The attached document consists of two parts:
Part I: Method Comparison – Conducted by Q Laboratories
Part II: Robustness – Conducted by 3M

PART I

Comparative Evaluation of the 3M™ Petrifilm™ Rapid Aerobic Count Plate for the Enumeration of Total Viable Count in a Variety of Foods

AOAC® Performance Tested Method℠ Method Developer Study

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A comparative evaluation of the 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate (St. Paul, MN) was conducted at Q Laboratories, Inc. (Cincinnati, OH). The 3M Petrifilm RAC Plate was compared to the FDA/BAM Chapter 3 for the enumeration of total viable count in raw ground beef, raw ground pork, raw ground turkey, chicken carcass rinsate, fresh swai, fresh tuna, fresh tiger shrimp, easy-peel shrimp, cherry tomato wash, frozen blueberries, Mediterranean apricots, creamy salad dressing and fresh pasta. In addition, the 3M Petrifilm RAC Plate was compared to the Standard Methods for the Examination of Dairy Products Chapter 6 for the enumeration of total aerobic count in vanilla ice cream, dry milk powder and pasteurized skim milk. Three different levels of microbial contamination (low, medium, high) were enumerated for each matrix, except pasteurized skim milk, which was artificially contaminated and included an uninoculated level. A total of five replicates per level were analyzed. The difference of means for each level for each matrix was determined. The 3M Petrifilm Rapid RAC Plate demonstrated reliability as a rapid and accurate alternative to the reference methods for aerobic plate enumeration in the food products evaluated.

This report presents the analytical results for the comparison of the 3M Petrifilm Rapid Aerobic Count (RAC) Plate method to the FDA/BAM Chapter 3 Aerobic Plate Count and to the Standard Methods for the Examination of Dairy Products Chapter 6 Microbiological Count Methods, Standard Plate Count reference methods. [1, 2] All analyses were conducted at Q Laboratories, Inc. (Cincinnati, OH). All 3M Petrifilm RAC plates were provided by 3M Food Safety (St. Paul, MN). The study was administered by the AOAC Research Institute.

Materials and Methods

Testing was conducted following the procedures outlined in the protocol provided by the AOAC Research Institute: *Comparative Evaluation of the* 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate *for the Enumeration of Total Viable Count in a Variety of Foods, June 2014 (Version 1)*. [3] The evaluation was conducted using paired samples with a variety of food matrices. Raw ground beef, raw ground pork, raw ground turkey, chicken carcass rinsate, fresh swai, fresh tuna, fresh tiger shrimp, easy-peel shrimp, cherry tomato wash, frozen blueberries, Mediterranean apricots, creamy salad dressing and fresh pasta were compared to the FDA BAM Chapter 3 *Aerobic Plate Count* reference method. Vanilla ice cream, dry milk powder and pasteurized skim milk were compared to the Standard Methods for the Examination of Dairy Products Chapter 6 *Microbiological Count Method, Standard Plate Count* reference method. For each food matrix, three different brands, or product lots, were obtained from local grocers to quantify three different levels of microbial contamination. All food matrices had various levels of microbial contamination, with the exception of pasteurized skim milk. For this matrix, artificial contamination was required. The target contamination levels for each matrix, whether natural or artificial, were as follows: a low level (≈ 10–100 CFU/g), a medium level (≈ 100–1,000 CFU/g) and a high level (≈ 1,000–10,000 CFU/g) with five replicates analyzed at each level. An uninoculated control level was also included for the pasteurized skim milk. Table A presents the matrix summary information.

Prior to inoculation of the pasteurized skim milk, a single colony of *Enterobacter aerogenes* ATCC 13048 from Tryptic Soy Agar with 5% Sheep Blood (SBA)was transferred to Brain Heart Infusion (BHI) broth at 32 ± 1°C for 18–24 hours. After incubation, the culture was heat stressed for 10 ± 1 minutes at 50 ± 1°C in a water bath. The heat stressed culture was plated onto a selective agar, Violet Red Bile (VRB) agar and a non-selective agar, Tryptic Soy Agar (TSA), to determine percent reduction. The plates were incubated at 32 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

Where n_{select} = number of colonies on selective agar and $n_{nonselect}$ = number of colonies culture on non-selective agar after the heat stress protocol. Using BHI broth as the diluent, the culture was diluted to achieve the three target contamination levels.

Table A: Study Summary

Matrix	Target Contamination Level	Replicates	Test Portion Size	Reference Method	3M™ Petrifilm™ Rapid Aerobic Count Plate Method
Raw Ground Beef	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Raw Ground Pork	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Raw Ground Turkey	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Chicken Carcass Rinsate	10–100 CFU/mL	5	50mL	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/mL	5			
	1,000–10,000 CFU/mL	5			

[1] FDA/BAM Chapter 3
[2] Standard Methods for the Examination of Dairy Products Chapter 6

Table A: Study Summary (continued)

Matrix	Target Contamination Level	Replicates	Test Portion Size	Reference Method	3M™ Petrifilm™ Rapid Aerobic Count Plate Method
Chicken Carcass Rinsate	100–1,000 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	1,000–10,000 CFU/g	5			
Fresh Swai	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Fresh Tuna	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Fresh Tiger Shrimp	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Easy-Peel Shrimp	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Cherry Tomato Wash	10–100 CFU/mL	5	50mL	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/mL	5			
	1,000–10,000 CFU/mL	5			
Frozen Blueberries	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Mediterranean Apricots	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Creamy Salad Dressing	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Fresh Pasta	10–100 CFU/g	5	50g	FDA/BAM[1] 48 ± 2 hours @ 35 ± 1°C	24 ± 2 hours @ 35 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Vanilla Ice Cream	10–100 CFU/g	5	11g	SMEDP[2] 48 ± 3 hours @ 32 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Dry Milk Powder	10–100 CFU/g	5	11g	SMEDP[2] 72 ± 3 hours @ 32 ± 1°C	48 ± 3 hours @ 32 ± 1°C
	100–1,000 CFU/g	5			
	1,000–10,000 CFU/g	5			
Pasteurized Skim Milk	0 CFU/mL	5	11mL	SMEDP[2] 48 ± 3 hours @ 32 ± 1°C	24 ± 2 hours @ 32 ± 1°C
	10–100 CFU/mL	5			
	100–1,000 CFU/mL	5			
	1,000–10,000 CFU/mL	5			

[1] FDA/BAM Chapter 3
[2] Standard Methods for the Examination of Dairy Products Chapter 6

Method Comparison

FDA/BAM Chapter 3

Five replicate test portions per level, consisting of 50 ± 1g each, were diluted with 450 ± 5mL of Butterfield's Phosphate Buffer (BPB) and homogenized by mechanically stomaching in filter stomacher bags for 2 minutes. From the diluted sample, 1.0mL was placed in duplicate into separate, sterile Petri dishes. Subsequent 10-fold serial dilutions were prepared by removing 10mL from the previous dilution and placing it into 90 ± 1mL BPB dilution bottles, shaking 25 times within seven seconds in a 30cm (1 ft) arc to homogenize thoroughly. From each dilution, 1.0mL was removed and placed in duplicate into separate, sterile Petri dishes and covered with 12–15mL of tempered Plate Count Agar (PCA) within 15 minutes. All plates were mixed thoroughly and uniformly by alternate rotation and back and forth motions of the plates on a flat surface, taking care to avoid spillage on the Petri dish lid. After the agar solidified, all plates were inverted and incubated at 35 ± 1°C for 48 ± 2 hours. Plates having colonies within the countable range of 30–300 per plate were enumerated using a Darkfield manual colony counter.

Standard Methods for the Examination for Dairy Products (SMEDP) Chapter 6

Five replicate test portions per contamination level, consisting of 11 ± 1mL each, were diluted into a dilution bottle containing 99 ± 1mL of Butterfield's Phosphate Buffer (BPB) and homogenized by shaking 25 times in a 30cm (1 ft) arc within seven seconds. From the diluted sample, 1.0mL was removed and placed in duplicate into separate, sterile Petri dishes then promptly covered with 12–15mL of tempered Standard Methods Agar (SMA). Subsequent 10-fold serial dilutions were prepared by removing 11mL from the previous dilution and placing it into a 99 ± 1mL BPB dilution bottle, shaking 25 times within seven seconds in a 30cm (1 ft) arc. From each dilution, 1.0mL was removed and placed in duplicate into separate sterile Petri dishes and covered with 12–15mL of tempered SMA within 15 minutes of the dilution originally performed. All plates were mixed thoroughly and uniformly by alternate rotation and back-and-forth motions on a flat surface, taking care to avoid spillage on the Petri dish lid. After the agar solidified, all plates for vanilla ice cream and pasteurized skim milk were inverted and incubated at 32 ± 1°C for 48 ± 3 hours. Plates for the dry milk powder were inverted and incubated at 32 ± 1°C for 72 ± 3 hours. Plates having colonies within the countable range of 25–250 per plate were enumerated using a Darkfield manual colony counter.

