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Technical Bulletin

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Manual Microbial Interpretation vs. Automated Interpretation using the 3M™ Petrifilm™ Plate Reader Advanced

Overview

Visually counting colonies on a plate is a time-consuming task which has made automated plate counters a valuable tool in a microbiology laboratory. The 3M™ Petrifilm™ Plate Reader Advanced automates the enumeration of nine 3M™ Petrifilm™ Plates, plus the 3M Petrifilm Staph Express Disk to aid lab analysts while performing this task.

The following study was conducted to demonstrate the accuracy of the 3M Petrifilm Plate Reader Advanced as compared to the results obtained by manual counting. The following 3M Petrifilm Plates were evaluated: *E. coli*/Coliform Count Plate (EC/CC), Rapid *E. coli*/Coliform Count Plate (REC), Coliform Count Plate (CC), Select *E. coli* Count Plate (SEC), Staph Express Count Plate (STX), Staph Express Disk (STX disk), *Enterobacteriaceae* Count Plate (EB), Aerobic Count Plate (AC) and Rapid Aerobic Count Plate (RAC).

Method

Diluent Study

Pure strains of bacteria cultures were isolated from purchased culture stocks or from 3M frozen stock cultures. Each strain was sub-cultured into a non-selective broth and incubated for 18 – 24 hours before diluting the culture out into each diluent that is listed in the corresponding product instructions. Different concentrations of each of the organisms listed in Table 1 were obtained in order to have counts at the low, medium and high ranges of each 3M Petrifilm Plate countable range (e.g. target CFU/mL for a plate with a countable range of 0-300 was 0-75 (low), 75-175 (medium) and 175-300 (high)). One mL of each dilution was plated on to the corresponding 3M Petrifilm Plates based on Table 1. The 3M Petrifilm Plates were then incubated according to their corresponding instructions for use. After incubation, the 3M Petrifilm Plates were fed through the 3M Petrifilm Plate Reader Advanced and subsequently were manually counted by a microbiologist to obtain the colony counts for comparison.

Table 1. Organisms Used for Each 3M Petrifilm Plate

Organisms	3M Petrifilm Plate
***Bacillus atrophaeus* ATCC 9372**	STX, STX disk, RAC
Bacillus spizizenii ATCC 6633	AC, RAC
Citrobacter *freundii* ATCC 43864	REC, SEC
***Enterococcus faecalis* ATCC 29212**	EC/CC, REC, CC, EB, RAC
***Enterococcus faecalis* ATCC 19433**	REC
***Enterococcus faecalis* ATCC 14506**	EB
***Enterobacter amnigenus* ATCC 51816**	EC/CC, REC, CC, EB
***Enterobacter amnigenus* ATCC 51818**	EC/CC, REC, CC, EB
***Escherichia coli* ATCC 11229**	EC/CC, SEC
***Escherichia coli* ATCC 25922**	EC/CC, CC, SEC, STX, STX disk, EB, AC, RAC
***Escherichia coli* ATCC 51813**	EC/CC, CC, SEC, AC
***Escherichia coli* ATCC 8739**	REC, CC, SEC, EB, AC, RAC
***Escherichia coli* 3M-FR8**	SEC
***Escherichia coli* NCTC 13216**	REC, SEC
***Escherichia coli* REC1**	REC, SEC
Flavobacterium species ATCC 51823	AC
***Hafnia alvei* ATCC 51815**	EC/CC, CC
Korcuria varians ATCC 51820	AC
***Klebsiella oxytoca* ATCC 51817**	EC/CC, REC, CC
Lactococcus lactis subsp. cremoris 19257	AC, RAC
Microbacterium testaceum ATCC 15829	AC
Micrococcus species ATCC 51819	AC
***Pseudomonas aeruginosa* ATCC 27853**	EC/CC, CC, RAC
***Pseudomonas aeruginosa* ATCC 35554**	EB
Pseudomonas species ATCC 51821	AC
Pseudomonas aeruginosa NCIMB 12469	RAC
***Salmonella Typhimurium* ATCC 51812**	EC/CC, REC, CC, EB
***Salmonella Typhimurium* ATCC 14028**	EB
***Staphylococcus aureus* ATCC 25923**	STX, STX disk, AC, RAC
***Staphylococcus aureus* ATCC 49476**	STX, STX disk
***Staphylococcus aureus* ATCC 6538**	STX, STX disk, AC, RAC
***Staphylococcus epidermidis* ATCC 12228**	STX, STX disk
Staphylococcus epidermidis ATCC 14990	STX, STX disk
Streptococcus agalactiae ATCC 27965	AC

