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PRIMARY VALIDATION OF THE GROWTH DIRECT ENVIRONMENTAL MONITORING SYSTEM AND MEDIA

INTRODUCTION

In 2015 Murphy and Schwedock published a paper outlining the statistics to be used to validate a Rapid Microbial Method. Since that time new versions of the USP and EP have been published with updated statistics. The current EP 5.1.6[6] chapter has more clearly defined the requirement for suppliers of Rapid Microbial Methods (RMM) to supply the formal validation data obtained using their method. The analytical testing is well defined in the chapter however the statistical analysis is not as clear. The USP has made a clear choice to focus on equivalence or non-inferiority for most of the validation parameters, however, are less clear on the role of the supplier. It is the goal of this paper to describe the validation methods for the Environmental Monitoring application performed using the Rapid Micro Biosystems Growth Direct system and associated consumables. Experimental data obtained has been analyzed using statistical methods that are in line with the European and United States pharmacopeia.

GROWTH DIRECT TECHNOLOGY

The Growth Direct System for rapid microbial enumeration is designed to automate the incubation of the EM media plates and enumerate any colonies present on the media. The instrument comprises two automated incubators, robotic sample transport systems, an advanced imaging system, two computers (one for system control, the other for image analysis) and associated hardware and staging required for the handling of up to 679 Growth Cassette™ products. The Growth Cassette products are plastic contact plate style cassettes with specific mechanical and optical features that facilitate the automated handling and imaging process. The Growth Cassette products incorporate standard media depending on application. Each test method requires the presence of a black mixed cellulose ester membrane, 0.45-micron pore size, to improve the signal to noise ratio for the detection system. Both white membranes and the media itself exhibit fluorescence at the critical wavelengths of the system and need to be removed or reduced.

During the incubation phase, images of each cassette are taken at intervals of 4 hours, allowing organisms and debris that are naturally fluorescent under the excitation blue light of the imager to be detected in the green spectrum. The images are recorded by a Charged-Coupled Device (CCD) camera every 4 hours. Analysis of the behavior of objects over the incubation time by proprietary growth rules of the vision analysis software allows the Growth Direct System to distinguish and enumerate the growing objects from the background and debris. Most of the Pharmacopoeial organisms are detected in <16 hours and accurately enumerated by 24 hours. At the end of an assay, the system reports the number of growing objects found on the surface of the membrane in the cassette. The technology is a Quantitative Enumeration method and will be validated as such.

MATERIAL & METHODS

The validation of the Growth Direct System and dedicated consumables was performed in accordance with the full requirements of EP 9.2; 5.1.6[6] and USP 38/NF33 Ch <1223>[5].

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The data presented in this paper supports the primary analytical performance validation of the Growth Direct system (e.g. validation at the supplier). For subsequent site implementations a subset of the analytical tests can be performed to verify the systems performance as an "automated compendial test". The test requirements for "automated compendial" are described in USP Ch <1223>, the PDA TR33[4] technical report and in literature (Jones et al 2018)[2].

Materials

Testing was performed on:

RMB TSA LP80 media part number ET80-100.
Control media BD RODAC TSA with Letheen and Tween 80, Part No 222207.
RMB TSA LP80HT media part number ET80HT-100
Control media was Heipha TSA w. LTHTh-ICR part 146069 (supplied via Millipore).

Incubation was performed on the Growth Direct System and the control method was incubated in standard incubators. All incubations were performed at 30-35°C for >48 hours.

- Challenge ATCC micro-organism, reconstituted commercial preparations from BioBall®.
- Stock EM cultures, freshly grown, for the EM isolates and titer from a colonial suspension.
- Stressed cells prepared by treatment with bleach to obtain >99% kill. Remaining 1% "stressed" cells then titrated for assay.

Methods: Validation Parameters

The parameters required to validate a quantitative analytical method according to the European and United States Pharmacopoeia are defined below:

Table 1 Required validation parameters according to EP and USP.

Parameter	EP 5.1.6	USP 1223
Accuracy	Yes	Yes
Intermediate Precision	Yes	No
Repeatability	Yes	Yes
Limit of Detection	No[a]	Yes
Limit of Quantification	Yes	Yes
Linearity	Yes	Yes
Range	Yes	Yes
Specificity	Yes	Yes
Ruggedness	Yes	Yes

[a]*For 5.1.6 the LOD can be obtained from accuracy data*

- Accuracy of the test method is defined by the ability to accurately recover organisms with respect to a gold standard method. This means that the Growth Direct system must accurately culture and enumerate colonies on

Growth Cassettes compared to results obtained on traditional media plates. We will investigate the accuracy of both the software (automated counts) and the manual counts.

