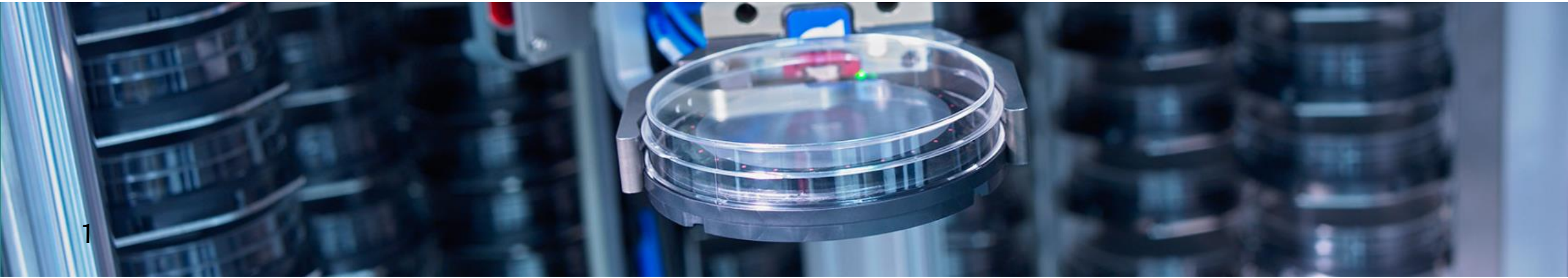
Validation and Implementation of an Automated Colony Counter for Product Testing in Biopharmaceutical Manufacturing

CMC Forum Fall 2020, October 15, 2020, Virtual Conference

Dr. Sven Deutschmann, Roche, Global QC, Global Analytical Science and Technology



Technology and Methodology
Expected Improvements

Automated Colony counting: Expectations

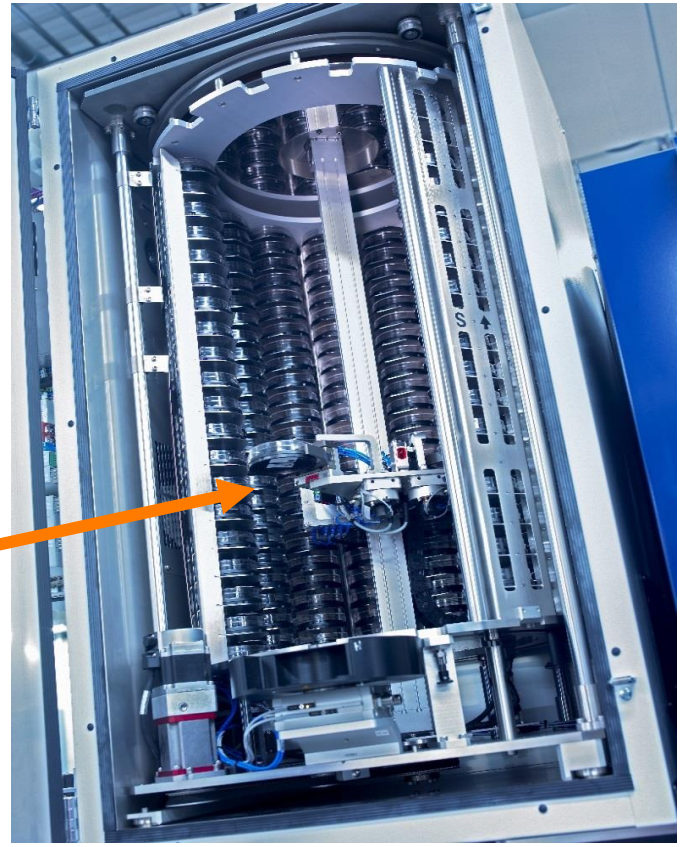
- **Data Integrity improvements:** with automated and validated result interface with LIMS and also with standardized readout per camera and not per human eye (variance per employee); also with capturing electronic raw data/images (audit trail and audit trail review possible)
- **Reduction of hands-on time and review time:** headcount savings
- **Enables real-time-release:** immediately reports results after 36 hours for IPC and DS release (note: per current Roche rtr definition)
- **Notification if growth is detected:** quicker response times to potential contamination in manufacturing

