GXP

Validation of the Growth Direct System to Perform Pharmaceutical In-process Bioburden Analysis

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Abstract

The validation of the Growth Direct™ system is described for the automated incubation and enumeration of microbial colonies derived from in-process bioburden testing at a biologic facility using TSA media plates. The validation strategy conforms to the recently updated USP Ch <1223>1 and sample data are given to demonstrate that the technology is accurate at enumerating microorganisms for in-process testing.

Introduction

Pharmaceutical in-process microbiological testing can be a significant percentage of the workload in the QC department. Each sample test is divided into 2 phases: first sampling/setting up the test and secondly reading and recording the test result(s). The second phase involves a trained analyst performing the very repetitive task of data collection and collation for which they may be overqualified. A reduction in the time to results, and the analyst involvement on the sample analysis would be a benefit to the micro QC department. Both of which can be obtained by the introduction of automation to this process. Linking automation to a 2-way LIMS system to control sample work-lists and result upload removes time-consuming, laborious tasks from the analyst.

The use of automated plate readers should not be considered an alternative microbiological test method and subject to full method validation but merely the automation of the incubation and reading of a compendial microbiological method, Jones and Cundell2. This view is supported by recent updates to both USP40/NF35 General Informational Chapter <1223> Validation of New Microbiological Testing Methods and industry practice as found in the 2013 PDA Technical Report 33 (Revised)3 Evaluation, Validation and Implementation of Alternative and Rapid Microbial Methods. Both these documents support a more limited verification of the automated bioburden methods.

The paper describes the validation of a Rapid Micro Method (RMM) system as an automated colony counter applied to Pharmaceutical in-process bioburden testing.

Growth Direct Technology

The Growth Direct system uses a proven technology that is based on the same principles and procedures as defined in the European and United States Pharmacopoeia Monographs for in-process testing using TSA or SDA at 30-35°C or 20-25°C respectively. The system automates the compendial incubation and visual colony counting method for in-process testing by replacing detection by the human eye with sensitive digital imaging. The technology uses the same media and sampling methods as the current testing methods and delivers faster results by detecting microscopic colonies well before they become visible to the naked eye. This is achieved by imaging the intrinsic cellular auto-fluorescence of the bacterial colony. The growth of microscopic colonies is detected by the Growth Direct system and the increase in their inherent auto-fluorescence tracked over time. The technology is based on the fact that all microbial cells emit yellow-green fluorescence when illuminated by blue light at 460-490nm due to the presence of fluorescent biomolecules inclusive of (ribo)flavins and flavoproteins. Like ATP or DNA, these molecules are ubiquitous in living systems in all the kingdoms of life including microorganisms. This permits the detection of the same broad range of species as are detected by the visual colony counting method.

Growing micro-colonies exhibit increasing auto-fluorescence and size over time which the system detects and records. The system discriminates growing microbial colonies from inanimate fluorescent debris as these objects do not change in signal intensity or size.

The imaging method does not harm the cells, and as such is a non-destructive method, thus the micro-colonies can grow into visible colonies for use in subsequent microbial identification.

Validation Rationale

USP40/NF35 General Notices 6 Testing Practices and Procedures provides guidance of the use of automated and alternative test methods.

6.20 Automated Procedures states “Automated and manual procedures employing the same basic chemistry are considered equivalent.” The statement is equally true for procedures employing the same basic microbiology such as a plate count and the Growth Direct™ System. Furthermore,

6.30 Alternative and Harmonized Methods and Procedures states “Alternative methods and/or procedures may be used if they have advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other specialized circumstances. Such alternative procedures and methods shall be validated as described in the USP40/NF35 general chapter Validation of Compendial Procedures <1225> and must be shown to give equivalent or better results.” However, the authors understand that the General Notices are being updated to be restated in terms of microbial methods as they are validated as described in <1223> Validation of Alternative Microbiological Methods not USP <1225>.

USP40/NF35 <1223> also states the following "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." This statement supports the view that the Growth Direct™ System is not an alternative method requiring method validation.