Method

Food Study

All foods were screened prior to the start of the study. Foods that contained a sufficient level of natural background flora to cover the low, medium and high countable ranges of the 3M Petrifilm Plates were not artificially spiked. Foods that did not contain a sufficient level of natural background flora were inoculated using pure strains of bacteria. Cultures were isolated from purchased culture stocks or from 3M frozen stock cultures. Each strain was sub-cultured into a non-selective broth and was incubated for 18 – 24 hours before diluting the culture out to appropriate levels. Different concentrations of each of the organisms listed in Table 3 were spiked into the foods listed in Table 2.

Each food matrix was diluted 1:10, 1:100 and 1:1000 and spiked to achieve the low, medium and high countable ranges of the 3M Petrifilm Plate. One mL of each dilution was plated on to the corresponding 3M Petrifilm Plate based on Table 2 and Table 3. The 3M Petrifilm Plates were then incubated according to their corresponding instructions for use. After incubation, the 3M Petrifilm Plates were fed through the 3M Petrifilm Plate Reader Advanced and subsequently were manually counted by a microbiologist to obtain the colony counts for comparison.

Table 2. Foods Tested for Each 3M Petrifilm Plate

Matrix	3M Petrifilm Plate
Raw Ground Beef	EC/CC, REC, CC, SEC, EB
Raw Milk	EC/CC, REC, CC, SEC, STX, STX disk, AC, RAC
Coconut	EC/CC, REC, CC, SEC, EB
Raw Chicken	EC/CC, REC, CC, SEC, EB
Ice Cream	EC/CC, REC, CC, SEC
Red Pepper Hummus	EC/CC, REC, CC, SEC
Alfalfa Sprouts	EC/CC, REC, CC
Offal's	SEC
Raw Sausage	STX, STX disk
Liquid Egg	STX, STX disk, EB
Chocolate Cake with Cream Filling	STX, STX disk
Potato Salad	STX, STX disk
Frozen Vegetable Dumplings	STX, STX disk
Chicken Nuggets	EB
Chocolate Milk	EB
Frozen Supreme Pizza	EB
Heavy Cream	AC, RAC
Yellow Fin Tuna	AC, RAC
Whey Powder	AC, RAC
Pasteurized Cheese	AC, RAC
Frozen Spaghetti Meal	AC, RAC
Frozen Spinach	AC, RAC
Processed Ham Spread	AC, RAC
Ground Turkey	RAC
Tomato Wash	RAC
Chocolate Sandwich Cookie	RAC

Table 3. Organisms Used to Spike Foods for Each 3M Petrifilm Plate

Organisms	3M Petrifilm Plate
***Bacillus atrophaeus* ATCC 9372**	STX, STX disk
***Enterobacter amnigenus* ATCC 51818**	EC/CC, REC, CC, SEC, EB
***Escherichia coli* ATCC 25922**	EC/CC, REC, CC, SEC, AC, RAC
***Escherichia coli* ATCC 51813**	EC/CC, REC, CC, SEC
***Hafnia alvei* ATCC 51815**	EB
***Klebsiella oxytoca* ATCC 51817**	REC, SEC
Lactobacillus fermentum ATCC 9338	RAC
***Pseudomonas aeruginosa* ATCC 27853**	AC, RAC
***Pseudomonas aeruginosa* ATCC 35554**	EB
***Salmonella Typhimurium* ATCC 51812**	EC/CC, REC, CC, SEC
***Staphylococcus aureus* ATCC 25923**	STX, STX disk, AC, RAC
***Staphylococcus cohnii* ATCC 35662**	STX, STX disk
***Shigella sonnei* U8**	EC/CC, REC, CC, SEC, EB

The diluent and the food study data were analyzed based on the three statements below using 3M Petrifilm Plates within the countable range, as described in the product instructions. The percentage of 3M Petrifilm Plates that met these criteria are displayed in Table 4 and Table 5 for the diluents study and foods study respectively.

