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Research Note

Comparison of a Rapid ATP Bioluminescence Assay and Standard Plate Count Methods for Assessing Microbial Contamination of Consumers' Refrigerators

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ABSTRACT

The feasibility of using an ATP bioluminescence assay for assessing microbial contamination of home refrigerators was evaluated and compared with the standard culture methods. Samples of refrigerator surfaces were collected from 123 households by swabbing an area of 100 cm^2 on three locations in the refrigerator with premoisturized sterile swabs. Microbial contaminations were determined by aerobic plate count (APC; incubated at 35°C for 48 h) and psychrotrophic plate count (PPC; incubated at 7°C for 10 days) on plate count agar. The results were compared to the readings from the microbial ATP (mATP) bioluminescence assay. The correlation coefficient (r) between mATP and PPC (r = 0.851) was slightly higher than that between mATP and APC (r = 0.823). Our results indicated a potential discrepancy in the population of mesophilic and psychrotrophic bacteria in the refrigerator samples. Nevertheless, mATP appeared to be a reliable indication of the average of APC and PPC (r = 0.895). The mATP bioluminescence assay would provide a rapid and convenient test for researchers in field studies to assess microbial contamination in refrigerators.

Consumers' handling and storage practices have a significant impact on the safety and quality of foods at home *(10, 17)*. Research has been focused on identifying the risks associated with consumers' handling and storage practices of refrigerated foods *(9)*. Visual inspection and microbiological evaluation of refrigerator conditions and in-person interviews with consumers are crucial for accessing these risks. For microbiological evaluation, the aerobic plate count (APC) is commonly used as an indication of microbial contamination *(12)* and the psychrotrophic plate count (PPC) is often used as an indicator of bacteria that grow under refrigeration temperature *(3)*. These standard culture methods require 2 to 10 days to complete the analysis. In addition, researchers often travel to multiple states for in-home interviews with participants. It is inconvenient to transport the samples back to the laboratory in a timely manner. In order to minimize the effects of transporting samples, a rapid assay is preferred for personnel with minimal training to perform the microbial analysis on sites.

ATP bioluminescence is a rapid assay that has become a widely accepted method to monitor the hygienic status of food production lines and verify effective cleaning procedures *(2, 5, 13, 15, 16)*. In the presence of a firefly enzyme system (luciferin and luciferase system), ATP will facilitate the reaction to generate light. The light can be measured by a luminometer and used to estimate the biomass of cells in a sample. Some ATP bioluminescence assays have been designed to measure ATP from bacteria and other microbes, so called microbial ATP (mATP) bioluminescence assays. In general, these assays incorporate reagents to lysis somatic cells and filtration to remove somatic ATP from the sample. Microbial ATP bioluminescence assays have been applied for rapid assessment of bacterial contamination on the surfaces of meat carcasses *(4, 19)*, poultry carcasses *(7, 20)*, whole cantaloupes *(21)*, and within drinking water *(6, 14)* and milk *(18)*. However, the validity of using the mATP bioluminescence assay to analyze samples from low-temperature environments, such as those from the interior surfaces of refrigerators, has not been evaluated.

This study examines the feasibility of using a rapid mATP assay as an alternative to the standard culture methods for rapid evaluation of microbial contamination in home refrigerators. Our objectives were to (i) characterize the performance of the rapid mATP assay in terms of its sensitivity, repeatability, and reproducibility, and (ii) correlate the mATP assay with APC and PPC, in order to establish a standard for assessing microbiological status of refrigerators.

