Simpler RMM Validation for Environmental Monitoring Using Current USP Ch <1223> Requirements

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Introduction

The question asked by many pharmaceutical microbiologists and regulators alike is whether the use of an automated colony counter should be considered as an alternative microbiological test method and subject to full method validation. As the process is automation of the incubation and reading of a traditional microbiological method, it may be subject to a more limited verification. Based on these discussions, a case will be made for method verification and not an alternative method validation strategy, a position justified by the 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) *Evaluation, Validation and Implementation of Alternative and Rapid Microbial Methods*.

Regulatory and Compendial Guidance for the Validation of Alternative Microbiological Test Methods

What is the USP position on alternative microbiological methods?

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**."* 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".*

Why should the Growth Direct ™ System NOT be considered an alternative method?

USP40/NF35 <1223> states:

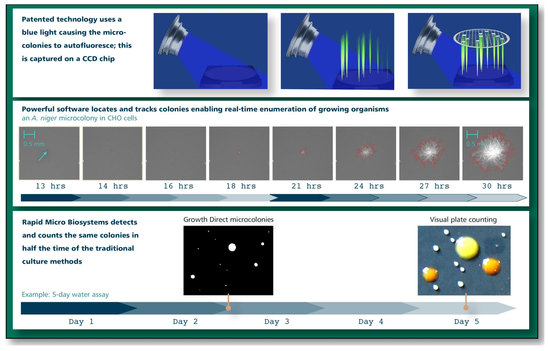
"*There are commercially-available enhancements to growth-based methods that allow colonies on solid media to be read more quickly, with substantially less incubation time, than is possible using only the unaided eye. In the implementation of these enhanced methods for the detection of colony growth,*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

This statement supports the view that the Growth Direct™ System is not an alternative method requiring method validation.

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.***"

The Growth Direct 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 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 used for drug product, environmental monitoring, and water testing. The media cassettes are incubated at the recommended temperatures for times customized to the local facility flora.

Table 2: Standard Media and Incubation Conditions used for Routine Microbial Enumeration in the Pharmaceutical Industry

Microbiological Culture Media	Target Microorganisms	Referenced Methods	Incubation Conditions
Soybean-casein digest agar	Total aerobic microbial count	USP <61> Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests and USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments	30-35°C for 3 to 5 days
Sabouraud dextrose agar	Total combined yeast and mold count	As above	20-25°C for 5 to 7 days
R2A agar	Waterborne bacterial count	USP <1231>Water for Pharmaceutical Purposes and AWWA/APHA Standard Methods	30-35°C for 48 to 72 hours

Recommendations for the Method Verification of the Growth Direct™ System

Prior to performing the method verification, a standard IOQ protocol would be completed to verify the performance of the equipment.

The method verification approach was to plate 6 replicates of representative microorganisms, as defined in USP40/NF35 <61> with representative environmental organisms in the countable range of <100 CFU per membrane. Enumerate the CFUs using the Growth Direct™ System and continue to incubate the test units for a total 3-5 days for bacteria and 5-7 days for fungi. Post incubation, multiple experienced microbiologists (3) count the colonies using the standard visual inspection procedure to minimize counting error. This testing is conducted in place of the typical Performance Qualification.

Results

The data below verifies the enumeration accuracy of the GD system and vision algorithms for the EM application using organisms defined in the USP and found in the environment. Standard Growth Promotion studies verify the nutritive properties of the media during incoming QC.

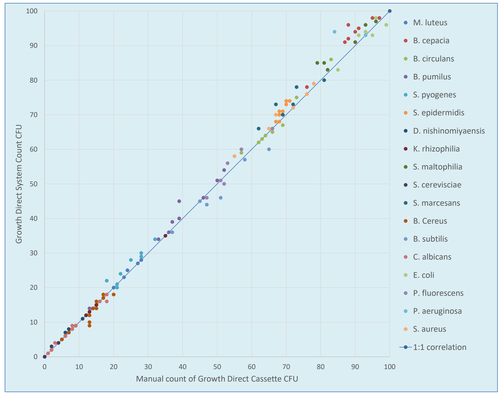


Figure 1: Verification of Enumeration Accuracy by The Growth Direct Software

Method Suitability Tests

The method suitability testing for EM must be met prior to routine testing. This requirement is independent of method validation or verification.

Cassettes used for air, surface, and personnel monitoring in a pharmaceutical facility, contain neutralizing agents for commonly used disinfectants. The recovery of microorganisms especially from facility and equipment surfaces with residual disinfectant needs to be validated. The acceptance criteria for the disinfectant residue neutralization should be between 50% and 200% recovery.

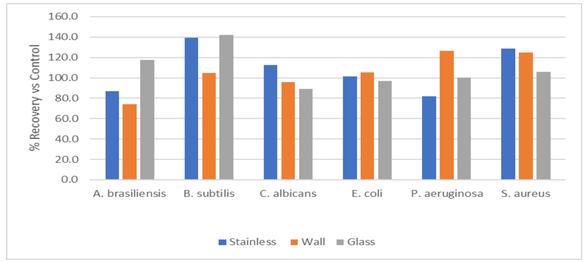


Figure 2a: Microbial recovery after sampling coupon surfaces coated with a Biguanide

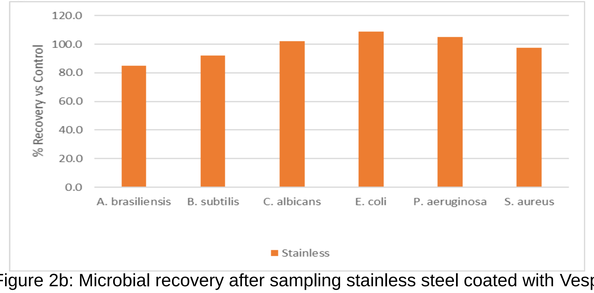


Figure 2b: Microbial recovery after sampling stainless steel coated with Vesphene

Conclusions

Following the USP guidance chapters, the Growth Direct™ System meets the conditions stated in USP <1223> and the industry practice document PDA Technical Report No. 33 that it is an automated system for the incubation and enumeration of the compendial microbial plate count method. As such, the system only requires verification of the counting method and method suitability to be performed.

The verification of the system colony enumeration and the method suitability for environmental sample testing was performed successfully.

References

1 USP 1223 USP40/NF35 General Informational Chapter <1223> *Validation of New Microbiological Testing Methods*
2 USP 61 USP40/NF35 *Microbiological Examination of Non Sterile products: Microbial Enumeration Tests*
3 TR33 2013 PDA Technical Report 33 (Revised) *Evaluation, Validation and Implementation of Alternative and Rapid Microbial Methods*
4 A. Cundell and D. Jones 2017 PDA Journal of Technology, In Press. *Method Verification Requirements for an Advanced Imaging System for Microbial Plate Count Enumeration*