Title: Validation of an Automated Method for Enumeration of Microbial Counts for Environmental Monitoring for an Allogeneic Cell-Based Therapy Product.

Authors: Daniel Lesnoy, Daniel Sutherby, Zorina Pitkin

Background: The three principal factors that ensure the safety of a cell-based product are: a) absence of microbial growth in final product testing, b) acceptable test results for in-coming materials, and c) control of the manufacturing process. The latter concerns rigorous environmental and personnel monitoring (EM) to demonstrate that all process controls are appropriate. The typical process takes up to 7 days of incubation for sufficient growth of microorganisms and requires two analysts to read the EM plates to comply with the data integrity requirement. All collected EM plates need to be assessed for microbial count to determine whether the established criteria are met; if they are not, then a product impact assessment is required. This conventional EM method presents a challenge for cellular therapies with a short shelf life.

Hypothesis: New automated microbial enumeration systems have gained acceptance by the biotech and pharmaceutical industries as new technologies have entered the marketplace. It is imperative, however, that the benefits of automated systems be demonstrated through carefully considered validation.

Methods: Organogenesis's Apligraf® is a 351 HCT/P cell-based commercial product with a 15-day shelf life. It is FDA approved for the treatment of venous leg ulcers and for the treatment of diabetic foot ulcers. Apligraf manufacturing process is rigorously monitored for viable and non-viable particles in clean rooms; up to 200,000 plates are collected annually. To expedite time to microbial detection prior to product release, the Growth Direct System (GDS, from Rapid Micro Biosystems) has been validated for the continuous microbial count detection and enumeration of the following samples: plated growth media samples collected for environmental and personnel monitoring within the ISO 8, ISO 7, and ISO 5 clean rooms and clean utility systems, and in-process bioburden samples. In transitioning from a manual to an automated system, a comprehensive risk-based validation strategy was successfully completed.

Results: The validation study demonstrated a significantly reduced time-to-detection, thus enhancing product safety profile. It enabled early excursion notifications, leading to rapid responses for exceptions, and it elevated compliance by eliminating human error and improving operator efficiency. The system was validated to detect microbial growth in classified areas in less than 2 days vs. conventional 5 days.

Conclusions: This validation challenged the GDS with an extensive panel of ATCC organisms and in-house isolates, and provided a high degree of confidence that the GDS is highly reliable in early detection and reporting of accurate plated media results, and is at least equivalent to the current manual method with the added benefit of earlier time-to-detection.

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ORGANOGENESIS

Validation of an Automated Method for Enumeration of Microbial Counts for Environmental Monitoring for an Allogeneic Cell-Based Therapy Product

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BACKGROUND

The three principal factors that ensure the safety of a cell-based product are: a) absence of microbial growth in final product testing, b) acceptable test results for in-coming materials, and c) control of the manufacturing process. The latter concerns rigorous environmental and personnel monitoring (EM) to demonstrate that all process controls are appropriate. The typical process takes up to 7 days of incubation for sufficient growth of microorganisms and requires two analysts to read the EM plates to comply with the data integrity requirement. All collected EM plates need to be assessed for microbial count to determine whether the established criteria are met; if they are not, then a product impact assessment is required. Utilization of new automated technologies presents an opportunity to improve the operational efficiency of the environmental monitoring process.

HYPOTHESIS

New automated microbial enumeration systems have gained acceptance by the biotech and pharmaceutical industries as new technologies have entered the marketplace. It is imperative, however, that the benefits of automated systems be demonstrated through carefully considered validation.

METHODS

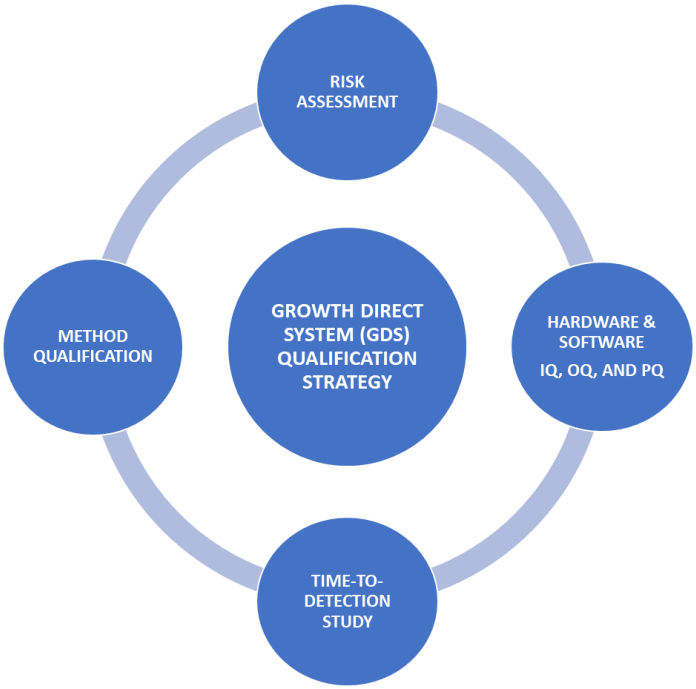
Organogenesis's Apligraf® is a 351 HCT/P cell-based commercial product with a 15-day shelf life. It is FDA approved for the treatment of venous leg ulcers and for the treatment of diabetic foot ulcers. Apligraf manufacturing process is rigorously monitored for viable and non-viable particles in clean rooms; up to 200,000 plates are collected annually. To expedite time to microbial detection prior to product release, the Growth Direct System (GDS, from Rapid Micro Biosystems) has been validated for the continuous microbial count detection and enumeration of the following samples: plated growth media samples collected for environmental and personnel monitoring within the ISO 8, ISO 7, and ISO 5 clean rooms and clean utility systems, and in-process bioburden samples. For reference, a picture of the Growth Direct® System (GDS) is provided in **Figure 1**.

