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Evaluation of Aerobic Plate Count Media for Consistency

Introduction

Aerobic Plate Count (APC) testing provides a food testing laboratory with a level of microorganisms present in the product being tested and can be used to determine sanitary quality, organoleptic acceptability, adherence to good manufacturing practices and to some extent, the safety of the product.[1, 2, 3] Since all of these requirements are critical to the ability of a food manufacturer to utilize the product, testing for APCs is a significant part of the daily routine in a food testing laboratory.

Selecting the appropriate method for APC testing can be challenging as there are various testing methods from rapid automated methods to traditional cultural methods. It's important for a laboratory to understand which method best fits their needs. Two of these options are Standard Methods Agar (SMA) plating and 3M™ Petrifilm™ Aerobic Count Plate. There are variations in formulation and production of SMA, but there are also many variations in how these media are used between laboratories. Traditional agar plating involves adding a sample to an empty Petri dish followed by the addition of tempered agar. The plates can be placed in a single layer on the laboratory bench or can be stacked prior to adding the tempered agar. The plates are then allowed to cool before being placed into the incubator. This study evaluated adding agar to samples with the plates stacked and non-stacked. The stacking method is often used to save time and bench space but studies have shown that the agar plates in the center of the stack do not cool at the same rate as the plates at the top and bottom of the stack.[4] This can lead to lower counts and ultimately inconsistent results if the stacked plates are replicates from the same sample. These variations can all add to the inconsistent performance of the aerobic plate count method selected by laboratory.

Three global SMA manufacturer's products, stacked and not stacked, were compared to 3M Petrifilm Aerobic Count Plate to determine if there are differences in each media's performance when evaluating consistency. The study was performed by three different analysts on different days. Each analyst conducted the protocol from start to finish including media preparation, sample preparation, sample plating and incubation.

Materials and Methods

Media and Reagents

- Peptone Saline Buffer, prepared according to manufacturer's instructions, 90mL
- Butterfield's Phosphate-Buffered Dilution Water, 90mL purchased prepared
- Buffered Peptone Water, 90mL purchased prepared
- 3M™ Petrifilm™ Aerobic Count Plate
- Standard Methods Agar A
- Standard Methods Agar B
- Standard Methods Agar C
- NSI Lab Solutions Multi-Organism Certified Reference Material (CRM)
  - *Escherichia coli* NCTC[A] 9001/ATCC[B] 11775
  - *Klebsiella oxytoca* NCTC 8167
  - *Staphylococcus aureus* NCTC 6571/ATCC 9144
  - *Candida albicans* NCPF[C] 3255/ATCC 2019

[A] National Collection of Type Cultures
[B] American Type Culture Collection
[C] National Collection of Pathogenic Fungi

Method

Sample preparation
The pH of each diluent was checked to ensure it was between 6.6 and 7.2. NSI Lab Solutions Multi-Organism CRM with yeast disk was equilibrated to room temperature for five minutes then aseptically added to 90mL of buffer (diluent). The diluent was swirled to mix and the disk was allowed to dissolve completely, approximately 15 minutes.

Plate preparation
SMA A, B and C were prepared per manufacturer's instructions. Petri dishes and 3M Petrifilm Aerobic Count Plates were labeled with buffer, replicate number and incubation temperature. There were 10 plates per buffer and corresponding incubation temperature (Table 1) for each plating option.

Table 1. Conditions for all media types.

Media Evaluated	Buffer (Diluent)	Incubation Temperature
Agar A, B, C, 3M™ Petrifilm™ Aerobic Count Plate	Peptone Saline Buffer	30°C
Agar A, B, C, 3M™ Petrifilm™ Aerobic Count Plate	Butterfield's Phosphate-Buffered Dilution Water	32°C
Agar A, B, C, 3M™ Petrifilm™ Aerobic Count Plate	Buffered Peptone Water	35°C

