Primary Validation for an Automated Colony Counter per EP 9.2, 5.1.6

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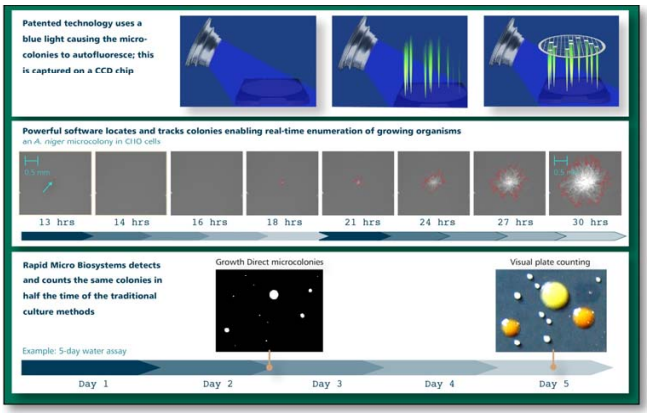
Introduction

The European Pharmacoepia (EP) 9.2 Chapter 5.1.6 on alternative methods was released in Jan 2017. This includes a clear structure for primary validation and validation of intended use together with directives for the validation process for both the technology supplier and the end user. Additionally, example method validations have been moved to the EDQM Website, which will be kept updated with new validated alternative methods, concise methods, and given acceptance specifications. The information described here shows the data required for the Primary Validation document to be supplied by the vendor.

Technology

The Growth Direct™ System is an automated rapid microbial enumeration platform suitable for in process product testing, environmental, and water monitoring that integrates digital imaging, robotic cassette handling, incubation, and software control. Samples are prepared and loaded into the incubators. Cassettes are removed from the incubator every four hours and illuminated by blue light. The green auto fluorescence from the microorganism is then captured by a camera to build up an image time series that differentiates growing micro-colonies from debris. Post imaging, the cassettes are automatically returned to the incubator by the robotic system.

The membranes employed in the system are 0.45 micron, mixed cellulose ester as used for compendial testing. The membranes are stained black to quench the auto fluorescence of the cellulose esters and the underlying media that may inhibit the auto fluorescence of the captured microorganisms. The membranes are placed on standard compendial microbiological growth media and the media cassettes are incubated at the recommended temperatures for times customized to the local facility flora.



Methodology

The organism panel used for the validation consisted of the standard EP species with a media- specific EM isolate, stressed or slow growing organism, and a mixed organism sample. Ten replicates of 6 concentrations from 0.5 to 750CFU for each organism were analyzed using the Rapid Micro Biosystems consumables and control commercial media for each media type. Organisms were spread plated for the EM media and added to Fluid A for filtration on the bioburden filtration methods. The 4 media used were R2A, TSA with Millipore Milliflex control, TSA LP80 with BD RODAC, and TSA LP80HT with Millipore (Heipha) contact plates. Incubations were performed either on the Growth Direct system or in traditional incubators for 48 to 120 hours at 30 to 35°C, depending on media and organism. Colonies were enumerated by 3 analysts to minimize colony count variation on control and test plates.

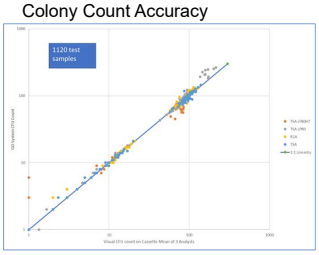
Parameter	Acceptance Criteria
Accuracy	System Count Accuracy: Growth Direct System count will be >90% of the visual count of the same cassette 90% of the time. (Fowler, J. L., W.S. Clark, J. F. Foster and A. Hopkins 1978. Standard Methods for the Examination of Dairy products. J. Food. Prot. 41(1): 4-7) Colony Counter specific. Accuracy of GD vs competitor method Alternative method should recover at least as many organisms as the Pharmacopeial method using appropriate statistical methods e.g. the TOST (two one sided T-test).
Repeatability	The Growth Direct variance shall not be significantly different to the compendial test
Limit of Detection	Equivalence to the compendial test at 1CFU. Use Accuracy data
Limit of Quantification	Alternative method must not be greater than Pharmacopeial method.
Linearity	Slope is significant and deviation from linearity is not significant.
Range	Determined by Accuracy, precision and linearity tests.
Specificity	Method detects colony growth of all relevant organisms, no false positives

Results

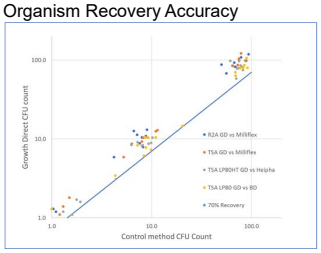
The following shows the results for the various tests required for the Primary Validation.