3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate Method

Using the diluted test portions for each of the reference methods, prepared as described above, 1.0mL of each dilution was placed onto a 3M Petrifilm RAC Plate by aseptically retracting the top film of the plate and placing the diluted sample into the center of the plate. The top film was gently lowered and the aliquot spread with the 3M™ Petrifilm™ Flat Spreader. Firm and even pressure was applied to the spreader to evenly distribute the sample onto the plate. All plates except those for seafood and dairy products were incubated at 35 ± 1°C for 24 ± 2 hours. Seafood and dairy products, except dry milk powder, were incubated at 32 ± 1°C for 24 ± 2 hours. Dry milk powder was incubated at 32 ± 1°C for 48 ± 3 hours. Plates having colonies within the countable range of 30–300 were enumerated using a Darkfield manual colony counter.

Results

Statistical analysis of all matrices was conducted for each contamination level. Logarithmic transformations of the bacterial counts (CFU/g or CFU/mL) were performed. The transformed data was analyzed for outliers by the Cochran and Grubbs' tests. No evidence of physical cause or suspicion of cause was noted, so all outliers identified were included in the statistical analysis for each matrix. The difference of means with 95% confidence intervals and the reverse transformed mean difference with confidence intervals (CFU/g or CFU/mL) for each contamination level were determined. [4] A mean difference value less than the standard alpha value of 0.5 indicated no statistical difference between the 3M Petrifilm RAC Plate method and either reference method. The results of the heat stress for the culture used to artificially contaminate the pasteurized skim milk is presented in Table 1 of the Appendix. Table 2 of the Appendix presents a summary of the logarithmically transformed data for each matrix. Tables 3–19 of the Appendix present the raw data, mean Log_{10}, repeatability (S_r), relative standard repeatability (RSD_r) values for each contamination level and difference of means values. Figures 1–17 present the square of the linear correlation coefficient (r^2).

Raw Ground Beef

For the low, medium and high levels, mean differences of -0.0572, -0.0456 and -0.0674 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low and high contamination levels, with S_r values of 0.1519 and 0.0783, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 3 and Figure 1 in the Appendix.

Raw Ground Pork

For the low, medium and high levels, mean differences of -0.2878, 0.2134 and -0.0012 were obtained, respectively. One data point was identified in the 3M Petrifilm RAC Plate method high contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low and medium contamination levels, with S_r values of 0.0852 and 0.0172, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 4 and Figure 2 in the Appendix.

Raw Ground Turkey

For the low, medium and high levels, mean differences of -0.2946, -0.7374 and -0.0170 were obtained, respectively. There was a significant difference between the two methods for the medium contamination level, with a mean difference of -0.7374. The 3M Petrifilm RAC Plate method produced a lower standard deviation value than the FDA/BAM method for the low contamination level, with a S_r value of 0.1721, indicating a more repeatable method when compared to the reference method. Detailed results are presented in Table 5 and Figure 3 in the Appendix.

Chicken Carcass Rinsate

For the low, medium and high levels, mean differences of -0.1380, -0.0202 and -0.0405 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced a lower standard deviation value than the FDA/BAM method for the medium contamination level, with a S_r value of 0.0474, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 6 and Figure 4 in the Appendix.

Fresh Swai

For the low, medium and high levels, mean differences of -0.0585, -0.2760 and 0.0080 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low and high contamination levels, with S_r values of 0.0237 and 0.0472, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 7 and Figure 5 in the Appendix.

Fresh Tuna

For the low, medium and high levels, mean differences of -0.6401, -0.4451 and 0.6271 were obtained, respectively. One data point was identified in the 3M Petrifilm RAC Plate method medium contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were significant differences between the two methods for the low and high contamination levels, with a mean difference of -0.6401 and 0.6271, respectively. The 3M Petrifilm RAC Plate method produced a lower standard deviation value than the FDA/BAM method for the low contamination level, with a S_r value of 0.3288, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 8 and Figure 6 in the Appendix.

Fresh Tiger Shrimp

For the low, medium and high levels, mean differences of 0.7970, 0.9457 and 1.0056 were obtained, respectively. One data point was identified in the FDA/BAM method medium contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were significant differences between the methods for all three contamination levels, with mean differences of 0.7970, 0.9457 and 1.0056 for the low, medium and high levels, respectively. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low, medium and high contamination levels, with S_r values of 0.3223, 0.0800 and 0.0959, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 9 and Figure 7 in the Appendix.

Easy-Peel Shrimp

For the low, medium and high levels, mean differences of 0.0415, 0.1536 and 0.0764 were obtained, respectively. One data point in the low level of the FDA/BAM method and another in the high contamination level of the FDA/BAM method were identified as outliers by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. Detailed results are presented in Table 10 and Figure 8 in the Appendix.

Cherry Tomato Wash

For the low, medium and high levels, mean differences of -0.2273, 0.0113 and 0.0117 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. Detailed results are presented in Table 11 and Figure 9 in the Appendix.

Frozen Blueberries

For the low, medium and high levels, mean differences of 0.0951, -0.0233 and 0.0202 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low and medium contamination levels, with S_r values of 0.1297 and 0.0531, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 12 and Figure 10 in the Appendix.

Mediterranean Apricots

For the low, medium and high levels, mean differences of 0.0137, -0.0185 and 0.0204 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced a lower standard deviation value than the FDA/BAM method for the high contamination level, with a S_r value of 0.0298, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 13 and Figure 11 in the Appendix.

Creamy Salad Dressing

For the low, medium and high levels, mean differences of 0.3703, 0.0919 and 0.0152 were obtained, respectively. One data point was identified in the FDA/BAM method low contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low, medium and high contamination levels, with S_r values of 0.1297, 0.0397 and 0.0588, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 14 and Figure 12 in the Appendix.

Fresh Pasta

For the low, medium and high levels, mean differences of -0.0087, -0.0026 and 0.0242 were obtained, respectively. One data point was identified in the FDA/BAM method low contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and FDA/BAM using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the FDA/BAM method for the low, medium and high contamination levels, with S_r values of 0.0523, 0.0270 and 0.0460, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 15 and Figure 13 in the Appendix.

Vanilla Ice Cream

For the low, medium and high levels, mean differences of 0.4124, -0.0193 and -0.0313 were obtained, respectively. One data point was identified in the 3M Petrifilm RAC Plate method high contamination level as an outlier by the Single Grubbs' test. However, no evidence of physical cause or suspicion of cause was noted and it was determined that it would be included in the statistical analysis. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and SMEDP using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced a lower standard deviation value than SMEDP method for the low contamination level, with a S_r value of 0.0971, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 16 and Figure 14 in the Appendix.

Dry Milk Powder

For the low, medium and high levels, mean differences of 0.0866, 0.0401 and 0.0823 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and SMEDP using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than the SMEDP method for the low and high contamination levels, with S_r values of 0.0300 and 0.0683, respectively, indicating higher repeatability when compared to the reference method. Detailed results are presented in Table 17 and Figure 15 in the Appendix.

Pasteurized Skim Milk

For the low, medium and high artificially contaminated levels, mean differences of 0.0426, 0.0312 and 0.0440 were obtained, respectively. There were no statistically significant differences determined between the 3M Petrifilm RAC Plate method and SMEDP using the difference of means at all three contamination levels. The 3M Petrifilm RAC Plate method produced lower standard deviation values than SMEDP method for the medium and high inoculation levels, with S_r values of 0.0267 and 0.0275, indicating higher repeatability when compared to the reference method. All uninoculated replicates produced results of <10 CFU/mL. Detailed results are presented in Table 18 and Figure 16 in the Appendix.

Discussion

The 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate is an efficient and easy to use plating method for detection and quantification of aerobic organisms in a variety of foods.

Under a top cover, the plate consists of a recessed sample area which contains a water-soluble gelling agent, nutrients and indicator dyes beneficial to the growth of microorganisms. Since the plated inoculum rehydrates the gel, there is no time or expense involving media preparation and pouring agar in Petri dishes. The compact size and thin design of the plates takes up less incubator and storage space than traditional Petri dishes, in addition to reducing biohazard waste.

The 3M Petrifilm RAC Plate is intended to reduce the total incubation time commonly associated with reference method aerobic plate count procedures. Of the seventeen matrices tested, only dry milk powder required 48 ± 3 hours of incubation using 3M Petrifilm RAC Plates, all other matrices were 24 ± 2 hours. For many end users, this can result in significant time savings and expedited release of results.

To assist in colony enumeration, the 3M Petrifilm RAC Plate employed two indicator dyes. One dye colored the colonies red, while the other colored the colonies blue. This biochemical and enzymatic detection system differentiates the organisms present from any food particulate matter, thereby increasing the accuracy of the plate count data generated. In addition, using a filtered stomacher bag when preparing samples reduced or eliminated the amount of particulate matter on the plate. Although the colonies were colored, a wide range of sizes, from pin-point to several millimeters across, were observed. The use of a magnified dark field colony counter aided in colony enumeration specifically as the analyst became more familiar with identifying variations in the size of the colonies.

Any diluted food product plated onto the 3M Petrifilm RAC Plate is required to have a pH greater than 5.0. Of the seventeen matrices evaluated only two, specifically frozen blueberries and Mediterranean apricots, required a pH adjustment using 1N NaOH.