Technology and Methodology

Technology

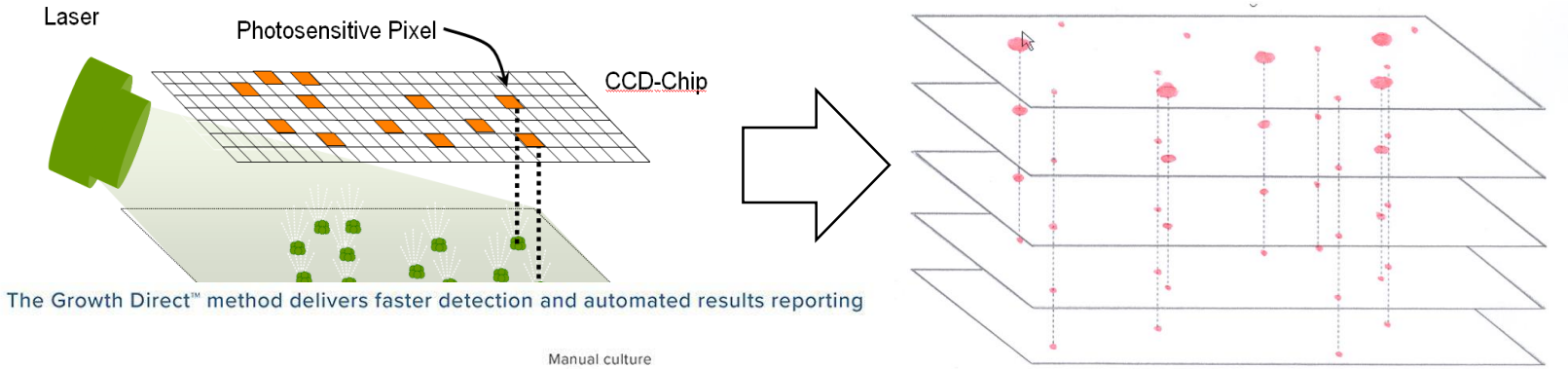
Upper and Lower Incubator

Robotic Arm

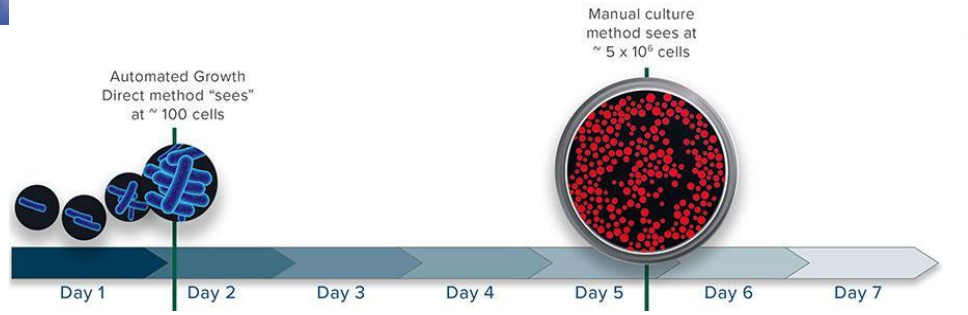


Technology and Methodology
Automated Colony Counting (1)

Automated system for bioburden counting using endogeneous autofluorescence of the cells (500-550 nm)

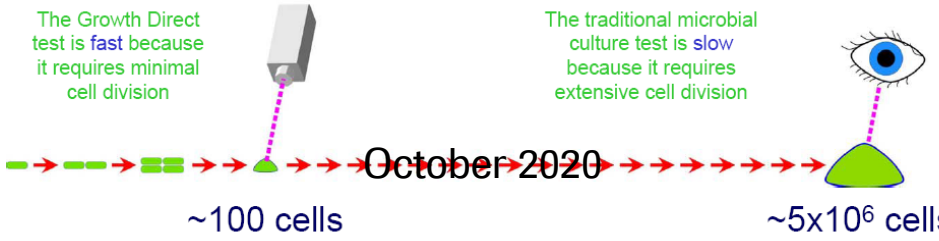


Stack of images



Growth Direct test

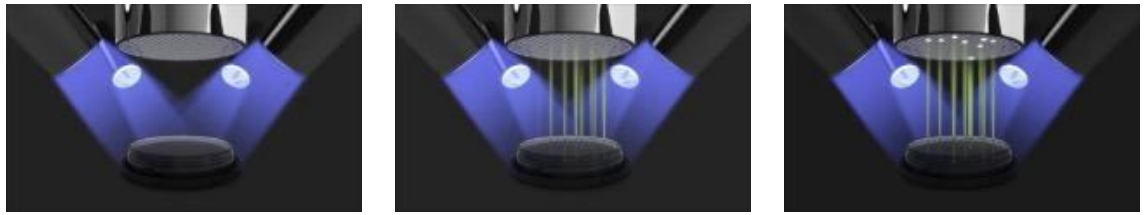
visual plate counting



Technology and Methodology

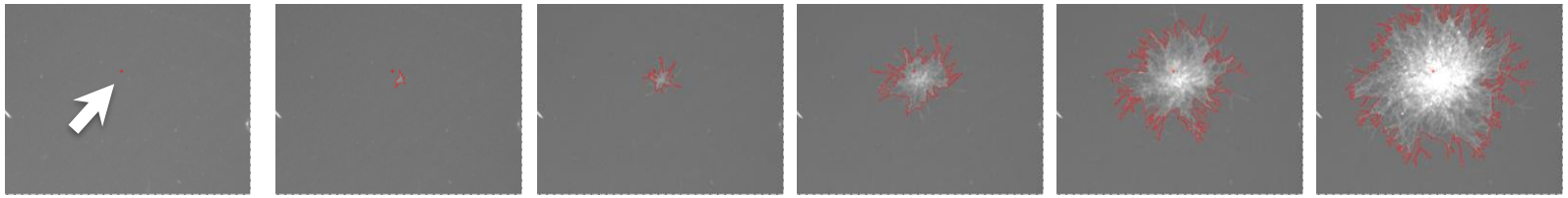
Automated Colony Counting (2)