- Precision consists of two separate components:
  - Repeatability represents the variability of the test method under constant conditions (i.e. when multiple replicates of the same test material are analyzed by one person at one time).
  - Intermediate Precision represents the variability of the test method when performed on multiple samples from the same test material under various routinely changing conditions (e.g. different operators, days).
- Linearity and Range define the lowest to highest CFU count that can be quantified with good precision and where the CFU counts are quantifiably or proportionally related to the sample dilution.
- The Limit of Detection (LOD) is the lowest level of CFU that can be confidently detected, while the Limit of Quantification (LOQ) is the lowest level of CFU that can be precisely quantified. The EP allows the Limit of Detection to be determined from the accuracy study. If the two methods are equivalent, the LOD of the rapid method will be the same as the compendial.
- Specificity is defined as the ability of the technology to accurately detect and enumerate the required range of micro-organisms without generating false positives. Mixed organisms and stressed micro-organisms are included in the validation study.
- Ruggedness is defined as the ability to change equipment, analysts and reagent lot number and still obtain comparable results. This part will coincide with intermediate precision.

Methods: Experimental Design

The experiment was designed to obtain all the required data in one series of experiments. The control compendial contact plates (Heipha LTHTh-ICR and BD TSA LP80) were used with manual analyst readings and incubations (CMM). For the Growth Direct method two data sets were obtained, one using a manual enumeration method (GDC) and the other using the system (GDS) as an automated method to perform the colony enumeration. To minimize any variability in the manual enumeration method three analysts read both the control samples, CMM, and the Growth Direct cassettes, GDC, after the plates completed the automated count, GDS. The system only generated one result per cassette.

The representative test microorganisms, see Table 2 below, were prepared to cover the following range 0.5CFU, 1CFU, 10CFU, 100CFU, 300CFU, 1000CFU in 50µl of diluent, Fluid A. The 0.5CFU dilution was created to extend the dilution range to increase the probability of obtaining 1CFU. With 10 replicates the expectation is 5 tests with 1 CFU and 5 tests with 0 CFU, average 0.5CFU. The dilutions were created from two stock solutions by taking aliquots of different volumes. Prepare ten replicates of each dilution on each media type by spread plating 50µl of test sample to the plate surface. Negative controls were performed for each run. Growth Direct cassettes had the vision lids attached and loaded to the Growth Direct system. Standard contact plates were capped and loaded to standard incubators. Upon completion of each test on the Growth Direct System the cassettes were collected for additional human enumeration of CFU. The manual read was performed by three independent analysts at the same time.

During execution with diluted cultures the experimental conditions were kept the same to minimize the possibility of cell numbers changing due to cell multiplication or death between test methods. The dilution series was divided between 3 operators for each series to minimize preparation time from start to finish and minimize any cell number changes.

Table 2. Microorganisms used to perform the study

Test Microorganism	ATCC Number	Incubation Temperature	Incubation Duration
Bacillus subtilis	6633	30 to 35°C	≥48 hours
Staphylococcus aureus	6538	30 to 35°C	≥48 hours
Escherichia coli	8739	30 to 35°C	≥48 hours
Pseudomonas aeruginosa	9027	30 to 35°C	≥48 hours
Candida albicans	10231	30 to 35°C	≥48 hours
Aspergillus brasiliensis	16404	30 to 35°C	≥48 hours
Mixed orgs -*S. aureus* and *C. albicans*	6538 & 10231	30 to 35°C	≥48 hours
EM Organism TSA LP80HT *B. cepacia*		30 to 35°C	≥48 hours
EM Organism TSA LP80 *S. epidermidis*		30 to 35°C	≥48 hours
Stressed – Bleach stressed *B. subtilis* spores		30 to 35°C	≥48 hours

For the intermediate precision study both GDC and GDS were evaluated with S. aureus at 2 levels and both media (TSA LP80HT, and TSA LP80). Three analysts measured two spiked levels (50 CFU and 150 CFU) on 5 different days with 10 replicates at each test point. Media lots (n=3) and Growth Direct system (n=3) were also varied across days.

Methods: Statistical Approach[1,3]

The analysis for the accuracy data is conducted for each combination of type of organism and medium. We limited the analysis to the first four spike levels, since only the GD system readings were collected above 100 CFU as the plates were TNTC for the human analysts to count.

For notational purposes we assume that observation Y_{hijk} is the count for the k^{th} test sample measured with method h ($h = 1$: CMM; $h = 2$: GDC; $h = 3$: GDS), at spike level i ($i = 1$: $\lambda_1 = 0.5$ CFU; $i = 2$: $\lambda_2 = 1.0$ CFU; $i = 3$: $\lambda_3 = 10$ CFU; $i = 4$: $\lambda_4 = 100$ CFU) for analyst or reader j ($j = 1$: analyst 1; $j = 2$: analyst 2; $j = 3$: analyst 3). For the GD system there are no analysts ($j = 1$). We have denoted the spike level by λ_i and we assume that it is independent of method, analyst and sample. The count data Y_{hijk} were analyzed using Poisson regression. We assume that the expected or mean count is given by

$$\mathbb{E}(Y_{hijk}) = p_h \lambda_i^{\eta_h} = \exp\{\log p_h + \eta_h \log \lambda_i\}, \quad (1)$$

With log the natural logarithm, p_h the "detection proportion" and η_h the "linearity coefficient" of method h. Enumeration of microbiological method h is perfect when the following three conditions are satisfied: the level of spike λ_i is exact, $p_h = 1$, and $\eta_h = 1$.