When performing the statistical analysis for the tiger shrimp, a significant difference was clearly evident in the counts between 3M Petrifilm RAC Plate and FDA/BAM. The mean differences for the low, medium and high levels were 0.7970, 0.9457 and 1.0056, respectively. It is speculated that the lower incubation temperature (32 ± 1°C) of the 3M Petrifilm RAC Plate compared to FDA/BAM (35 ± 1°C) may have been a contributing factor to the higher bacterial recovery by the candidate method.

Overall, the 3M Petrifilm RAC Plate method produced aerobic plate count data in a variety of food matrices that was comparable to the FDA/BAM or SMEDP procedures, but approximately 24 hours less than the standard methods. The 3M Petrifilm RAC Plate also had higher repeatability in 26 out of 51 contamination levels evaluated. The results of this evaluation indicate that the 3M Petrifilm Rapid Aerobic Count Plate method is a rapid and accurate alternative to the reference methods for enumeration of aerobic bacteria in the food products tested.

References

(1) Food and Drug Administration Bacteriological Analytical Manual Chapter 3: *Aerobic Plate Count*. January, 2001. (Accessed August 2014) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

(2) Standard Methods for the Examination of Dairy Products Chapter 6: *Microbiological Count Method*, 17th Edition.

(3) AOAC Research Institute Performance tested Methods Program. *Comparative evaluation of the 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate for the Enumeration of Total Viable Count in a Variety of Foods*. June 2014. Version 1.

(4) Least Cost Formulations, Ltd., AOAC International Interlaboratory Study Workbook Paired Method Analysis for Micro Testing, Version 1.0 (2010) (Accessed August 2014)

Appendix

Table 1. Inoculum Heat Stress Results for *Enterobacter aerogenes* ATCC[1] 13048 in Pasteurized Skim Milk

Matrix	Inoculating Organism	Violet Red Bile Agar Count (CFU/mL)	Tryptic Soy Agar Count (CFU/mL)	Percent Injury
Pasteurized Skim Milk	*Enterobacter aerogenes* ATCC[1] 13048	1.5×10^8	4.5×10^8	66.7

[1] American Type Culture Collection

Table 2. Summary of Mean Differences and Reverse Transformed Mean Differences between 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and Reference Method

Matrix	Contamination Level	Reference Method	Mean Difference[a] (Log_{10})	95% Confidence Intervals (LCL, UCL) (Log_{10})	Reverse Transformed Mean Difference (CFU/g or CFU/mL)	95% Confidence Intervals (LCL, UCL) (CFU/g or CFU/mL)
Raw Ground Beef	Low	FDA/BAM	-0.0572	-0.3316, 0.2171	0.8766	0.4660, 1.6485
	Medium		-0.0456	-0.2876, 0.1963	0.9003	0.5157, 1.5714
	High		-0.0674	-0.1695, 0.0347	0.8562	0.6769, 1.0832
Raw Ground Pork	Low	FDA/BAM	-0.2878	-0.5067, -0.0690	0.5155	0.3114, 0.8531
	Medium		0.2134	0.1246, 0.3022	1.6346	1.3323, 2.0054
	High		-0.0012	-0.0837, 0.0812	0.9972	0.8247, 1.2056
Raw Ground Turkey	Low	FDA/BAM	-0.2946	-0.8394, 0.2503	0.5075	0.1447, 1.7795
	Medium		-0.7374	-1.0588, 0.4161	0.1831	0.0873, 2.6068
	High		-0.0170	-0.0792, 0.0451	0.9616	0.8333, 1.1094
Chicken Carcass Rinsate	Low	FDA/BAM	-0.1380	-0.2916, 0.0156	0.7278	0.5110, 1.0366
	Medium		-0.0202	-0.0703, 0.0299	0.9546	0.8506, 1.0713
	High		-0.0405	-0.0916, 0.0106	0.9110	0.8098, 1.0247
Raw Turkey Sausage	Low	FDA/BAM	-0.1541	-0.2536, -0.0547	0.7013	0.5577, 0.8817
	Medium		-0.3279	-0.4280, -0.2277	0.4700	0.3732, 0.5920
	High		-0.3802	-0.4599, -0.3005	0.4167	0.3468, 0.5006
Fresh Swai	Low	FDA/BAM	-0.0585	-0.1434, 0.0264	0.8740	0.7188, 1.0627
	Medium		-0.2760	-0.4981, -0.0539	0.5297	0.3176, 0.8833
	High		0.0800	-0.0794, 0.0953	1.2023	0.8329, 1.2454
Fresh Tuna	Low	FDA/BAM	-0.6401	-1.1007, -0.1795	0.2290	0.0793, 0.6615
	Medium		-0.4451	-1.2297, 0.3395	0.3588	0.0589, 2.1852
	High		0.6271	0.3274, 0.9268	4.2374	2.1252, 8.4489

[a] A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods

Table 2. Summary of Mean Differences and Reverse Transformed Mean Differences between 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and Reference Method (cont.)

Matrix	Contamination Level	Reference Method	Mean Difference[a] (Log_{10})	95% Confidence Intervals (LCL, UCL) (Log_{10})	Reverse Transformed Mean Difference (CFU/g or CFU/mL)	95% Confidence Intervals (LCL, UCL) (CFU/g or CFU/mL)
Fresh Tiger Shrimp	Low	FDA/BAM	0.7970	0.4484, 1.1456	6.2661	2.8080, 13.9830
	Medium		0.9457	0.6387, 1.2527	8.8247	4.3521, 17.8937
	High		1.0056	0.8848, 1.1263	10.1298	7.6701, 13.3752
Easy-Peel Shrimp	Low	FDA/BAM	0.0415	-0.0463, 0.1293	1.1003	0.8989, 1.3468
	Medium		0.1536	-0.4433, 0.7506	1.4243	0.3603, 5.6312
	High		0.0764	0.0074, 0.1453	1.1923	1.0172, 1.3973
Cherry Tomato Wash	Low	FDA/BAM	-0.2273	-0.3133, -0.1412	0.5925	0.4861, 0.7224
	Medium		0.0113	-0.0758, 0.0984	1.0264	0.8398, 1.2543
	High		0.0117	-0.0811, 0.1045	1.0273	0.8297, 1.2720
Frozen Blueberries	Low	FDA/BAM	0.0951	-0.0756, 0.2657	1.2448	0.8402, 1.8437
	Medium		-0.0233	-0.1910, 0.1445	0.9478	0.6442, 1.3948
	High		0.0202	-0.0549, 0.0952	1.0476	0.8813, 1.2451
Mediterranean Apricots	Low	FDA/BAM	0.0137	-0.0490, 0.0764	1.0320	0.8933, 1.1923
	Medium		-0.0185	-0.1387, 0.1017	0.9583	0.7266, 1.2639
	High		0.0204	-0.0029, 0.0438	1.0481	0.9933, 1.1061
Creamy Salad Dressing	Low	FDA/BAM	0.3703	0.1627, 0.5780	2.3458	1.4545, 3.7844
	Medium		0.0919	0.0048, 0.1789	1.2357	1.0111, 1.5097
	High		0.0152	-0.0307, 0.0612	1.0356	0.9318, 1.1513
Fresh Pasta	Low	FDA/BAM	0.0087	-0.1299, 0.1472	1.0202	0.7415, 1.4035
	Medium		-0.0026	-0.0368, 0.0316	0.9940	0.9188, 1.0755
	High		0.0242	-0.0730, 0.1215	1.0573	0.8453, 1.3228
Vanilla Ice Cream	Low	SMEDP	0.4124	0.1288, 0.6960	2.5846	1.3452, 4.9659
	Medium		-0.0193	-0.0988, 0.0602	0.9565	0.7965, 1.1487
	High		-0.0313	-0.0699, 0.0073	0.9305	0.8513, 1.0170
Dry Milk Powder	Low	SMEDP	0.0866	0.0132, 0.1601	1.2207	1.0309, 1.4458
	Medium		0.0401	-0.0852, 0.1654	1.0967	0.8219, 1.4635
	High		0.0823	-0.0664, 0.2310	1.2086	0.8582, 1.7022
Pasteurized Skim Milk	Low	SMEDP	0.0426	-0.0330, 0.1181	1.1031	0.9268, 1.3125
	Medium		0.0312	-0.0557, 0.1181	1.0745	0.8796, 1.3125
	High		0.0440	0.0068, 0.0812	1.1066	1.0158, 1.2056

