Excerpted from *Food Quality* magazine, Dec/Jan 2006

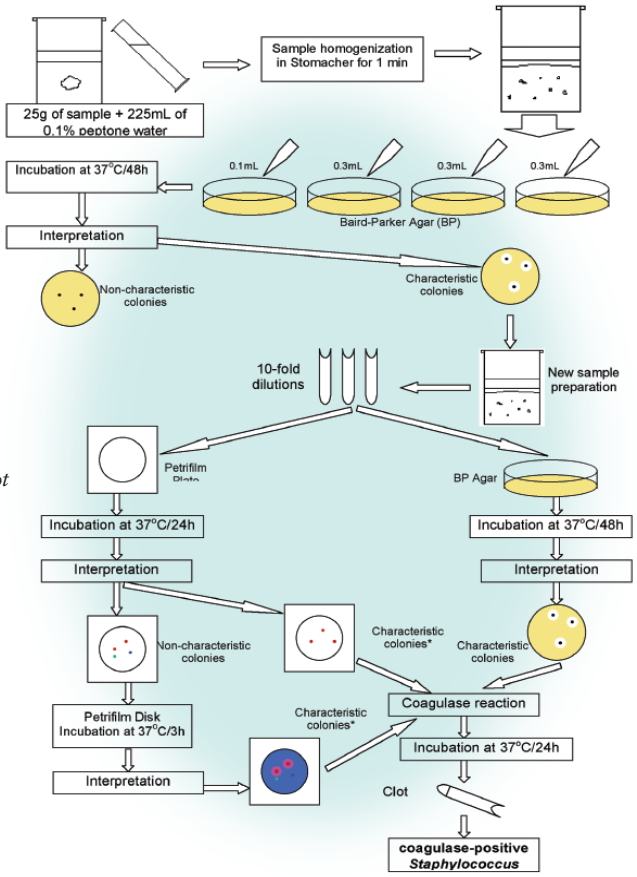


Fig. 1: Flow chart of the used methodology

**Coagulase reaction is not a necessary step when using STX System.*

Express Evaluation

An evaluation of 3M Petrifilm Staph Express Count System for enumerating coagulase-positive *Staphylococcus*

BY **A.R. TASSINARI, P.K. NODA, G.M. FRANCO, M. LANDGRAF AND M.T. DESTRO**

IN THE LAB

Classical methodologies used for enumerating *Staphylococcus* in food are time consuming taking up to 78 hours. The 3M Petrifilm Staph Express Count System (STX) is a rapid test that has recently been commercialized in order to shorten the time. When using STX, the population of *S. aureus* in food samples can be determined in as soon as 22 hours because the identification of *S. aureus* is not based on coagulase production.

LAB ANALYSIS

The objective of this study was to evaluate the efficiency STX on enumerating coagulase-positive *Staphylococcus* in naturally contaminated foods samples. The study used 128 food samples that were first screened for *Staphylococcus* by plating dilutions onto Baird Parker (BP) agar. Samples showing characteristic colonies were re-sampled and simultaneously plated onto STX plates, according to manufacturer's instructions, and onto BP according to FDA-BAM procedure. The results were compared, submitted to variance analysis (p>0.05) (Minitab Release14 Statistical Software) and linear regression (Log BP= -0.1545+1.00LogSTX).

Among the 77 samples with characteristic colonies in the screening evaluation, 62 (80 percent) showed similar results by both methods (p>0.05) indicating their equivalence (0.951). The number of coagulase-positive *Staphylococcus* was higher in the STX System than BP for 9 (11.7 percent) samples and was higher in BP than the STX System for 6 (7.8 percent) samples. A higher proportion of coagulase-negative colonies were observed amongst typical colonies isolated from BP (52 percent) than from the STX System (13 percent). No atypical colony was coagulase positive. Therefore, the STX System may be an alternative to the FDA-BAM protocol for enumeration of coagulase-positive *Staphylococcus*.