Patented technology uses a blue light causing the micro-colonies to autofluoresce: this is captured on a CCD chip



Powerful software starts to detect colonies within hours, enabling real-time enumeration of organisms

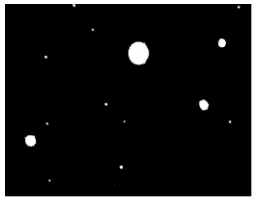
A. brasiliensis microcolony in CHO cells



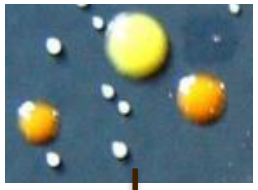
12 hrs | 16 hrs | 20 hrs | 24 hrs | 28 hrs | 32 hrs

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

Technology and Methodology
Comparison of Readout Methods

Manual/Visual AND Automated Workflows:

1st Step: Membrane Filtration per Ph. Eur., USP, JP

Manual

Automated

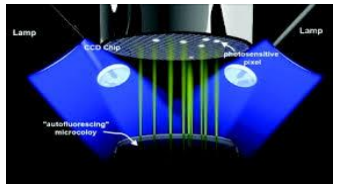
2nd Step: Incubation

3rd Step: Count colonies

3 - 5 days

3 b

3 a



CCD camera reads every 4hrs, results in ~36 hrs*

*Determined by Time to Results (TTR) studies

Auto-generated LIMS and Alerts

3a) Automated readout
3b) Manual readout

Regulatory Guidance
Equipment Qualification / Method Validation Parameter (1)

1. General Consideration

- The Growth Direct System technology uses **standard media** for microbial growth and **standard incubation temperatures** to allow colony forming units **(CFU) to grow and be counted**. Sampling and testing methodology is per **standard microbiological methods**, using the Growth Cassette products instead of traditional consumables. The media can be qualified as described in the relevant pharmacopeia, e.g. **USP <61> and Ph. Eur. 2.6.12.**

- The core component of the test, the cassette, uses the **same growth media** held in a polystyrene cassette as is used in traditional Petri plates. The test for viability is thus the same as determined using the traditional method.

- **The Growth Direct System can be defined as growth-based bioburden test with automated colony counting or readout** for the incubation and enumeration of colonies grown on standard microbiological media.

Regulatory Guidance
Equipment Qualification / Method Validation Parameter (2)

Criterion	Quantitative test
Accuracy	+
Precision	+
Specificity	+
Detection limit	-(2)
Quantitation limit	+
Linearity	+
Range	+
Robustness	+
Suitability testing	+
Equivalence testing	+

- **Ph. Eur. 2.6.12 and USP<61>:** *"Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeia method has been demonstrated."*
- **Ph. Eur. 5.1.6 (01/2008)** *"Validation of this application would, therefore, require validation of the recovery system employed rather than the entire test."*
- **USP<1223>** *"In the implementation of these enhanced methods for the detection of colony growth, only the detection capability of the method requires verification."*
- **PDA Technical Report TR33**
  - *"Some alternative or rapid technologies may be considered as automated traditional or compendial microbiological test methods, …*
  - *"A risk assessment should be performed to determine the required testing that would support the validation of the alternative or rapid technology."*

Conclusion: The two key parameters to evaluate during the equipment qualification are thus defined as Accuracy and Precision. Both parameters apply to the automated count of a specific sample compared to the human count for the same sample.