Accuracy

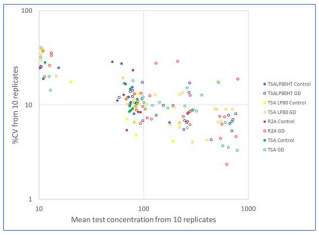
Colony Count Accuracy



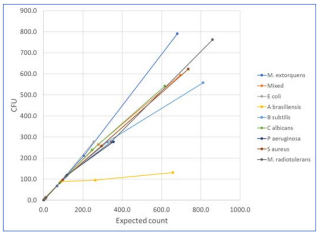
Organism Recovery Accuracy



Precision Profile



Linearity and Range for R2A



Linearity and range 0.5 to 300CFU

	R2A		TSA		TSA LP80HT		TSA LP80	
Organism	Correlation	Slope	Correlation	Slope	Correlation	Slope	Correlation	Slope
mixed *S. aureus* & *C. albicans*	1.000	0.951	0.997	0.825	0.999	1.111	0.998	0.860
E coli	1.000	1.091	0.998	1.261	0.998	0.865	0.997	0.825
A brasiliensis (to 100cfu)	0.999	0.986	0.999	0.984	0.999	0.985	1.000	0.998
B subtilis	0.998	0.854	0.998	0.836	1.000	1.019	1.000	0.921
C albicans	1.000	1.154	0.999	1.169	1.000	1.070	1.000	0.975
P aeruginosa	0.996	0.781	1.000	0.985	1.000	0.935	1.000	0.921
S aureus	0.999	0.875	1.000	0.979	1.000	0.962	1.000	0.946
M. extorquens	1.000	1.031						
M. radiotolerans	0.997	0.806						
S. epidermidis					1.000	1.018	1.000	0.922
Stressed *B subtilis*					1.000	0.948	0.991	0.732

Limit of Detection

Determined by analyzing 10 replicates spiked at 0.5 or 1CFU for test and control media

		TSA LP80HT		TSA LP80		R2A		TSA	
Organism	Expected CFU	Heipha Control CFU	Growth Direct CFU	BD Control CFU	Growth Direct CFU	Milliflex Control CFU	Growth Direct CFU	Milliflex Control CFU	Growth Direct CFU
mixed *S. aureus*	0.5	0.5	0.4	0.5	0.5	0.3	0.3	0.7	0.7
& *C. albicans*	1.0	0.8	0.7	0.8	0.5	0.2	0.7	1.1	0.4
	0.5	0.1	0.1	0.0	0.0	0.3	0.2	0.1	0.4
E coli	1.0	0.4	0.6	0.0	0.0	0.3	0.3	0.2	0.4
	0.5	0.4	0.3	0.7	0.3	0.7	0.7	0.8	0.6
A brasiliensis	1.0	1.3	1.2	0.9	0.9	1.0	1.3	1.5	1.8
	0.5	0.9	0.5	0.4	0.7	0.6	0.3	0.2	0.3
B subtilis	1.0	0.9	1.3	1.0	1.3	1.0	1.3	0.5	1.0
	0.5	0.7	0.2	0.3	0.3	0.2	0.5	0.2	0.5
C albicans	1.0	1.6	1.1	0.7	0.3	1.1	1.2	0.9	0.4
	0.5	0.4	0.7	0.3	0.3	0.5	1.0	0.5	0.7
P aeruginosa	1.0	1.8	1.7	0.7	1.1	0.8	1.7	1.3	1.4
	0.5	0.2	0.4	0.2	0.4	0.5	0.3	0.5	0.4
S aureus	1.0	0.2	1.1	0.7	0.4	0.7	1.1	1.2	1.1
	0.5	0.8	1.1	0.9	0.6				
S. epidermidis	1.0	1.9	1.6	0.8	0.5				
	0.5					N/A	0.2		
M. radiotolerans	1.0					N/A	1.1		
	0.5					0.7	0.3		
M. extorquens	1.0					0.7	0.7		
stressed *B subtilis*	0.5			0.1	0.4				
	1.0			0.9	0.9				

Summary

The data generated for the four media formulations demonstrate that, when analyzed as defined by the Pharmacopeia, the colony counting method of the Growth Direct complies with the assay performance requirements defined in the EP Ch 5.1.6. Equivalence was demonstrated with the compendial method using media from two alternative suppliers Millipore and BD for both bioburden and EM testing formats.

From the Primary Validation data, the user is required to verify key parameters as part of their validation. It is important to note that not all tests are required to be repeated by the user depending on the application.

For Harmonization to the USP40/NF35 Ch <1223> states:

"There are commercially-available enhancements to growth-based methods that allow colonies on solid media to be read more quickly, with substantially less incubation time, than is possible using only the unaided eye. In the implementation of these enhanced methods for the detection of colony growth, only the detection capability of the method requires verification."

Growth Direct is classified as an automated colony counter with a compendial growth-based assay, therefore a smaller subset of tests can be used, specifically Accuracy and Precision of the enumeration method. The comprehensive Primary Validation by the supplier in addition to the user's accuracy and precision tests and subsequent method suitability validation facilitates a comprehensive validation with a reduced time line.