The analysis of the precision study assumes a mixed effects Poisson regression model for the observed counts. The combination of analyst, day, and medium lot were taken together as one factor called 'analytical run', since this combined factor may affect precision in routine testing. Analysis is conducted per spike level and medium. For each combination of spike level and medium, the observation Y_{hij} is the count for the j^{th} test sample measured with method h ($h = 1$: GDC; $h = 2$: GDS) at analytical run i ($i = 1, 2, \ldots, 15$). Given the effect Z_{hi} of analytical run i for method h, it is assumed that the distribution of the count data Y_{hij} is Poisson distributed with expected or mean count given by

$$\mathbb{E}(Y_{hijk}) = \exp\{\alpha_h + Z_{ih}\} \qquad (2)$$

The effect Z_{ih} of analytical run i for method h is considered random having a normal distribution with mean zero and variance σ_h^2. It quantifies the variability in enumeration that is additional to the Poisson variability from test samples, i.e. run-to-run variability. The Wald test statistic will be used to test the null hypothesis $H_0: \alpha_1 = \alpha_2$ (equality of mean counts) and the likelihood ratio test will be used to test the null hypothesis $H_0: \sigma_1 = \sigma_2$ (equality of run-to-run variability).

An explorative comparison of precision of the GD RMM with the compendial method is performed with the accuracy study. Here the counts are transformed to $U_{hijk} = [Y_{hijk} + 3/8]^{1/2}$, since the transformed counts are more normally distributed that the original counts with a variance equal to approximately 0.25 when Y_{hijk} is Poisson distributed having an expected count that is large enough (Anscombe, 1946). We will calculate the variance of the transformed data over the 10 test samples and 3 analysts per method h and spike level i for each media and organism (excluding the stressed organism). For each media, the variances are then pooled over organisms for each method and spike level and accompanied with a 95% confidence interval. The individual variances are plotted in a boxplot.

Methods: Quantifying Validation Parameters

Based on the mathematical form of the expected counts, the microbiological method is linear in the log scale, i.e. the logarithm of the expected count is linear in the logarithm of the spike. To investigate the linearity, we will use the likelihood ratio test to compare the expected counts in (1) with expected counts of the form $\mathbb{E}(Y_{hijk}) = \mu_{hi}$ (the saturated Poisson regression model). We will evaluate this goodness-of-fit on linearity for all three microbiological methods, to determine whether linearity or non-linearity is consistent across the three methods (likelihood ratio test). The expected counts in (1) will also be compared with the observed counts using the R^2-value to quantify goodness-of-fit and evaluate more practically possible violations of non-linearity in the log scale. An R^2 larger than 90% indicates a good prediction of the log linear model (even if the likelihood ratio test indicate a lack-of-fit). We will also investigate the linearity coefficient, i.e. test with the Wald test statistic null hypothesis $H_0: \eta_h = 1$ for method h. If this null hypothesis is not rejected, the expected counts is proportional to the spike level when the goodness-of-fit is not violated. We will also visualize the estimated expected counts in (1) with respect to the spiked levels.

Based on the statistical model in (1), the accuracy of the RMM is considered non-inferior with respect to the CMM whenever the expected count in (1) for RMM ($h = 2, h = 3$) is at least 70% of the expected counts for CMM ($h = 1$) with 95% confidence. We will determine the range of spiked levels λ that would satisfy this definition of non-inferiority on the accuracy. The expected counts in (1) for the three methods will be visualized in one graph simultaneously in its original scale, for each organism and medium separately.

The limit of quantitation for method h is determined by the lowest level of spike λ for which an observed count is unlikely (say 1%) to be equal to zero. For the Poisson regression analysis with expected counts in (1), the limit of quantitation is given by

$$QL_h = \exp\{[\log(-\log(1-q)) - \log p_h]/\eta_h\},$$

with q the level of confidence (0.99 IOR 99%). This limit will be estimated for all three methods rounded upwards to the nearest integer.

