Automated Colony Counter

Global Implementation

PDA Parenteral Drug Association Midwest Chapter

Our top-tier customer base includes the majority of top 20 global pharma*

>75 MANUFACTURING SITES

125+
cumulative instruments placed

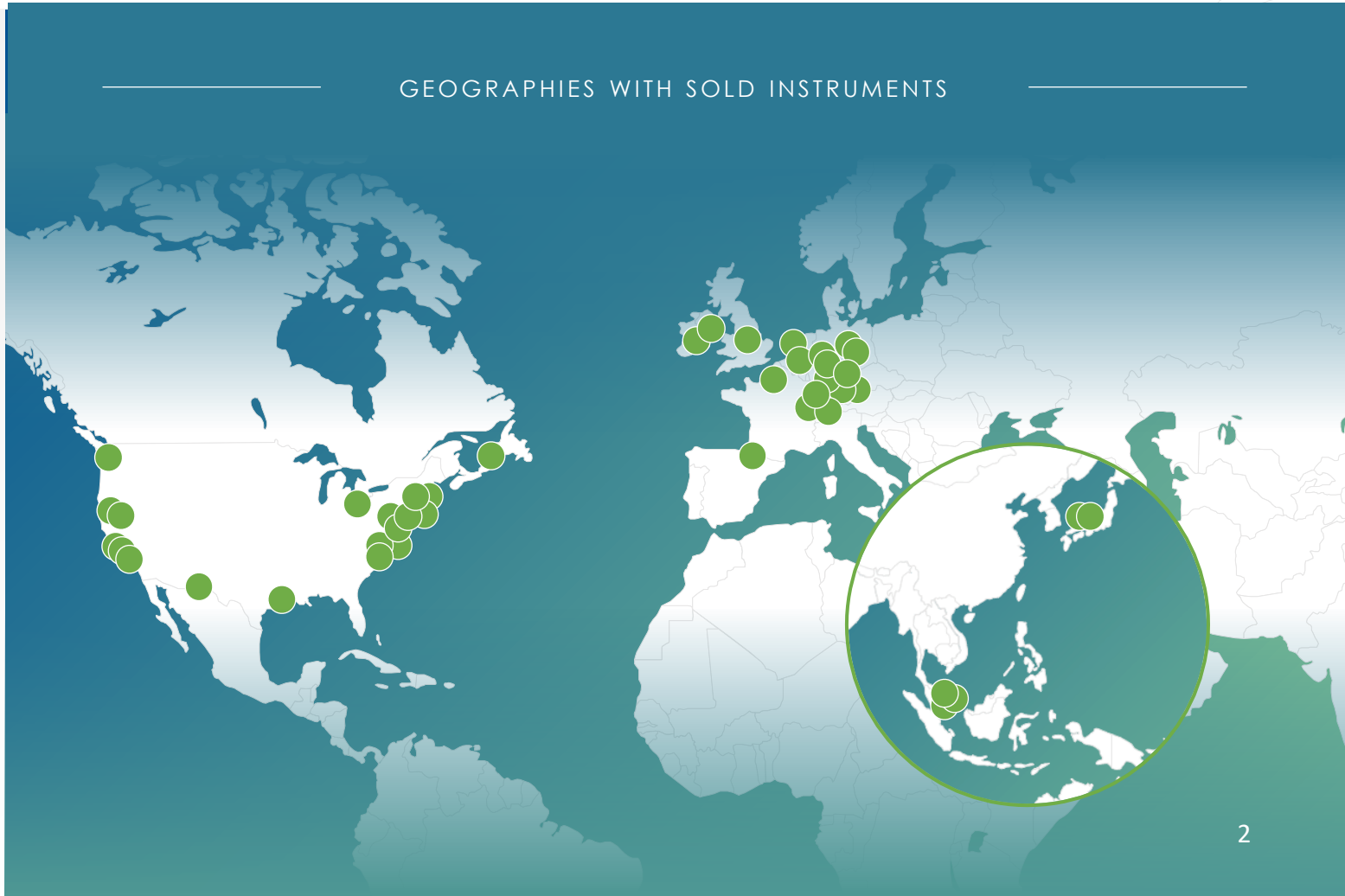
3M+
cumulative consumables sold

>45%
customers with multi-site deployments

>55%
customers with multiple systems

CUSTOMER SEGMENTS WITH ESTABLISHED USE

- Biologics
- Cell & Gene Therapy/ CAR-T
- CDMO
- Small Molecules
- 503B Compounders
- Personal Care Products



* By revenue

Why an Increased Interest in Colony Counters?