1. The log value of the reader count is within ± 10% of the value of the log of the human count, assuming the plate has more than 10 colonies.
2. The total number of false positives and false negatives cannot exceed 10% of the log of the human count.
3. The difference between the human to reader count cannot exceed 2 colonies for plates containing 0-10 colonies, including 2 total false positives and false negatives.

Results – Diluents Study

11 Diluents were tested in total resulting in 1,705 plates tested across the 8 3M Petrifilm Plates plus the 3M Petrifilm Staph Express Disk. The percent of plates for which the 3M Petrifilm Plate Reader Advanced met the outlined criteria is summarized in Table 4.

Table 4. Percentage of 3M Petrifilm Plate Reader Advanced counts that met the criteria for the diluents study.

Method	Colony Types Counted	3M Petrifilm Plate Result	# of Plates	Accuracy for Reader #1 (%)	Accuracy for Reader #2 (%)
EC/CC	1. Red with gas and blue with gas	Total Coliform Count	156	96	97
	2. Blue with gas	Total *E. coli* Count		99	99
	3. Blue with and without gas	N/A		100	99
REC	1. All red colonies with gas and all blue colonies	Total Coliform Count (FDA-BAM Method)	165	100	99
REC	2. All red colonies and all blue colonies with or without gas	Total Coliform Count	165	100	100
	3. All blue colonies	Total *E. coli* Count		100	100
CC	1. All red colonies with gas	Total Coliform Count (FDA-BAM Method)	165	98	100
	2. All red colonies with and without gas	Total Coliform Count		98	100
SEC	All blue-green colonies	Total *E. coli* Count	186	99	98
STX	All red-violet colonies	Total *S. aureus*	104	100	100
STX disk	All pink zones	Total *S. aureus*	101	97	100
EB	All red colonies producing acid and/or gas	Total *Enterobacteriaceae*	181	100	99
AC	All colonies	Total Aerobic Count	272	97	96
RAC	All colonies	Total Aerobic Count	210	94	97

Results – Food Study

26 foods were tested in total resulting in 1,503 plates tested across the 8 3M Petrifilm Plates plus the 3M Petrifilm Staph Express Disk. The percent of plates for which the 3M Petrifilm Plate Reader Advanced met the outlined criteria is summarized in Table 5.

Table 5. Percentage of 3M Petrifilm Plate Reader Advanced counts that meet the criteria for the foods study.

Method	Colony Types Counted	3M Petrifilm Plate Result	# of Plates	Accuracy for Reader #1 (%)	Accuracy for Reader #2 (%)
EC/CC	1. Red with gas and blue with gas	Total Coliform Count	195	98	98
	2. Blue with gas	Total *E. coli* Count		96	96
	3. Blue with and without gas	NA		97	95
REC	1. All red colonies with gas and all blue colonies	Total Coliform Count (FDA-BAM Method)	123	98	99
	2. All red colonies and all blue colonies with or without gas	Total Coliform Count		96	96
	3. All blue colonies	Total *E. coli* Count		98	99
CC	1. All red colonies with gas	Total Coliform Count (FDA-BAM Method)	126	98	99
	2. All red colonies with and without gas	Total Coliform Count		98	98
SEC	All blue-green colonies	Total *E. coli* Count	151	97	93
STX	All red-violet colonies	Total *S. aureus*	149	97	95
STX disk	All pink zones	Total *S. aureus*	154	95	90
EB	All red colonies producing acid and/or gas	Total *Enterobacteriaceae*	140	96	93
AC	All colonies	Total Aerobic Count	204	95	93
RAC	All colonies	Total Aerobic Count	261	95	96

Conclusion

Of the 3M Petrifilm Plates that were tested with 7 to 13 different isolates in the diluents study, all of them achieved an accuracy of at least 94% on Reader 1 and 96% on Reader 2. Similarly, the 3M Petrifilm Plates that were tested across 6-8 representative food matrices achieved an accuracy of at least 95% on Reader 1 and 90% on Reader 2.

Notes and References:

1. Standard Methods for the Examination of Dairy Products, 17th Edition. American Public Health Association. 2004.

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