MATERIALS AND METHODS

Collection of samples from home refrigerators. Swab samples from household refrigerators that are in routine usage were collected during the visits to participants' homes for in-person interviews. The purpose of the interview was to assess the consumer's knowledge and practice on handling and storing refrig-

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erated foods at home. A total of 123 households in Nashville, Tenn., participated in this study. Three swabs were taken from the interior surfaces in each refrigerator, including shelves, and bottoms of meat and vegetable drawers. The sampling area was delineated with an alcohol cleaned and dried plastic template frame (10 by 10 cm) to confine an area of 100 cm^2. The surface was then rubbed slowly and thoroughly using an ATP-free Dacron sterile swab premoisturized in 5 ml of neutralizing buffer in a capped vial (Hardy Diagnostics, Santa Maria, Calif.). Swabs were placed in a cooler with icepacks and were transported back to the laboratory within 4 h after collection, where samples were analyzed within 2 h after receiving.

Rapid mATP bioluminescence assay. The bioluminescence assay was performed using a microluminometer NHD model 3560 and PROFILE-1 reagent kit (New Horizons Diagnostic, Columbia, Md.). The reagent kit includes the Filtravette, somatic cell–releasing agent, bacterial-releasing agent, and luciferin-luciferase. The swab in the sample vial was first vortexed for 30 s. The suspension was then transferred using a sterile pipette tip to the Filtravette, a combined device of filter and cuvette with a pore size of 0.45 μm, underlined with a blotter paper to absorb the filtrate. The suspension was then pushed through the Filtravette by a positive pressure device (a 3-ml syringe with o-ring attached to the top). The assay volumes were between 0.2 and 1.0 ml, based on the filterability of the suspension. After the desired volume was passed through the Filtravette, three drops of somatic cell–releasing agent were added and the mixture was pushed through the Filtravette by the positive pressure device. Three more drops of somatic cell–releasing agent were added and pressure filtered to ensure the removal of interfering substances, free ATP, and somatic cell ATP. The Filtravette was then placed into the drawer slide of the microluminometer. Two drops of bacterial releasing agent were added into the Filtravette to extract the microbial ATP. Immediately after the addition of the bacterial releasing agent, 50 μl of luciferin-luciferase was added and mixed by aspirating the fluid up and down three times. The drawer slide was closed immediately; light emission was measured with integration over 10 s. Bioluminescence from mATP was measured directly from the microluminometer digital readout as relative light unit (RLU). For most of the samples, it was not difficult to pass 1.0 ml of the suspension through the Filtravette. However, for some severely clouded samples, the Filtravette became clogged before the required 1.0 ml was reached. Subsequently, the readings from these samples were corrected for the actual assay volume used.

Preliminary validation of mATP bioluminescence assay. The performance of the mATP bioluminescence assay was tested in our laboratory. Calibration curves were prepared by serial dilutions made from ATP stocks (2 mg/ml) in distilled water. Calibration standards (0.2 to 20,000 pg) in 10 μl of distilled water were added to the Filtravette without filtering and immediately combined with 50 μl of bacterial-releasing agent. The rest of the procedures were the same as described in the previous section. Three levels of ATP controls (2, 200, and 20,000 pg) were prepared daily. Repeatability of the assay was determined by 10 repeated measurements of the controls using the luciferin-luciferase reagent from the same vial, while reproducibility of the assay was determined by measurements of the control samples in different days using the luciferin-luciferase reagent from different vials. Variations of the determinations were calculated and expressed as percent coefficient of variation (% CV).

APC and PPC. In addition to mATP, APC and PPC were also determined for each of the swab samples. Serial dilutions