Similarly, the PDA Technical Report No. 33 (Revised) dated September 2013, states the following: “Some alternative or rapid technologies may be considered automated traditional or compendial microbiological methods, especially when the results are in colony-forming units (CFU). These technologies may be qualified for their intended use without the need for demonstrating certain method validation requirements as specified in Section 5.0 of the Technical Report. For these technologies, at least accuracy and precision assessments should be performed, in addition to method suitability and equivalence/comparability studies.” The view expressed in USP <1223> was fully supported in this industry practice document.

All analytical equipment, including that used for microbiological testing methods, is subject to industry-standard instrument qualification requirements. The revised chapter <1223> cites USP40/NF35 <1058> Analytical instrument qualification for general guidance in this area. This latter chapter includes the development of user requirement specifications and the well-known elements of equipment method qualification, installation qualification, operational qualification and performance qualification. The authors support the standard installation qualification (IQ), and operational qualification (OQ) approaches to the Growth Direct™ System but as outlined in the next section, not the standard performance qualification (PQ). A simple method verification approach would be substituted for the standard performance qualification approach.

The validation steps of the GD system can be divided into the following sections.

1. The Installation/Operational Qualification (IOQ) phase concentrates on the validation of the system’s hardware and software components to confirm that they are all functioning according to the design specification. This

includes the calibration and temperature mapping of the incubators.

2. The Performance Qualification (PQ) is subsequently carried out to validate the microbiological method in accordance with those requirements of USP 1223 that apply to the Growth Direct technology.
3. The Time to Result Qualification (TTRQ) phase consists of the determination of the timepoint at which the system result is shown to give the maximal colony detection count. For this work, the natural flora from the facility should to be tested as well as the USP/Ph Europa organisms. Stressed organisms should be run in this phase as the stressor usually results in a cell with an extended lag phase. The stressed cells may either be those that exist in the natural product or cells that have been exposed to a stressor that is seen in the process e.g. pH or dehydration. Some of these may have a long lag phase and slow doubling time that could lead to false negatives if the TTR was set from the speed of the pharmacopoeia organism growth rates.
4. The final phase of method suitability for the product evaluates interference, either to the growth of the organisms or interference of the product on the detection system of the Growth Direct technology. Sections 2, 3 and 4 can be merged into the same experimental design if required.

This paper describes the approach used and data generated during the performance of a bioburden test validation.

Installation Operation Qualification (IOQ)

The component testing performed during the IOQ are shown in Table 1. Much of the testing focuses on the performance of the software to verify the correct steps and sequences. All menu interactions, incubation temperature and timings, report and error outputs are verified in this phase. The key hardware test consists of temperature mapping of the two incubators. The mapping was performed with 10 Ellelab probes placed throughout the incubator with both a full and empty load pattern of test cassettes. The temperature profiles for the empty incubator at 22.5 and 32.5°C are shown in Table 2. The temperature range seen for both incubators over 24 hours at all test points was <±1.0°C. The same range was seen when testing the full load pattern.

Table 1 IOQ content

Table 2. Temperature mapping profiles for the two incubators at their specified set points. Data from all probes placed in each incubator

	13-14560: set @ 22.5°C			**13-14413: set @32.5°C**		
	Mean Temp (°C)	Min Temp. (°C)	Max Temp. (°C)	Mean Temp. (°C)	Min Temp. (°C)	Max Temp(°C)
Incubator Empty	20.7	20.1	22.6	32.5	32.0	33.1
Incubator Full	20.8	20.1	21.6	32.3	31.9	33.3

Performance Qualification (PQ)

The PQ phase of the validation utilizes microorganisms for the qualification of growth promotion and system accuracy for colony detection. The organisms were presented in a neutral buffer such as Fluid A, buffered saline, or Peptone broth. The USP/EP suite of organisms was used in conjunction with organisms derived from EM or Bioburden testing. (Table 3).

The accuracy of the CFU count was determined by filtering 5 replicates of each of the organisms through the Bioburden Filtration kit, transferring the membrane filter onto the TSA Growth cassettes and incubate the cassettes on the system for 36 hours at 30-35°C to generate a system CFU count. The cassettes were then removed and the colonies on the membrane surface counted by the analyst. Due to analyst to analyst variation for enumeration of colonies, the cassette colony count was performed by three analysts for the PQ. The mean value obtained was then compared to the system count for each cassette. The results of the system count and the mean of the analyst count were then directly compared.