[a] A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods

Table 3. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Raw Ground Beef

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
	1	1.0×10^2	2.0000				8.0×10^1	1.9031				
	2	1.8×10^2	2.2553				4.5×10^2	2.6532				
Low	3	2.0×10^2	2.3010	2.2453	0.1519	6.7652	1.7×10^2	2.2304	2.3025	0.3119	13.5461	-0.0572
	4	2.6×10^2	2.4150				3.8×10^2	2.5798				
	5	1.8×10^2	2.2553				1.4×10^2	2.1461				
	1	9.5×10^3	3.9777				8.1×10^3	3.9085				
	2	7.8×10^3	3.8921				6.0×10^3	3.7782				
Medium	3	9.4×10^3	3.9731	3.8474	0.1507	3.9169	9.1×10^3	3.9590	3.8930	0.0931	2.3915	-0.0456
	4	4.2×10^3	3.6232				1.0×10^4	4.0000				
	5	5.9×10^3	3.7709				6.6×10^3	3.8195				
	1	7.6×10^5	5.8808				7.8×10^5	5.8921				
	2	1.1×10^6	6.0414				1.0×10^6	6.0000				
High	3	9.0×10^5	5.9542	5.9829	0.0783	1.3087	1.3×10^6	6.1139	6.0503	0.1176	1.9437	-0.0674
	4	1.2×10^6	6.0792				1.6×10^6	6.2041				
	5	9.1×10^5	5.9590				1.1×10^6	6.0414				

Table 4. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Raw Ground Pork

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
	1	1.3×10^4	4.1139				2.0×10^4	4.3010				
	2	1.0×10^4	4.0000				1.1×10^4	4.0414				
Low	3	8.8×10^3	3.9445	4.0409	0.0852	2.1084	2.3×10^4	4.3617	4.3287	0.1763	4.0728	-0.2878
	4	1.0×10^4	4.0000				3.0×10^4	4.4771				
	5	1.4×10^4	4.1461				2.9×10^4	4.4624				
	1	5.1×10^5	5.7076				3.2×10^5	5.5051				
	2	5.2×10^5	5.7160				2.5×10^5	5.3979				
Medium	3	5.0×10^5	5.6990	5.7124	0.0172	0.3011	3.0×10^5	5.4771	5.4990	0.0810	1.4730	0.2134
	4	5.5×10^5	5.7404				4.2×10^5	5.6232				
	5	5.0×10^5	5.6990				3.1×10^5	5.4914				
	1	3.3×10^6	6.5185				3.6×10^6	6.5563				
	2	3.2×10^6	6.5051				3.6×10^6	6.5563				
High	3	3.2×10^6	6.5051	6.5994	0.1460	2.2123	3.2×10^6	6.5051	6.6007	0.0911	1.3802	-0.0012
	4	4.2×10^6	6.6232				4.5×10^6	6.6532				
	5	7.0×10^6	6.8451*				5.4×10^6	6.7324				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

* Grubbs' test outlier

Table 5. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Raw Ground Turkey

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	2.0×10^1	1.3010	1.2760	0.1721	13.4875	6.0×10^1	1.7782	1.7143	0.1791	10.4474	-0.2946
	2	1.0×10^1	1.0000				8.0×10^1	1.9031				
	3	3.0×10^1	1.4771				5.0×10^1	1.6990				
	4	2.0×10^1	1.3010				3.0×10^1	1.4771				
	5	2.0×10^1	1.3010				$<1.0 \times 10^1$	<1.0000				
Medium	1	2.6×10^2	2.4150	2.0369	0.2773	13.6138	6.0×10^2	2.7782	2.7747	0.0462	1.6650	-0.7374
	2	1.2×10^2	2.0792				5.9×10^2	2.7709				
	3	1.4×10^2	2.1461				7.0×10^2	2.8451				
	4	7.0×10^1	1.8451				5.2×10^2	2.7160				
	5	5.0×10^1	1.6990				5.8×10^2	2.7634				
High	1	4.2×10^6	6.6232	6.6852	0.0584	0.8736	4.3×10^6	6.6335	6.7023	0.0458	0.6833	-0.0170
	2	4.5×10^6	6.6532				4.8×10^6	6.6812				
	3	5.2×10^6	6.7160				5.4×10^6	6.7324				
	4	5.9×10^6	6.7709				5.2×10^6	6.7160				
	5	4.6×10^6	6.6628				5.6×10^6	6.7482				

Table 6. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Chicken Carcass Rinsate

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/mL	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/mL	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	1.6×10^4	4.2041	4.3192	0.0878	2.0328	3.4×10^4	4.5315	4.4571	0.0489	1.0971	-0.1380
	2	2.0×10^4	4.3010				3.0×10^4	4.4771				
	3	2.0×10^4	4.3010				2.6×10^4	4.4150				
	4	2.8×10^4	4.4472				2.8×10^4	4.4472				
	5	2.2×10^4	4.3424				2.6×10^4	4.4150				
Medium	1	4.2×10^5	5.6232	5.5935	0.0474	0.8474	4.3×10^5	5.6335	5.6137	0.0599	1.0670	-0.0202
	2	4.4×10^5	5.6435				4.2×10^5	5.6232				
	3	3.6×10^5	5.5563				3.6×10^5	5.5563				
	4	3.4×10^5	5.5315				3.6×10^5	5.5563				
	5	4.1×10^5	5.6128				5.0×10^5	5.6990				
High	1	1.7×10^6	6.2304	6.2140	0.0300	0.4828	1.7×10^6	6.2304	6.2545	0.0288	0.4605	-0.0405
	2	1.5×10^6	6.1761				1.7×10^6	6.2304				
	3	1.8×10^6	6.2553				1.8×10^6	6.2553				
	4	1.6×10^6	6.2041				2.0×10^6	6.3010				
	5	1.6×10^6	6.2041				1.8×10^6	6.2553				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

Table 7. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Fresh Swai

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	1.3×10^3	3.1139	3.1134	0.0237	0.7612	1.3×10^3	3.1139	3.1719	0.0666	2.0997	-0.0585
	2	1.4×10^3	3.1461				1.8×10^3	3.2553				
	3	1.3×10^3	3.1139				1.3×10^3	3.1139				
	4	1.3×10^3	3.1139				1.4×10^3	3.1461				
	5	1.2×10^3	3.0792				1.7×10^3	3.2304				
Medium	1	1.4×10^4	4.1461	4.0643	0.2003	4.9283	2.9×10^4	4.4624	4.3402	0.1429	3.2925	-0.2760
	2	1.8×10^4	4.2553				1.7×10^4	4.2304				
	3	1.4×10^4	4.1461				2.6×10^4	4.4150				
	4	1.1×10^4	4.0414				2.8×10^4	4.4472				
	5	5.4×10^3	3.7324				1.4×10^4	4.1461				
High	1	4.0×10^7	7.6021	7.6682	0.0472	0.6155	4.0×10^7	7.6021	7.6602	0.0531	0.6932	0.0080
	2	4.7×10^7	7.6721				5.0×10^7	7.6990				
	3	4.8×10^7	7.6812				5.0×10^7	7.6990				
	4	4.5×10^7	7.6532				5.0×10^7	7.6990				
	5	5.4×10^7	7.7324				4.0×10^7	7.6021				

Table 8. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Fresh Tuna

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	8.0×10^1	1.9031	1.8179	0.3288	18.0868	2.0×10^2	2.3010	2.4586	0.4561	18.5512	-0.6401
	2	8.0×10^1	1.9031				5.8×10^2	2.7634				
	3	1.6×10^2	2.2041				3.1×10^2	2.4914				
	4	6.0×10^1	1.7782				9.1×10^2	2.9590				
	5	2.0×10^1	1.3010				6.0×10^1	1.7782				
Medium	1	1.4×10^5	5.1461*	4.4030	0.4430	10.0613	3.8×10^4	4.5798	4.8481	0.2024	4.1748	-0.4451
	2	1.0×10^4	4.0000				1.2×10^5	5.0792				
	3	2.4×10^4	4.3802				7.2×10^4	4.8573				
	4	2.2×10^4	4.3424				5.3×10^4	4.7243				
	5	1.4×10^4	4.1461				1.0×10^5	5.0000				
High	1	2.9×10^5	5.4624	5.2523	0.1873	3.5661	3.4×10^4	4.5315	4.6253	0.1311	2.8344	0.6271
	2	1.4×10^5	5.1461				5.6×10^4	4.7482				
	3	1.8×10^5	5.2553				6.1×10^4	4.7853				
	4	1.0×10^5	5.0000				3.2×10^4	4.5051				
	5	2.5×10^5	5.3979				3.6×10^4	4.5563				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

* Grubbs' test outlier

Table 9. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Fresh Tiger Shrimp

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	3.6×10^5	5.5563	5.7699	0.3223	5.5859	8.6×10^4	4.9345	4.9729	0.0467	0.9391	0.7970
	2	2.0×10^6	6.3010				1.1×10^5	5.0414				
	3	6.6×10^5	5.8195				1.0×10^5	5.0000				
	4	4.8×10^5	5.6812				8.6×10^4	4.9345				
	5	3.1×10^5	5.4914				9.0×10^4	4.9542				
Medium	1	1.1×10^6	6.0414	6.1705	0.0800	1.2965	1.2×10^5	5.0792	5.2248	0.2326	4.4518	0.9457
	2	1.4×10^6	6.1461				4.2×10^5	5.6232*				
	3	1.6×10^6	6.2041				1.1×10^5	5.0414				
	4	1.7×10^6	6.2304				1.5×10^5	5.1761				
	5	1.7×10^6	6.2304				1.6×10^5	5.2041				
High	1	1.2×10^7	7.0792	7.1559	0.0959	1.3402	1.0×10^6	6.0000	6.1503	0.1226	1.9934	1.0056
	2	1.1×10^7	7.0414				1.2×10^6	6.0792				
	3	1.5×10^7	7.1761				2.1×10^6	6.3222				
	4	1.6×10^7	7.2041				1.4×10^6	6.1461				
	5	1.9×10^7	7.2788				1.6×10^6	6.2041				