Precision of the GD RMM is quantified by relative standard deviations (expressed in percentages). The repeatability is determined by the variability between test samples when all conditions can be seen constant and the intermediate precision is the variability in test samples under controlled by variable conditions (analyst, day, and media lot). Due to the assumption of the mixed effects Poisson model, repeatability and intermediate precision are quantified as

$$R_h(\%) = 100\%\exp\{-\alpha_h/2\}.$$

$$IP_h(\%) = 100\%\left[\exp\{-\alpha_h - \sigma_h^2/2\} + \exp\{\sigma_h^2\} - 1\right]^{1/2}$$

RESULTS

Results on Accuracy, Linearity, Range, LOD, and LOQ were all determined from the accuracy experiment, while Repeatability and Intermediate Precision were determined from the precision experiment.

Linearity

The expected counts of statistical model (1) at different concentrations are visualized for the different organisms for TSA LP80 in Figure 1.

Tables 3a (TSA LP80) and 3b (TSA LP80HT) contain the results on the linearity and goodness-of-fit of the Poisson regression analysis with the log linear model (i.e. a linear relationship between the log expected count and the log spike). The column P-LRT indicate if the log-linear model fits properly to the data and indicate if the linearity condition in the log scale is appropriate. The column P-LC provides the p-value on proportionality, indicating if the expected counts are linear with spike level (i.e. $\eta_h = 1$, with h referring to CMM, GDC, and GDS). The R^2 (observed in percentage) indicates how well the log linear model predicts the observed counts.

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Table 3a: The goodness-of-fit (linearity) of log linear model per organism for TSA LP80

Organism	GDC			GDS			CMM		
	P-LRT	P-LC	R^2	P-LRT	P-LC	R^2	P-LRT	P-LC	R^2
A. brasiliensis	0.448	0.314	96.7	0.825	0.318	98.4	0.109	<0.001	97.6
B. subtilis	0.864	<0.001	98.3	0.709	0.291	98.5	0.087	0.002	98.0
C. albicans	0.003	0.086	98.0	0.151	0.202	97.7	0.915	0.015	98.6
E. coli	<0.001	<0.001	98.2	0.064	<0.001	98.8	<0.001	<0.001	98.2
P. aeruginosa	0.011	<0.001	98.3	0.264	0.013	98.3	0.074	0.031	88.3
S. aureus	0.168	0.368	94.9	0.597	0.328	95.2	0.025	0.054	97.8
S. epidermidis	0.041	0.290	98.3	0.345	0.399	97.9	0.004	0.275	97.5
mixed	0.001	0.036	98.7	0.112	0.169	98.7	0.637	0.165	99.5
stressed *B. subtilis*	0.235	<0.001	92.7	0.980	<0.001	97.9	<0.001	0.004	98.6

Table 3b: The goodness-of-fit (linearity) of log linear model per organism for TSA LP380HT

Organism	GDC			GDS			CMM		
	P-LRT	P-LC	R^2	P-LRT	P-LC	R^2	P-LRT	P-LC	R^2
A. brasiliensis	0.108	0.033	96.9	0.356	<0.001	97.7	0.077	<0.001	58.8
B. subtilis	0.085	0.229	96.6	0.366	0.425	96.1	0.073	<0.001	97.9
C. albicans	0.015	0.007	98.5	0.291	0.219	98.4	<0.001	0.018	98.8
E. coli	<0.001	<0.001	97.7	0.073	<0.001	97.6	0.321	<0.001	97.8
P. aeruginosa	0.101	<0.001	99.3	0.403	0.027	99.3	0.001	<0.001	89.6
S. aureus	0.359	0.287	98.1	0.687	0.423	98.5	<0.001	0.019	96.8
B. cepacia	0.150	<0.001	98.1	0.138	<0.001	97.0	0.113	<0.001	98.2
mixed	0.338	0.364	97.6	0.827	0.369	97.8	0.086	0.277	98.0

Linearity (in the log scale) is never rejected for the Growth Direct GDS at significance level of 0.05. For the CMM and the GDC series, linearity in the log scale was violated several times at significance level of 0.05 (CMM: 12 times; GDC: 13 times; Both CMM & GDC: 6 times). The linearity coefficient is often different from one, indicating that the expected counts are not proportional to the spike level (CMM: 18 times; GDC: 17 times; GDS: 12 times). However, all of the R^2-values for the GD RMM (GDC≥ 92.7%; GDS ≥ 95.2%) show that the log linear model is a (very) good approximation for describing the counts. For many conditions, the R^2-values for the compendial method are at the same level as the GD RMM, but not for testing the conditions: A.brasiliensis TSA LP80 (58.8%) and P. aeruginosa with medium TSA LP80 (88.3%) and TSA LP380HT (89.6%).