Colony counters are a recent Pharma focus due to:

- Concerns on the integrity of data with the compendial method using single manual read and disposal of evidence
  - Resulted in the 4-eyes counting guideline
  - However, 4-eyes is not easy to implement

Errors around manual data transfer to batch records or LIMS systems, automation has “paperless” benefits

MHRA & PDA Guidance Documents Have Highlighted the Risk from Manual Results in Microbiology Testing

Technical Report No. 80: Data Integrity Management System for Pharmaceutical Laboratories - Released Aug 2018

Potential Data Integrity Risk Matrix for Microbiological Testing

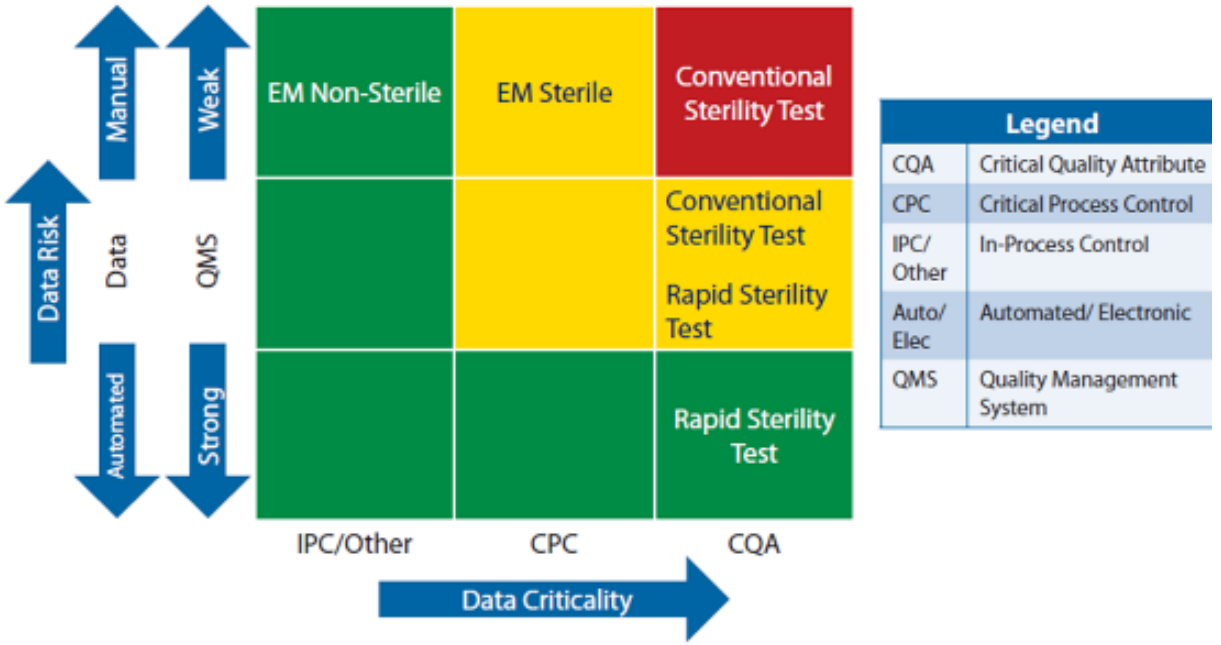


Figure 7.2-1 Risk Matrix Example for Microbiological Testing

Manual observations in critical quality tests such as sterility, environmental monitoring, and bioburden testing represent risk. Guidance documents state that computer and automated systems represent ways to reduce that risk.

