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3M™ Petrifilm™ Staph Express Count Plate and Disk
Performance Summary

3M™ Petrifilm™ Staph Express Count Plate and Disk

Staphylococcus aureus (S. 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 40% of *S. aureus* strains produce at least one type of heat-stable enterotoxin that can cause food poisoning.[1] The 3M™ Petrifilm™ Staph Express Count (STX) 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 3M Petrifilm STX Plate and Disk 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 3M Petrifilm STX Plate and Disk method gives similar, quantitative results in approximately one-third the time of the Baird-Parker agar plus tube-coagulase method.

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Experiment

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. Strains were confirmed by biochemical analysis.

Preparation of Artificially Inoculated Food Samples

Food samples were prepared according to ISO 6887-1. Briefly, a 10g food sample was added to 90mL peptone salt diluent before homogenizing by stomaching for 1–2 minutes. 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 two levels of inoculation. Samples were artificially contaminated to achieve 50–150 organisms/1mL of plate inoculum.

Microbiological Analyses

3M Petrifilm Staph Express (STX) Plate and Disk Method

- Plates were inoculated with 1.0mL portions.
- Plates were incubated for 24±2 hours at 37±1°C.
- Red-violet colonies on the plate were enumerated and recorded as *S. aureus*.
- 3M Petrifilm Staph Express Disk was inserted and the plate and disk were incubated for 3 hours at 37±1°C.
- Pink zones were enumerated as *S. aureus*. Colonies without pink zones were considered to be atypical and non-*S. aureus*.
- Colonies associated with a pink zone were selected for tube-coagulase testing.

Microbiological Analyses (cont.)

Baird-Parker Agar Plus Tube-Coagulase Reference Method

- Three BPA plates were inoculated with 0.3, 0.3 and 0.4mL portions.
- Plates were incubated for 45–48 hours at 37±1°C.
- Colonies were identified according to ISO 6888-1.
  - Typical colonies were counted.
  - Presumptive colonies were selected for tube-coagulase testing.

Tube-Coagulase Test Method

- Five presumptive colonies were streaked to tryptic soy agar (TSA) plates.
- Plates were incubated overnight at 37±1°C.
- Colonies from TSA plate were transferred to tubes containing brain heart infusion broth (BHI).
- BHI tubes were incubated for 24±2 hours at 37±1°C.
- 0.1mL of the BHI culture was added to 0.3mL of reconstituted rabbit plasma with ethylene diamine tetraacetic acid (EDTA).
- Tubes were re-incubated for 6 hours at 37±1°C.
- Coagulase tests were read for clotting.

Study Design

Approximately 35 samples from foods (Appendix 3) were prepared and plated as described above. These samples were used in the comparison of the BPA plus tube-coagulase test method and 3M Petrifilm STX Plate with the Staph Express Disk method.

After overnight incubation in tryptic soy broth, cultures of *S. aureus* and non-*S. aureus* (Appendices 1 and 2) were diluted in tryptone salt buffer to a concentration of approximately 100 organisms/mL. Three BPA plates were inoculated as described above; 3M 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.

Data Analysis

The sensitivity rate and the specificity rate were calculated for both the 3M Petrifilm STX Plate and Disk 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:

$$\textbf{Sensitivity} = \frac{\text{positive by the method}}{\text{total } \textit{S. aureus}}$$

$$\textbf{Specificity} = \frac{\text{negative by the method}}{\text{total non-}\textit{S. aureus}}$$

A paired t-test by inoculation level was used to compare the 3M Petrifilm STX Plate and Disk 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

Approximately 35 samples from foods (Appendix 3) were prepared and plated as described on the previous page. These samples were used in the comparison of the BPA plus tube-coagulase reference method and 3M Petrifilm STX Plate with the 3M Petrifilm Staph Express Disk method. Table 1 shows the comparisons between the 3M Petrifilm STX Plate and Disk method and the BPA method with artificially contaminated foods (see Appendix 3). The mean log *S. aureus* counts per gram were not significantly different between the methods.