Sample Inoculation
The pipette tip was changed prior to inoculation of each plate; the inoculated buffer was mixed every 10 plates; and the plating order of each 3M Petrifilm Aerobic Count Plates and the SMA plates were randomized. For the 3M Petrifilm Aerobic Count Plate,[5] 1mL aliquots of sample were added to 10 plates for each of the three buffer/temperature combinations listed in Table 1 by three analysts. For agar plates,[1] 1mL aliquots of sample were added to 10 Petri dishes by three analysts. Within 15 minutes, approximately 12–15mL of SMA, tempered in a water bath set to 45°C ± 1°C for 1–3 hours, was added and the samples were mixed by alternate rotation and back-and-forth movement of plates. Two different approaches were used to add the agar to the plates: either the agar was added to the plates while they were in a single layer on the laboratory bench (not stacked) or as the plates were stacked in groups of six. See Figure 1 for a detailed diagram of the study.

Incubation
SMA plates were kept at room temperature to allow the agar to solidify, then incubated in stacks of no more than six plates. 3M Petrifilm Aerobic Count Plates were incubated in stacks of no more than 20 plates. Plates were incubated at the temperature indicated in Table 1.

Enumeration and Data
At the end of the appropriate incubation period, 3M Petrifilm Aerobic Count Plates and agar plates were removed from the incubator then enumerated manually.

Colony forming units (CFU/mL) were determined for all methods. Standard deviation, test for significant difference (P-Value), coefficient of variation were calculated and individual plots of raw counts were prepared (Figures 2–4, Tables 2–3) for each diluent/temperature combination.

Results and Discussion

This study compared 3M Petrifilm Aerobic Count Plates to a variety of SMA plates when focusing on the consistency of test results. Three different diluents were paired up with the most appropriate temperature where those diluents are typically incubated — Peptone Saline Buffer/30°C, Butterfield's Phosphate-Buffered Dilution Water/32°C and Buffered Peptone Water/35°C. Each SMA brand was evaluated after adding tempered agar to samples in a single layer of plates as well as in stacks of six.

When reviewing the standard deviations for the 3M Petrifilm Aerobic Count Plate as compared to Agars A, B and C, the standard deviations for 3M Petrifilm Aerobic Count Plates were equivalent or the lowest for all temperatures, diluents and stacking procedures. The standard deviation of the 3M Petrifilm Aerobic Count Plate was significantly lower ($P<0.05$) than Agar B for all diluent/temperature/stacking combinations. With the exception of not stacked at 32°C using Butterfield's Phosphate-Buffered Dilution Water, the standard deviation of Agar A was statistically equivalent to 3M Petrifilm Aerobic Count Plates. With the exception of Peptone Saline Buffer/30°C/not stacked, Agar C had a significantly higher standard deviation than 3M Petrifilm Aerobic Count Plate.