Regulatory Guidance
Product-Specific Method Validation

Product-Specific Compendial Method Suitability Test

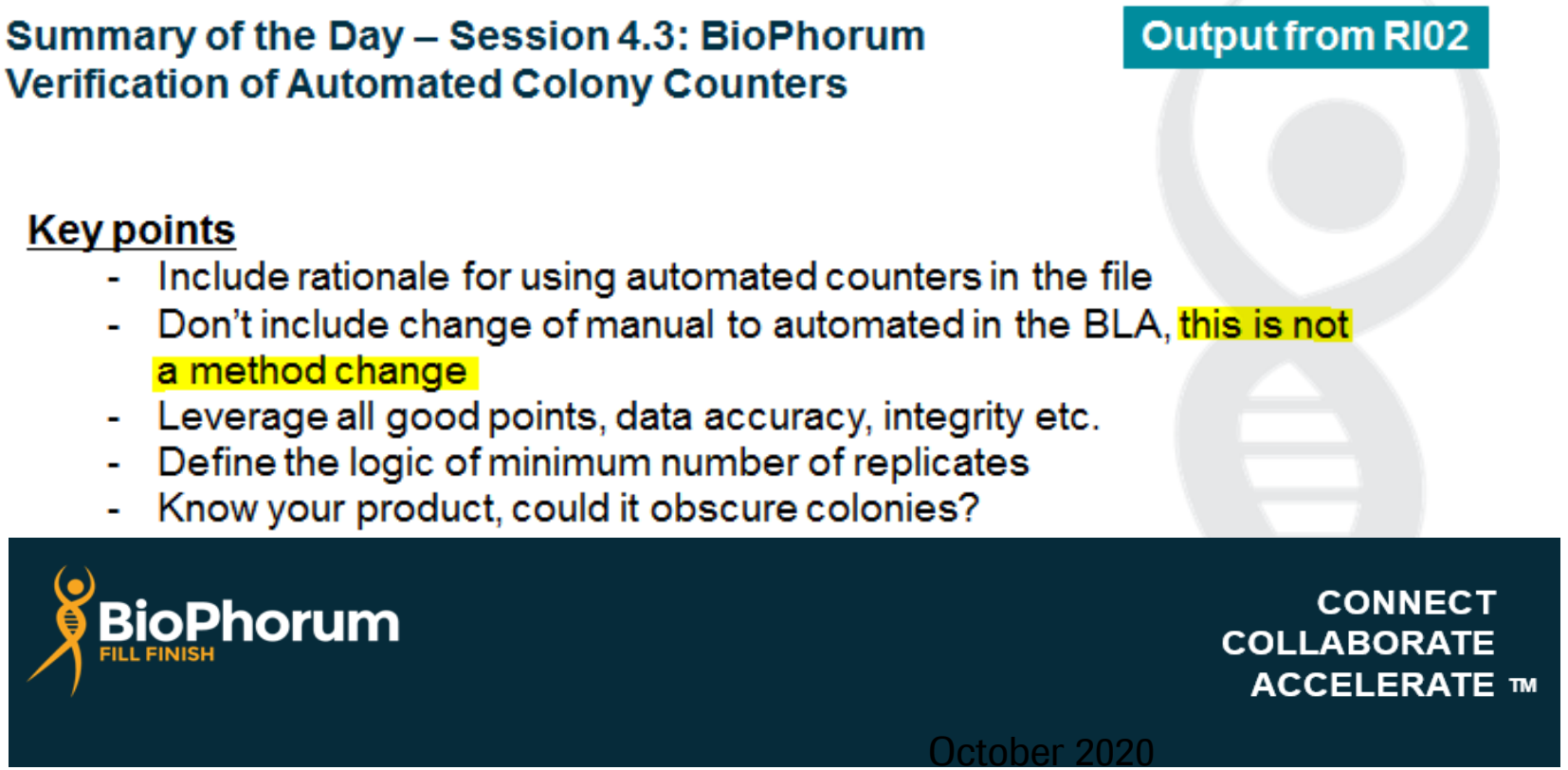
- The Growth Direct technology uses standard membrane and media for microbial growth and standard incubation temperatures to allow CFU to grow and be counted. As these methods and materials comply with the compendia this technology is not defined as an alternative method. As such many of the requirements of USP <1223>, Ph. Eur. 5.1.6 and PDA TR 33 (alternative methods sections) are not applicable and the technology can be validated using standard USP and Ph. Eur. methods.
- The key system parameters for evaluation of the bioburden test are those that are defined in the USP Chapters <61>, and EP 2.6.12.
- The parameters to test will be:
  1. Spike and recovery of micro-organisms to the matrix of interest, Accuracy, and Precision. (note: these are the requirements defined in TR33 for an "*Automated Compendial Method*").
  2. Growth inhibition assay with and without product (Method Suitability Test). Acceptance Criterion: 50 – 200 % recovery of the microorganisms spiked into the sample to be tested.
- Care should be taken to ensure that any liquid samples are readily filterable and do not leave residues on the filter that could obscure colonies or otherwise interfere with the CCD chip-based readout technology.

Regulatory Guidance

Assessment: Alternative Method or NOT? (1)

FDA's Feedback:

- **Minutes FDA-BPOG f2f meeting on 19-OCT-2018 in Bethesda (s. screenshot)**



Regulatory Guidance
Assessment: Alternative Method or NOT? (2)

Feedback from European Authorities (1):

- **PEI/ECA Joint Workshop on Alternative Microbiological Methods on 13-FEB-2019 in Langen (s. screenshot)**

Participants:

- In sum 30 participants
- 15 participants from different competent authorities (reviewer or GMP-inspectors) representing 9 different European countries

Outcome:

- No uniform position of the representatives of the authorities
- Follow-up as part of the f2f Meeting BPOG and EMA/EDQM in Rome (Italy), 27th June 2019