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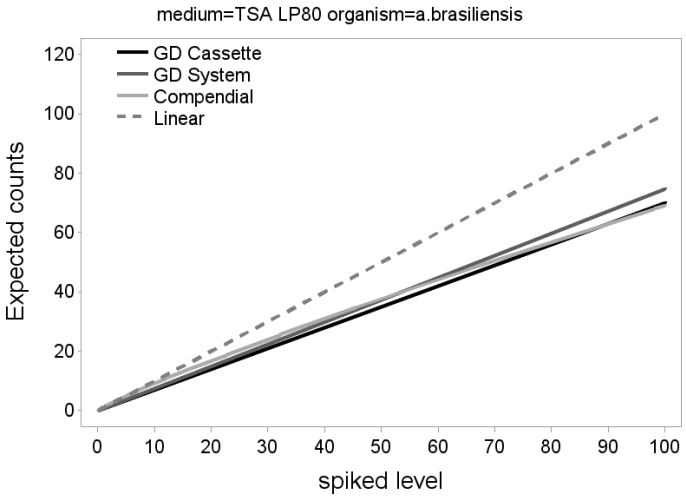

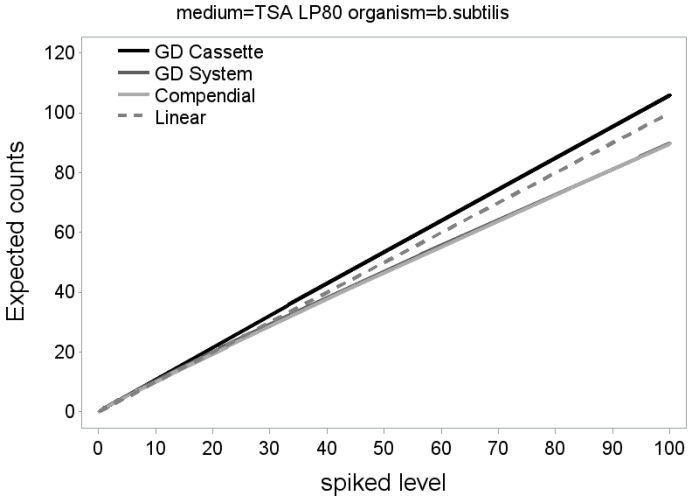

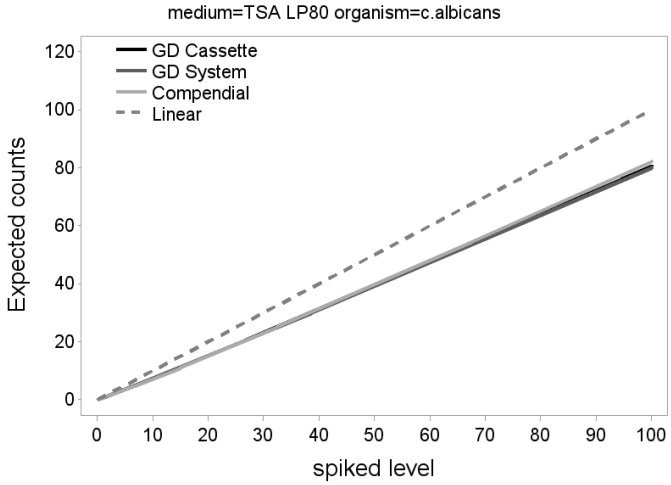

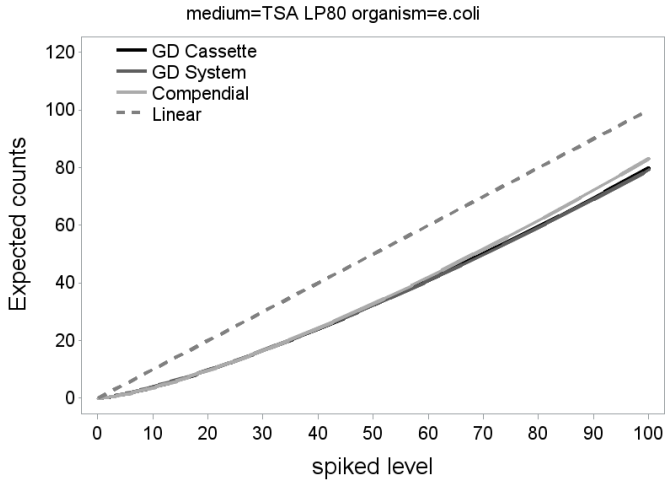

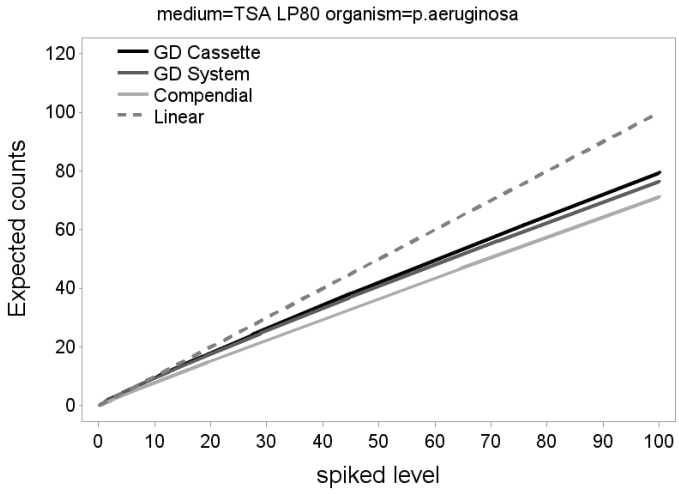

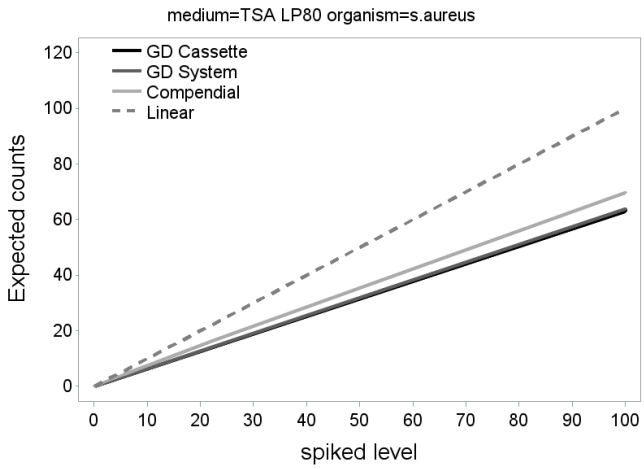

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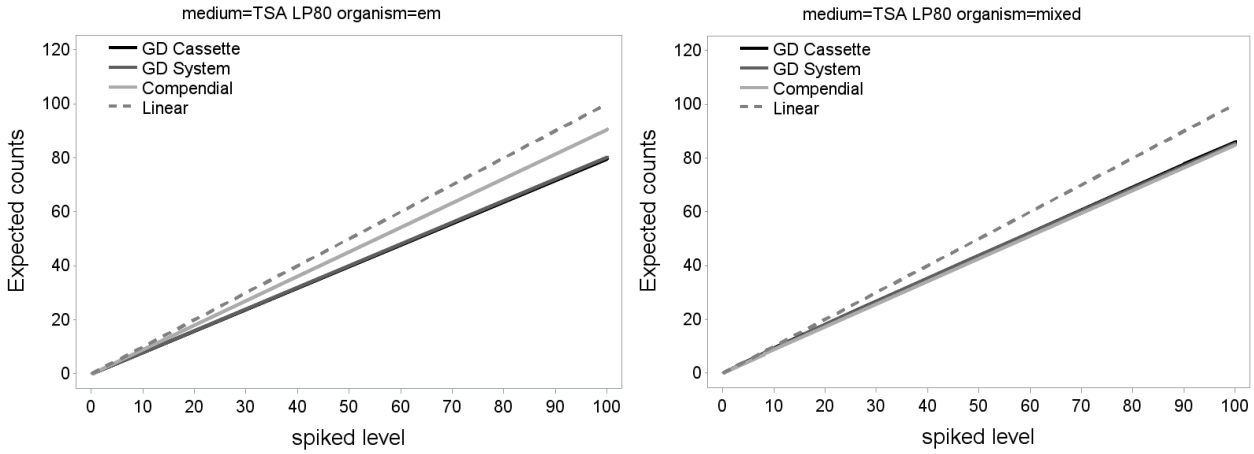

Figure 1: Visualization of expected counts at different spike levels for several organisms on the TSA LP80

Non-inferiority for accuracy

Table 4 reports results on non-inferiority for accuracy. It contains the range of spike levels for which the recovery of the GDC and GDS with respect to CMM is at least 70% with 95% confidence. It also shows that in most conditions a recovery of 70% or more is obtained at very low spike levels but guaranteed for all conditions at spike levels of at least 20 CFUs.

Table 4: Minimal spike levels for which a recovery of at least 70% can be demonstrated.

Organism	TSA LP380HT		TSA LP80	
	GDC	GDS	GDC	GDS
A. brasiliensis	1.7	1.8	10.2	13.1
B. subtilis	1.6	2.8	0.1	0.5
C. albicans	0.1	1.1	0.1	0.4
E. coli	13.6	19.2	0.1	3.1
P. aeruginosa	0.8	1.4	0.1	0.1
S. aureus	0.1	0.1	6.9	16.3
S. epidermidis	4.1	1.6	4.3	8.9
mixed	0.1	0.1	0.1	0.1
stressed *B. subtilis*	NA	NA	7.4	8.0

Limit of detection and quantitation

Table 5 reports the limit of detection and limit of quantitation. They are the lowest estimated level of CFU for which it is unlikely (with 5% for LOD and 1% for LOQ) to observe zero counts when the test sample contains this level of organisms on average. The results show that the limits of detection and quantitation are very similar for the three microbiological methods. The GDC and GDS have identical quantitation limits for all conditions, but the LOD is in some cases lower for GDC compared to GDS. The CMM has a higher estimated limit of quantitation than the GD RMM in 5 of the 16 conditions, a lower estimated limit of quantitation in 5 of 16 conditions, and an equal limit of quantitation in 6 of 16 conditions. When the CMM has a lower limit of quantitation, the difference with GD RMM is rarely larger than one. Similar results are observed for the LOD.