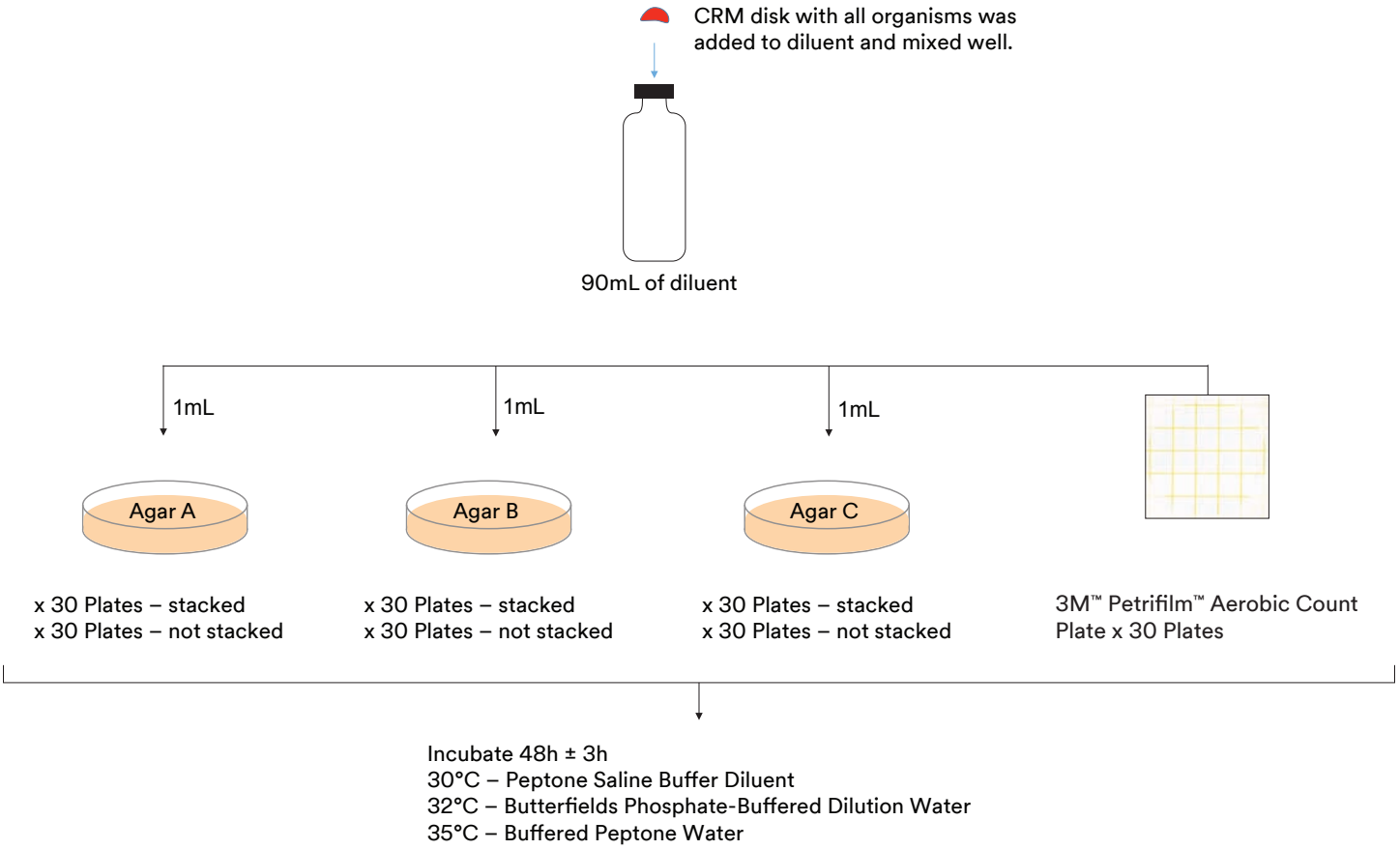
When evaluating the Coefficient of Variation (CV), 3M Petrifilm Aerobic Count Plates resulted in the smallest percentage as compared to the SMA. For the Peptone Saline Buffer/30°C the CV was 11.15% (Agars: 11.46% to 21.12%); for the Butterfield's Phosphate-Buffered Dilution Water/32°C the CV was 9.70% (Agars: 12.10% to 16.98%); and for the Buffered Peptone Water/35°C the CV was 10.43% (Agars: 11.80% to 17.73%).

Figures 2–4 show how 3M Petrifilm Aerobic Count Plates are able to provide more consistent results as compared to the SMA brands evaluated. There is clear clustering with the results from the 3M Petrifilm Aerobic Count Plates where as the results from Agars A, B and C are more scattered. This is important to food testing laboratories as most participate in proficiency testing and daily process controls. They want to be able to provide proof to their customers that the methods they have selected for their laboratory will provide consistent and accurate results. As the food industry continues to grow, with a projection of becoming a $1.6 billion dollar industry by 2020,[6] laboratories will need to be able to rely on their chosen methods to keep up with demands and satisfy their customers. Ultimately, providing a laboratory with an aerobic plate count method that is able to provide consistent results offers them confidence in their decisions to release or hold product.

While there were no significant differences between the SMA plates that were stacked during plating as compared to those that were poured in a single layer in this study, the performance of the 3M Petrifilm Aerobic Count Plate method is better as compared to the agar plate methods (Tables 2–3). It is possible that with a larger data set there would be a clear differentiation between the preparation methods for the SMA.