ECA Academy
Your GMP/GDP Information Source

PEI und ECA Joint Workshop on Alternative Microbiological Methods

Schedule, 13 February 2019, Paul-Ehrlich Institut, Langen, Germany

08.30 – 09.00 h	**Welcome and Introduction**
09.00 – 09.20 h	**Look to the Neighbourhood – The Validation Guidance in Food** Barbara Gerten, Chairwoman DIN Working Group Microbiological Food Testing incl. Rapid Methods
09.20 – 09.40 h	**Implementation of AMM's - Expectations of an Authority** Oleg Krut, Paul-Ehrlich Institut,
09.40 – 10.00 h	**Automated Colony Counter – Alternative Method or not?** Dr. Sven Deutschmann, Roche
10.00 – 10.15 h	Short Wrap-Up
10.15 – 10.45 h	Coffee-break
10.45 – 11.10 h	**Cooperation Roadmap on AMM Implementation Roche/GSK/MSD/J&J/Astra Zeneca** Philip Breugelmans, Janssen, Sven Deutschmann, Roche, Chairman ECA Microbiology Working Group,
11.10 – 11.35 h	**CAR-T cells? Challenges with Patients specific Lot Release** Stefan Merkle, Janssen,
11.35 – 12.00 h	**Testing of ATMP** Antonio Rodríguez, Cell Manufacturing Unit. Regional University Hospital, Malaga-IBIMA. GMP Network of the Andalusian Initiative for Advanced Therapies, Spain,
12.00 – 13.00 h	Lunch
13.00 – 13.30 h	**EP Chapter 5.1.6.** Sébastien Jouette, EDQM
13.30 - 14.00 h	**USP 1071** David Roesti, Novartis, Member USP Expert Group
14.00 – 14.15 h	Short Break
14.15 – 15.15 h	**Round Table Discussions**
15,15 – 15.30 h	Short Break
15.30 – 16.30 h	Final Plenum Q&A Session

Regulatory Guidance
Assessment: Alternative Method or NOT? (3)

Feedback from European Authorities (2):

- **EMA/EDQM-BPOG f2f meeting on 27-JUNE-2018 in Rom**

Participants:

- 5 colleagues representing the Agencies (1x EDQM / Ph. Eur.-Department, 4x EMA)
- 18 colleagues representing 11 different pharmaceutical companies
- 2 BPOG-moderator

Outcome:

- Automated colony counting is considered as a change to traditional colony counting methods
- EDQM's representative prefers the following definition for Growth Direct System:
  → ***"Growth-based bioburden test with automated colony counting or readout"***

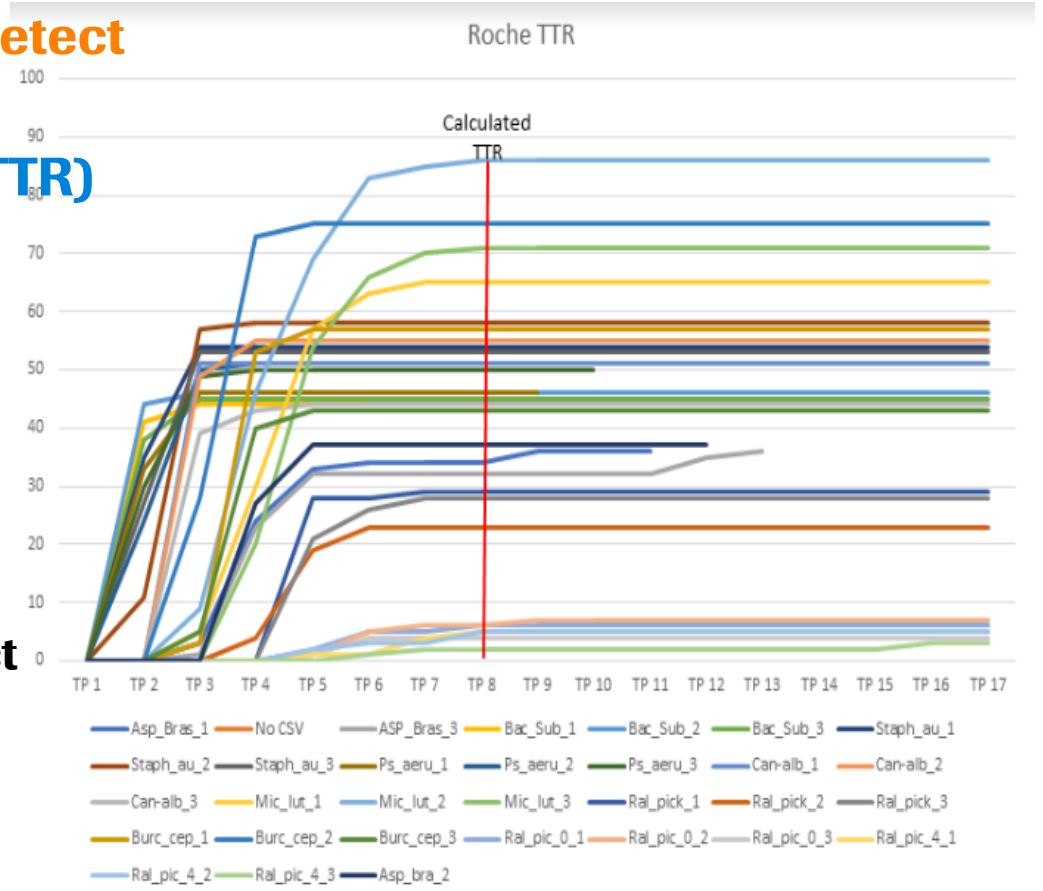
Equipment Qualification / Method Validation

Time-to-Result-Determination

Does the Shorter Incubation Time Impact Ability to Detect Slow-Growing Organisms?