Colony counter technology

Colony Counter Evolution

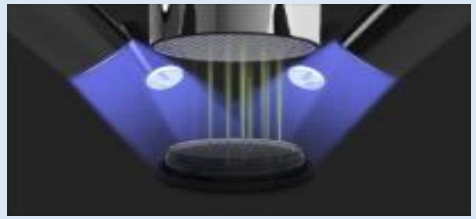
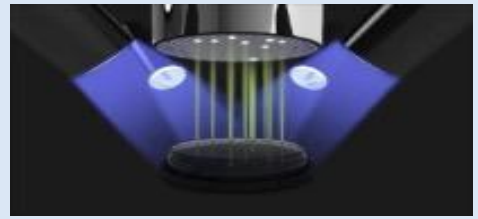




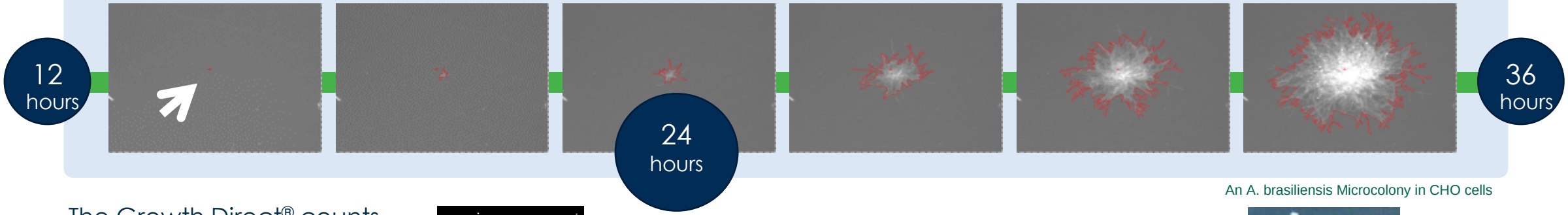
	Manual	Semi Automated	Semi Automated	Automated Low capacity	Automated Higher capacity	Fully automated Higher capacity
Enumeration	Yes End Point	Yes End Point	Yes End Point	Yes Kinetic (10min)	Yes Kinetic (60min)	Yes Kinetic (4 hour)
Incubation	No	No	No	Yes Single temp	Yes Single temp	Yes Dual temp
Capacity	1 plate	1 plate	100	12-24	300	680

Kinetic Colony Counting Technology

Patented technology uses blue light causing the micro-colonies to auto-fluoresce, the fluorescence is captured on a CCD chip

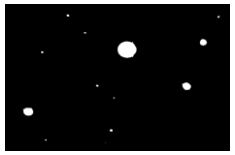




Powerful algorithms detect colonies within hours, enabling real-time enumeration of organisms



An A. brasiliensis Microcolony in CHO cells

The Growth Direct® counts the same colonies in half the time of the traditional method.