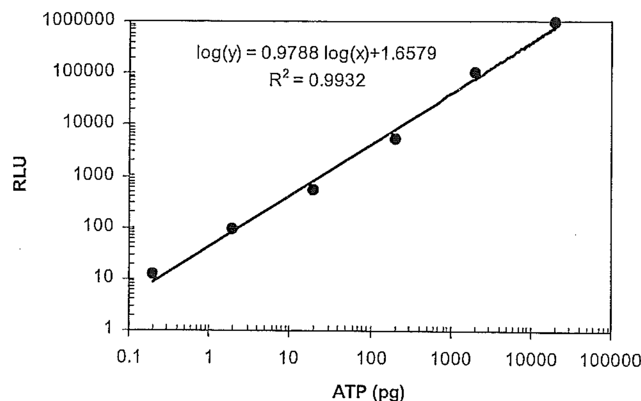

FIGURE 1. *Dose-response curve of mATP bioluminescence assay.*

(10^{-1} to 10^{-6}) of the suspensions were prepared in Butterfield's phosphate buffer; enumerations were performed on plate count agar (BD, Franklin Lakes, N.J.) using the pour plate method according to the standard protocol *(1)*. APC plates were incubated at 35°C for 48 h while PPC plates were incubated at 7°C for 10 days. Counting of the colonies was performed using an automatic colony counter (Synbiosis, Frederick, Md.).

Statistical analyses. Readings of mATP from the microluminometer were converted to log RLU, and the APC and PPC were converted to log CFU per sample before statistical analyses were performed. Correlation and linear regression were performed on mATP versus APC and PPC using SPSS 12.0 for Windows (SPSS, Chicago, Ill.).

RESULTS

Performance of mATP bioluminescence assay. Dose response of the assay was calibrated with various quantities of ATP. A log-linear relation of the bioluminescence RLU and ATP level was established within the range of 0.2 and 20,000 pg. A typical dose-response curve is shown in Figure 1; the slope of the regression line (0.9788) approaching 1.0 indicated that the increase of RLU is directly proportional to the increase of the ATP quantity. Analytical sensitivity (limit of detection) of the assay was 0.2 pg ATP as determined by serial dilutions of calibration standards. Three levels of ATP controls were applied to determine the performance of the assay. Repeatability and reproducibility of the assay were calculated as % CV of the measurements. Repeatability of the assay as determined from repeated measurements of control samples in the same day was 16.2, 5.9, and 13.7% for low, medium, and high controls, respectively (Table 1). Reproducibility of the assay as determined from repeated measurements of control samples in 15 days was 23.8, 11.6, and 18.6% for low, medium, and high controls, respectively.

Microbial contamination of home refrigerators. APC and PPC from a total of 369 swab samples were determined. The average APC and PPC for all the samples was 3.9 and 3.3 log CFU per sample, respectively. The comparison of APC and PPC showed inconsistencies between these two counts (Table 2). In general, APC tends to be higher than PPC when PPC is $<10^5$; on the other hand, APC tends to be lower than PPC when PPC is $>10^5$. These results indicated a potential discrepancy in the population

TABLE 1. *Repeatability and reproducibility of mATP bioluminescence assay measured from three levels of control samples*

	Control	ATP (pg)	*n*	Mean (RLU)[a]	% CV[b]
Repeatability	Low	2	10	62 ± 10	16.2
	Medium	200	10	6,765 ± 396	5.9
	High	2,000	10	143,843 ± 19,666	13.7
Reproducibility	Low	2	15	73 ± 17	23.8
	Medium	200	15	6,209 ± 723	11.6
	High	2,000	15	124,813 ± 23,261	18.6

[a] Values are presented as the mean ± standard deviation of determination.
[b] % CV, percent coefficient of variation.

of mesophilic and psychrotrophic bacteria in the refrigerator samples. The low correlation coefficient ($r = 0.778$) between APC and PPC also support this observation.

Correlations of the mATP bioluminescence assay and standard plate counts. Scatter plots of mATP to APC and PPC are presented in Figure 2. The correlation between mATP and PPC ($r = 0.851$) was slightly higher than that between mATP and APC ($r = 0.823$). Assuming 1 CFU is originally from a single bacterium, the level of ATP in a bacterial cell as estimated from the regression equation and the ATP calibration curve was in a range from 0.0010 to 0.0043 pg with an average of 0.0025 pg for APC and from 0.0022 to 0.0051 pg with an average of 0.0033 pg for PPC. These values are consistent with the reported ATP quantity in bacterial cells *(8)*. A correlation was sought to compare the mATP to the average of APC and PPC (expressed as log[APC + PPC]/2); the correlation coefficient of mATP to the average of APC and PPC was 0.895. It appears that mATP is a better indication of the average of APC and PPC than the individual APC or PPC. Our results are comparable with other previous studies, which reported correlation coefficients of 0.91 between mATP and APC in samples from beef carcasses *(4, 19)*, 0.90 between mATP and APC in samples from pork carcasses *(4, 19)*, 0.82 between mATP and APC in samples from poultry carcasses *(20)*, and 0.84 between mATP and heterotrophic plate count in samples of drinking water *(14)*.