→ Incubation time is determined by Time to Result (TTR) studies

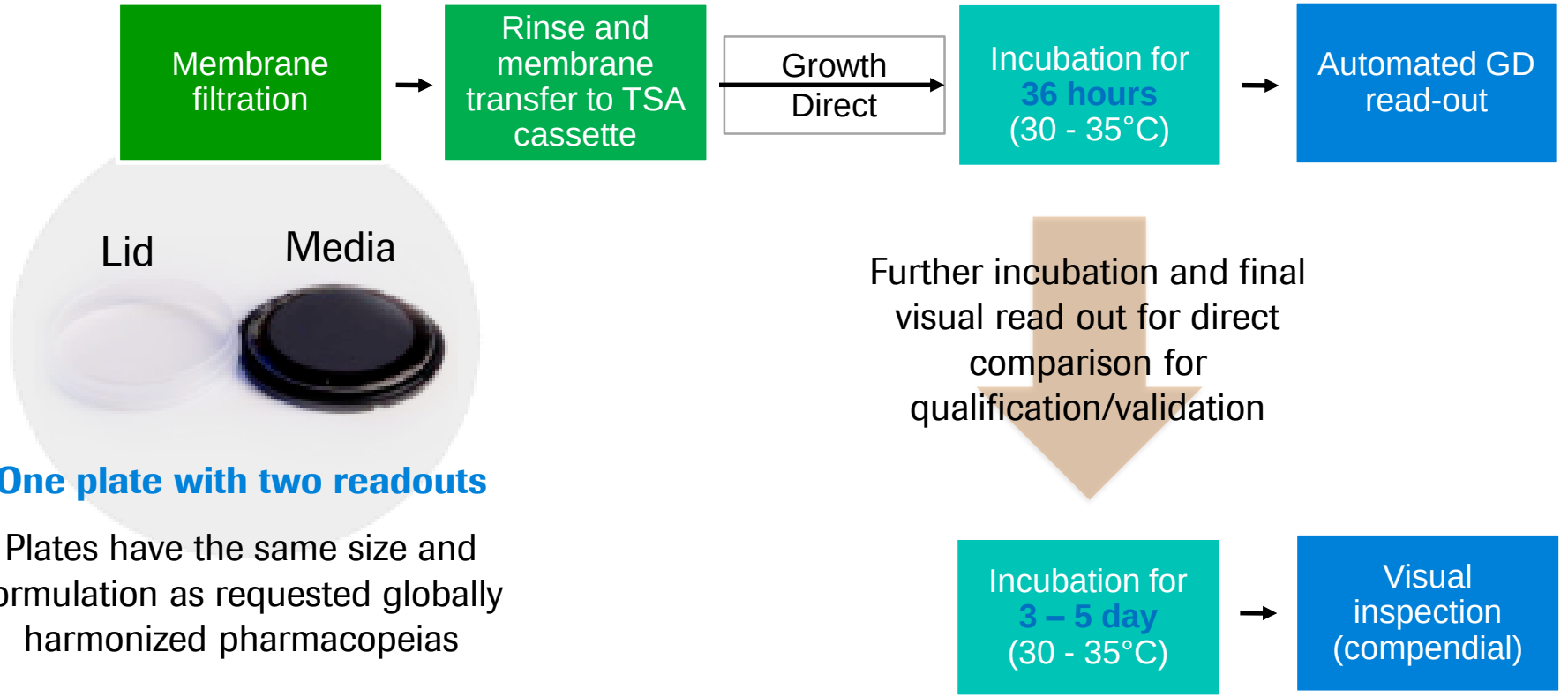
- Independent of whether the readout is visual or automated, **TTR studies determine the appropriate incubation time**
- Including **slow-growing, stressed or sub-lethal damaged organisms** in the TTR study ensures incubation time will detect them during routine testing
- **Conclusion: Automated colony counting does not impact the ability to detect slow-growing organisms.**



TTR-study for product testing

Equipment Qualification / Method Validation

Experimental Setup



One plate with two readouts

Plates have the same size and formulation as requested globally harmonized pharmacopeias

Equipment Qualification / Method Validation

Accuracy & Precision

Design of Experiments:

- For comparison of **Growth Direct (GD)** and **REF-method** (visual counting) consider separately 7 organisms at 60 cfu / 70 cfu level.
- **Per 7 microorganism:**
  - 5 independent analytical runs (different analysts, different lots)
  - 6 replicate samples per run (12 dependent results)
  - Each replicate sample measured twice (GD / REF)
- Number of runs/replicates determined by a power study for accuracy / precision hypothesis testing (minimum power 0.8)

Microorganism X	Growth Direct Replicate Sample (Repeats)						Reference Method Replicate Sample (Repeats)					
Run	1	2	3	4	5	6	1	2	3	4	5	6
1												
2												
3												
4												
5												