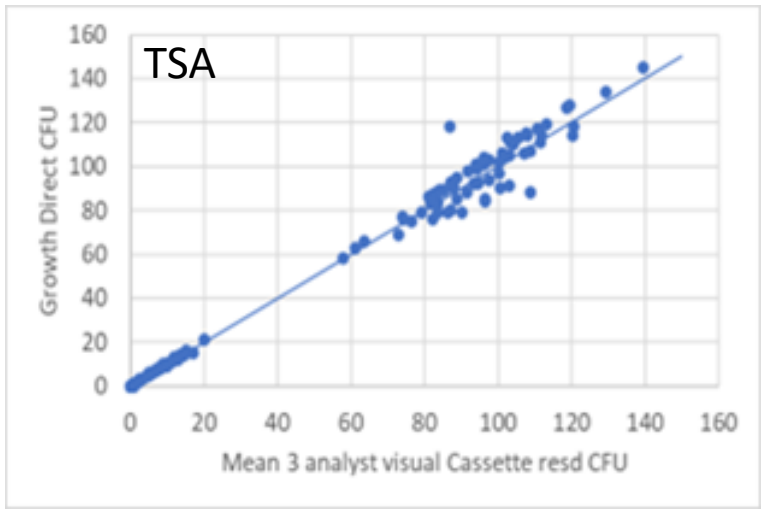
Growth Direct® Imaging

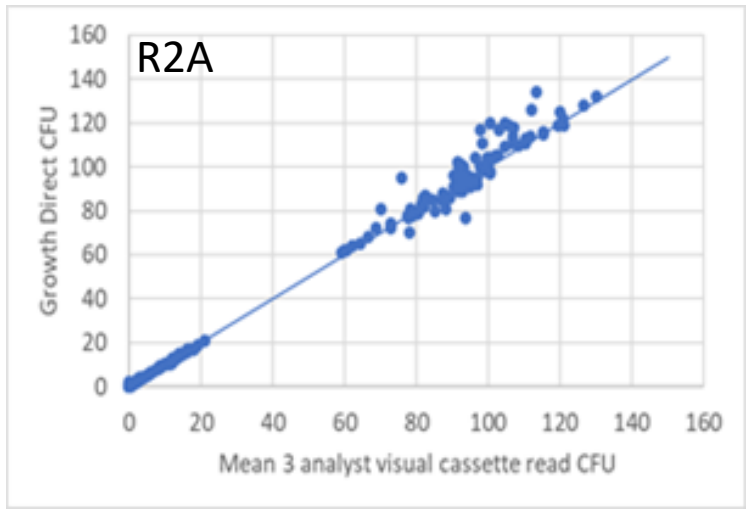
Visual Plate Counting

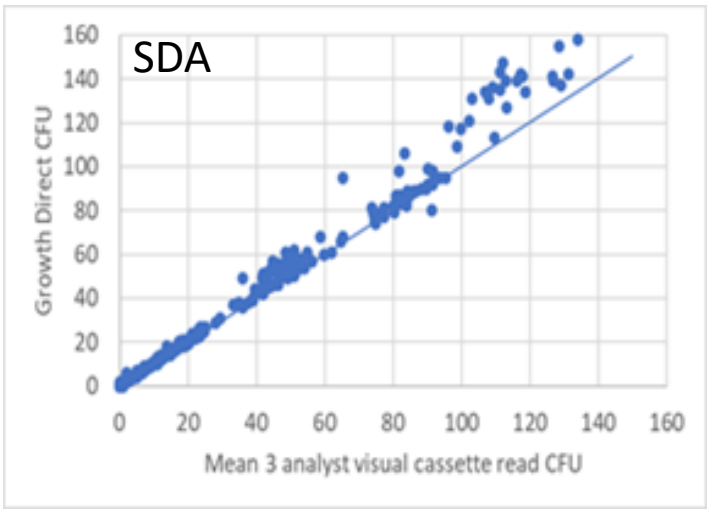
Day 1 | Day 2 | Day 3 | Day 4 | Day 5

Colony Enumeration Accuracy- USP Organisms

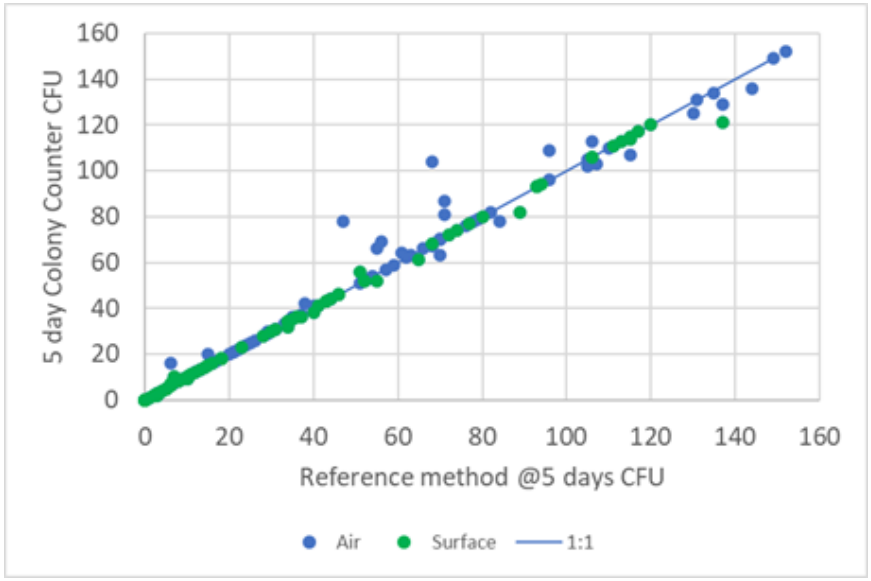
Bioburden Media	R^2 value	Slope value
TSA	0.9964	1.0129
R2A	0.9972	1.0314
SDA	0.9980	1.0998

