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Comparison of adenosine triphosphate (ATP) bioluminescence and aerobic plate counts (APC) on plastic cutting boards*

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Abstract

The objective of this study was to compare the adenosine triphosphate (ATP) bioluminescence method with aerobic plate count (APC). Three plastic cutting boards (30×35 cm) divided into 42 equal areas of 5×5 cm were used as food-contact surfaces. A culture of *Lactobacillus rhamnosus* ATCC 7469 with a starting concentration of 10^9 colony-forming units (cfu/mL) was used as a source of cells and ATP. The culture was diluted in phosphate buffer saline to obtain four more initial target concentrations 10^7, 10^5, 10^3 and 10^1 cfu/mL. After inoculation of the boards, recovery of microorganism was achieved by swabbing the surface with sterile cotton-tipped swabs for the APC assay, and PocketSwab Plus (Charm Sciences Inc., Lawrence, MA) for the bioluminescence test. Recovery after 15 min of inoculation gave average bioluminescence readings of 1.08 $\log_{10}$, 2.99 $\log_{10}$, 4.97 $\log_{10}$ and 5.84 $\log_{10}$ relative light units that corresponded to average recovered APC readings of 3.71×10^3, 5.39×10^5, 7.44×10^7 and 9.30×10^9 cfu on a 25-cm^2 surface area. Controls taken prior to the inoculation of the boards gave estimated average APC readings of <10 cfu and bioluminescence readings equal to zero. The correlation coefficient, $r = 0.98$, suggested a strong relationship between the two methods. A linear regression analysis showed a positive linear relationship between the two methods in the range of 10^3–10^7 cfu. A sensitive ratio test with a value of 1.016 gave an indication that ATP is as good as APC in evaluating sanitation on plastic cutting boards.

Introduction

Adenosine triphosphate (ATP) bioluminescence is a rapid sanitation test that has been gaining growing acceptance in the food industry as a fast, easy way to monitor sanitation programs. This technique may be extended to the restaurant and foodservice industry as a way to prevent potential cross-contamination of foods by using contaminated cutting boards. Aerobic plate count (APC) is the approved standard microbiology test to measure sanitation levels on food-contact surfaces.

Unsanitary surfaces in contact with food represent a high risk for cross-contamination. Cross-contamination of bacterial and viral pathogens in restaurant and foodservice establishments is thought to be a major factor for sporadic and

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epidemic foodborne illness outbreaks (USHHS 1999; Chen *et al.* 2001; FDA 2005). Microorganisms normally present in raw foods can be transferred to various surfaces, such as cutting boards, during food handling and preparation of ready-to-eat products (Chen *et al.* 2001; Montville & Schaffner 2004).

Inspections of foodservice establishments are an important tool to ensure food safety (Montville & Schaffner 2004). Most local health departments utilize visual examination when inspecting food service operations to assess sanitization (Kassa *et al.* 2001). While visual inspection of restaurant kitchens is important for reducing the risk of foodborne disease outbreaks, microbiological evaluation of selected kitchen areas, such as food-contact surfaces, has been suggested as a tool for obtaining additional information for reducing outbreaks (Kassa *et al.* 2001). However, microbiological methods take up to 48 h to express a result. By the time potential contamination has been detected, the food has already been served. A sanitation test that promises results within a few minutes is more likely to be implemented in restaurant and food service inspection plans (Bautista *et al.* 1992; Ogden 1993; Bell *et al.* 1994; Torre *et al.* 1998; Girotti *et al.* 2003).

Adenosine 5′-triphospahte (ATP) is present in all plant, animal and microbial cells (Garret & Grisham 1999). The use of the firefly enzyme luciferase to measure levels of ATP, and therefore, levels of biological residue, has been investigated (Ogden 1993). The breakthrough idea was the approach of using ATP to look for all biological residues as an indicator of cleanliness rather than to try to ascertain the number of microorganisms. Even though food residue may not be inherently dangerous, its presence indicates that a surface has not been thoroughly cleaned and has the potential to contain hazardous material. In addition, the food residues provide a nutrient source for the subsequent growth of microorganisms (Ehrenfeld *et al.* 1996).

ATP bioluminescence technology is now widely recognized as a real-time test for measuring the efficacy of cleaning and sanitation of food-processing equipment and food-contact surfaces (Ogden 1993; Bell *et al.* 1996; Griffith *et al.* 1997; Corbitt *et al.* 2000; Moore & Griffith 2002; Larson *et al.* 2003). This test quickly validates effective cleaning and sanitation procedures. As a sanitation-monitoring test, it has been successfully used to monitor critical control points that are relevant to Hazard Analysis and Critical Control Points plans.