This study, overall, provided data demonstrating that the 3M Petrifilm Aerobic Count Plate method provides more consistent results when used under the common incubation conditions — Peptone Saline Buffer/30°C, Butterfield's Phosphate-Buffered Dilution Water/32°C and Buffered Peptone Water/35°C.

Figure 1. Aerobic test plating flowchart.



Figures 2a and 2b
Scatterplot comparing the counts from Agars A, B and C to 3M™ Petrifilm™ Aerobic Count Plate after plating cultures with Peptone Saline Buffer and incubating at 30°C. Plates were either stacked during the pouring of agar or in a single layer on the laboratory bench (not stacked).

Figure 2a.

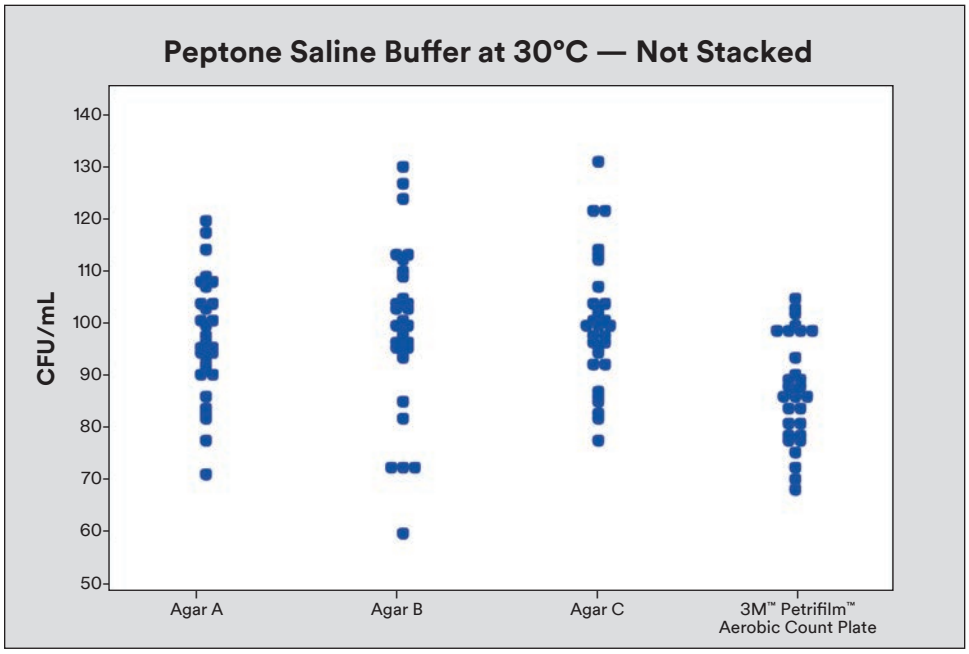
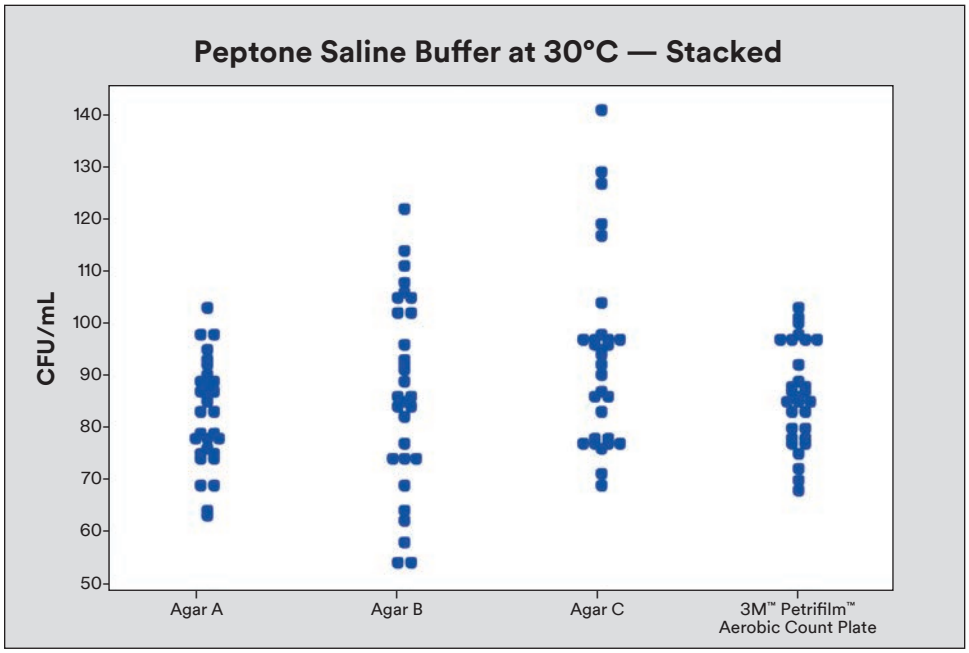


