A Research Note

Adenosine Triphosphate Bioluminescence for Hygiene Monitoring in Health Care Institutions

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ABSTRACT

An investigation was conducted to assess the practical use of an adenosine triphosphate (ATP) bioluminescence assay to evaluate the effectiveness of cleaning and sanitizing meat slicers in eight health care institutions. The ATP bioluminescence assay was compared to conventional swabbing techniques using standard plate count to enumerate microbial load. Assays were performed on meat slicers before use, after slicing a meat product and after sanitizing. There was a general overall agreement in results obtained by both methods but the ATP assay gave a better indication of cleanliness of the meat slicer as it was able to detect the presence of meat residues left on the blade after improper sanitation. Results were available within 5 min using the ATP bioluminescence method, thus providing an opportunity for immediate remedial action.

Key Words: ATP, bioluminescence, hygiene monitoring, meat

Hygiene monitoring is an important procedure to evaluate whether high standards are being maintained during the preparation of food. Various methods are used to determine the effectiveness of cleaning and sanitizing regimes. Many large food processing plants have on-site quality control personnel who monitor critical control points during production to ensure the safety and quality of the product. However, in food service settings, such as restaurants and health care institutions, most operators do not have the funds for this type of monitoring. Rapid methods for detecting potential sources of contamination of foods are needed in the food service sector; especially since incidents of foodborne illness are mainly associated with mishandling of food service establishments, institutions and caterers (6). Strict monitoring of food preparation practices is even more critical in health care institutions where the resident’s health may be already compromised putting them at greater risk. A quick, efficient method for the detection of microorganisms and food residues would help in implementing a more effective sanitation and Hazard Analysis Critical Control Points (HACCP) program. Immediate feedback on the effectiveness of a cleaning procedure would motivate staff to maintain sanitizing efficiency. At the same time, cross-contamination and bacterial loading of foods would be reduced.

Rapid methods for hygiene monitoring based on ATP bioluminescence are available from a number of companies and are routinely used in the food processing sector (2,4). They can be carried out in less than 5 min and the availability of portable instrumentation and constant-light-output reagents makes them suitable for on-site testing. However, there appears to be little published information on the use of these rapid hygiene monitoring kits in the food service sector. This study investigates the practical use of an ATP bioluminescence hygiene monitoring kit in different health care institutions.

MATERIALS AND METHODS

Sampling methodology

Hygiene testing was conducted at four nursing homes and four hospitals in Ontario. Swabs were taken from the feeding tray and the blade of meat slicers upon arrival, unannounced, in six of the health care institutions. On a separate visit to five of the institutions, swabs were taken from the meat slicers before use, after slicing a meat product and following the cleaning and sanitizing procedure. In all 69 surfaces were sampled.

Swabs were taken from a 25 cm² area using a clear, sterile acetate template (5 cm x 5 cm) to mark the section to be sampled. Swabs for ATP and plate count analyses were taken from adjacent areas of the slicer. As swabbing techniques provide a representative sample of the surface tested, it was deemed unnecessary to take duplicate swabs.

Adenosine triphosphate hygiene monitoring assay

Adenosine triphosphate bioluminescence hygiene monitoring kits and accessories were obtained from Biotrace™ Inc. (Biotrace™ Inc., Plainsboro, NJ). The assay was carried out according to the manufacturer’s instructions (1,2) immediately after swabbing at the health care institution. Light output from the reaction was measured using a Biotrace™ M3 luminometer.

A reagent blank was obtained using a sterile swab as the control, and any surface that gave a reading greater than three times the reagent control was considered unsatisfactory.
HYGIENE MONITORING WITH ATP BIOLUMINESCENCE

Swabs (Double Integral Sanitation, Ltd., Cooksville, Ontario) were transported at 5°C to the regional Public Health Laboratory, Palmerston, Ontario for analysis within 24 h of sampling. After vigorous shaking, samples of swab diluent (1 ml) were poured onto Bacto Plate Count Agar (Difco Laboratories, Detroit, MI) and incubated at 32°C for 48 ± 3 h before counting. When necessary, ten-fold serial dilutions of the swab diluent sample were prepared in sterile peptone water (Difco).