ATP bioluminescence has had limited use in the restaurant and foodservice industry. However, the test could be used to evaluate the sanitary conditions of a food-contact surface within minutes, thus providing an opportunity for immediate remedial action. ATP bioluminescence also provides an easy-to-use method at a reasonable cost compared to APC. Food service operations generally rely on plastic and wood cutting boards as direct food-contact surfaces. Therefore, plastic cutting boards were selected to study the performance of the bioluminescence test, and determine how well it correlates with the APC, which has been the traditional surface sanitation assessment test. Thus, the purpose of this study was to compare APC, the traditional sanitation monitoring technique, with ATP bioluminescence (ATP) as an alternative rapid test for monitoring sanitation.

Materials and methods

Bacterial strain and culture media

A lactic acid bacteria culture, *Lactobacillus rhamnosus* ATCC 7469, was used as the contaminant for this study. The culture was obtained in a freeze-dried state from the American Type Culture Collection (ATCC) (Manassas, VA). The culture was reconstituted in 5.0 mL of Lactobacilli broth (Difco Becton Dickinson, Sparks, MD). This suspension was used to inoculate a second tube of broth, and incubation of this culture was carried out at 35°C for 36 h. This culture became the stock or mother culture (MC) and was maintained at 5°C.

From the MC, slant cultures were prepared using Lactobacilli DeMan-Rogosa-Sharpe (MRS) agar (Difco BD), and in this form, the culture was stored at 5°C. Every 3 days, the culture was transferred to a new 10-mL MRS agar slant tube.

For the purpose of this investigation, bacterial cultures of five different concentrations were prepared: 10^1, 10^3, 10^5, 10^7 and 10^9 colony-forming

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units (cfu)/mL. To achieve this goal, a culture material was obtained using a loop and was transferred into 10 mL of Lactobacilli broth. The culture was then incubated for 24 h at 35°C. After this first incubation, 0.1 mL was transferred to 9.9 mL of fresh sterile MRS broth and was incubated at 35°C for 18 h. The cells were separated by centrifugation for 20 min at 5°C at 2095 × g (Allegra X-12R, Beckman Coulter Inc., Palo Alto, CA). The supernatant was discarded, and each harvested pellet was washed and then reconstituted using sterile phosphate buffer saline (PBS; final pH 7.3).

To obtain an initial target inoculum of 10^9 cfu/mL, four pellets were suspended in 10 mL of PBS to obtain approximately 2.7×10^9 cfu/mL. To prepare the remaining target concentrations of 10^7, 10^5, 10^3 and 10^1 cfu/mL, appropriate dilutions, using PBS, were completed from the 10^9 cfu/mL culture. These target concentrations became the inocula.

Food-contact surfaces

Three new plastic cutting boards (Super Board, Texas International, Carrollton, TX) were used as food-contact surfaces. Plastic boards were 0.5-inch thick, double-sided super high-density polyethylene. The surface texture was slightly rough. However, the surface can be described as nonporous and nonabsorbent. Each board was resized as a rectangle of 35 × 30 cm. On the top surface of each board, a grid consisting of 42 squares was carefully drawn. Each square was 5 × 5 cm, which represent a 25-cm^2 surface area.

Before each experiment was carried out, the boards were washed using a commercial dish soap and running water at 40°C. The boards were rinsed with distilled lukewarm water and air-dried for 5 min. After drying, the boards were disinfected with bath of 10% chlorinated water (v/v) followed by a final rinse with 75% (v/v) ethyl alcohol. The boards were air-dried for 10 min. Each board was double wrapped in aluminum foil, and then autoclaved at 121°C for 15 min.

Inoculation of cutting boards

To inoculate the cutting boards, the target bacterial cultures were gently mixed for 7 s to suspend the bacterial cells before inoculation. Then, a total of 4.2 mL of each initial bacterial target concentration was evenly spread on each board to cover each 25-cm^2 surface area with approximately 0.1 mL of inoculum.

