Gage R&R Study Comparison of Variability in two Measurement Systems, 3M™ Petrifilm™ Plate Readers and Trained Technicians, to Enumerate Counts below Plate Count Ranges

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ABSTRACT

Accurate enumeration and reporting of microbial results is important in the food industry. The 3M™ Petrifilm™ Plate Reader is a compact computerized image analyzer designed and validated to accurately read and report microbial results within the counting ranges of three validated methods: 3M™ Petrifilm™ Aerobic, Coliform and E. coli/Coliform Count Plate methods. Increased demands for safe food, have led many food processors to set product specifications at sensitivities below the validated counting ranges of approved methods.

In this study, Petrifilm Coliform and E. coli/Coliform Count plates were inoculated with pure strains of coliform organisms including *Escherichia coli,* at levels below the validated counting ranges of these methods. Three Petrifilm Plate Readers and three trained technicians counted four replicates of 33 Petrifilm Coliform plates, and four replicates of 33 Petrifilm E. coli/Coliform plates. A Gage R&R (Repeatability and Reproducibility) study was used to demonstrate variation due to each of the measurement systems used: enumeration of Petrifilm Plates by humans, and enumeration of Petrifilm Plates by the Petrifilm Plate Reader.

Analysis of data following logarithmic transformation showed that counts enumerated by the Petrifilm Plate Reader were not statistically different ($p>0.05$) from counts enumerated by the trained technicians. The difference between the percent of variability due to the measurement systems for enumeration of Petrifilm Coliform and E. coli/Coliform plates was no more than 2.03%. Results suggest enumeration of low levels of colonies on Petrifilm Coliform and E. coli/Coliform Count plates using the Petrifilm Plate Reader show similar variability to enumeration by trained technicians.

INTRODUCTION

One of the more time consuming aspects of microbiological plating methods, is the time it takes to read and record results. When many plates are read at one time, eye fatigue, interruptions, data transcription with subsequent manual transfer of data to computers, can result in errors, which can mean financial consequences to food processors.

The Petrifilm Plate Reader provides fast, automated reading of Petrifilm plates. Results are automatically exported into a Microsoft® Excel spreadsheet and/or a log file that can not be edited, thereby eliminating the potential of human error. This unchangeable log file provides a historical record of data for audit purposes to ensure FDA 21CFRPart 11 compliance.

Food processors are looking for low, or no levels of bacteria in their product, and are therefore pressed to provide microbial results that often fall below the statistical range of most methods available today. All plate methods have defined counting ranges, representing the counting area in which statistically, counts can be replicated and reproduced. Outside of these ranges, chances are statistically less likely to produce a similar count each time a sample is plated[1]. Data collected in this study provides additional support of the ability of the Petrifilm Plate Reader to consistently read low level colony counts on Petrifilm plate methods.

MATERIALS and METHODS

A Gage R&R (Repeatability and Reproducibility) study measures what percent of the total variation in a process, is a result of the variation in the measurement system. In this multi-variable Gage R&R study, two measurement systems (Petrifilm Plate Readers and trained technicians) were compared in their enumeration of Petrifilm Coliform and E. coli/Coliform Plates.

It is important in a Gage R&R study, to use samples that represent full, but typical, process variation. To provide this "full and typical" variation, the following variables were incorporated into this study:

1. **Specific organisms** were selected to exhibit a variety of key characteristics on Petrifilm plates (see Table 1).

Table 1: ORGANISM Selection based on growth characteristics

Organism	Colony Size	β-glucuronidase Production	Lactose fermentation
Escherichia coli ATCC 51813	Large	Strong positive	Positive
Klebsiella oxytoca ATCC 51817	Large	Negative	Positive
Enterobacter amnigenus ATCC 51818	Small	Negative	Positive
Shigella sonnei food isolate	Small	Strong positive	Negative
Escherichia coli food isolate	Large	Weak positive	Negative

Organisms were grown overnight in Tryptic Soy Broth incubated at 35˚C. Plating preparations were made by inoculating Butterfield's Phosphate Diluent (BPD) with micro liter amounts of the growth cultures, and diluting to levels that would provide counts below the counting ranges of Petrifilm E. coli and Coliform Count Plate methods (< 15 colonies/ mL).

2. **Inoculated plates exhibiting specifically defined combinations of growth characteristics** (see Table 2) were selected to provide challenging plates for both reader types. The following growth characteristic variables were included: colony size; strong or weak gas production from lactose; the location of the colony and/ or gas production (near the edge of the plate, in the center of the plate, next to other colonies); and strong, weak or no β-glucuronidase reaction to 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) indicator in the plate medium.