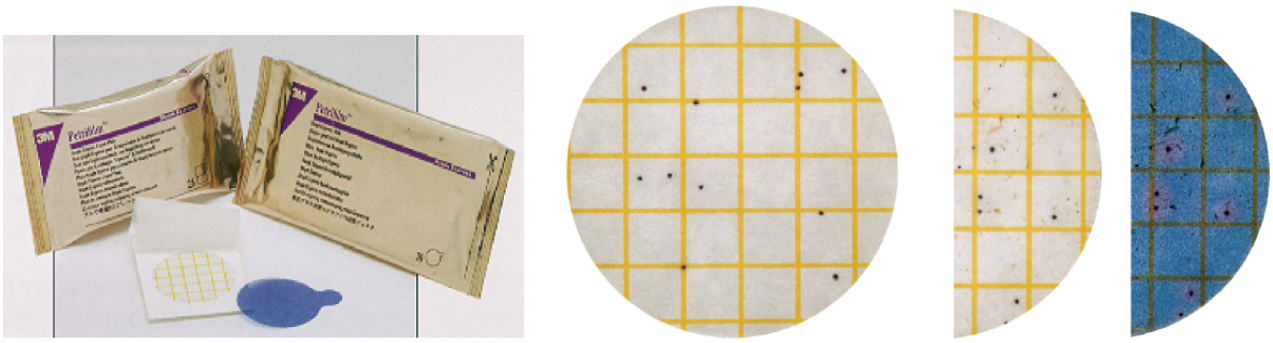
Food poisoning caused by *Staphylococcus* is the second cause of foodborne illness in the Latin America countries, including Brazil (Panalimentos, 2004).

Classical methodologies used for enumerating *Staphylococcus* in food are time consuming, taking up to more than three days. The most used method is the FDA-BAM and it consists of plating samples on Baird Parker Agar (BP) that contains in its composition, as selective agents, potassium tellurite and lithium chloride. The addition of egg yolk allows the visualization of the proteases and lipases reaction, produced by microorganisms. The suspect colonies of *S. aureus* are circular, black colored, brilliant, surrounded by opaque zone and frequently with an outer clear zone.

According to this methodology, the suspect colonies must be tested for coagulase production, however the use of thermo nuclease (TNase) is mentioned as being a simple, easy and quick alternative way for the usual identification of *S. aureus*.

The STX system consists of a Petrifilm Staph Express Count plate and a Petrifilm Staph Express disk, which are package separately. The Petrifilm Staph Express plate is a sample-ready culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for *Staphylococcus aureus*. The Express disk contains toluidine blue-O that facil-

These samples were bought in bakeries, supermarkets, snack bars and open markets in the cities of Sao Paulo and Mogi das Cruzes cities, Sao Paulo State.



a. b. c.

a. 3M™ Petrifilm Staph Express System; *b.* Petrifilm™ Staph Express plate showing characteristics colonies; *c.* Petrifilm™ Staph Express plate showing characteristics and non-characteristics colonies and using the Petrifilm™ Staph Express disk.

Fig. 2: **Percentage of coagulase-producer colonies isolated from Petrifilm STX System and BP agar**

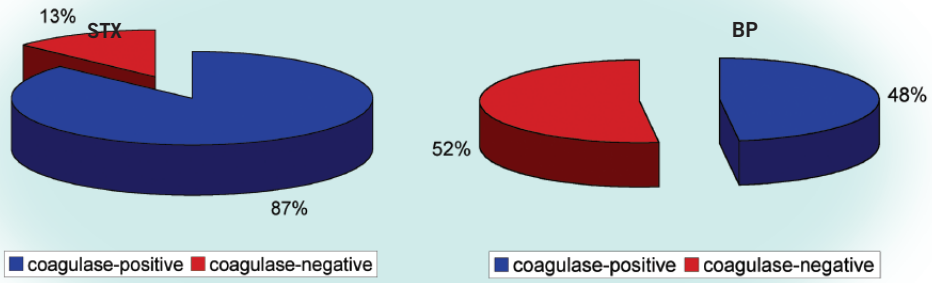
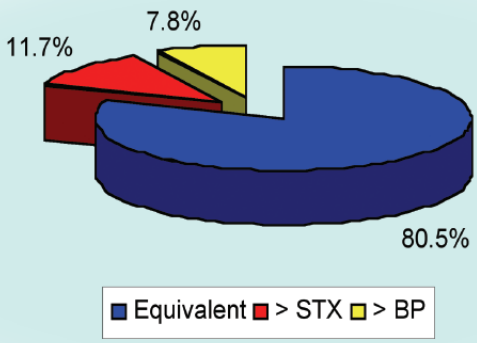


Fig. 3: **Relation between Petrifilm STX and BP methods for the analysis of coagulase-positive *Staphylococcus***



itates the visualization of deoxyribonuclease (DNase) reactions, according to the manufacturer. The DNase-positive results are claimed to be sufficient for the identification of coagulase-positive producers *Staphylococcus*, removing the need forthe coagulase production test. DNase-positive organisms detected on the Express plate, according to the manufacturer, are *S. aureus*, *S. hyicus e S. intermedius*. These three organisms comprise the majority of the group of organisms commonly known as coagulase-positive *staphylococci*. The total time spent in the analysis is, approximately, 27 hours.

