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TECHNICAL REPORT

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TITLE: Statistical analysis of a collaborative study comparing Petrifilm to TSA plate count for four analytes.

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ABSTRACT: A multicollaborator study was undertaken to validate the equivalence of TSA agar plates vs. Petrifilm methods of quantifying bacteria. The two quantitation methods are based upon different media, have different physical sizes of plate units and different means of identifying colonies. Four representative analytes were used (*B. subtilis, S. aureus, S. enterica, P. aeruginosa*), measured in 4 replicates on each of 3 carriers at each of five different laboratories (one laboratory did not report data). The equivalency criterion was that the mean difference of the candidate and reference methods must have a 95% confidence interval falling entirely within (-0.5, +0.5) in $\log_{10}$(concentration). Based on matched-by-laboratory analysis, for all analytes the 95% confidence interval for the mean difference of $\log_{10}$(Concentration) fell within (-0.2, +0.2), well within the (-0.5, +0.5) allowed for equivalence. The mean difference pooled across analytes was –0.012 with a 95% confidence interval of (-0.090, +0.066). The between-carrier standard deviation was 0.139 and the between-replicate standard deviation was 0.050. The carrier with single replicate repeatability was estimated to be 0.148.

KEYWORDS: 1) PETRIFILM 2) MICRO 3) EQUIVALENCE
4) TSA 5) COLLAB

REL.DOC.: TR302, TR307

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INTRODUCTION

Petrifilm has been proposed as a substitute for the reference standard plate count method (trypticase soy agar or 'TSA') of quantifying bacteria on stainless steel carriers. The two methods are based upon different media, have different physical sizes of units and different means of identifying colonies. Before the candidate method can be allowed as a substitute for the reference method, the two must be shown to have acceptably equivalent results. The means of proving this is via a multicollaborator comparison study on 4 different representative analytes. In TR307, for a 3 laboratory study, it was found that: 1) the $\log_{10}$-transform of the count data satisfactorily normalized the data; 2) The best dilution for each laboratory should be used, maximizing the number of plates with counts between 30 and 300; and 3) The between method difference based on 3 collaborators had a 95% confidence interval was approximately (-0.2, +0.2), and fell within the (-0.5, 0.5) criterion for acceptability.

STUDY DESIGN

Six different collaborating laboratories undertook to compare the two methods on *in vitro* suspensions in replicate for each analyte. One laboratory did not report data.

Each laboratory was supplied with an inoculum the same strain of each analyte. The inoculum was then added to the specified broth for each method and growth amplified under specified conditions to a high level of concentration (+8E CFU/mL or more). Decimal serial dilutions were then made, and each dilution quantified in 4 replicates each on 3 carriers each by each method. Each collaborator had separate enrichments, so final concentrations were not expected to be the same. (I.e., the "laboratory" effect includes an enrichment offset plus any idiosyncratic quantitation effect.)

The relevant dilution for *each* laboratory with the highest counts per unit (plate) for which most or all replicates fall within the range 30 to 300 colonies is used. Note that different laboratories have *different* chosen dilutions.

The four analytes used were: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella enterica*.

Note that Method (TSA vs. Petrifilm) is matched on Laboratory, but not on Carrier nor Replicate.

EQUIVALENCY CRITERION

The two methods will be considered "equivalent" to the reference method if the mean difference in $\log_{10}$(concentration) results has a 95% confidence interval which falls entirely within (-0.5, 0.5).

LOG$_{10}$(CONCENTRATION)

The values of $\log_{10}$(concentration) are calculated using the actual count/plate plus a 0.1 count offset, in order to avoid problems arising from the transform of zero counts.

RESULTS

The first analyte used was *B. subtilis*. The mean values of log-transformed concentrations, average across carriers and replicates, were:

	Organism	Lab	Method	Dilution	Count	Conc	logConc
1	Bsubtilis	BIOSCIE	Petrifilm	3	60.66667	60666.67	4.757185
2	Bsubtilis	BIOSCIE	TSA	3	53.83333	53833.33	4.691044
3	Bsubtilis	DOW	Petrifilm	3	78.25000	78250.00	4.890275
4	Bsubtilis	DOW	TSA	3	59.25000	59250.00	4.728607
5	Bsubtilis	ECOLAB	Petrifilm	4	30.75000	307500.00	5.397394
6	Bsubtilis	ECOLAB	TSA	4	29.00000	290000.00	5.387011
7	Bsubtilis	EPAMLB	Petrifilm	3	70.33333	70333.33	4.830348
8	Bsubtilis	EPAMLB	TSA	3	73.00000	73000.00	4.854552
9	Bsubtilis	MCRBAC	Petrifilm	5	26.83333	2683333.33	6.417011
10	Bsubtilis	MCRBAC	TSA	5	30.83333	3083333.33	6.468818

Note that there are substantial differences among laboratories, but not between methods.