Figure 1. Picture of a single standalone unit of the Growth Direct® System

METHODS (cont.)

In transitioning from a manual to an automated system, a comprehensive risk-based validation strategy was successfully completed per the illustration in **Figure 2**.

Figure 2. Growth Direct® System Validation Strategy



RESULTS

The EM Time to Results (TTR) validation study demonstrated a significantly reduced time-to-detection, thus enhancing product safety profile. It enabled early excursion notifications, leading to rapid responses for exceptions, and it elevated compliance by eliminating human error and improving operator efficiency. The system was validated to detect microbial growth in classified areas in less than 2 days vs. conventional 5 days.

The TTR validation consisted of a total of sixty (60) samples, surface and active air samples, which were collected throughout the Organogenesis facility during three sampling sessions over three consecutive days.

Any differences in colony counts between visual and GD of greater than 10% were investigated.

The TTR for the sample pool of organisms collected from the Organogenesis facility was determined on the crossing point where at least 85% of the total colonies were detected as compared to visual counts of the same samples.

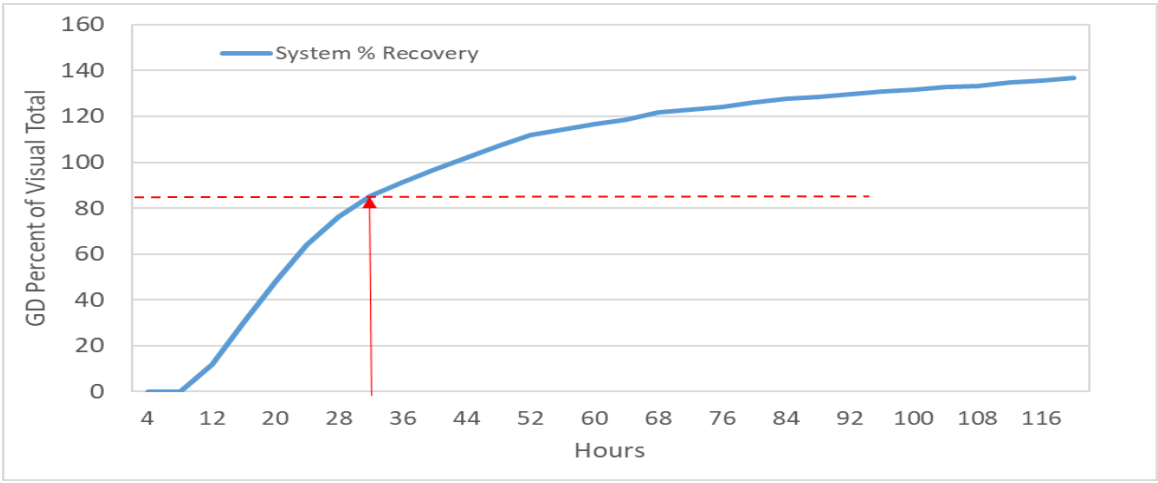
As shown in **Figure 3**, percent system recovery was plotted versus incubation time in order to determine the incubation time where this intersection occurs. Based on this analysis, a TTR of 32 hours was established as the minimum incubation time for purposes of method qualification. Where overcounts represent worst-case, Organogenesis established a more conservative TTR of 48 hours for subsequent qualification/validation activities.

An environmental monitoring (EM) method qualification was designed to ensure that all process steps of quality control testing and incubation do not interfere with the enumerating technology employed by the GDS. This EM method qualification was designed to evaluate the four (4) main performance requirements of the GDS when used for EM. More specifically, the associated performance characteristics evaluated under this method qualification included:

a) Disinfectant neutralization
b) Capture and recovery
c) Active Air Comparison
d) EM Program Equivalence

All acceptance criteria were satisfied for each separate characteristic.

