Staphylococcus aureus has been recognized as a cause of foodborne illness since the late 19th century. Identification of this potential pathogen is important for food safety because approximately 35% of S. aureus strains produce at least one type of heat-stable enterotoxin that can cause food poisoning. The 3M™ Petrifilm Staph Express Count Plate and the 3M Petrifilm Staph Express Disk have been developed to accurately enumerate S. aureus in foods.

The performance of the method was demonstrated by comparing the counts from 96 pure strains and from 33 foods using both the Petrifilm Staph Express plate method and the reference method, the Baird-Parker agar plus tube-coagulase test (BPA). Sensitivity and specificity values are similar for both methods. Analysis of variance showed that there was no statistical difference between the methods. These results indicate that the Petrifilm plate method gives similar, quantitative results in approximately one-third the time of the Baird-Parker agar plus tube-coagulase method.

### Test Organisms
Bacteria for the study of pure cultures were derived from lyophilized preparations purchased directly from the American Type Culture Collection or were taken from frozen stock cultures of food isolates that had been identified by reference methods before freezing.

### Preparation of Artificially Inoculated Food Samples
Food samples were prepared according to ISO 6887-1. Briefly, a 10-g food sample was added to 90 mL peptone salt diluent before homogenizing. Diluted samples were adjusted to pH 6.0-8.0. Additional dilutions were made in serial 1:10 increments.

The S. aureus isolate used for artificial inoculation was ATCC 8095. The isolate was added at 2 levels of inoculation.

### Microbiological Analyses

<table>
<thead>
<tr>
<th>Petrifilm Staph Express plate method</th>
<th>Baird-Parker agar plus tube-coagulase reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Food samples were artificially contaminated with 50-150 organisms/mL.</td>
<td>• Food samples were artificially contaminated with 50-150 organisms/mL.</td>
</tr>
<tr>
<td>• Plates were inoculated with 1.0 mL portions.</td>
<td>• Three BPA plates were inoculated with 0.3, 0.3, and 0.4 mL portions.</td>
</tr>
<tr>
<td>• Plates were incubated at 37 ± 1°C for 24 ± 2 h.</td>
<td>• Plates were incubated at 37 ± 1°C for 45 - 48 h.</td>
</tr>
<tr>
<td>• Red-violet colonies on the plate were enumerated and recorded as S. aureus.</td>
<td>• Suspect colonies were identified according to ISO 6888-1.</td>
</tr>
<tr>
<td>• Petrifilm Staph Express disk was inserted and the plate and disk were incubated at 37 ± 1°C for 3 h.</td>
<td>1. Colonies were counted.</td>
</tr>
<tr>
<td>• Pink zones were enumerated as S. aureus. Colonies without pink zones were considered to be atypical and non-S. aureus.</td>
<td>2. Colonies were selected for tube-coagulase testing.</td>
</tr>
<tr>
<td>• Colonies associated with a pink zone were selected for tube-coagulase testing.</td>
<td><strong>Coagulase tests</strong></td>
</tr>
<tr>
<td></td>
<td>• Colonies were streaked to tryptic soy agar (TSA) plates.</td>
</tr>
<tr>
<td></td>
<td>• Plates were incubated overnight at 37 ± 1°C.</td>
</tr>
<tr>
<td></td>
<td>• Colonies from TSA plate were transferred to tubes containing brain heart infusion broth (BHI).</td>
</tr>
<tr>
<td></td>
<td>• BHI tubes were incubated at 37 ± 1°C for 24 ± 2 h.</td>
</tr>
<tr>
<td></td>
<td>• 0.1 mL of the BHI culture was added to 0.3 mL of reconstituted rabbit plasma with ethylene diamine tetraacetic acid (EDTA).</td>
</tr>
<tr>
<td></td>
<td>• Tubes were re-incubated at 37 ± 1°C for 6 h.</td>
</tr>
<tr>
<td></td>
<td>• Coagulase tests were read for clotting.</td>
</tr>
</tbody>
</table>
**Experiment (continued)**

**Study Design**
After overnight incubation in tryptic soy broth, cultures of *S. aureus* and non-*S. aureus* (Appendices 1 & 2) were diluted in diluent to a concentration of approximately 100 organisms/mL. Three BPA plates were inoculated; Petrifilm plates were inoculated from the same suspension used to inoculate BPA plates. Plates were incubated, then colonies were counted and tested for coagulase as described above.

Approximately 35 samples from foods (Appendix 3) were prepared and plated as described above. These samples were used in the comparison of the BPA and Petrifilm Staph Express plate method.

**Data Analysis**
The sensitivity rate and the specificity rate were calculated for both the Petrifilm Staph Express plate method and the BPA plus tube-coagulase method using the samples inoculated with pure strains. The sensitivity rate and the specificity rate are defined by the following formulae:

\[
\text{Sensitivity} = \frac{\text{positive by the method}}{\text{total } S. \text{ aureus by biochemical analysis}}
\]

\[
\text{Specificity} = \frac{\text{negative by the method}}{\text{total non - } S. \text{ aureus by biochemical analysis}}
\]

A paired t-test by inoculation level was used to compare the Petrifilm Staph Express plate method with the BPA plus tube-coagulase method using the samples from artificially inoculated foods. The raw counts were first converted to log_{10} counts to more nearly match the underlying assumption of normality.

**Results**

Ninety-six isolates were tested using the Petrifilm Staph Express plate method and the BPA method. Table 1 identifies *S. aureus* by both methods compared to the true state, which was determined by biochemical analysis.