Figure 2b.



Figures 3a and 3b
Scatterplot comparing the counts from Agars A, B and C to 3M™ Petrifilm™ Aerobic Count Plate after plating cultures with Butterfield's Phosphate-Buffered Dilution Water and incubating at 32°C. Plates were either stacked during the pouring of agar or in a single layer on the laboratory bench (not stacked).

Figure 3a.

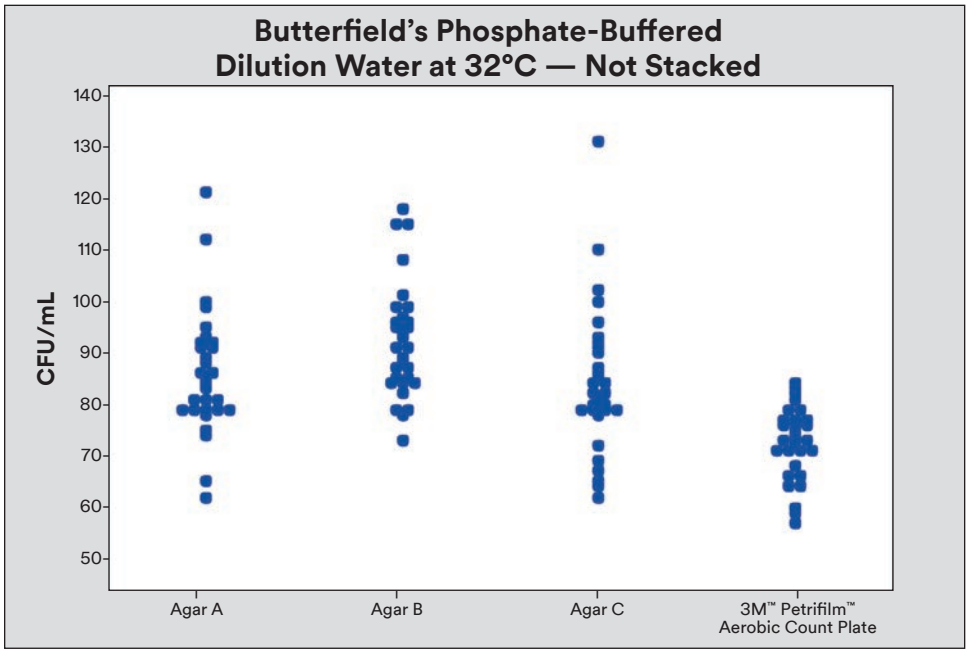
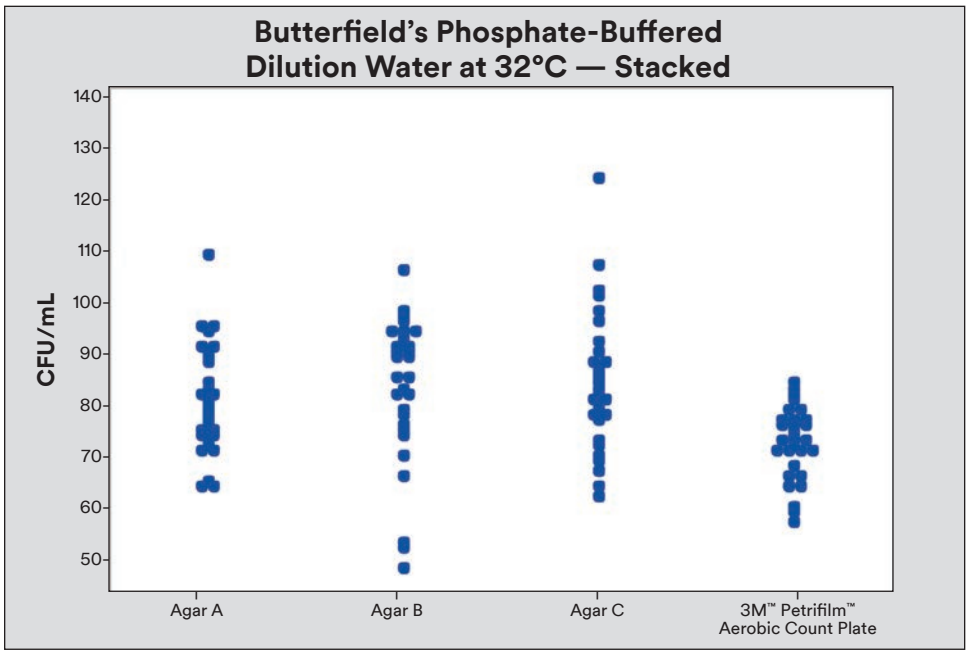