System Count Accuracy

The results for the enumeration accuracy performed during PQ for USP and environmental organisms are shown in Fig 1. A very good correlation was seen between the manual and system count for the test organisms. The data verifies the enumeration accuracy of the Growth Direct for organisms relevant to the site micro flora testing.

Table 3 Test organisms for use in the PQ

Test Micro-organism	ATCC Number
USP Organisms	
Bacillus subtilis	6633
Staphylococcus aureus	6538
Pseudomonas aeruginosa	9027
Candida albicans	10231
Aspergillus brasiliensis	16404
Mixed *C. albicans / S. aureus*	10231/6538
Environmental organisms	
Staphylococcus epidermidis	EM
Bacillus cereus/thuringiensis	EM
Paenibacillus glucanolyticus	EM
Ralstonia pickettii	EM
Penecillium spp.	EM

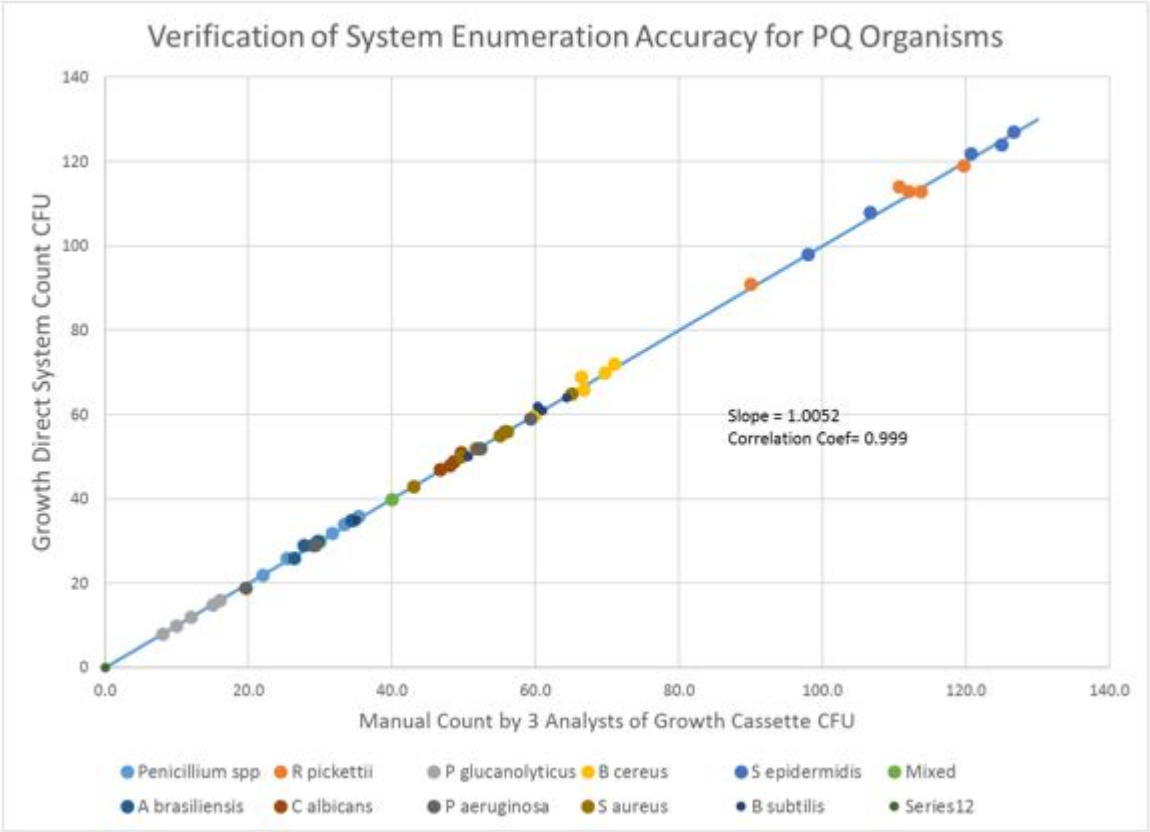

Fig 1 Correlation of System CFU counts for USP and EM microorganisms compared to the mean CFU count of 3 analysts on the same cassette

Time to Result Qualification

Following confirmation of the efficacy of the performance of the instrument, the time required to confirm a negative result was defined. A Time to Result (TTR) Qualification was carried out.