7 Microorganisms per run:

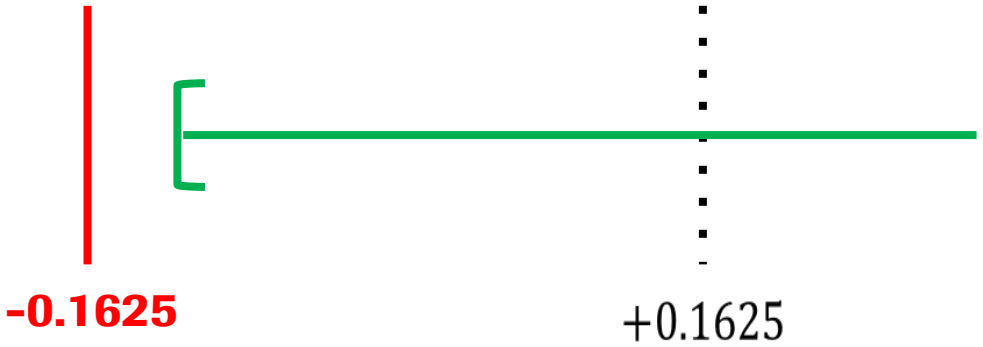
- *A. brasilienis*
- *B. subtilis*
- *C. albicans*
- *P. aeruginosa*
- *S. aureus*
- *M. luteus*
- *R. pickettii*

Equipment Qualification / Method Validation

Accuracy & Precision: Non-Inferiority Testing & Acceptance Criteria

Accuracy:

Non-inferiority is accepted if lower bound one-sided 95% confidence interval for the difference of means is **> -0.1625**

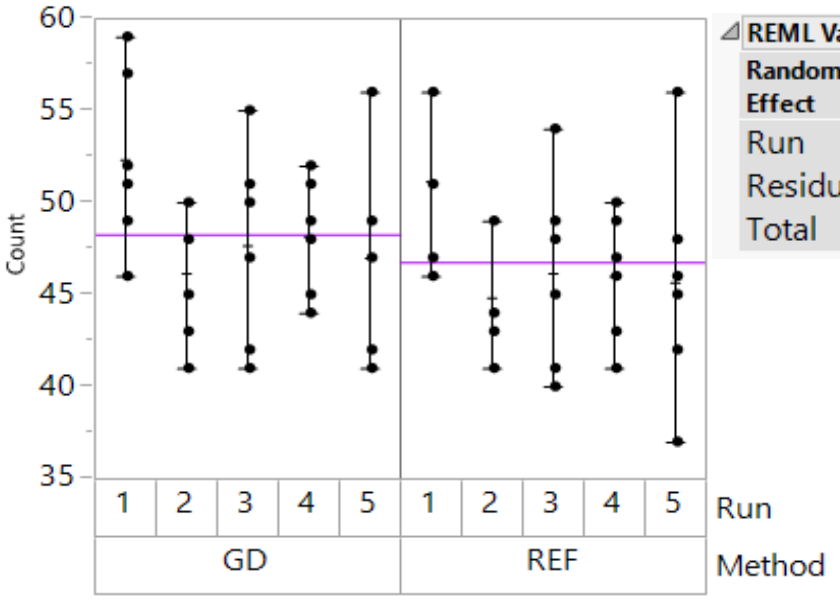


Precision:

Non-inferiority is accepted if upper bound one-sided 95% confidence interval for the variance component „Total“ is **< (0.7)^2 = 0.49**

Equipment Qualification / Method Validation

Validation Example: B. subtilis



REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	90% Lower	90% Upper	Pct of Total
Run	0,0912445	0,0008362	0,0017263	-0,002003	0,0036757	8,362
Residual		0,0091646	0,0025921	0,006085	0,0156806	91,638
Total		0,0100008	0,0027312	0,0067234	0,016752	100,000

Accuracy: **-0.1625 <** X = 0.016 → **PASS**

Precision: 0.017 = X **< 0.49** → **PASS**

LSMeans Differences Student's t

α= 0,100

Mean[i]-Mean[j] / Std Err Dif / Lower CL Dif / Upper CL Dif	LSMean[j] GD	LSMean[j] REF
LSMean[i] GD	0	0,0321
	0	0,00936
	0	0,01621
	0	0,048
LSMean[i] REF	-0,0321	0
	0,00936	0
	-0,048	0
	-0,0162	0

Summary:

- All 5 pharmacopeia and 2 in-house microorganisms passed the accuracy and precision acceptance criteria
- Equivalency (= Non-Inferiority) between automated Growth Direct readout and manual readout can be concluded with statistical significance with this method validation

Equipment Qualification / Method Validation

Validation Results: Summary Accuracy

Accuracy:

Organism	Ratio geom. Mean GD/REF (original scale)	Ratio geom. Mean GD/REF (log scale)	LCL (log scale)*	Acceptance Criterion pass/fail
Aspergillus brasiliensis	1.03	0.0271	**0.0055**	**Pass**
Bacillus subtilis	1.03	0.0321	**0.0162**	**Pass**
Candida albicans	1.01	0.0051	**0.0004**	**Pass**
Pseudomonas aeruginosa	1.00	-0.0026	**-0.0139**	**Pass**
Staphylococcus aureus	1.00	-0.0011	**-0.0054**	**Pass**
Micrococcus luteus	1.00	0.0023	**-0.0019**	**Pass**
Ralstonia pickettii	0.97	-0.0301	**-0.0416**	**Pass**