DISCUSSION

The ATP bioluminescence assay has become a widely accepted method to monitor the hygienic status of food production lines and verify effective cleaning procedures *(2, 5, 13, 16)*. Hygienic ATP bioluminescence detects ATP from both microbial cells and food residues; therefore, it does not provide a good correlation to standard culture methods *(2)*. Our purpose was to select an assay that could serve as an alternative to standard culture methods for evaluating microbial contamination of refrigerators in field studies. An assay that is able to differentiate the ATP of microbes from food residuals is essential for our application. The mATP assay used in this study involves procedures of disrupting somatic cells by a lysis reagent and filtration to remove ATP from nonmicrobial sources (e.g., food residuals commonly found in refrigerator surfaces). In addition, filtration procedures also eliminate cleaning agents and chemical sanitizers in the samples, which have been reported to interfere with the ATP bioluminescence assay *(11, 13)*.

The mATP assay provides a wide range of detection (0.2 to 20,000 pg) without the need of tedious dilutions as required in the standard culture methods. Based on our data, the ATP level per CFU is between 0.001 and 0.005 pg; therefore, it was estimated that the detection limit of 0.2 pg would be equivalent to about 40 to 200 CFU, which converted to a detection limit of 200 to 1,000 CFU per sample of the refrigerator surface. With the current protocol, the mATP assay allowed for a rapid analysis of up to 10^8 CFU without the need of sample dilution. Reproducibility of the assay was within acceptable range for the intended applications.

Large variations in mATP were apparent between and within the refrigerators. Variable mATP results are likely

TABLE 2. *Comparison of APC and PPC from 369 refrigerator samples*

APC (CFU)	No. of samples with PPC (CFU) of:								Total no. of samples
	<10	$\geq10^{1}$–$<10^{2}$	$\geq10^{2}$–$<10^{3}$	$\geq10^{3}$–$<10^{4}$	$\geq10^{4}$–$<10^{5}$	$\geq10^{5}$–$<10^{6}$	$\geq10^{6}$–$<10^{7}$	$\geq10^{7}$	
<10									0
$\geq10^{1}$–$<10^{2}$	3	18	4						25
$\geq10^{2}$–$<10^{3}$	9	40	29	9	2		1		90
$\geq10^{3}$–$<10^{4}$	1	19	37	24	8	3	1		93
$\geq10^{4}$–$<10^{5}$	1	5	18	21	24	10	2		81
$\geq10^{5}$–$<10^{6}$		2	4	7	11	12	6	3	45
$\geq10^{6}$–$<10^{7}$		1		3	2	4	9	6	25
$\geq10^{7}$								10	10
Total	14	85	92	64	47	29	19	19	369