The USP <61> states that plates for Bioburden testing should be incubated for between 3 to 5 days at 30-35°C, therefore these timings should form the basis for the TTR Qualification.

Test samples of the organisms of interest from standard USP and in-house slow growing environmental organisms were prepared and run on the system at 30-35°C. The colony detection profiles from the Growth Direct system were downloaded and analyzed. The Time to Result is taken as the point at which the recovery of the slowest growing organisms is acceptable. For the test samples in the study the TTR determined to be 36 hours as shown in Fig 2.

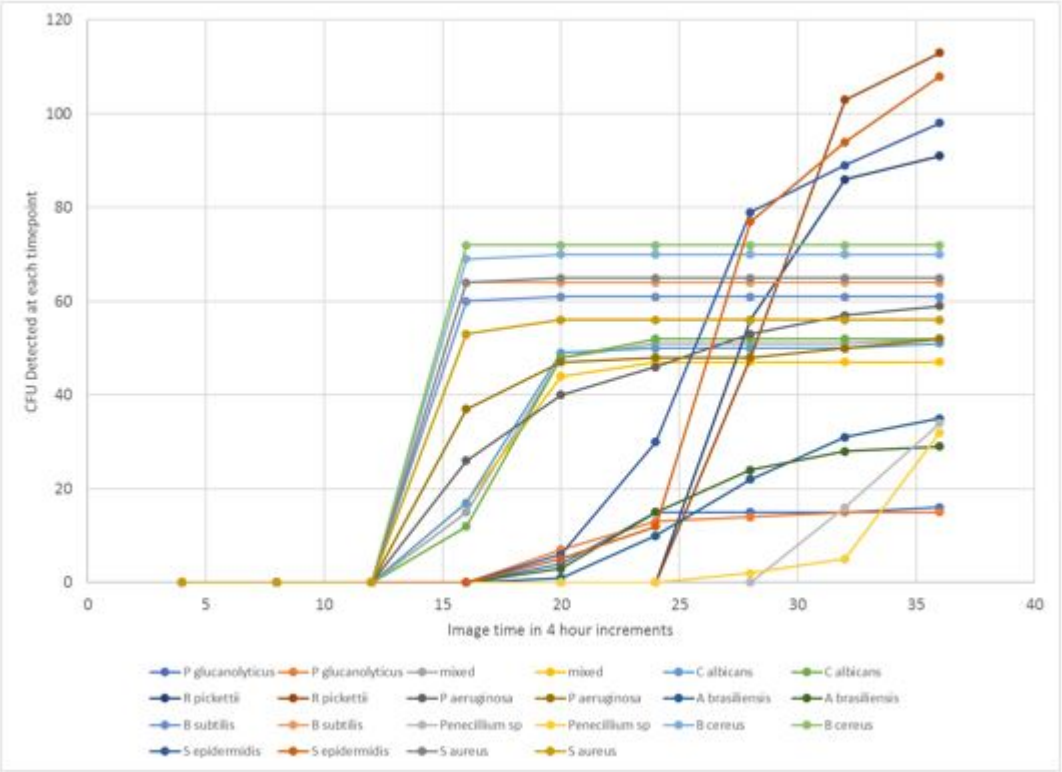

Fig 2 Estimation of Time to Results (TTR) from test samples run on the Growth Direct System. Each line represents the colony detection curve for each individual test sample.

Method Suitability

The method suitability testing for individual pharmaceutical ingredients and drug products as required in USP <61> must be met prior to routine testing. This would demonstrate the recovery of the challenge microorganisms in the presence of any product residues after the rinsing of the membrane filter with a diluent such as buffered saline or peptone water containing the appropriate neutralizing agents. There should no more than a two-fold difference between the recovery with and without the product, i.e., between 50% and 200% recovery.

