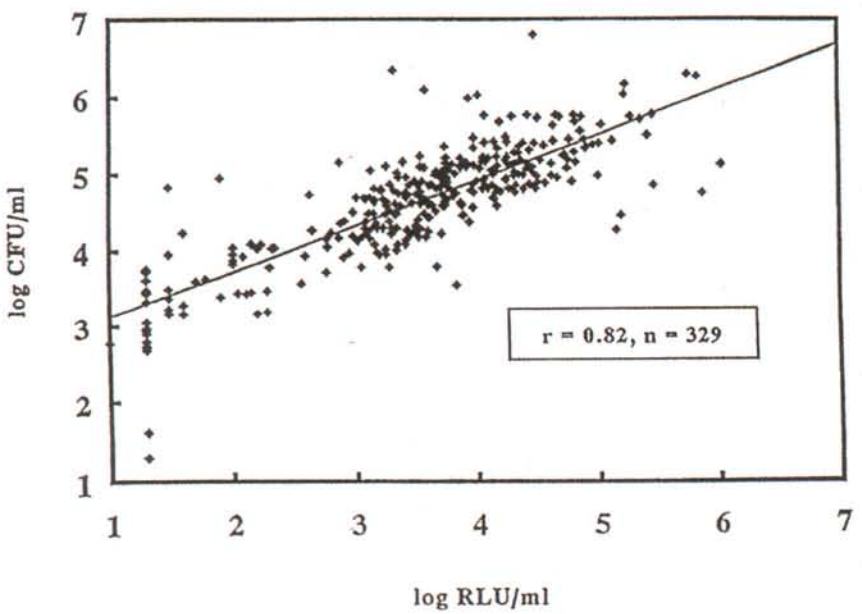


Figure 7. Scatterplot of R-mATP assay values (log RLU/ml) and aerobic plate counts (log CFU/ml) from poultry-carcass in-plant samples. The solid line is the regression including all data points.



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