Table 10. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Easy Peel Shrimp

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	1.4×10^2	2.1461	2.4060	0.1630	6.7747	1.3×10^2	2.1139*	2.3645	0.1407	5.9505	0.0415
	2	2.3×10^2	2.3617				2.7×10^2	2.4314				
	3	3.1×10^2	2.4914				2.6×10^2	2.4150				
	4	2.9×10^2	2.4624				2.6×10^2	2.4150				
	5	3.7×10^2	2.5682				2.8×10^2	2.4472				
Medium	1	8.0×10^2	2.9031	3.0883	0.4497	14.5614	9.5×10^2	2.9777	2.9347	0.0805	2.7430	0.1536
	2	8.0×10^2	2.9031				1.0×10^3	3.0000				
	3	6.0×10^3	3.7782				6.8×10^2	2.8325				
	4	1.8×10^3	3.2553				1.0×10^3	3.0000				
	5	4.0×10^2	2.6021				7.3×10^2	2.8633				
High	1	1.4×10^5	5.1461	5.1253	0.0439	0.8565	1.1×10^5	5.0414	5.0490	0.0169	0.3347	0.0764
	2	1.4×10^5	5.1461				1.1×10^5	5.0414				
	3	1.2×10^5	5.0792				1.2×10^5	5.0792*				
	4	1.2×10^5	5.0792				1.1×10^5	5.0414				
	5	1.5×10^5	5.1761				1.1×10^5	5.0414				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

* Grubbs' test outlier

Table 11. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Cherry Tomato Wash

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/mL	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	1.1×10^3	3.0414	3.0125	0.0461	1.5303	1.7×10^3	3.2304	3.2398	0.0283	0.8735	-0.2273
	2	1.2×10^3	3.0792				1.6×10^3	3.2041				
	3	9.4×10^2	2.9731				1.8×10^3	3.2553				
	4	9.8×10^2	2.9912				1.9×10^3	3.2788				
	5	9.5×10^2	2.9777				1.7×10^3	3.2304				
Medium	1	3.3×10^4	4.5185	4.5606	0.0550	1.2060	3.0×10^4	4.4771	4.5493	0.0482	1.0595	0.0113
	2	3.2×10^4	4.5051				3.8×10^4	4.5798				
	3	3.8×10^4	4.5798				4.0×10^4	4.6021				
	4	3.6×10^4	4.5563				3.6×10^4	4.5563				
	5	4.4×10^4	4.6435				3.4×10^4	4.5315				
High	1	4.0×10^5	5.6021	5.5613	0.0486	0.8739	3.0×10^5	5.4771	5.5496	0.0451	0.8127	0.0117
	2	3.4×10^5	5.5315				3.6×10^5	5.5563				
	3	3.1×10^5	5.4914				3.6×10^5	5.5563				
	4	4.0×10^5	5.6021				3.6×10^5	5.5563				
	5	3.8×10^5	5.5798				4.0×10^5	5.6021				

Table 12. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Frozen Blueberries

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	4.0×10^1	1.6021	1.6475	0.1297	7.8725	2.0×10^1	1.3010	1.5521	0.2402	15.4758	0.0951
	2	4.0×10^1	1.6021				4.0×10^1	1.6021				
	3	6.0×10^1	1.7782				6.0×10^1	1.7782				
	4	3.0×10^1	1.4771				2.0×10^1	1.3010				
	5	6.0×10^1	1.7782				6.0×10^1	1.7782				
Medium	1	9.3×10^3	3.9685	3.9573	0.0531	1.3418	8.0×10^3	3.9031	3.9806	0.1266	3.1804	-0.0233
	2	8.4×10^3	3.9243				8.5×10^3	3.9294				
	3	8.0×10^3	3.9031				1.4×10^4	4.1461				
	4	1.1×10^4	4.0414				1.2×10^4	4.0792				
	5	8.9×10^3	3.9494				7.0×10^3	3.8451				
High	1	4.8×10^4	4.6812	4.6333	0.0833	1.7979	3.8×10^4	4.5798	4.6131	0.0650	1.4090	0.0202
	2	3.4×10^4	4.5315				3.4×10^4	4.5315				
	3	5.2×10^4	4.7160				4.5×10^4	4.6532				
	4	3.6×10^4	4.5563				4.0×10^4	4.6021				
	5	4.8×10^4	4.6812				5.0×10^4	4.6990				

[1] SD = Standard Deviation
[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$
[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

Table 13. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Mediterranean Apricots

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	3.5×10^3	3.5441	3.5373	0.0431	1.2184	3.4×10^3	3.5315	3.5236	0.0118	0.3349	0.0137
	2	3.1×10^3	3.4914				3.3×10^3	3.5185				
	3	4.0×10^3	3.6021				3.2×10^3	3.5051				
	4	3.5×10^3	3.5441				3.4×10^3	3.5315				
	5	3.2×10^3	3.5051				3.4×10^3	3.5315				
Medium	1	5.9×10^4	4.7709	4.7248	0.0555	1.1746	4.8×10^4	4.6812	4.7433	0.0442	0.9318	-0.0185
	2	4.4×10^4	4.6435				6.2×10^4	4.7924				
	3	5.0×10^4	4.6990				5.8×10^4	4.7634				
	4	5.4×10^4	4.7324				5.8×10^4	4.7634				
	5	6.0×10^4	4.7782				5.2×10^4	4.7160				
High	1	1.4×10^6	6.1461	6.1263	0.0298	0.4864	1.3×10^6	6.1139	6.1059	0.0386	0.6322	0.0204
	2	1.2×10^6	6.0792				1.1×10^6	6.0414				
	3	1.3×10^6	6.1139				1.3×10^6	6.1139				
	4	1.4×10^6	6.1461				1.4×10^6	6.1461				
	5	1.4×10^6	6.1461				1.3×10^6	6.1139				

Table 14. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Creamy Salad Dressing

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	6.0×10^1	1.7782	1.7327	0.1297	7.4854	4.0×10^1	1.6021*	1.3612	0.1346	9.8883	0.3703
	2	6.0×10^1	1.7782				2.0×10^1	1.3010				
	3	8.0×10^1	1.9031				2.0×10^1	1.3010				
	4	4.0×10^1	1.6021				2.0×10^1	1.3010				
	5	4.0×10^1	1.6021				2.0×10^1	1.3010				
Medium	1	2.0×10^2	2.3010	2.3290	0.0397	1.7046	1.9×10^2	2.2788	2.2371	0.0623	2.7849	0.0919
	2	2.4×10^2	2.3802				1.8×10^2	2.2553				
	3	2.2×10^2	2.3424				2.0×10^2	2.3010				
	4	1.9×10^2	2.2788				1.6×10^2	2.2041				
	5	2.2×10^2	2.3424				1.4×10^2	2.1461				
High	1	9.8×10^4	4.9912	4.9774	0.0588	1.1813	1.1×10^5	5.0414	4.9621	0.0708	1.4268	0.0152
	2	7.6×10^4	4.8808				7.1×10^4	4.8513				
	3	1.1×10^5	5.0414				1.0×10^5	5.0000				
	4	9.8×10^4	4.9912				9.2×10^4	4.9638				
	5	9.6×10^4	4.9823				9.0×10^4	4.9542				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

* Grubbs' test outlier

Table 15. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the FDA/BAM Reference Method for Fresh Pasta

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					FDA/BAM APC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	2.8×10^5	5.4472	5.4057	0.0523	0.9675	1.9×10^5	5.2788*	5.3971	0.0703	1.3026	0.0087
	2	2.9×10^5	5.4624				2.6×10^5	5.4150				
	3	2.3×10^5	5.3617				2.7×10^5	5.4314				
	4	2.2×10^5	5.3424				2.9×10^5	5.4624				
	5	2.6×10^5	5.4150				2.5×10^5	5.3979				
Medium	1	2.5×10^6	6.3979	6.4209	0.0270	0.4205	2.6×10^6	6.4150	6.4235	0.0388	0.6040	-0.0026
	2	2.9×10^6	6.4624				2.8×10^6	6.4472				
	3	2.5×10^6	6.3979				2.3×10^6	6.3617				
	4	2.6×10^6	6.4150				2.7×10^6	6.4314				
	5	2.7×10^6	6.4314				2.9×10^6	6.4624				
High	1	1.3×10^8	8.1139	8.1741	0.0460	0.5628	1.1×10^8	8.0414	8.1499	0.0953	1.1693	0.0242
	2	1.5×10^8	8.1761				1.4×10^8	8.1461				
	3	1.7×10^8	8.2304				1.9×10^8	8.2788				
	4	1.6×10^8	8.2041				1.2×10^8	8.0792				
	5	1.4×10^8	8.1461				1.6×10^8	8.2041				