Microbiological test

Six 25-cm^2 surface areas, selected at random on each cutting board, were swabbed with sterile cotton-tipped swabs (Fisher, Pittsburgh, PA). Contaminated swabs were placed into 9.9-mL buffered peptone water (Difco BD) tubes, and kept at 5°C until the time of assay. The organisms collected from the surface were resuspended from the swab by a mild mixing, using a vortex, for 7 s. The fluid was serially diluted in buffered peptone water (Difco BD). Duplicate pour plates using Lactobacilli MRS agar (Difco BD) were prepared at the proper dilutions, and incubated for 36 h at 35°C. Only those plates with colony numbers in the range of 25–250 cfu (AOAC 2000; Downes & Ito 2001) were counted using a standard Quebec Dark-filed Colony Counter (Leica, Buffalo, NY).

Bioluminescence test

Six additional 25-cm^2 surface areas were randomly selected, in addition to those squares already selected for the microbiological assay, on each of the boards. Each square was swabbed using ATP swabs, and analyzed within the next minute. Swabs were removed from the refrigerator 5 min before they were used for readings. The measurement of ATP was carried out by means of the commercially available PocketSwab Plus single-shot swab device (Charm Sciences Inc., Lawrence, MA), and readings were obtained as relative light units (RLUs).

Statistical analysis

The experiment was a completely randomized design with subsampling with the four target inocula as treatments (the ATP bioluminescence read zero for 10^1 cfu/mL because it was not sensitive at that level and was left out of the model). There were a total of 12 experimental units that correspond to the 12 different days the experiment was conducted, resulting in three

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replications of each target concentration. Each experimental unit was composed of three subsamples that correspond to the three boards measured on a given day. Thirty-six observations were used in the analysis.

The ATP and APC values for an observation were obtained by calculating the average of the six measurements taken by either of the methods on each board. Averages of plate count results or luminescence readings per 25-cm^2 surface areas on each board, each day, were transformed to the decimal logarithmic scale (log_{10}). The conversion of scales was performed before the statistical analysis was completed.

Data were analyzed based on a mixed model for repeated measures procedure in SAS (SAS Institute 2004).

Sample averages, SDs and 95% confidence intervals were calculated for each concentration tested. Estimates of the correlation coefficient, coefficient of determination and sensitivity ratio (SR) were also obtained. The SR test (Mandel 1964) is a scale-independent approach for comparing two measurement methods when the relationship is unknown. The correlation between log_{10}APC and log_{10}ATP was estimated using SAS (SAS Institute 2004). All tests were conducted with a significance level of $P < 0.05$.

Results

Averages, SDs and 95% confidence intervals for APC and ATP values are listed in Table 1. Data were transformed to decimal logarithmic scale (log_{10}) before the statistical analysis was completed (Table 2).

An initial mixed model analysis to estimate the calibration curve was conducted using data from concentrations 10^3, 10^5, 10^7 and 10^9 cfu/mL. The ATP bioluminescence read zero for 10^1 cfu/mL. A significant F test ($P < 0.0001$) showed a linear component of the relationship ($\alpha = 0.05$). However, data obtained from concentration 10^9 cfu/mL did not follow the linear trend observed for concentrations 10^3, 10^5 and 10^7 cfu/mL.

A second mixed model analysis was conducted to test if observations from concentration

Table 1 Aerobic plate count and adenosine triphosphate bioluminescence relative light units (RLUs) of plastic cutting boards inoculated with Lactobacillus

Target inoculum (cfu/mL)	**Actual inoculum (cfu/25 cm^2)**	**Confidence intervals (95%) (cfu/25 cm^2)**	**Actual inoculum* (RLU/25 cm^2)**	**Confidence intervals (95%) (RLU/25 cm^2)**
10^3	$5.23 \times 10^3 \pm 1.01 \times 10^3$	3.81×10^3–6.91×10^3	6.89 ± 11.71	0–28
10^5	$2.70 \times 10^5 \pm 1.18 \times 10^5$	1.37×10^5–4.32×10^5	1036.11 ± 428.36	584–1925
10^7	$2.81 \times 10^7 \pm 5.83 \times 10^7$	1.67×10^7–3.76×10^7	98 753.89 ± 36 295.81	54 728–165 035
10^9	$2.06 \times 10^9 \pm 2.98 \times 10^9$	1.64×10^9–2.57×10^9	694 951.67 ± 63 958.12	543 314–738 678

*Means 36 samples.