Table 2: PLATE selection based on growth characteristics

Plate #	β-glucuronidase Reaction	Colony Size	Colony with Gas	Colony Clustered
1	Strong	Large	Gas	Not Close
2	Strong	Large	Gas	Not Close
3	Strong	Large	Gas	Too Close
4	Strong	Large	Gas	Too Close
5	Strong	Large	No Gas	Not Close
6	Strong	Large	No Gas	Not Close
7	Strong	Large	No Gas	Too Close
8	Strong	Large	No Gas	Too Close
9	Strong	Small	Gas	Not Close
10	Strong	Small	Gas	Not Close
11	Strong	Small	Gas	Too Close
12	Strong	Small	Gas	Too Close
13	Strong	Small	No Gas	Not Close
14	Strong	Small	No Gas	Not Close
15	Strong	Small	No Gas	Too Close
16	Strong	Small	No Gas	Too Close
17	Weak	Large	Gas	Not Close
18	Weak	Large	Gas	Not Close
19	Weak	Large	Gas	Too Close
20	Weak	Large	Gas	Too Close
21	Weak	Large	No Gas	Not Close
22	Weak	Large	No Gas	Not Close
23	Weak	Large	No Gas	Too Close
24	Weak	Large	No Gas	Too Close
25	Weak	Small	Gas	Not Close
26	Weak	Small	Gas	Not Close
27	Weak	Small	Gas	Too Close
28	Weak	Small	Gas	Too Close
29	Weak	Small	No Gas	Not Close
30	Weak	Small	No Gas	Not Close
31	Weak	Small	No Gas	Too Close
32	Weak	Small	No Gas	Too Close
33	No Growth (NG)	NG	NG	NG

3. **Four Petrifilm Plate Readers,** obtained from different manufactured lots, were used to represent variance that could normally occur between Petrifilm Plate Readers. Calibration readings were recorded for each Petrifilm Plate Reader before and after enumeration.

4. **Three Trained Technicians** were selected for this study, based on their expertise in reading Petrifilm plates, to represent the "reference" counting method. The trained technicians were "calibrated" by reviewing together, the interpretation rules described in each of the corresponding Petrifilm plate interpretation guides and product inserts, to eliminate as much subjectivity as possible.

Plates for both Petrifilm Coliform and E. coli/Coliform Plate methods were incubated at 35˚C for 24±2 hours and /or 48±3 hours (as appropriate per method). Thirty-three Petrifilm E. coli /Coliform Plates, and 33 Petrifilm Coliform plates exhibiting the criteria defined in Table 2, were selected for enumeration by both enumeration methods. Five counting repetitions were made of each plate by both Petrifilm Plate Readers and trained technicians, using random run order.

The Gage R&R study was first conducted for the Petrifilm Coliform Plate, and repeated in its entirety with the Petrifilm E. coli/Coliform Plate the following week.

The logarithms_{10} of colony counts were used for the Gage R&R analysis under the assumption that the transform numbers would be normally distributed and of homogenous variance.

RESULTS

Gage R&R analysis results for each Petrifilm plate method are listed in Tables 3, 4, and 5. The percent variance for REPEATABILITY represents the percent of the variability in the measurement system caused by the SAME reader, reading the SAME plate repeatedly. The percent variance for REPRODUCIBILITY represents

the percent of the variability in the measurement system caused by DIFFERENT readers, reading the SAME plate.

Table 3 shows the Gage R&R analysis using data from the Petrifilm Coliform Plate for both the trained technician and Petrifilm Plate Reader enumeration methods.

Table 3: Analysis of variance for the Petrifilm Coliform Plate method @ 24 hrs

Trained Technician variability at 24 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	**0.067877**	**0.34957**	**15.05**
Repeatability	0.024784	0.12764	5.49
Reproducibility	0.063191	0.32543	14.01

Petrifilm Plate Reader variability at 24 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	0.056086	0.28884	**13.65**
Repeatability	0.050216	0.25861	12.22
Reproducibility	0.024980	0.12865	6.08

Repeatability for the trained technicians for the Petrifilm Coliform Plate was lower than Reproducibility. That means, the variability of a trained technician reading the SAME plate repeatedly was very low. The greater variability in the measuring system (enumeration) was observed BETWEEN trained technicians. The opposite was true for the Petrifilm Plate Reader. Here, the variability of the Petrifilm Plate Reader reading the SAME plate repeatedly was very low, and greater variability in the measuring system (enumeration) was observed BETWEEN Petrifilm Plate Readers.

For Gage R&R studies, it is recommended the percent variability for the Total Gage R&R of a measurement system be < 30%[2]. Total Gage R&R variability for both trained technicians and Petrifilm Plate Readers when enumerating the Petrifilm Coliform plate, were well below the recommended level.

Tables 4 and 5, show Gage R&R analysis using the data from the Petrifilm E. coli/Coliform Plate for both trained technician and Petrifilm Plate Reader enumeration methods following 24 and 48 hours of incubation, respectively.

Table 4: Analysis of variance for the Petrifilm E.coli /Coliform Plate method @ 24 hrs

Trained Technician variability at 24 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	0.040873	0.21050	**8.99**
Repeatability	0.039797	0.20495	8.76
Reproducibility	0.009318	0.04799	2.05

Petrifilm Plate Reader variability at 24 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	0.043708	0.22510	**9.92**
Repeatability	0.043708	0.22510	9.92
Reproducibility	0.000000	0.00000	0.00

Table 5: Analysis of variance for the Petrifilm E.coli /Coliform Plate method @ 48 hrs

Trained Technician variability at 48 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	0.038687	0.19924	**10.08**
Repeatability	0.035912	0.18495	9.36
Reproducibility	0.014386	0.07409	3.75

Petrifilm Plate Reader variability at 48 hours

Source	StdDev (SD)	Study Var (5.15 * SD)	%Study Var (%SV)
Total Gage R&R	0.048734	0.25098	**12.11**
Repeatability	0.040483	0.20849	10.06
Reproducibility	0.027133	0.13973	6.74

For the Petrifilm E. coli/Coliform Plate, the repeatability for SAME enumeration system to read the SAME plates at both incubation temperatures was greater than the variation observed in the variability of the DIFFERENT systems to read the SAME plate.