Table 16. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the SMEDP Reference Method for Vanilla Ice Cream

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					SMEDP SPC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	1.0×10^2	2.0000	1.9053	0.0971	5.0963	2.0×10^1	1.3010	1.4919	0.2044	13.7006	0.4124
	2	7.0×10^1	1.8451				4.0×10^1	1.6021				
	3	1.0×10^2	2.0000				3.0×10^1	1.4771				
	4	8.0×10^1	1.9031				6.0×10^1	1.7782				
	5	6.0×10^1	1.7782				2.0×10^1	1.3010				
Medium	1	1.1×10^4	4.0414	3.9883	0.0345	0.8650	9.4×10^3	3.9731	4.0076	0.0323	0.8060	-0.0193
	2	1.0×10^4	4.0000				1.0×10^4	4.0000				
	3	9.0×10^3	3.9542				1.1×10^4	4.0414				
	4	9.6×10^3	3.9823				9.6×10^3	3.9823				
	5	9.2×10^3	3.9638				1.1×10^4	4.0414				
High	1	1.3×10^5	5.1139	5.1303	0.0580	1.1305	1.5×10^5	5.1761	5.1616	0.0552	1.0694	-0.0313
	2	1.2×10^5	5.0792				1.2×10^5	5.0792				
	3	1.3×10^5	5.1139				1.5×10^5	5.1761				
	4	1.7×10^5	5.2304*				1.7×10^5	5.2304				
	5	1.3×10^5	5.1139				1.4×10^5	5.1461				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

* Grubbs' test outlier

Table 17. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the SMEDP Reference Method for Dry Milk Powder

Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					SMEDP SPC					Mean Log_{10} Difference[3]
		CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Low	1	3.8×10^2	2.5798	2.6204	0.0300	1.1449	3.4×10^2	2.5315	2.5337	0.0549	2.1668	0.0866
	2	4.4×10^2	2.6435				4.2×10^2	2.6232				
	3	4.2×10^2	2.6232				3.4×10^2	2.5315				
	4	4.5×10^2	2.6532				3.0×10^2	2.4771				
	5	4.0×10^2	2.6021				3.2×10^2	2.5051				
Medium	1	4.0×10^5	5.6021	5.6111	0.0636	1.1335	3.5×10^5	5.5441	5.5710	0.0442	0.7934	0.0401
	2	4.4×10^5	5.6435				3.4×10^5	5.5315				
	3	5.0×10^5	5.6990				3.6×10^5	5.5563				
	4	3.8×10^5	5.5798				3.8×10^5	5.5798				
	5	3.4×10^5	5.5315				4.4×10^5	5.6435				
High	1	6.4×10^6	6.8062	6.7440	0.0683	1.0128	3.5×10^6	6.5441	6.6617	0.0739	1.1093	0.0823
	2	6.4×10^6	6.8062				5.4×10^6	6.7324				
	3	5.6×10^6	6.7482				4.5×10^6	6.6532				
	4	5.2×10^6	6.7160				4.6×10^6	6.6628				
	5	4.4×10^6	6.6435				5.2×10^6	6.7160				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

Table 18. Method Comparison Results Between the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method and the SMEDP Reference Method for Pasteurized Skim Milk

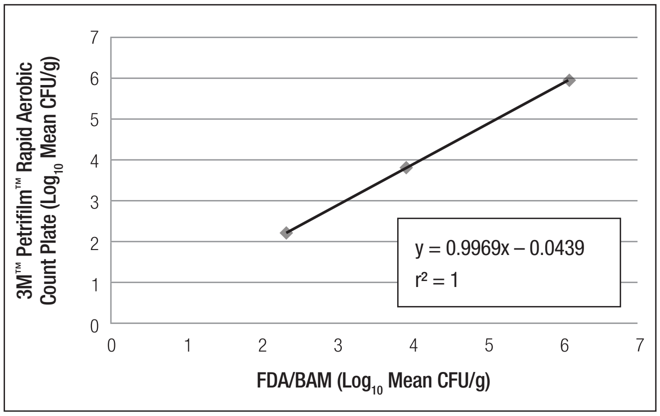
Contamination Level	Sample Replicate	3M™ Petrifilm™ Rapid Aerobic Count Plate Method					SMEDP SPC					Mean Log_{10} Difference[3]
		CFU/mL	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	CFU/g	Log_{10}	Log_{10} Mean	SD[1] (S_r)	RSD_r[2]	
Uninoculated	1	<10	<1.0000	—	—	—	<10	<1.0000	—	—	—	N/A
	2	<10	<1.0000				<10	<1.0000				
	3	<10	<1.0000				<10	<1.0000				
	4	<10	<1.0000				<10	<1.0000				
	5	<10	<1.0000				<10	<1.0000				
Low	1	1.6×10^3	3.2041	3.1689	0.0390	1.2307	1.4×10^3	3.1461	3.1263	0.0298	0.9532	0.0426
	2	1.5×10^3	3.1761				1.3×10^3	3.1139				
	3	1.3×10^3	3.1139				1.4×10^3	3.1461				
	4	1.6×10^3	3.2041				1.2×10^3	3.0792				
	5	1.4×10^3	3.1461				1.4×10^3	3.1461				
Medium	1	4.9×10^4	4.6902	4.7269	0.0267	0.5649	5.7×10^4	4.7559	4.6957	0.0606	1.2905	0.0312
	2	5.8×10^4	4.7634				5.4×10^4	4.7324				
	3	5.4×10^4	4.7324				5.2×10^4	4.7160				
	4	5.2×10^4	4.7160				4.7×10^4	4.6721				
	5	5.4×10^4	4.7324				4.0×10^4	4.6021				
High	1	1.1×10^6	6.0414	6.0010	0.0275	0.4583	9.8×10^5	5.9912	5.9570	0.0472	0.7923	0.0440
	2	1.0×10^6	6.0000				9.2×10^5	5.9638				
	3	1.0×10^6	6.0000				1.0×10^6	6.0000				
	4	1.0×10^6	6.0000				8.9×10^5	5.9494				
	5	9.2×10^5	5.9638				7.6×10^5	5.8808				

[1] SD = Standard Deviation

[2] RSD_r = Relative Standard Deviation = $\frac{SD}{MEAN} \times 100$

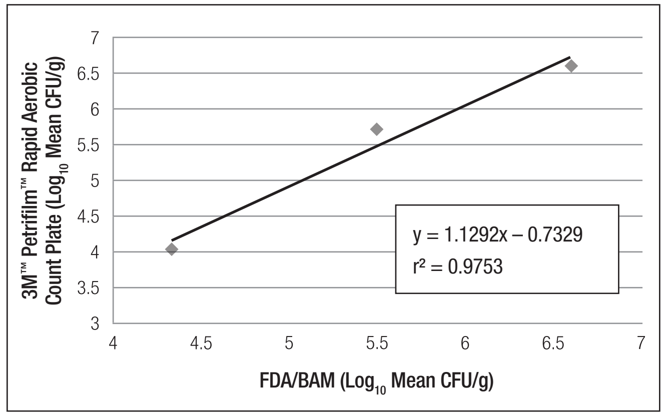
[3] Mean Difference = Candidate Log Mean – Reference Log Mean (A mean difference absolute value of greater than 0.5 indicates a statistical significant difference between methods)

Figure 1. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Raw Ground Beef



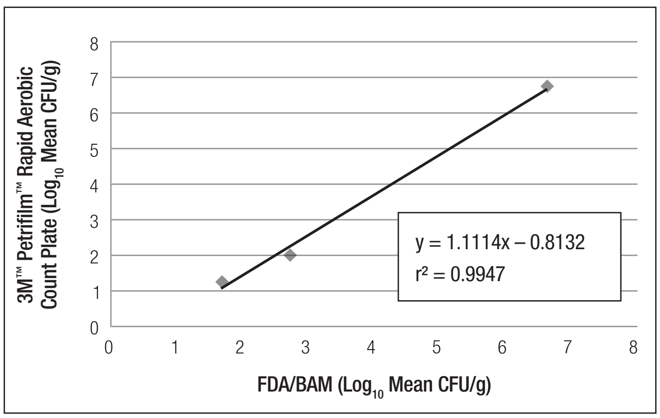
r^2 = Linearity Correlation Coefficient

Figure 2. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Raw Ground Pork



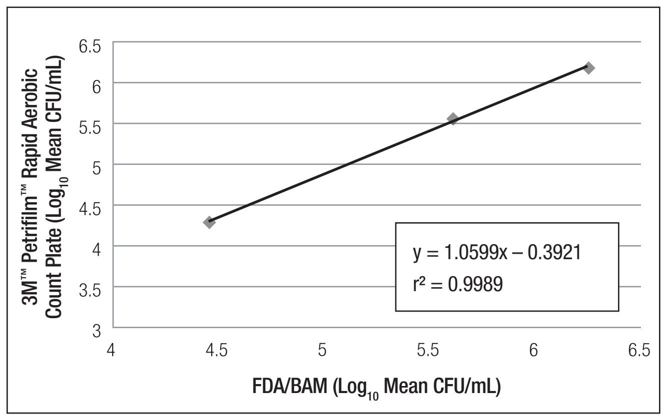
r^2 = Linearity Correlation Coefficient

Figure 3. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Raw Ground Turkey



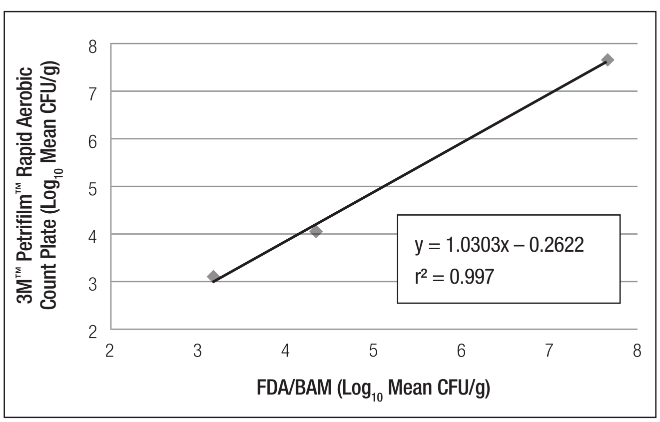
r^2 = Linearity Correlation Coefficient

Figure 4. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Chicken Carcass Rinsate