Table 2 Log_{10} aerobic plate count and log_{10} adenosine triphosphate bioluminescence relative light unit (RLU) values of plastic cutting boards inoculated with *Lactobacillus rhamnousus*

Target inoculum (log_{10}cfu/mL)	**Actual inoculum (log_{10}cfu/25 cm^2)**	**Confidence intervals (95%) (log_{10}cfu/25 cm^2)**	**Actual inoculum* (log_{10}RLU/25 cm^2)**	**Confidence intervals (95%) (log_{10}RLU/25 cm^2)**
3	3.7 ± 0.08	3.58–3.84	1.08 ± 0.20	0.90–1.48
5	5.39 ± 0.20	5.14–5.64	2.99 ± 0.17	2.77–3.28
7	7.44 ± 0.10	7.22–7.58	4.97 ± 0.15	4.74–5.22
9	9.30 ± 0.06	9.21–9.41	5.84 ± 0.06	5.74–5.87

*Means 36 samples.

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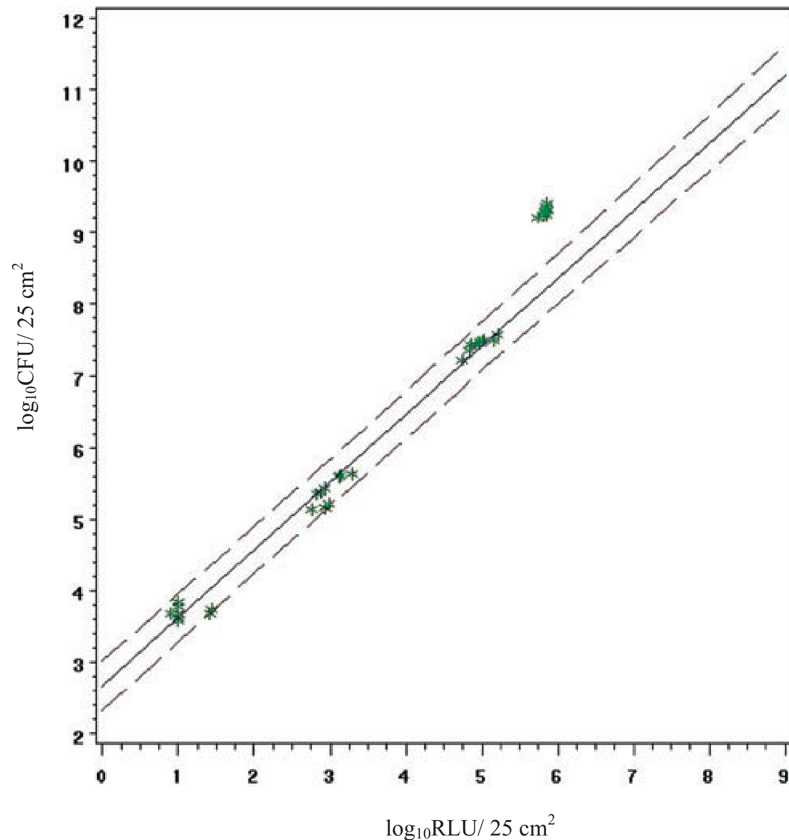

Figure 1 Prediction intervals for the linear regression analysis between total plate count (TPC) and ATP bioluminescence (ATP) tests assayed on 25-cm^2 plastic surfaces, where $\log_{10}$ATP is the dependent variable and $\log_{10}$TPC is the response variable.

10^9 cfu/mL were deviating significantly from the linear calibration curve based on concentrations 10^3, 10^5 and 10^7 cfu/mL. A second F test ($P <$ 0.0001) showed that the deviation of inoculum 10^9 cfu/mL was significant ($\alpha = 0.05$).

Therefore, the third mixed model analysis was conducted using data from concentrations 10^3, 10^5 and 10^7 cfu/mL to estimate the final calibration curve.

The final equation for the calibration curve was found to be

$$APC = 465.16 * ATP^{0.9480}$$

Prediction intervals were calculated for the calibration curve (Fig. 1). These intervals accounted for 95% of the points in the calibration curve.

To express the degree of association between $\log_{10}$ATP and $\log_{10}$APC, a correlation analysis was performed. The Pearson correlation coefficient was $r = 0.98$ ($P < 0.0001$). Therefore, a strong association existed between the $\log_{10}$ATP and $\log_{10}$APC readings, consistent with the linear regression analysis.

The sample coefficient of determination was used to indicate the proportion of the variability in $\log_{10}$APC explained by the linear bivariate association with $\log_{10}$ATP. From the data analyzed, it was found that $r^2 = 0.97$. Thus, 97% of the variability among $\log_{10}$APC can be explained on the basis of the linear relationship between $\log_{10}$ATP and $\log_{10}$APC.