Table 5: LoQ (LoD) for the two methods with different organisms and EM media.

Organisms	TSA LP380HT			TSA LP80		
	CMM	GDC	GDS	CMM	GDC	GDS
A. brasiliensis	6 (4)	6 (4)	6 (4)	5 (3)	7 (5)	7 (5)
B. subtilis	5 (3)	5 (4)	5 (4)	5 (3)	5 (3)	5 (3)
C. albicans	6 (4)	6 (4)	6 (4)	7 (5)	7 (5)	7 (5)
E. coli	10 (8)	12 (9)	12 (9)	12 (9)	12 (8)	12 (9)
P. aeruginosa	5 (3)	4 (3)	4 (3)	6 (4)	5 (3)	5 (3)
S. aureus	7 (5)	6 (4)	6 (4)	6 (4)	8 (5)	8 (6)
S. epidermidis	4 (3)	5 (3)	5 (3)	5 (4)	6 (4)	6 (4)
mixed	7 (5)	6 (4)	6 (4)	6 (4)	5 (4)	5 (4)
stressed *B. subtilis*	NA	NA	NA	3 (2)	4 (3)	4 (3)

Precision & Ruggedness

Table 6 reports the P-values for the two hypothesis testing on equality of the two GD RMM methods. The null hypothesis on the expected counts is never rejected.

Table 6: P-values for hypothesis testing on equality of mean counts and run-to-run variability

Parameter	TSA LP380HT		TSA LP80	
	50 CFU	150 CFU	50 CFU	150 CFU
Expected counts	0.836	0.394	0.599	0.466
Run-to-run variability	0.826	0.900	0.824	0.535

Table 7 reports the measures of precision for the different conditions. The measures of precision across GD RMMs are very similar. The intermediate precision is less than twice the repeatability, indicating a limited influence of run-to-run variability. Note that relative standard deviations below 30% is typically considered appropriate for microbiological methods and in-vivo bioassays (due to the expected Poisson variation).

Table 7: Measures of precision in relative standard deviations (expressed in percentages)

Media	Repeatability				Intermediate Precision			
	50 CFU		150 CFU		50 CFU		150 CFU	
	GDC	GDS	GDC	GDS	GDC	GDS	GDC	GDS
TSA LP80HT	16.0	16.0	9.5	9.5	20.4	20.1	16.6	16.6
TSA LP80	15.9	16.0	9.6	9.6	21.0	20.7	17.3	16.8

Comparison of the GDS against the CMC on precision was obtained from the accuracy study and the variances of the transformed counts are visualized in Figure 2 and the pooled variances over organisms are reported in Table 8.