For the purposes of this study, a count of < 40 CFU/cm² was considered acceptable and indicative of a clean surface (3).

RESULTS AND DISCUSSION

Examination of meat slicers at health care institutions when visit was unannounced

Feeding trays and blades from meat slicers in the kitchens of six health care institutions were examined before use during an unannounced visit. For 8 of 11 samples (72.7%), there was agreement between results obtained by both ATP and plate count methods (Table 1). Where there was disagreement, the ATP result for one slicer blade (institution 1) was only a marginal failure and the other two samples failed by the ATP assay but were acceptable when analyzed by plate count. This was probably because the ATP method also detected ATP present in food residues remaining on the surface which may not have been contaminated with microorganisms (2,4). In many cases, plate counts of < 4 CFU/cm² were obtained and this should be considered an achievable target.

The disagreements all arose with samples from slicer blades. These are notoriously difficult to clean and a method to detect inadequate removal of meat residues, as provided by the ATP bioluminescence assay, is of great value in ensuring proper sanitization.

Slicers at institutions 1, 3 and 4 had been used earlier on the day of sampling and cleaned and sanitized. All these machines were considered contaminated, as indicated by high ATP readings for swab samples from either blades and/or feeding trays. The slicer from institution 3 was used later in the day of sampling. Thus, the potential for contamination could have been eliminated by re-cleaning the machine in response to the results obtained by the ATP assay.

Adenosine triphosphate bioluminescence assay to evaluate effectiveness of sanitization of meat slicers

In the second part of the study, five health care institutions were visited by prior appointment. Where possible, meat slicers were examined before use, immediately after slicing and after cleaning and sanitization. A suitable time period was allowed for dissipation of the sanitizing agents before using the ATP hygiene monitoring kit to reduce the risk of inhibition of the luciferase.

For blade swabs, there was 72.7% agreement (8 of 11 samples) in results obtained with the ATP and plate count methods. A similar value (81.8%; 9 of 11 samples) was found when feeding tray swabs were analyzed (Table 2). Three samples (two blade and one feeding tray) failed by the ATP assay but the plate count was within the recommended standard. This may have been due to the presence of food residues not detected by plate count. However, in all these samples the plate count was relatively high. Two samples gave acceptable ATP levels but high plate counts. This may have been due to the presence of sporeforming organisms. It is known that spores contain negligible amounts of ATP (5).

When swabs were taken immediately after the machine was used, all were considered unacceptable by the ATP assay (Table 2). However, when tested by plate count, two swabs (1 blade and 1 feeding tray sample) came within the guidelines for acceptable cleanliness. After cleaning and sanitizing, all the slicers fell within acceptable limits for the ATP assay, but one feed tray swab showed appreciable levels of microbial contamination upon plating. Appropriately sanitized or combination detergent-sanitizers were being used in each institution, but dosage rates did not seem to be a priority for the staff when they were questioned. Institution 3 used a combination of sodium hypochlorite and detergent and this was effective (Table 2). However, the same slicer was found to be contaminated by both the ATP bioluminescence method and plate count when sampled during the unannounced visit (Table 1). This may have been due to inconsistencies in the cleaning regime. The person who normally cleaned the equipment was absent at the time of the second sampling, but it clearly illustrates the usefulness of the immediate results provided by the ATP hygiene monitoring kit.

For all the surfaces tested, there was an overall agreement of 74% between results obtained using ATP bioluminescence and plate count (Fig. 1).

CONCLUSIONS

The ATP bioluminescence assay is a reliable alternative to plate counting for evaluating the cleanliness of food contact surfaces in food service kitchens. The results showed

TABLE 1. Comparison of ATP bioluminescence hygiene monitoring kit and conventional plate count method for examining swabs taken from the feeding tray and blade of meat slicers before use during an unannounced visit to six health care institutions.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Feeding tray</th>
<th>Slicer blade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP (RLU)</td>
<td>Plate count (CFU/cm²)</td>
</tr>
<tr>
<td>1</td>
<td>10 (P)</td>
<td>8 (P)</td>
</tr>
<tr>
<td>2</td>
<td>147 (F)</td>
<td>116 (F)</td>
</tr>
<tr>
<td>3</td>
<td>130 (F)</td>
<td>68 (F)</td>
</tr>
<tr>
<td>4</td>
<td>110 (F)</td>
<td>NA *</td>
</tr>
<tr>
<td>5</td>
<td>23 (P)</td>
<td>&lt;4 (P)</td>
</tr>
<tr>
<td>6</td>
<td>16 (P)</td>
<td>4 (P)</td>
</tr>
</tbody>
</table>