Materials and Methods

The goal of the study was to evaluate the efficiency of the Staph Express System, for enumeration of coagulase-producers *Staphylococcus*, comparing the results to those obtained by the reference method. Amongst the 128 food samples examined, 82 samples were considered in this study, and include:

Cheese – 34
Cakes and pastries – 32
Different sausages – 14
Ready- to-eat sandwich – 1
Finger food – 1

Results

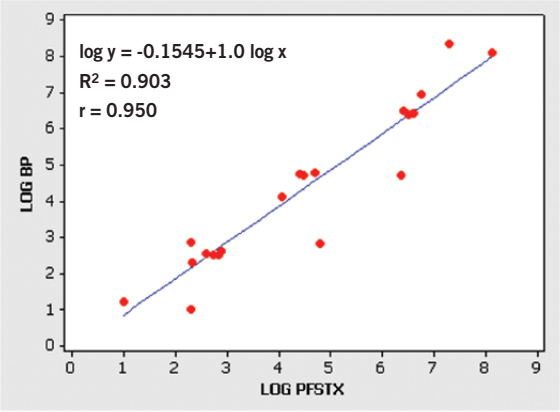
A total of 85 colonies, originated from 49 samples, were submitted to the coagulase test. Of these, 54 colonies were isolated from BP agar and 31 colonies were isolated from Petrifilm™ STX. 33 samples had no characteristic colonies onto BP agar, neither onto Petrifilm STX.

The percentage of compliance between Petrifilm STX and the reference method was calculated for 77 out of the 82 samples considered in this study. The results of the 5 discharged samples could not be used because of technical problems. Of the 77 results, 62 were equivalents and the percentage of compliance was 80.5 percent. This level is considered excellent for a new method. Amongst the discordant results, 9 samples (11.7 percent) presented higher counts of coagulase-positive *S. aureus* when tested with Petrifilm STX and 6 (7.8 percent) when using BP method.

There was no significant difference ($p > 0.05$) between the results of the counts obtained by both methods, being the correlation coefficient (r) equal to 0.950, slope equal to 1.00 and the intercept equal to -0.1545, as shown in the Figure 4.

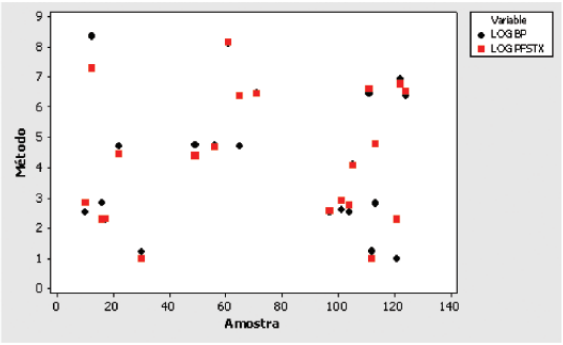
Using paired T- Test, the average count was 4.16 log using BP method and 4.32 log using Petrifilm STX, getting a difference of -0.154571 between the averages. The standard deviation was 2.26512 for BP method and 2.15304 for Petrifilm STX. The T-value (-1.01) and p-value (0.326), related to the test, also indicate that the methods did not present statistically significant difference, allowing the acceptance of the hypothesis that, BP method and Petrifilm STX methods are equivalent

Fig. 4: **Fitted Line Plot**



PFSTX = Petrifilm Staph Express; BP = Baird-Parker

Fig. 5: **Scatterplot of Log BP vs Log PFSTX**



PFSTX = Petrifilm Staph Express; BP = Baird-Parker

Conclusion

The results obtained in this study indicate that the 3M Petrifilm Staph Express System may be an alternative method to the FDA-BAM protocol for enumeration of coagulase-positive *Staphylococcus*. ■

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B.D. Gombossy, P.K. Noda, G.M. Franco, M. Landgraf and **M.T. Destro** are members of the of Pharmaceutical Sciences Faculty at the, University of Sao Paulo, Brazil Faculty, while **A.R. Tassinari** is from 3M do Brasil Ltda. For more information, contact Vicki Moss, sr. communications administrator, 3M Microbiology, 651-733-1213.