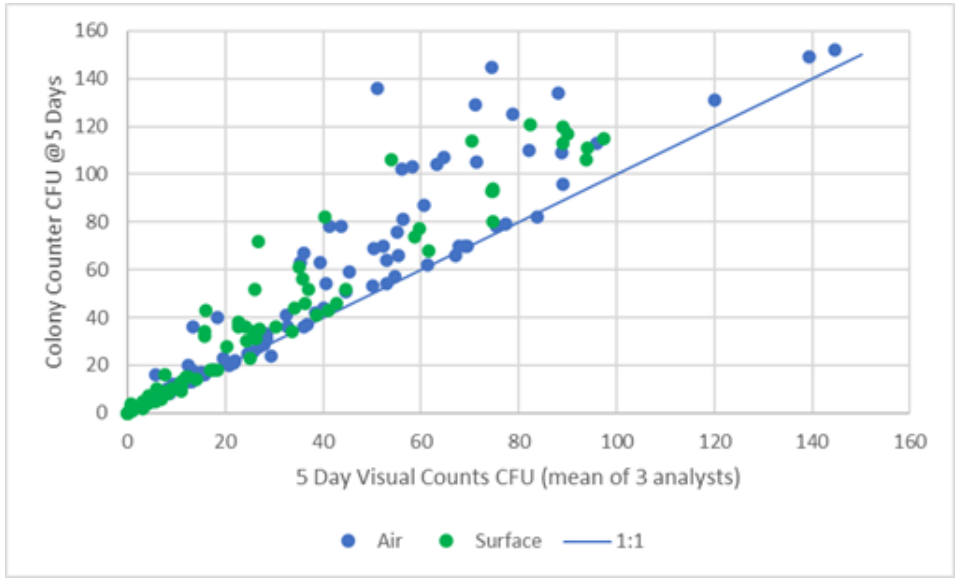






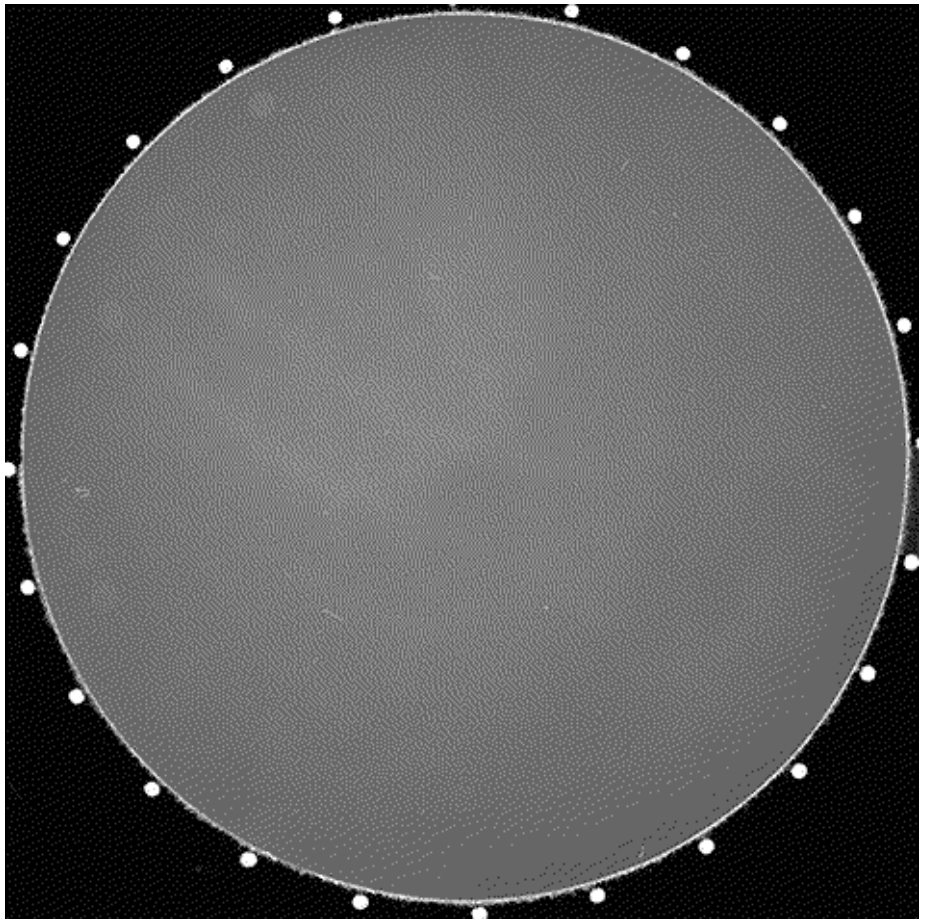
Colony Enumeration Accuracy- EM Sampling