Samples were taken from 7 points in the manufacturing process from 3 lots of product:

Ultafiltration (UF)	**10mL**
Viral Filtration (VF)	10mL
Column 1	10mL
Column 2	10mL
Column 3	10mL
Single Pass Tangential Flow Filtration (SPTFF)	10mL
Bulk Drug Substance (BDS)	10mL

Samples of the in-process material and a control buffer series were filtered through Rapid Micro Biosystems filtration funnels and rinsed with Fluid A containing <100 CFU of the test organisms. The membranes were then loaded to

TSA media cassettes and incubated at 30-35°C for 36 hours. The recovery of microorganisms from the test matrices was compared to the control counts. Data shown in Fig 3. All matrices and organisms passed the acceptance criteria.

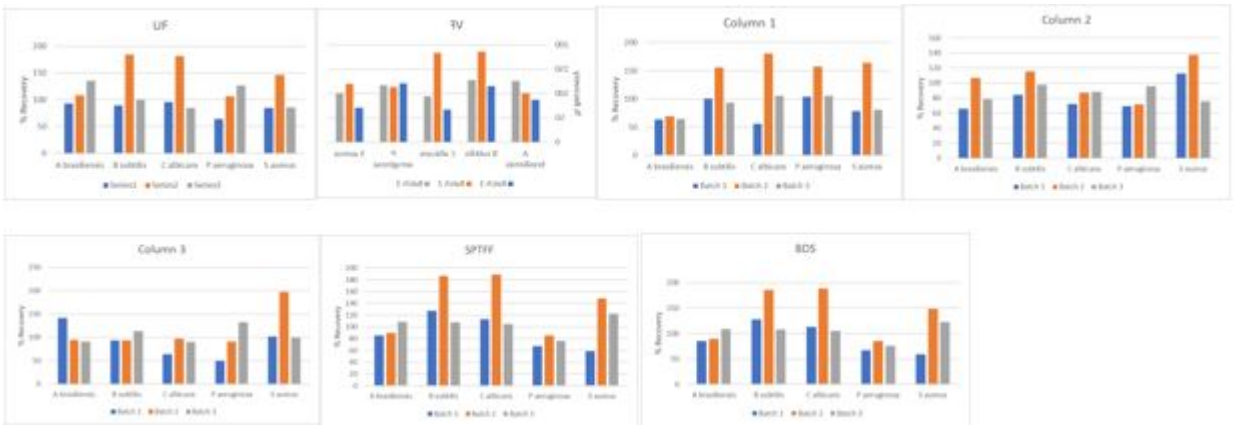

Fig 3 Percentage recovery of USP organisms spiked to seven in-process sample and analyzed using TSA media incubated for 36 hours at 30-35°C on the Growth Direct. Three batches were tested with all organisms and each indicated by bar color in the charts.

Discussion

The paper introduces the validation strategy for the Growth Direct system, an automated colony counter, for the analysis of in-process samples in a Biopharmaceutical environment. As the system is based on the compendia test method for bioburden the verification focuses primarily on those components that are different e. g. the colony detection and enumeration algorithms and the automated incubation step. For the verification of the method a simple approach to test the accuracy of the method was chosen as suggested in the current USP <1223> monograph.

The data show that, the camera detection and associated algorithms, accurately enumerate the microorganism colonies on the membrane surface. The test organisms cover the range of expected colony morphologies seen in the facility.

With the GD system a result can be obtained by 36 hours rather than the 3-5 days used in the traditional in-house method at the site. If the action level were 1 CFU, detection of an issue could be determined as early as 16 hours, this would be flagged by the system to the analyst.

Using the parameters developed in the PQ phase the method suitability was performed on 7 samples from 3 lots of product. For all samples run, with all the test organisms, there was no apparent matrix effect on the organism recovery. All passed the defined acceptance criteria.

The method is deemed to be verified for application with the product and in-process tests used for the study.

In summary the Growth Direct system has used a proven strategy with a minimal verification approach following the process defined in the USP <1223> to show acceptability for in-process product testing.

References

1. USP 38/NF 33 General Chapter <1223>, Validation of Alternative Microbial Methods, page 7667-7681
2. D.L. Jones, T. Cundell, Method Verification Requirements for an Advanced Imaging System for Microbial Plate Count Enumeration, PDA Journal of Pharmaceutical Technology (in press)

3. PDA Technical Report 33 (Revised), 2013, *Evaluation, Validation and Implementation of Alternative and Rapid Microbial Methods*