Figure 3b.



Figures 4a and 4b
Scatterplot comparing the counts from Agars A, B and C to 3M™ Petrifilm™ Aerobic Count Plate after plating cultures with Buffered Peptone Water and incubating at 35°C. Plates were either stacked during the pouring of agar or in a single layer on the laboratory bench (not stacked).

Figure 4a.

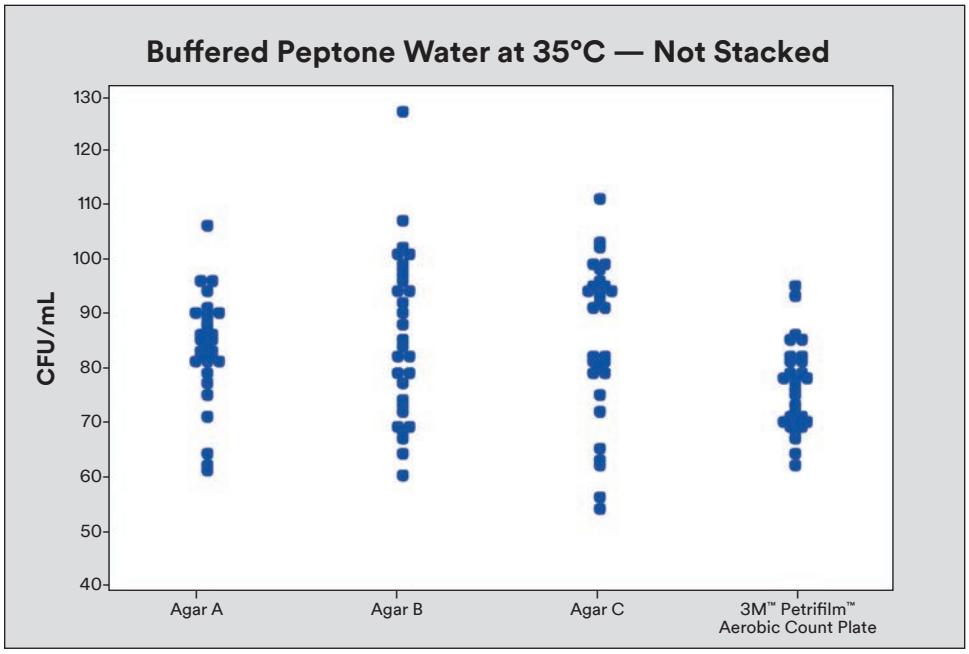


Figure 4b.