The second analyte was *S. aureus*. The mean values of log-transformed concentrations, average across carriers and replicates, were:

	Organism	Lab	Method	Dilution	Count	Conc	logConc
1	Saureus	BIOSCIE	Petrifilm	4	25.16667	251666.7	5.391379
2	Saureus	BIOSCIE	TSA	4	22.50000	225000.0	5.337823
3	Saureus	DOW	Petrifilm	3	149.16667	149166.7	5.170647
4	Saureus	DOW	TSA	3	146.83333	146833.3	5.163605
5	Saureus	ECOLAB	Petrifilm	4	155.91667	1559166.7	6.155108
6	Saureus	ECOLAB	TSA	4	154.00000	1540000.0	6.152793
7	Saureus	EPAMLB	Petrifilm	4	143.16667	1431666.7	6.132415
8	Saureus	EPAMLB	TSA	4	139.33333	1393333.3	6.119743
9	Saureus	MCRBAC	Petrifilm	5	22.00000	2200000.0	6.296586
10	Saureus	MCRBAC	TSA	5	22.75000	2275000.0	6.295286

Note again that there are substantial differences among laboratories, but not between methods.

The third analyte was *S. enterica*. The mean values of log-transformed concentrations, average across carriers and replicates, were:

	Organism	Lab	Method	Dilution	Count	Conc	logConc
1	Senteric	BIOSCIE	Petrifilm	3	10.666667	10666.667	4.024327
2	Senteric	BIOSCIE	TSA	3	9.583333	9583.333	3.958670
3	Senteric	DOW	Petrifilm	3	93.750000	93750.000	4.963231
4	Senteric	DOW	TSA	3	100.583333	100583.333	4.992404
5	Senteric	ECOLAB	Petrifilm	4	44.083333	440833.333	5.483020
6	Senteric	ECOLAB	TSA	4	58.416667	584166.667	5.604001
7	Senteric	EPAMLB	Petrifilm	4	39.333333	393333.333	5.590504
8	Senteric	EPAMLB	TSA	4	44.916667	449166.667	5.638773
9	Senteric	MCRBAC	Petrifilm	5	23.333333	2333333.333	6.346457
10	Senteric	MCRBAC	TSA	5	20.833333	2083333.333	6.267073

Again that there are substantial differences among laboratories, but not between methods.

The third analyte was *P. aeruginosa*. The mean values of log-transformed concentrations, average across carriers and replicates, were:

	Organism	Lab	Method	Dilution	Count	Conc	logConc
1	Paerug	BIOSCIE	Petrifilm	4	50.41667	504166.7	5.695972
2	Paerug	BIOSCIE	TSA	4	48.33333	483333.3	5.675163
3	Paerug	DOW	Petrifilm	5	28.75000	2875000.0	6.453155
4	Paerug	DOW	TSA	5	25.16667	2516666.7	6.387186
5	Paerug	ECOLAB	Petrifilm	5	60.00000	6000000.0	6.772067
6	Paerug	ECOLAB	TSA	5	58.33333	5833333.3	6.761088
7	Paerug	EPAMLB	Petrifilm	4	54.08333	540833.3	5.728516
8	Paerug	EPAMLB	TSA	4	55.33333	553333.3	5.738308
9	Paerug	MCRBAC	Petrifilm	5	54.66667	5466666.7	6.734558
10	Paerug	MCRBAC	TSA	5	58.16667	5816666.7	6.763795

Again that there are substantial differences among laboratories, but not between methods.

TSA vs. Petrifilm Difference in log10(Concentration)

Analyte	Mean Difference	95% LCL	95% UCL	P-value	Carrier Std. Dev.	Carrrier D.f.	Replicate Std. Dev.	Replicate D.f.	Carrier Reproducibility
B. subtilis	-0.032	-0.138	0.073	0.440	0.134	20	0.060	90	0.147
S. aureus	-0.015	-0.042	0.012	0.190	0.113	20	0.029	90	0.117
S. enterica	0.011	-0.093	0.114	0.789	0.168	20	0.046	90	0.175
P. aeruginosa	-0.012	-0.056	0.033	0.505	0.134	20	0.060	90	0.147
Pooled:	-0.012	-0.090	0.066		0.139	80	0.050	360	0.148

NOTES:

1. The differences of methods by laboratory were analyzed by a matched-pairs t test and confidence interval. There were 4 d.f.
2. All C.I.s on the mean difference in log10(Concentration) between TSA and Petrifilm fell within (-0.02, +0.02), well within the required (-0.50, +0.50). The two methods may be considered equivalent.
3. The Replicate standard deviation was less than 1/2 the Carrier standard deviation. Carriers should be considered the measurement unit, and Replicates are pseudo-replicates of little value. A single Replicate per Carrier would suffice.
4. "Carrier Reproducibility" is the between-Carrier standard error, given a single replicate per Carrier is done.