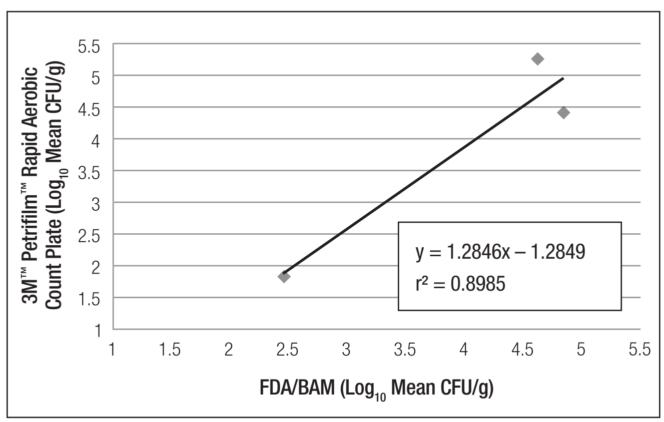
r^2 = Linearity Correlation Coefficient

Figure 5. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Fresh Swai



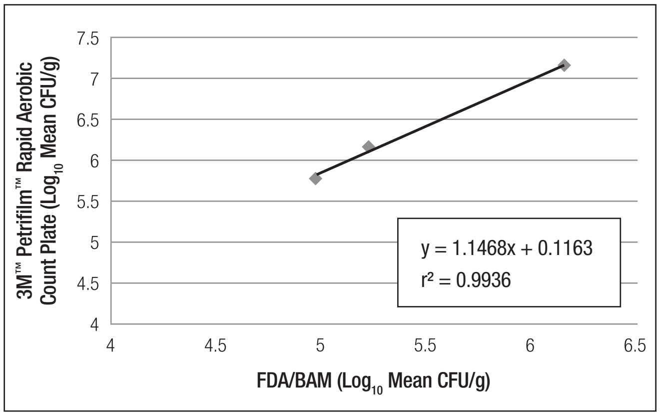
r^2 = Linearity Correlation Coefficient

Figure 6. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Fresh Tuna



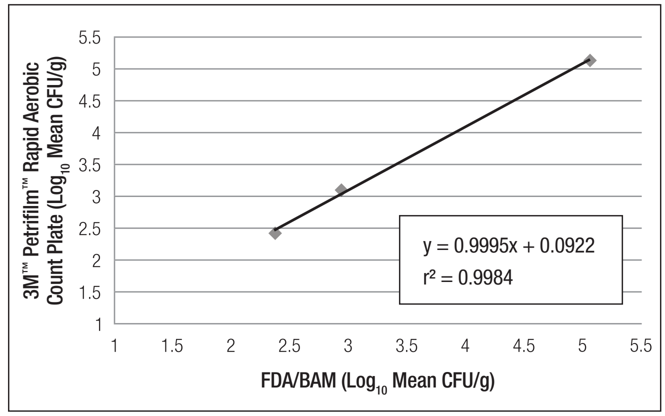
r^2 = Linearity Correlation Coefficient

Figure 7. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Fresh Tiger Shrimp



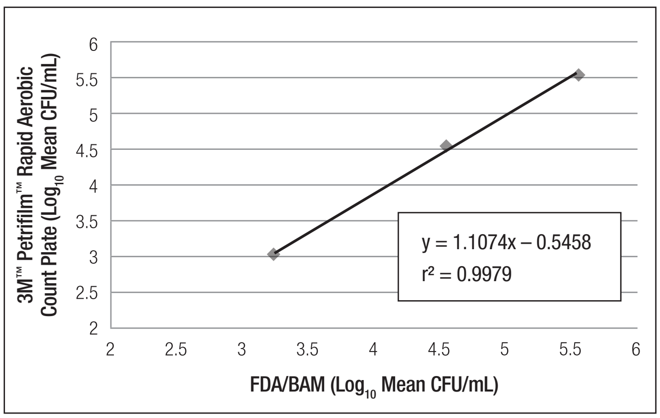
r^2 = Linearity Correlation Coefficient

Figure 8. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Easy-Peel Shrimp



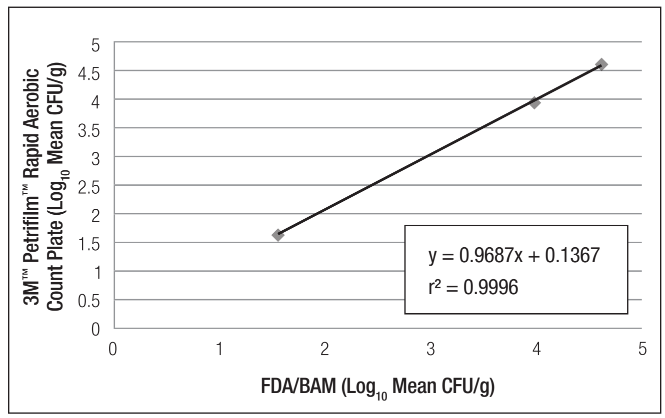
r^2 = Linearity Correlation Coefficient

Figure 9. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Cherry Tomato Wash



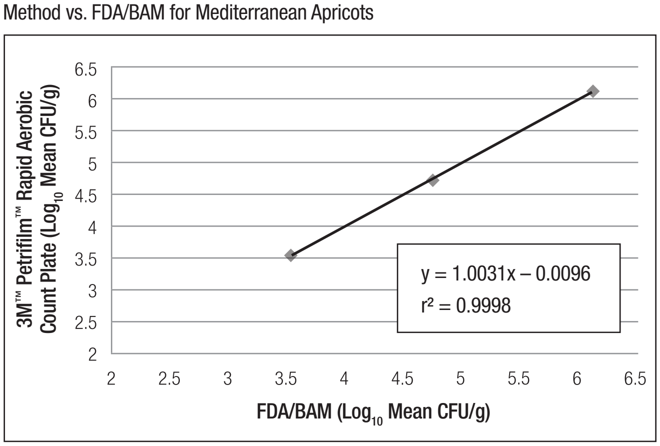
r^2 = Linearity Correlation Coefficient

Figure 10. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Frozen Blueberries



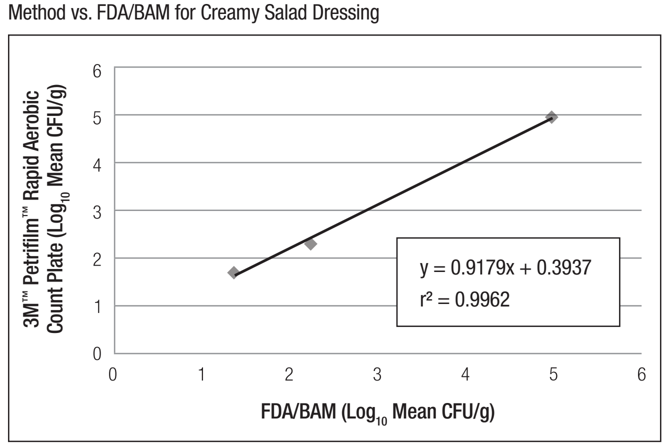
r^2 = Linearity Correlation Coefficient

Figure 11. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Mediterranean Apricots



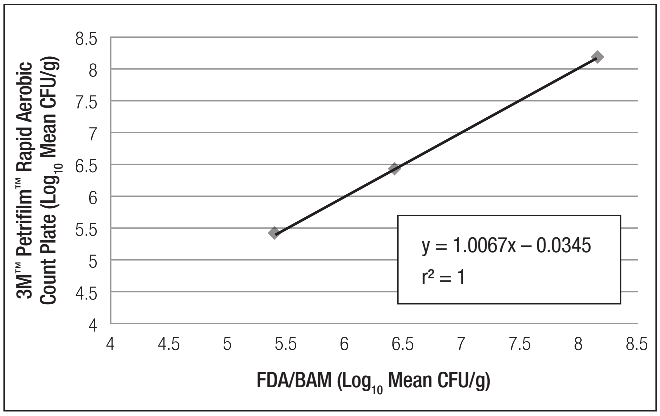
r^2 = Linearity Correlation Coefficient

Figure 12. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Creamy Salad Dressing