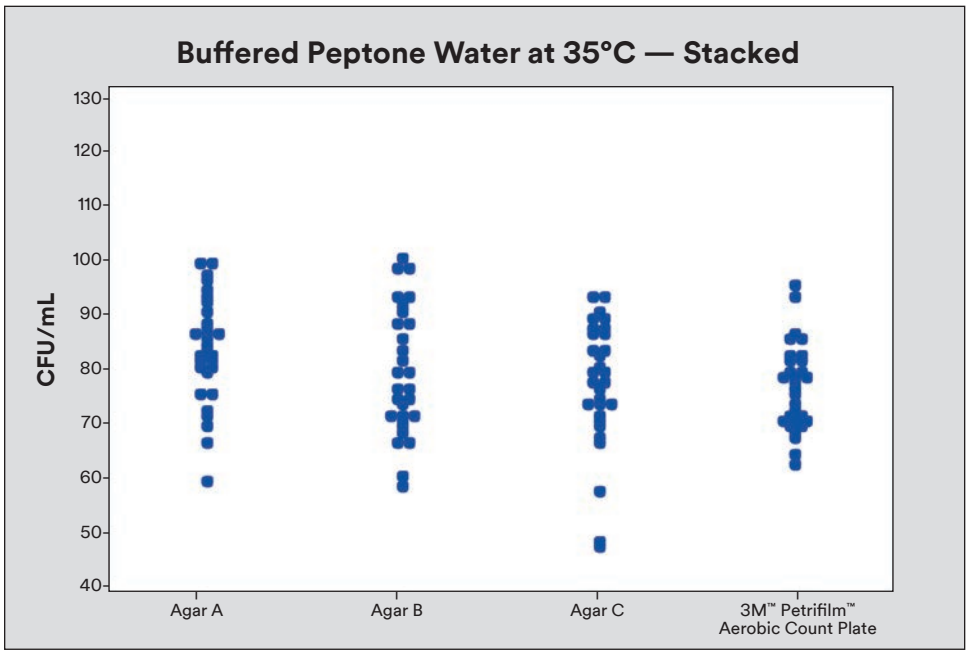


Table 2. Statistical analysis of methods after Standard Methods Agar plates were not stacked during plating.

Standard Deviation — Not Stacked			
Standard Media	**Peptone Saline Buffer at 30°C**	**Butterfield's Phosphate-Buffered Dilution Water at 32°C**	**Buffered Peptone Water at 35°C**
Agar A	11	12*	10
Agar B	15*	11*	15*
Agar C	12	14*	15*
3M™ Petrifilm™ Aerobic Count Plate	10	7	8

Percent Coefficient of Variation (%CV) — Not Stacked			
Standard Media	**Peptone Saline Buffer at 30°C**	**Butterfield's Phosphate-Buffered Dilution Water at 32°C**	**Buffered Peptone Water at 35°C**
Agar A	11.46%	14.02%	11.88%
Agar B	15.78%	12.10%	17.73%
Agar C	12.02%	16.98%	17.18%
3M™ Petrifilm™ Aerobic Count Plate	11.15%	9.70%	10.43%

*Statistically significant difference as compared to 3M™ Petrifilm™ Aerobic Count Plates.

Table 3. Statistical analysis of methods after Standard Methods Agar plates were stacked during plating.

Standard Deviation — Not Stacked			
Standard Media	**Peptone Saline Buffer at 30°C**	**Butterfield's Phosphate-Buffered Dilution Water at 32°C**	**Buffered Peptone Water at 35°C**
Agar A	10	10	10
Agar B	18*	14*	12*
Agar C	18*	13*	12*
3M™ Petrifilm™ Aerobic Count Plate	10	7	8

Percent Coefficient of Variation (%CV) — Not Stacked			
Standard Media	**Peptone Saline Buffer at 30°C**	**Butterfield's Phosphate-Buffered Dilution Water at 32°C**	**Buffered Peptone Water at 35°C**
Agar A	12.23%	12.92%	11.80%
Agar B	21.12%	16.95%	14.69%
Agar C	19.13%	16.02%	15.31%
3M™ Petrifilm™ Aerobic Count Plate	11.15%	9.70%	10.43%

*Statistically significant difference as compared to 3M™ Petrifilm™ Aerobic Count Plates.

References

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