* Letters in parenthesis refer to whether the sample was of an acceptable standard [e.g., pass (P) (ATP <3x control reading; plate count <40 CFU/cm²)], or was unacceptable [e.g., fail (F) (ATP >3x control reading; plate count >40 CFU/cm²)].
✓ indicates that the results obtained by the ATP method and plate count were in agreement.
X indicates that the results obtained by the ATP method and plate count were not in agreement.
TABLE 2. Comparison of ATP bioluminescence and plate count methods for examining swabs taken from the feeding tray and blade of meat slicers before use, after slicing and after cleaning/sanitizing at five health care institutions.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Blade</th>
<th>Feeding tray</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before use</strong></td>
<td><strong>ATP</strong></td>
<td><strong>SPC</strong></td>
</tr>
<tr>
<td>Institution 1</td>
<td>7 (P)</td>
<td>160 (F)</td>
</tr>
<tr>
<td></td>
<td>42 (F)</td>
<td>5200 (F)</td>
</tr>
<tr>
<td>Institution 2</td>
<td>39 (F)</td>
<td>28 (P)</td>
</tr>
<tr>
<td></td>
<td>18 (P)</td>
<td>&lt;4 (P)</td>
</tr>
<tr>
<td>Institution 3</td>
<td>8 (P)</td>
<td>&lt;4 (P)</td>
</tr>
<tr>
<td></td>
<td>8 (P)</td>
<td>4 (P)</td>
</tr>
<tr>
<td>Institution 7</td>
<td>76 (F)</td>
<td>44 (F)</td>
</tr>
<tr>
<td></td>
<td>15 (P)</td>
<td>12 (P)</td>
</tr>
<tr>
<td>Institution 8</td>
<td>19 (P)</td>
<td>4 (P)</td>
</tr>
<tr>
<td></td>
<td>17 (P)</td>
<td>16 (P)</td>
</tr>
</tbody>
</table>

* ATP bioluminescence method (RLU).
  1 SPC = Plate count (CFU/cm²).
  2 Letters in parenthesis refer to whether the sample was of an acceptable standard [e.g., pass (P) (ATP <3x control reading; plate count <40 CFU/cm²)], or was unacceptable [e.g., fail (F) (ATP >3x control reading; plate count >40 CFU/cm²)].
  3 X indicates that the results obtained by the ATP method and plate count were not in agreement.
  4 ✓ indicates that the results obtained by the ATP method and plate count were in agreement.
  5 NA = not available.

A 74% overall agreement between the two methods when using a pass/fail cut-off of 3x control values for ATP assay and 40 CFU/cm² for plate count. The ATP method had a greater failure rate than plate counts, primarily because the former detected non-microbial, as well as microbial, sources of contamination. Thus, a better indication of cleanliness was given by the ATP hygiene monitoring kit.

Results suggested that sanitizing regimes could be improved at health care institutions. Although there was, generally, a good sanitizing effectiveness when cleaning the feeding tray, blade cleaning was less effective. This was presumably due to the inability to remove the blade and caution in handling to prevent injury. Using the ATP bioluminescence assay on-site would help staff and management develop and monitor a consistent, effective cleaning/sanitizing regime.

A review of the current plate count standard for environmental hygiene monitoring of <40 CFU/cm² was indicated. A plate count from swabs of <4 CFU/cm² was achievable in 50% of the institutions monitored and a standard closer to this should be considered.

The benefits of the ATP assay seem evident for use during any HACCP audit and has educational value in training and motivating staff to carry out cleaning/sanitizing procedures. The ability to assess a critical control point during food preparation, and make on the spot corrective changes before proceeding, is of inestimable value. It would certainly be cost effective if a quality problem was identified before products were returned or a foodborne outbreak occurred.
ACKNOWLEDGMENTS

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REFERENCES