Benefits of kinetic imaging

- Image from an EM sample plate read every 4 hours over a traditional 120 hour incubation at 30-35°C.
  Note, for routine EM the incubation time would be 72hrs
- 14 colonies on plate but 2 molds overgrow all but 1 of the bacterial colonies by the end of incubation
- System remembers all colonies detected so gives the correct count at end of incubation



Relevant guidance chapters

PDA TR33 published 2013

Any new microbiology methods and RMMs are fully or semi-automated with regard to sample testing and/or data acquisition. TR33 discussed the differences between a new method that represents the automation of a current compendial or conventional method, or whether the new method is a scientific or technological alternative to a current compendial or conventional method.

TR33 2013 introduced the notion of an “Automated Compendial method” and described a reduced analytical validation of Accuracy and Precision– “Some alternative or rapid technologies may be considered as automated traditional or compendial microbiological test 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 this Technical Report. For these technologies, at least accuracy and precision assessments should be performed, in addition to method suitability and equivalence / comparability studies.”

Original version published in 2000, currently undergoing revision for 2023/24 release

USP Ch <1223> published 2015

First iteration of USP <1223> was in 2006

> *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*

Following verification of the systems detection capability a standard method qualification would be performed on any products to be tested.

Reference: *Method Verification Requirements for an Advanced Imaging System for Microbial Plate Count Enumeration*, Jones D, Cundell A, PDA Journal of Pharmaceutical Science and Technology 2018

Colony counters are defined as automated compendial tests which allows for a simplified validation

Facilitating Technology uptake

BioPhorum (biophorum.com) Over 160 manufacturers collaborating to improve pharma activities

Created working group for RMM covering, Sterility, Mycoplasma and Colony counters.

Top level document produced:

“A framework for the evaluation, validation and implementation of alternative and rapid microbiological testing methods”

12 companies contributed to the creation of this document in 2020

https://www.biophorum.com/download/alternative-and-rapid-micro-methods-armm-a-framework-for-the-evaluation-validation-and-implementation-of-alternative-and-rapid-microbiological-testing-methods/

Follow on case studies include-

“A Structured Approach for the Evaluation, Validation and Implementation of Automated Colony Counting Systems”

9 companies contributed to the creation of this document in 2022

https://journal.pda.org/content/76/6/509/tab-references

Implementation of a consistent validation approach

- Rapid Micro Biosystems works closely with their customer base to ensure consistent methods are used across the installed base.
- Through our Customer Advisory Board and the EU Growth Direct user group a consensus was obtained for the validation process to be used, titled Modular Validation
- Through use of the agreed method there is a consistent approach to lower the concerns of any auditor
- RMB have made 5 presentations to FDA reviewer and compliance groups over the years to ensure they are aware of the technology and validation approach

Modular Validation Approach

- Generic approach taken by all sites to validate the colony counter as an “automated compendial test”. Combines definitions from USP<1223> and PDA TR33.
- Modular approach also supported by peer reviewed papers validating EM, Water and in process sample types.

IOQ		
Instrument PQ		
TTR Water	TTR EM	TTR Product
MQ Water	MQ EM	MQ Product
		MST Product

Contributing Authors

Eight companies combined their data from the colony counter validations for EM and bioburden (water and in process)

- ROCHE
- NOVARTIS
- BI — EU SITES
- LONZA
- JANSSEN

- GSK
- KITE — USA SITES
- BIOGEN

Modular Performance Qualification PQ

- 3-6 Morphologically distinct microbial species are incubated, 3 to 7, days on the media of choice.
- Following incubation, three independent analysts enumerate the colonies.
- The colony counter result compared to the mean analyst count confirms accuracy of the software algorithms. (acceptance criteria ≥85%)