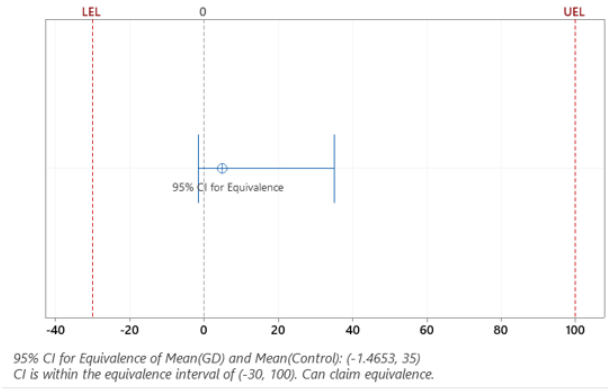
Figure 3. Determination of TTR for sample pool where at least 85% of the total colonies were detected.



The EM Program Equivalence arm of the EM method qualification was intended to test the recovery capabilities of the GD plates at the Organogenesis Inc. site on a cross-section of surfaces. A total of eighteen (18) sites across the facility (e.g., doors, ladders, carts, chairs, desks, signs, trash bins, etc.) were sampled with both GDS and standard contact plates then incubated according to the associated GDS and compendial test methods. Percent recoveries were determined for the GDS and compendial methods based on GDS recovery as a percent of the Control sample.

Appropriate statistical analysis of the entire population was performed to assess overall equivalency of the EM program. A non-parametric (Mann-Whitney) Equivalency Test was applied to compare the data populations from the GDS and compendial methods. As shown in **Figure 4**, the 95% CI for Difference of Mean of the GDS – Mean of the Control was determined establishing the Lower and Upper Equivalence limits (LEL and UEL, respectively) of -30 and 100, respectively. With the 95% CI for Equivalence of the Means for the GDS and Control being -1.4653 and 35, the CI was within the LEL and UEL. As a result, equivalence between the two methods has been demonstrated.

Figure 4. Equivalency test between visual and GDS



A Performance Qualification (PQ) was executed to validate the performance of the GDS by evaluating the equivalence of the GDS by comparison of time-to-results for the current manual visual counting method versus the automated Growth Direct System for environmental monitoring (3 to 5 days), water systems (5 to 7 days) and in-process product bioburden (5 to 7 days), at 48 hours incubation on the GDS. The PQ tested samples using ATCC microorganisms and facility isolates, six organisms per test method with six replicates per GDS (n=3), per microorganism. Control plates consisted of three replicates per organism/test method. All tests met the recovery of 50-200% when compared to the control (current media/method). See **Figure Nos. 5 to 6** (EM and Bioburden) for summary charts of recovery rates microorganisms at a target of 50 CFU. Sensitivity was also demonstrated at a target of 10 CFU.

Figure 5. PQ Results Summary (EM)

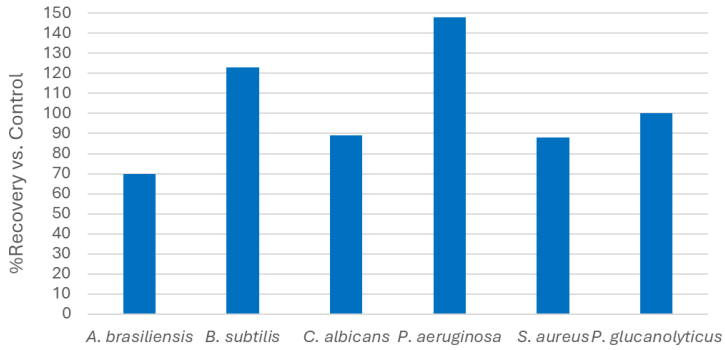
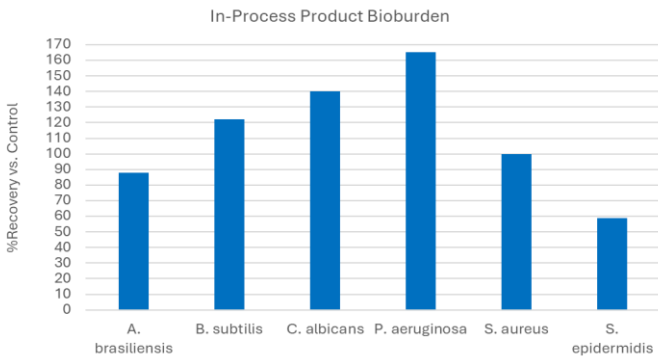


Figure 6. Bioburden PQ Results Summary



CONCLUSION

This validation challenged the GDS with an extensive panel of ATCC organisms and in-house isolates, and provided a high degree of confidence that the GDS is highly reliable in early detection and reporting of accurate plated media results, and is at least equivalent to the current manual method with the added benefit of earlier time-to-detection.