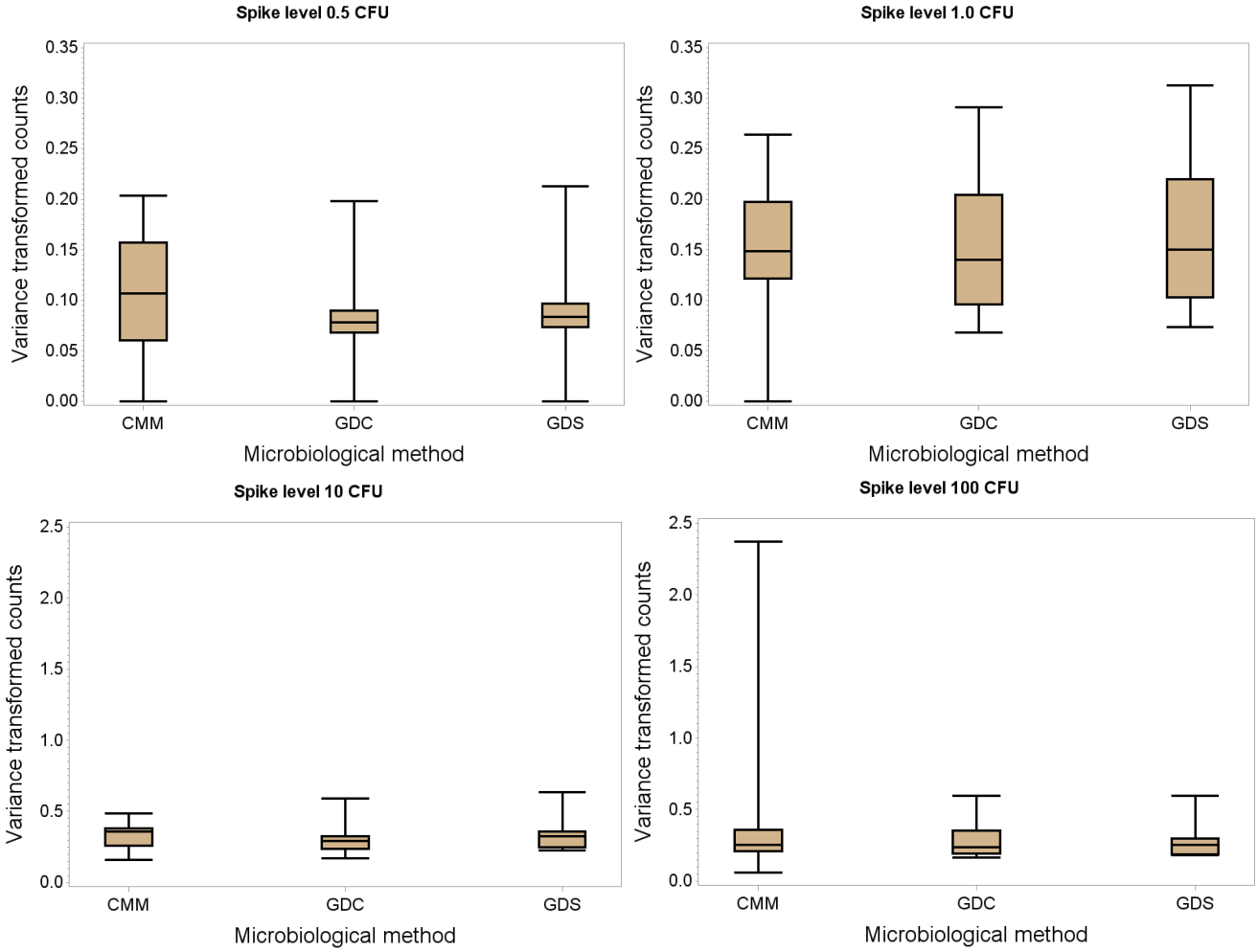

Figure 2: Visualization of the variances for transformed counts at the four spike levels for TSA LP80

It shows that the GD RMM has similar or better results than the compendial, demonstrating appropriate precision. The pooled estimate for the compendial at spike level 100 for TSA LP380HT is large due to an outlier variance at the organism *A. brasiliensis*.

Table 8: Pooled variance over organisms of the transformed counts

Spike level	TSA LP380HT			TSA LP80		
	CMM	GDC	GDS	CMM	GDC	GDS
0.5 CFU	0.12 [0.10; 0.14]	0.09 [0.07; 0.11]	0.14 [0.10; 0.19]	0.11 [0.09; 0.13]	0.08 [0.07; 0.10]	0.09 [0.07; 0.13]
1.0 CFU	0.17 [0.15; 0.21]	0.16 [0.14; 0.19]	0.18 [0.14; 0.26]	0.15 [0.13; 0.18]	0.15 [0.13; 0.19]	0.17 [0.12; 0.23]
10 CFU	0.25 [0.21; 0.30]	0.22 [0.18; 0.26]	0.25 [0.18; 0.34]	0.33 [0.28; 0.40]	0.31 [0.26; 0.37]	0.34 [0.26; 0.46]
100 CFU	2.02 [1.71; 2.44]	0.29 [0.25; 0.35]	0.32 [0.24; 0.45]	0.51 [0.43; 0.61]	0.29 [0.25; 0.35]	0.28 [0.21; 0.39]

Results for Ruggedness are also shown in Table 7 in that the intermediate precision data was collected using 3 lots of reagent, 3 analysts and 3 growth direct systems. Results show that the precision obtained was well within expected values showing no impact of any variable on the final result.

CONCLUSION

The data presented in this paper supports the Primary Validation of the Growth Direct system's EM media, TSA LP80 and TSA LP80HT, for the quantitative detection of micro-organisms relevant to the pharmaceutical industry. The experimental design and analysis were performed according to the requirements of the EP 5.1.6 and the USP Ch <1223>. The technology has passed all the key parameters to validate a Rapid Micro Method for use in the pharmaceutical QC testing arena. It also demonstrates equivalent or better results than the compendial.

Within each of the pharmaceutical Alternative Microbiological Methods chapters there is a reference to the performance of a risk assessment to determine the validation required for the implementation of a new method. Both chapters allow for a reduced validation/verification with adequate justification, see EP 5.1.6 Section 3-2-3 Primary Validation, "Depending on the type of alternative method, relevant validation criteria shall be selected from the list below:" In the USP the Growth Direct technology is classed as an automated compendial method, requiring only a verification of the counting method followed by a method suitability study. It should be noted that in the USP the Growth Direct technology is classed as an automated compendial method requiring only a verification of the counting method followed by a method suitability study.

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