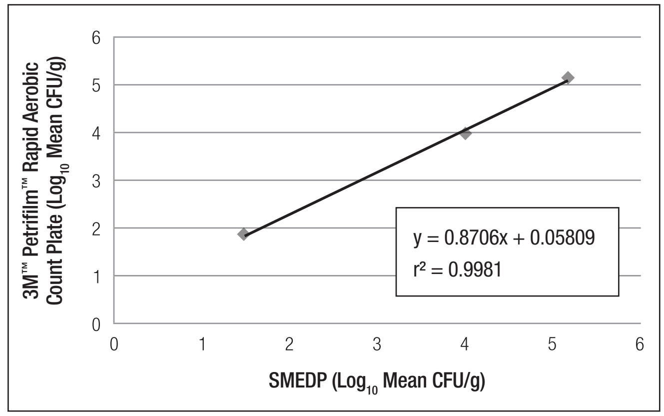
r^2 = Linearity Correlation Coefficient

Figure 13. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. FDA/BAM for Fresh Pasta



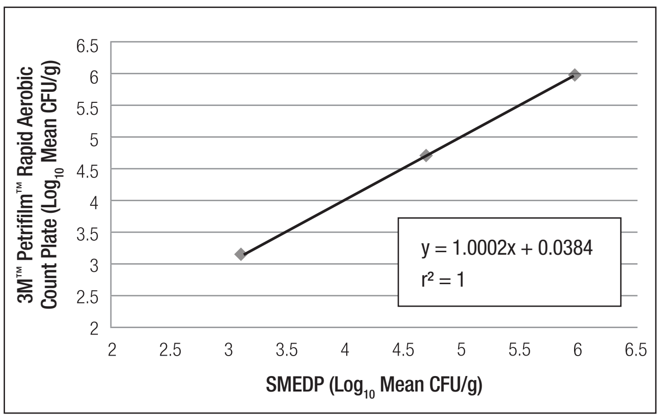
r² = Linearity Correlation Coefficient

Figure 14. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. SMEDP for Vanilla Ice Cream



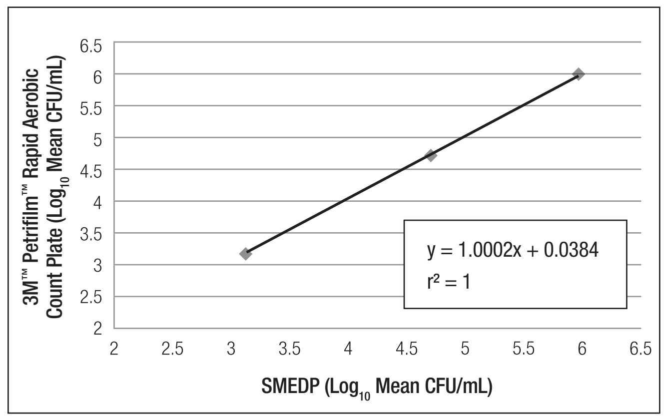
r² = Linearity Correlation Coefficient

Figure 15. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. SMEDP for Dry Milk Powder



r² = Linearity Correlation Coefficient

Figure 16. Method Comparison Results of 3M™ Petrifilm™ Rapid Aerobic Count Plate Method vs. SMEDP for Pasteurized Skim Milk



r² = Linearity Correlation Coefficient

3M™ Petrifilm™ Rapid Aerobic Count Plate — Robustness Study

Conducted by 3M Food Safety

This robustness study was conducted according to AOAC guidelines outlined in the AOAC General Referee approved harmonized PTM/OMA validation protocol.

Robustness Testing Methodology

This study evaluated the ability of the 3M™ Petrifilm™ Rapid Aerobic Count Plate to remain unaffected by variations in method parameters that might be expected to occur when the method is performed by an end user. The effects of perturbations in three method parameters were investigated:

1) **Incubation time** of the 3M Petrifilm RAC Plate (suggested 23–25 hours)*: 22, 24 and 26 hours.

2) **Incubation temperature** of the 3M Petrifilm RAC Plate method (suggested for dairy 31–33°C, suggested for Other Foods 34–36°C)**: Dairy — 30, 32 and 34°C, Other Foods — 33, 35 and 37°C.

3) **Various diluents:** Butterfield's phosphate buffer, 0.1% peptone water, peptone salt diluent, buffered peptone water, saline solution (0.85–0.90%), bisulphite-free letheen broth and distilled water.

Testing was conducted with vanilla ice cream and raw ground beef.

*AOAC suggested times. **AOAC suggested temperatures.

Robustness Testing Results

The log transformed results of the changes in incubation time, incubation temperature and diluent for both matrices (vanilla ice cream and raw ground beef) were analyzed by a nested analysis of variance (ANOVA). The data were calculated using the log counts from each plate, each replicate for each robustness parameter. The mean log difference data are presented in Tables 1 and 2.

Table 1. Summary of the Mean Log Difference for the Robustness Parameters for Vanilla Ice Cream

		Mean Log Difference*		
	Robustness Parameters	High	Medium	Low
Diluent	0.1% Peptone Water	0.024	-0.003	0.005
	Buffered Peptone Water	-0.046	-0.026	-0.030
	Butterfield's Phosphate Buffer	-0.008	0.000	-0.047
	Letheen Broth	-0.007	-0.002	0.018
	Peptone Salt	0.032	0.015	0.061
	Saline Solution	0.023	0.003	0.034
	Sterile Water	-0.017	0.013	-0.041
Time	22 Hours	0.016	0.101	0.015
	24 Hours	-0.006	-0.002	-0.004
	26 Hours	-0.010	0.001	-0.011
Temperature	30°C	0.030	-0.016	0.033
	32°C	-0.026	-0.048	-0.031
	34°C	-0.004	0.064	-0.003

*Mean Log Difference: Difference in overall mean values at contamination level minus the individual mean for variable tested at the corresponding contamination level.

Table 2. Summary of the Mean Log Difference for the Robustness Parameters for Raw Ground Beef

	Robustness Parameters	Mean Log Difference* High	Medium	Low
Diluent	0.1% Peptone Water	0.021	-0.051	0.054
	Buffered Peptone Water	-0.111	-0.096	-0.175
	Butterfield's Phosphate Buffer	0.122	0.059	0.116
	Letheen Broth	**-0.312**	**-0.288**	**-0.229**
	Peptone Salt	0.013	0.003	0.065
	Saline Solution	0.093	0.030	0.047
	Sterile Water	0.172	**0.345**	0.120
Time	22 Hours	0.010	0.012	0.041
	24 Hours	0.002	0.006	-0.008
	26 Hours	-0.012	-0.019	-0.034
Temperature	33°C	-0.065	-0.065	-0.098
	35°C	-0.011	0.006	0.032
	37°C	0.074	0.060	0.065

*Mean Log Difference: Difference in overall mean values at contamination level minus the individual mean for variable tested at the corresponding contamination level.
NOTE: The values (except those bolded) are ≤ 0.2 logs.

Discussion

In this robustness study, three parameters were evaluated: incubation time, incubation temperature and diluents according to the factorial design outlined in the approved protocol. The testing was done with two food matrices: vanilla ice cream and raw ground beef. The data was analyzed using a nested, one way ANOVA. In conducting the analysis, we first determined if there were any statistically significant differences. If statistically significant differences were found, mean log difference was calculated to determine if the differences were practically different. Practical difference is typically ≤ 0.2 logs.

For vanilla ice cream, incubation time was not significant at any time at the low, medium or high contamination levels. Incubation temperature was significant at all three contamination levels ($p = 0.000$ at all three levels). However, there were no practical differences at any of the three contamination levels. Similarly, diluents were also significant at all three contamination levels ($p = 0.000$, 0.001 and 0.000 respectively) but there were no practical differences for any diluent.

For raw ground beef, incubation time was not significant at any time at the low, medium or high contamination levels. Incubation temperature was significant at all three contamination levels ($p = 0.000$). However, there were no practical differences at any of the three contamination levels. Diluents were also significant at all three contamination levels ($p = 0.000$ at all three levels). There were practical differences for Letheen broth at all three contamination levels (recovering more organisms) and for sterile water (recovering fewer organisms) at the medium contamination level. Letheen broth has a high nutritive content compare to the other diluents tested. In addition, the medium also contains lecithin and Tween 80 which assist in breaking clumps and chains of bacteria resulting in higher recovery. These factors may contribute to the superior recovery by Letheen broth.

For either of the matrices and any of the robustness parameters evaluated, if the parameter under investigation was either not statistically significantly different, or the magnitude if the statistical difference was less than the limit for practical difference (≤ 0.2 logs), the method is considered robust with respect to the parameter.

The 3M™ Petrifilm™ Rapid Aerobic Count Plate Method is considered a robust method with respect to the influences of incubation time, incubation temperature and various diluents tested.

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