As was observed with the Petrifilm Coliform Plate, the Gage R&R variability for both trained technicians and Petrifilm Plate Readers when enumerating the Petrifilm E.coli/Coliform plate, were again, well below the recommended level.

Table 6 provides a summary of the overall difference between the variability of the two measurement systems for each of the plate types. The Gage R&R analysis results show that variability in the "system" a trained technician uses to make decisions to enumerate colonies on a Petrifilm Plate, is *very* similar to the variability the Petrifilm Plate Reader system uses to make enumeration decisions.

Table 6: Percent Variability for each PLATE per READER TYPE

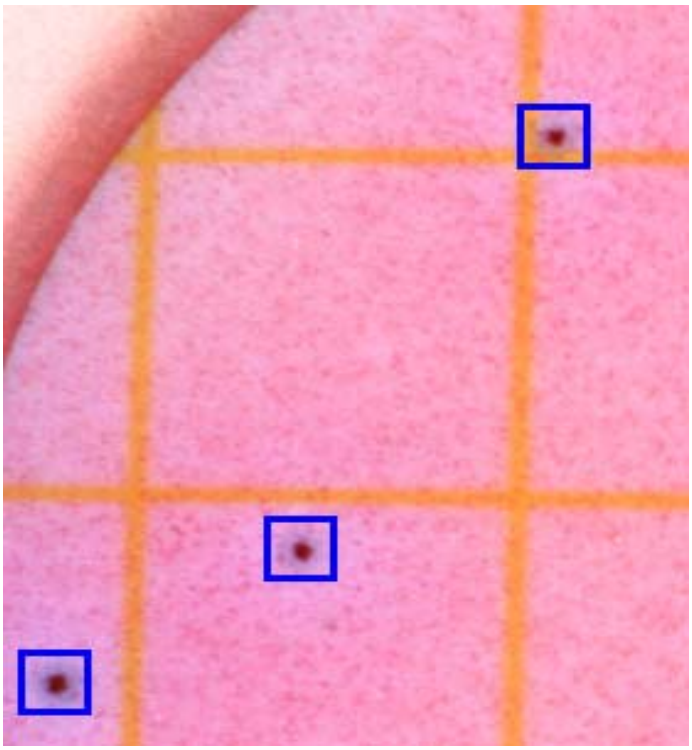
Reader Type	Coliform @ 24 ± 2 hours	E. coli/Coliform @ 24 ± 2 hours	E. coli @ 48 ± 3 hours
Trained Technician	15.05	8.99	10.08
Petrifilm Plate Reader	13.65	9.92	12.11
Variability between Reader Types	1.40	1.06	2.03

OBSERVATION

For some plates, slight differences were observed between trained technicians when reading weakly positive β-glucuronidase colonies on Petrifilm E. coli/Coliform Plates. In these cases, the Petrifilm Plate Reader was generally more consistent.

An enlarged example of the type of colonies seen where differences were recorded between the trained technicians and Petrifilm Plate Readers is shown in Figure 1.

Figure 1: Enlargement of weakly positive β-glucuronidase colonies



In this enlarged image, it is obvious the colonies themselves are not yet blue in color, but the Petrifilm Plate Reader has counted these colonies as blue.

On the Petrifilm E. coli/Coliform Plate, β-glucuronidase positive organisms will first form a blue precipitate around the colony, and as the colony grows, more precipitate is formed until the precipitate surrounds the colony, causing the colony to appear blue. Some trained technicians are able to detect this early color development and correctly enumerate *E. coli* at 24 hours of incubation; some technicians may need the additional 24 hours of incubation before they can confidently enumerate colonies as *E. coli*.

The Petrifilm Plate Reader correctly identified these colonies as blue, at 24 hours of incubation.

CONCLUSIONS

- Enumeration of Petrifilm Coliform and E. coli/Coliform Plates by the Petrifilm Plate Reader was as consistent as enumeration of these same plates by trained technicians. When the Petrifilm Plate Reader provided a different number than a technician, it was just as likely that a different technician would also provide a different number.
- The difference in the percent variability between the two measurement systems to enumerate Petrifilm Coliform and E. coli/Coliform Plates using the Gage R&R statistical tool was found to be *very* low: = 2.03%.
- In some cases, weakly positive ß-glucuronidase colonies may be detected more easily by the Petrifilm Plate Reader, than by technicians.

The automatic counting, recording and transfer of data into a Microsoft Excel sheet as provided by the Petrifilm Plate Reader system may further reduce possible variability from technicians due to eye fatigue, transcription and data transfer errors.

References:

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