To make a comparison between the two methods, we looked first at the coefficient of variation (CV). The CV for the $\log_{10}$ATP was equal to 5.28, and the CV for $\log_{10}$APC was found to be 2.69. Consequently, it may appear that APC did a better job estimating sanitation on plastic cutting boards compared with ATP bioluminescence. However, the comparison of two different methods using the CV is based on the assumption that one method is directly or inversely proportional to the other, and the relationship between APC and ATP bioluminescence was not clearly known. Therefore, the SR test is a better method of comparing APC and ATP bioluminescence tests when evaluating the sanitation

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levels on a food-contact surface. The estimated SR was equal to 1.016, which is very close to 1, indicating that there was a little difference between APC and ATP bioluminescence. In other words, ATP bioluminescence was as good as APC in evaluating sanitation on plastic cutting boards.

Discussion

Monitoring and evaluation of food-contact surface cleanliness are priorities in formulating an integrated sanitation program. Even though APC has been the traditional method of assessing the cleanliness of food-contact surfaces, the disadvantages of this method lie in the long time needed to obtain a microbial count, and that nonmicrobial residues cannot be detected. Information concerning microbial and food residue contamination obtained immediately after cleaning would be beneficial for real-time correction of cleaning efficiency problems. ATP bioluminescence is a fast, simple approach to surface sanitation monitoring.

After performing regression analyses, a linear model best described the relationship between APC and ATP bioluminescence. The linear trend was found when testing culture concentrations in the range of 10^3–10^7 cfu/25 cm^2. However, it was also important to note that culture inoculum of 10^9 cfu/25 cm^2 did not appear to follow the linear relationship. A generalized F-statistic was then obtained to test the lack of fit hypothesis that was statistically significant. The lack of fit associated with concentration 10^9 cfu/mL may be because of the firefly luciferase enzymatic reaction reaching a 'saturation' point at this high culture concentration. Thus, it was inferred that 10^7 cfu/mL/25 cm^2 was the sensitivity upper threshold for the ATP bioluminescence test.

A concentration of 10^3 cfu/25 cm^2 appeared to be the minimum sensitivity threshold for the ATP bioluminescence test. Additional tests using concentration 10^1 cfu/mL were conducted. Even though results were obtained by means of the APC method (estimated < 10 cfu/25 cm^2), the ATP bioluminescence test was recording zero readings. These findings led to the conclusion that APC and ATP bioluminescence were closely related, and did follow a linear relationship when levels of microbial load were higher or equal to 10^3 cfu but lower or equal to 10^7 cfu on 25-cm^2 plastic surface areas.

Determination of surface sanitation by ATP bioluminescence is increasingly used as a rapid test of proven value in food manufacturing environments (Ogden 1993), where most of the equipment and surfaces are made of stainless steel. Surface monitoring of different sites within the plant on a regular basis will provide the ability to establish ATP threshold levels appropriate to the specific process. Regular and frequent testing of control points will allow the observation of trends in levels of sanitation (Powell & Attwell 1997). However, the ATP bioluminescence test does not give an indication of the types of microorganisms present on food-contact surfaces. Microbiological tests are useful in determining the types of bacteria recovered, providing a microbial profile of the surface to aid in proper sanitation programs (Illsley *et al.* 2000).

Davidson *et al.* (1999) used either *Staphylococcus aureus* or *Escherichia coli* to inoculate 100-cm^2 stainless steel surfaces. The minimum bacterial detection limit for the ATP test was found to be 10^4 cfu/100 cm^2 for both organisms. Comparing their lower limit (10^4 cfu/100 cm^2) with our own data (10^3 cfu/25 cm^2), it can be inferred that the bioluminescence test used in our experiment was more sensitive when evaluating the sanitation of plastic cutting board surfaces.

General guidance on threshold limits for surface sanitation monitoring has been suggested as 'good' when estimated less then 10 cfu/plate or less than 200 RLU; 'pass' when 11–10 cfu/plate or 201–1000 RLU; and 'fail' when 11–50 cfu/plate or 1001–5000 RLU are recorded (Ogden 1993). Comparing this scale with the results found in our investigation, it could be implied that 'good' results will be achieved using the ATP bioluminescence test when a minimum of 1.9×10^4 cfu/25 cm^2 contamination level is present on the plastic surface.