Table 1.
Comparison of 3M Petrifilm Staph Express Count Plate method and BPA plus tube-coagulase reference method for the enumeration of *S. aureus* in artificially inoculated foods

Inoculation Level[a]	Comparison[b]	N[c]	Mean Log Difference[d]	SE[e]	t-value	p-value
Low	Red-Violet	35	0.01	0.02	0.77	0.44
	Pink Zones	35	0.01	0.01	1.00	0.32
High	Red-Violet	36	0.03	0.02	0.83	0.41
	Pink Zones	36	0.01	0.02	1.40	0.17

a Low inoculation level = about 30–50 organisms/1mL plate inoculum; high inoculation level = about 120–160 organisms/1mL plate inoculum
b Comparison of the BPA method to the 3M Petrifilm STX Plate method counting red-violet colonies and to the 3M Petrifilm Staph Express plate method counting pink zones
c Number of samples used in the analysis
d Mean log difference: log BPA count – log 3M Petrifilm STX Plate count
e Standard error of the mean log difference

Sensitivity and Specificity

Ninety-six isolates were tested using the 3M Petrifilm STX Plate method and the BPA plus tube-coagulase reference method. Table 2 displays the identification of *S. aureus* by both methods compared to reference method, ISO 6888-1;1999.

Table 2.
Identification of *S. aureus* by 3M Petrifilm Staph Express Count Plate method and BPA plus tube-coagulase method versus the true state as determined by biochemical analysis.

		True State	
Method	Results	*S. aureus*	non-*S. aureus*
3M Petrifilm Staph Express Plate Method	positive	36	5
	negative	0	55
Baird-Parker Agar Method (plus tube-coagulse)	positive	35	4
	negative	1	56

The 3M Petrifilm STX Plate and Disk 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 two *Staphylococcus schleiferi* isolates grew as black colonies and produced pink DNase zones when the disk was added (thus, a positive result) using the 3M Petrifilm STX 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.

Conclusion

Results with the 3M Petrifilm Staph Express Count Plate with the 3M Petrifilm Staph Express Disk method were similar to those of the BPA plus tube-coagulase method with pure cultures of organisms and artificially inoculated foods. The 3M Petrifilm STX Plate and Disk method had a sensitivity rate and a specificity rate of 100% and 92%, respectively. The mean log *S. aureus* counts per gram were not significantly different from the log colony counts of the BPA plus tube-coagulase method. The 3M Petrifilm STX Plate and Disk method gave final results in approximately one-third of the time required for the reference method, and the results were truly quantitative. The 3M Petrifilm STX Plate and Disk 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 Clinical
ATCC 6538
ATCC 12598
ATCC 12600
ATCC 25923

ATCC Source Unknown
ATCC 9144
ATCC 13301
ATCC 27659
ATCC 27660
ATCC 27661

Appendix 2

Non-*S. aureus* Strain Sources

Non-*Staphylococcus* Strains

Lab Strains
Bacillus species
Enterococcus faecalis
Escherichia coli
Serratia liquifaciens

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

Non-*Staphylococcus aureus* Strains

Lab Strains
S. capitis
S. carnosus
S. cohnii (3 isolates)
S. epidermidis (5 isolates)
S. hominis (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
S. auricularis ATCC 33753
S. caprae ATCC 35538
S. carnosus ATCC 51365
S. caseolyticus ATCC 13548
S. cohnii ATCC 35662
S. epidermidis ATCC 14990
S. equorum ATCC 43958
S. intermedius ATCC 49052
S. intermedius ATCC 51874
S. intermedius ATCC 29663
S. kloosii ATCC 43959
S. lentus ATCC 49574
S. lugdunensis ATCC 49576
S. schleiferi ATCC 43808
S. schleiferi ATCC 49545
S. warneri ATCC 49454
S. xylosus ATCC 29971

Appendix 3

Food Matrices 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 fettuccine
Frozen Prepared Foods: meat dumplings, chicken dinner, Salisbury steak dinner, hash brown potatoes, french-fried potatoes

References

1. Holekova B, Holoda E, Fotta M, Kalinacova V, Gondol J, Grolmus J. 2002 occurrence of enterotoxigenic *Staphylococcus aureus* in food. Ann Agric Environ Med. 9 (2): 179–82.
2. ISO 6887 Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
3. ISO 6888 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase positive staphyococci (**Staphylococcus aureus** and other species)

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