<table>
<thead>
<tr>
<th></th>
<th>TRUE STATE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>non-<em>S. aureus</em></td>
<td></td>
</tr>
<tr>
<td>Petrifilm Staph Express plate method</td>
<td>positive</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TRUE STATE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>non-<em>S. aureus</em></td>
<td></td>
</tr>
<tr>
<td>BPA plus tube-coagulase method</td>
<td>positive</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>1</td>
<td>56</td>
</tr>
</tbody>
</table>

The Petrifilm Staph Express plate method had a sensitivity rate and a specificity rate of 100% and 92%, respectively. The BPA plus tube-coagulase method had a sensitivity rate and a specificity rate of 97% and 93%, respectively.

Three *Staphylococcus intermedius* isolates and 2 *Staphylococcus schleiferi* isolates grew as black colonies and produced pink DNase zones when the disk was added (thus, a positive result) using the Petrifilm Staph Express plate method. The same three *S. intermedius* and one of the *S. schleiferi* gave positive tube-coagulase results using the BPA method. One *S. aureus* isolate from hash browns failed to grow on BPA.
Table 2 gives the comparisons between the Petrifilm Staph Express plate method and the BPA method with artificially contaminated foods (see Appendix 3). These foods were tested in an external laboratory in Japan and in 3M’s lab in the United States. The mean log \( S. \) \textit{aureus} counts per gram were not significantly different between the methods.

### Table 2. Comparison of Petrifilm Staph Express plate method and BPA method for the enumeration of \( S. \) \textit{aureus} in artificially inoculated foods.

<table>
<thead>
<tr>
<th>Inoculation Level(^b)</th>
<th>Comparison(^b)</th>
<th>( N )</th>
<th>Mean log Difference(^d)</th>
<th>SE(^c)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>Red-violet</td>
<td>35</td>
<td>0.01</td>
<td>0.02</td>
<td>0.77</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Pink zones</td>
<td>35</td>
<td>0.01</td>
<td>0.01</td>
<td>1.00</td>
<td>0.32</td>
</tr>
<tr>
<td>high</td>
<td>Red-violet</td>
<td>36</td>
<td>0.03</td>
<td>0.02</td>
<td>0.83</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Pink zones</td>
<td>36</td>
<td>0.01</td>
<td>0.02</td>
<td>1.40</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\(^a\) Low inoculation level = about 30-50 organisms/mL; high inoculation level = about 120-160 organisms/mL  
\(^b\) Comparison of the BPA method to the Petrifilm Staph Express plate method counting red-violet colonies and to the Petrifilm Staph Express plate method counting pink zones  
\(^c\) Number of samples used in the analysis  
\(^d\) Mean log difference: \( \log \) BPA count – \( \log \) Petrifilm Staph Express plate count  
\(^e\) Standard error of the mean log difference

### Conclusion

Results with the Petrifilm Staph Express plate method were similar to those of the BPA plus tube-coagulase method with pure cultures of organisms and artificially inoculated foods. The Petrifilm Staph Express plate method had a sensitivity rate and a specificity rate of 100% and 92%, respectively. The mean log \( S. \) \textit{aureus} counts per gram were not significantly different from the log colony counts of the BPA plus tube-coagulase method. The Petrifilm Staph Express plate method gave final results in approximately one-third of the time required for the reference method, and the results were truly quantitative. The Petrifilm Staph Express plate method provides the added advantages of a labor-saving, space-saving, waste-reducing, and sample-ready medium which can be used with a variety of diluents.
Appendix 1

*S. aureus* strain sources

**Lab Strains**
sweetener, spicy chili with beans, Italian alfredo sauce, banana nut muffin, beef patty, cream of broccoli soup, environmental frozen entrée plant, nutrition candy bar, breaded chicken, minestrone soup, chicken vegetable soup, hash brown potatoes (4 isolates), milk powder (3 isolates), hamburger patty (3 isolates), environmental pasta plant (3 isolates)

**ATCC food strains**
ATCC 8095 cream pie
ATCC 51740 margarine
ATCC 13565 ham

**ATCC source unknown**
ATCC 9144
ATCC 13301
ATCC 27659
ATCC 27660
ATCC 27661

**ATCC clinical**
ATCC 6538
ATCC 12598
ATCC 12600
ATCC 25923

Appendix 2

**Non-*S. aureus* strain sources**

**non-staph strains**
Bacillus species
Enterococcus faecalis
Escherichia coli
Serratia liquefaciens

**staphylococci yet non-aureus strains**
*S. capitis*
*S. carnosus*
*S. cohnii* (3 isolates)
*S. epidermidis* (5 isolates)
*S. hominus* (5 isolates)
*S. hyicus* (2 isolates)
*S. saprophyticus* (4 isolates)
*S. sciuri* (4 isolates)
*S. simulans*
*S. warneri* (5 isolates)
*S. xylosus* (2 isolates)

**ATCC strains**
Bacillus circulans ATCC 61
Bacillus subtilis ATCC 9372
Enterococcus durans ATCC 11576
Enterococcus faecalis ATCC 14506
Enterococcus faecium ATCC 6569
Listeria monocytogenes ATCC 15313

Appendix 3

**Food Tested**

**meat** - raw hamburger, raw round beef roast, raw ground pork, sliced deli ham, raw chicken thighs and breasts;
**fish** - king salmon, raw tuna, dried fish;
**seafood** - raw scallops, raw shrimp, raw squid;
**dried foods** - beef and pork gravy powders, mashed potato flakes;
**fresh vegetables** - cucumber and carrot;
**liquid eggs**

**cheese** - pasteurized, shredded mozzarella;
**fresh prepared foods** - rice balls, sandwich, lunch box meal, tuna macaroni salad, Italian pasta salad, potato salad with egg, chocolate custard pastry, fresh fettuccini;
**frozen prepared foods** - meat dumplings, chicken dinner, salisbury steak dinner, hash brown potatoes, french-fried potatoes.