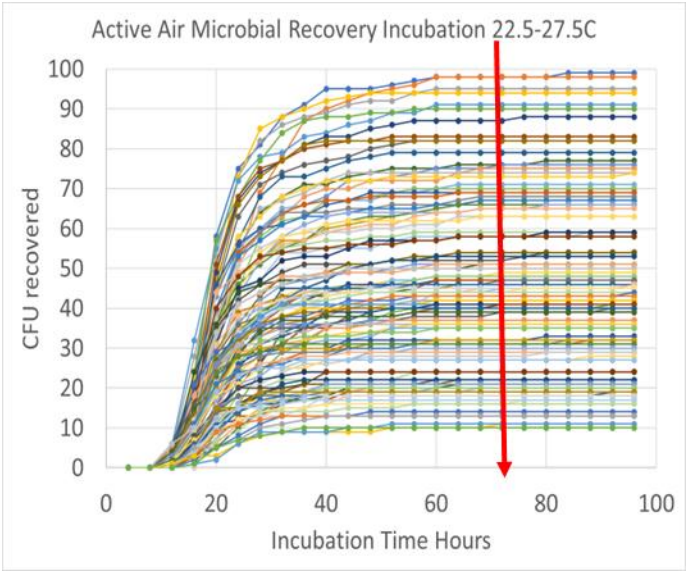
Site	*B. subtilis* ATCC 6633	*E. coli* ATCC 8739	*A. brasiliensis* ATCC 16404	*P. aeruginosa* ATCC 9027	*S. aureus* ATCC 6538	*C. albicans* ATTC 10231
Site 1	Pass	Pass	Pass	Pass	Pass	Pass
Site 2	Pass	Pass	Pass			
Site 3	Pass	Pass	Pass	Pass	Pass	Pass
Site 4	Pass	Pass	Pass			
Site 5	Pass	Pass	Pass			
Site 6	Pass	Pass	Pass	Pass	Pass	Pass
Site 7	Pass	Pass	Pass			
Site 8	Pass	Pass	Pass			

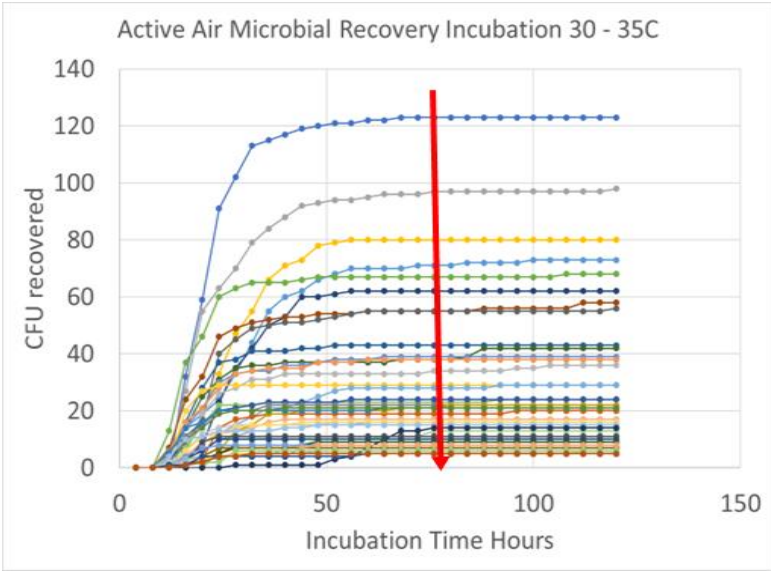
Hot Topic- EM Incubation Temperature: mold detection