There are no standards for food surface sanitation, and setting a level at which a test 'fails' has proven difficult. Cleaning was judged to be satisfactory if 10^3 cfu or less were recovered, or 400 RLU or less were detected by the ATP test on surface areas equal to 100 cm^2 (Tebbut 1999). Comparing our results obtained on plastic cutting boards, 400 RLU will represent approximately a

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microbial load of 3.7×10^4 cfu/25 cm^2. Bacterial contamination levels of 10^4 cfu are still high if foodborne pathogens are present on the surfaces.

Agreement between the microbiological technique and the ATP bioluminescence test was investigated. Illsley *et al.* (2000) reported that a surface was considered acceptable for processing food when less than or equal to 100 cfu/100 cm^2 were found with traditional methods. This level of contamination represented less than 200 or 2.5 RLU in the bioluminescence tests. Tests that failed the plate count methods and passed the ATP bioluminescence test probably resulted from the presence of low numbers of microorganisms sufficient to yield a failing result by the plate count criterion (>100 cfu/100 cm^2), but containing insufficient ATP to be detected by the bioluminescence test.

A 98% agreement was found between ATP and APC when tested on plastic cutting boards using a single bacterial culture (Bell *et al.* 1994), however, the authors stated that a high correlation should not be expected between ATP results and the microbiological assessments because of the former indicating the presence of ATP from sources additional to that of microorganisms. When stainless steel milk tankers surfaces (100 cm^2) were assessed for sanitation, results indicate that 93–98% of surfaces (tanker roof, side walls and end walls) were apparently ‘clean’ when examined using the microbiological swab technique (Bell *et al.* 1994). However, only 63–89% of these surfaces passed the criteria set as ‘clean’ for the ATP systems.

When surfaces were sampled before they had been cleaned, there was a significant difference between the results obtained using different methods of sanitation monitoring (Moore & Griffith 2002). Most notably, surfaces sampled were deemed unclean by the ATP bioluminescence test (44% of the time) but judged as being clean using traditional microbiology (Dipslides, Fisher). After the cleaning procedures, the level of agreement between ATP bioluminescence and traditional microbiology improved. Only 28.8% of the surfaces were deemed unacceptable by the ATP test despite being passed as clean by the Dipslides (Moore & Griffith 2002).

Daily routine cleaning and sanitation should reduce the contamination load below a threshold level. The specific physical properties of the surface will affect the ATP readings observed. The cleaning efficiency, such as the percentage of fall in ATP after cleaning, may indicate the effectiveness of sanitation control procedures. However, it is the final level of cleanliness achieved that is more important than the percent of contamination reduced. In most cases, cleaning appears to be effective in reducing ATP readings by up to 92.3% (Powell & Attwell 1997).

In the restaurant and foodservice industry, the use of ATP bioluminescence could be important because it allows real-time evaluation of the sanitation condition of cutting boards and work surfaces. Corrective actions could be taken immediately to avoid cross-contamination because of handling and preparation of foods on contaminated boards. Validation of the ATP bioluminescence using wooden surfaces is needed, because this material is also used in cutting boards. Testing the ATP bioluminescence *in situ* is also important. During different food service operations, the presence of a mixed flora is expected. The high correlation found between APC and ATP bioluminescence when using a pure culture may not necessarily be found.

Even though ATP bioluminescence proved to be as good as the microbiological methods in assessing contamination levels on plastic surfaces, a limitation of this study was the ability to obtain results with the luminescence test below 10^3 cfu/25 cm^2. This inoculum level still represents a high risk of contamination if pathogenic microorganisms were present on direct food-contact surfaces. It will be very useful to verify the ATP bioluminescence test using swabs that are more sensitive. The ideal situation will be to achieve ATP readings equal to zero RLU when there is an absence of microbiological contamination that would prevent false positive or false negative results.

The close relationship ($r = 0.98$) between APC and ATP bioluminescence assays in our study gave evidence that these two methods are in agreement (SR = 1.016) when measuring the sanitary condition of plastic surfaces. Therefore, ATP bioluminescence could be successfully used to monitor food-contact sanitation for plastic surfaces in restaurant and food service operations, and prevent cross-contamination of foods, especially ready-to-eat foods. ATP bioluminescence is

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an easy, rapid, cost-effective method for food service managers to evaluate the sanitation in their operations. In addition, managers could use ATP bioluminescence as a training tool for effective cleaning and sanitation methods.

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