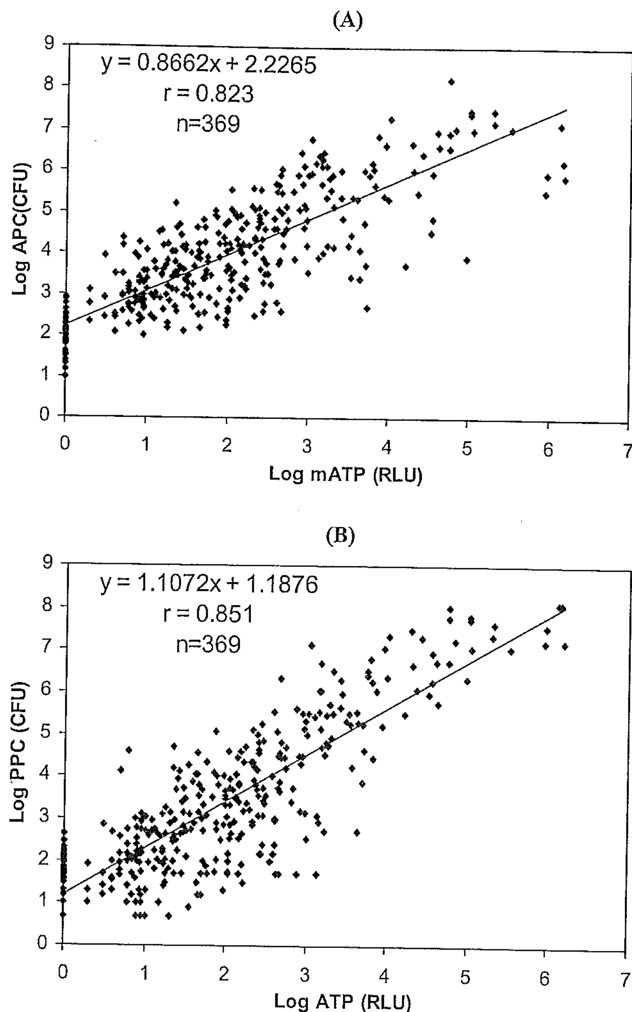

FIGURE 2. *Scatter plots of mATP to APC (A) and mATP to PPC (B). Data points at 0 log mATP indicate ATP levels below the detection limit of the assay.*

due to the random nature of both microbial contamination and growth and the sampling procedure. For example, the sampling area was 100 cm^2 regardless of whether the shelf surface was a metal grid or solid glass; consequently, there was significant difference of the actual surface between solid and grid under the same sampling area. In addition, the bacteria are not evenly distributed on the surface (they tend to cluster in a few spots); therefore, we did not intend to report the results as the bacteria number per actual unit of surface (CFU/100 cm^2) but interpreted the results as the bacteria numbers found on similar sizes of areas in the refrigerators (CFU per sample).

There is no published microbiological standard for refrigerator surfaces; therefore, researchers often have to set standards for evaluating and interpreting their data *(12)*. In our study, the higher mATP indicated a higher microbial load and consequently a higher risk for contamination of foods stored in the refrigerator. For the purpose of data analyses, RLU results reflecting mATP were divided into five categories: $\leq$20, 21 to 2,000, 2,001 to 20,000, 20,001 to 200,000, and $\geq$200,000; these categories are equivalent to $\leq 10^3$, 10^3 to 10^5, 10^5 to 10^6, 10^6 to 10^7, and $\geq 10^7$ CFU, respectively, based on regression analysis.

We have demonstrated that the mATP bioluminescence assay is an efficient and reliable method to determine microbial contamination of refrigerators comparable to standard culture methods. This assay can be performed on-site and the results are obtained within 5 min, thus avoiding the efforts of transporting samples back to the laboratories. This assay would provide a rapid and convenient test for researchers in field studies to assess microbial contamination in refrigerators.