"Optimal" Growth Temp				
22°C	22°C to 28°C	28°C	28°C to 32°C	32°C
Alternaria sp EM	*Penicillium notatum*	*Penicillium citrinum*	*Trichoderma asperellum*	*Curvularia hominis*
Alternaria species	*Aspergillus versicolor*	*Curvularia verruculosa*	*Fusarium keratoplasticum*	*Aspergillus fumigatus*
Arthrinium arundinis	*Penicillium Camemberti*	*Chaetomium globosum*	*Aspergillus flavus*	*Aspergillus terreus*
Aspergillus basiliensis	*Penicillium verhogenii*	*Curvularia pallescens*	*Curvularia lunata*	*Curvularia pseudobrachyspors*
Cladosporium herbarum	*Penicillium rubens*	*Chaetomium indicum*		*Hamigera insecttiola*
Penicillium roqufortii	*Penicillium chrysogenum*			*Cladosporium cladosporoides*
Exophiala xenobiotica	*Aspergillus caesiellus*			*Tricophyton interdigitale*
Exophiala lecanii-cori	*Aurobasidium pullulans*			*Tricophyton rubrum*
Ramichloridium ancep	*Epicoccum nigram*			*Phialemonium obovatum*
Geomyces pannorum	*Saccharomyces cerevisiae*			*Acremonium chrysogenum*
Geomyces asperulatus				*Aspergillus niger*
Penecillium olsonii				

Modular Time To Results (TTR)

- TTR depends on media type and incubation temperature
- Selected organisms or known contaminated sites were tested. All sites used a single incubation regime however the temperatures ranged from 22-35°C
- Colonies were enumerated by 3 independent analysts and recovery compared to the colony counter result
- The TTR is defined as the incubation time when the system cfu count is >85% of the final visual count.





Time to Results Site Summary

Site	Media	Time to Results
Site 1	TSA R2A TSA + LP80	Product: 36 hours Rinse Water 44 hours EM: 60 hours
Site 2	TSA + LP80	EM: 36 hours
Site 3	TSA + LP80	EM 44 hours
Site 4	R2A TSA + LP80 TSA	Water: 116 hours EM: 76 hours Product: 52 hours
Site 5	R2A TSA + LP80HT	EM: 56 hours Water: 116 hours
Site 6	TSA + LP80HT	EM: 72 hours
Site 7	TSA + LP80HT R2A TSA	EM: 52 hours Water: 64 and 108 hours Product: 36 hours
Site 8	TSA + LP80 R2A	EM: 68 hours Water: 100 hours

TTR summary:

EM 36 to 76 hours

Bioburden 36 to 52 hours

Water 44 to 116 hours

Modular Method Qualification MQ

The MQ module contains testing for

- Accuracy and Precision of the test method.
- Equivalence of results to standard method for EM and water bioburden is included

Test sample numbers:

- For water, sample volumes ranged from 0.1 mL to 200 mL depending on water type with 2 to 25 test sites yielding 192 to 600 test replicates.
- For EM air sample volumes ranged from 200 to 1000 liters from 18 to 83 sites yielding 54 to 215 samples and for contact plates 6 to 132 sites yielding 36 to 216 samples.

All sites passed the acceptance criteria for accuracy, precision and equivalence for all applications tested, water, product bioburden and EM.

Modular Method Suitability MS

- Method suitability criteria for individual pharmaceutical ingredients and drug products, as required in USP <61> Microbiological examination of non-sterile products: There should be no more than a two-fold difference between the organism recovery with and without the presence of product, i.e., between 50% and 200% recovery
- Two biologic sites performed method suitability for BLA submission in US and EU following the modular validation protocols. Submissions were accepted with no adverse comments.

Paper Reference

The data summarized in the presentation is published in the PDA Journal. Currently as the early electronic form but should be in press Q2 2023:

- Multisite Qualification of a Fully Automated Colony Counter for Environmental and Bioburden Applications in Pharmaceutical Microbiology

Hans Joachim Anders[1]; Daniel Männle[1]; William Carpenter[2]; Wolfgang Eder[3]; Ivana Heckel[4]; Tobias Götzen[4]; Corinne Oechslin[4]; Cedric Joossen[5]; Maria Eugenia Giribets Parra[6]; Jason Rose[7]; Vaishali Shah[8]; David L Jones[9]

- [1] Novartis Pharma Stein AG, Stein CH4332, Switzerland; [2] Biogen, Durham, North Carolina, USA; [3] Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany; [4] Lonza, Visp, Valais, Switzerland; [5] Janssen Pharmaceutica NV, Beerse, Belgium; [6] Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany; [7] GlaxoSmithKline, Upper Merion, PA, USA; [8] Kite Pharma, Santa Monica, California, USA; [9] Rapid Micro Biosystems, Massachusetts, USA.

- https://journal.pda.org/content/early/2022/11/15/pdajpst.2022.012742

THANK YOU AND QUESTIONS