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REFERENCES

1. Association of Official Analytical Chemists. 1990. Official methods of analysis, 15th ed. AOAC, Arlington, Va.
2. Aycicek, H., U. Oguz, and K. Karci. 2006. Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *Int. J. Hyg. Environ. Health* 209:203–206.
3. Byun, J. S., J. S. Min, I. S. Kim, J. W. Kim, M. S. Chung, and M. Lee. 2003. Comparison of indicators of microbial quality of meat during aerobic cold storage. *J. Food Prot.* 66:1733–1737.
4. Cutter, C. N., W. J. Dorsa, and G. R. Siragusa. 1996. A rapid microbial ATP bioluminescence assay for meat carcasses. *Dairy Food Environ. San.* 16:726–736.
5. Davidson, C. A., C. J. Griffith, A. C. Peters, and L. M. Fielding. 1999. Evaluation of two methods for monitoring surface cleanliness—ATP bioluminescence and traditional hygiene swabbing. *Luminescence* 14:33–38.
6. Delahaye, E., B. Welte, Y. Levi, G. Leblon, and A. Montiel. 2003. An ATP based method for monitoring the microbiological drinking water quality in a distribution network. *Water Res.* 37:3689–3696.
7. Ellerbroek, L., and C. Lox. 2004. The use of neck skin for microbial process control of fresh poultry meat using the bioluminescence method. *Dtsch. Tierarztl. Wochenschr.* 111:181–184.
8. Fung, D. Y. C. 2002. Rapid methods and automation in microbiology. *Comp. Rev. Food Sci. Food Saf.* 1:3–22.
9. Godwin, S., and R. Coppings. 2005. Analysis of consumer food handling practices from grocer to home including transport and storage of selected foods. *J. Food Dist. Res.* 36:55–62.
10. Godwin, S., R. Coppings, L. Speller-Henderson, and L. Pearson. 2005. Study finds consumer food safety knowledge lacking. *J. Fam. Cons. Sci.* 97:40–44.
11. Green, T. A., S. M. Russell, and D. L. Fletcher. 1999. Effect of chemical cleaning agents and commercial sanitizers on ATP bioluminescence measurements. *J. Food Prot.* 62:86–90.
12. Henroid, D. H., A. F. Mendonca, and J. Sneed. 2004. Microbiological evaluation of food contact surfaces in Iowa schools. *Food Prot. Trends* 24:682–685.
13. Lappalainen, J., S. Loikkanen, M. Havana, M. Karp, A. M. Sjoberg, and G. Wirtanen. 2000. Microbial testing methods for detection of residual cleaning agents and disinfectants—prevention of ATP bioluminescence measurement errors in the food industry. *J. Food Prot.* 63:210–215.
14. Lee, J., and R. A. Deininger. 1999. A rapid method for detecting bacteria in drinking water. *J. Rapid Meth. Auto. Microbiol.* 7:135–145.
15. Oulahal-Lagsir, N., A. Martial-Gros, M. Bonneau, and L. J. Blum. 2000. Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment—validation and application to a dairy factory. *J. Appl. Microbiol.* 89:433–441.
16. Paez, R., M. Taverna, V. Charlon, A. Cuatrin, F. Etcheverry, and L.

H. da Costa. 2003. Application of ATP-bioluminescence technique for assessing cleanliness of milking equipment, bulk tank and milk transport tankers. *Food Prot. Trends* 23:308–314.
17. Redmond, E. C., and C. J. Griffin. 2003. Consumer food handling in the home: a review of food safety studies. *J. Food Prot.* 66:130–161.
18. Samkutty, P. J., R. H. Gough, R. W. Adkinson, and P. McGrew. 2001. Rapid assessment of the bacteriological quality of raw milk using ATP bioluminescence. *J. Food Prot.* 64:208–212.
19. Siragusa, G. R., C. N. Cutter, W. J. Dorsa, and M. Koohmarale. 1995. Use of a rapid microbial ATP bioluminescence assay to detect contamination on beef and pork carcasses. *J. Food Prot.* 7:770–775.
20. Siragusa, G. R., W. J. Dorsa, C. N. Cutter, L. J. Perino, and M. Koohmarale. 1996. Use of a newly developed rapid microbial ATP bioluminescence assay to detect microbial contamination on poultry carcasses. *J. Biolumin. Chemilumin.* 11:297–301.
21. Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2001. Bioluminescence ATP assay for estimating total plate counts of surface microflora of whole cantaloupe and determining efficacy of washing treatments. *J. Food Prot.* 64:813–819.