*Note: acceptance criterion = lower bound one-sided 95% confidence interval for the difference of means is **> -0.1625**

Equipment Qualification / Method Validation

Validation Results: Summary Precision

Precision:

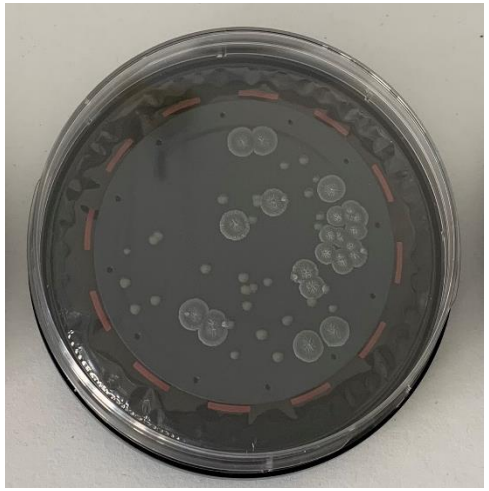
Organism	Growth Direct Method					
	RSD (%) (original scale)	Intermediate Precision				Repeatability
		SD = $\sqrt{VAR}$ (log scale)	VAR (log scale)	UCL* (log scale)	Acceptance Criterion pass/fail	SD = $\sqrt{VAR}$ (log scale)
Aspergillus brasiliensis	11.63	0.1245	0.0155	**0.0255**	**Pass**	0.1230
Bacillus subtilis	9.97	0.1000	0.0100	**0.0168**	**Pass**	0.0957
Candida albicans	6.96	0.0742	0.0055	**0.0097**	**Pass**	0.0674
Pseudomonas aeruginosa	8.52	0.0867	0.0075	**0.0129**	**Pass**	0.0867
Staphylococcus aureus	8.21	0.0821	0.0067	**0.0112**	**Pass**	0.0797
Micrococcus luteus	16.03	0.1683	0.0283	**0.0795**	**Pass**	0.1054
Ralstonia pickettii	18.08	0.1775	0.0315	**0.0558**	**Pass**	0.1598

*Note: acceptance criterion = upper bound one-sided 95% confidence interval for the variance component „Total“ is **< (0.7)^2 = 0.49**

Equipment Qualification / Method Validation

Supportive Studies: Mixed Cultures

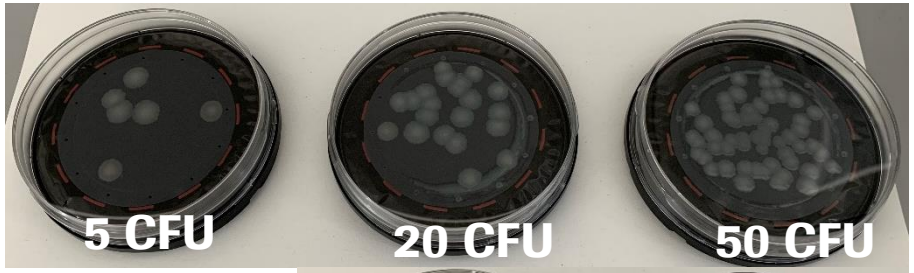
	Growth Direct / [cfu/membrane]	Visual / [cfu/membrane]	Species
Plate 1	42 CFU	38 CFU	*B. subtilis* (18), *R. pickettii (*20)
Plate 2	59 CFU	55 CFU	*B. subitilis* (21), *R. pickettii* (34)
Plate 3	59 CFU	61 CFU	*B. subitilis* (28), *R. pickettii* (33)



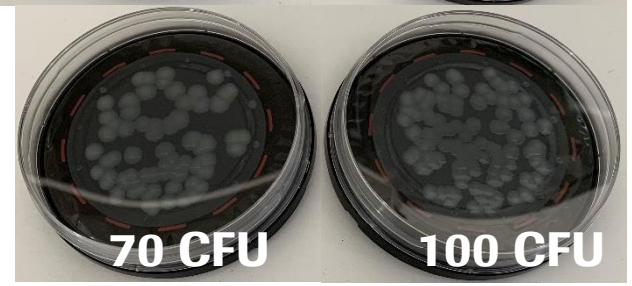
	Growth Direct / [cfu/membrane]	Visual / [cfu/membrane]	Species
Plate 1	98 CFU	101 CFU	*S. aureus* (23), *R. pickettii* (7), *B. subtilis* (31), *C. albicans* (28), *A. brasiliensis* (14)
Plate 2	101 CFU	98 CFU	*S. aureus* (25), *R. pickettii* (9), *B. subtilis* (28), *C. albicans* (22), *A. brasiliensis* (14)
Plate 3	104 CFU	96 CFU	*S. aureus* (26), *R. pickettii* (8), *B. subtilis* (27), *C. albicans* (25), *A. brasiliensis* (10)

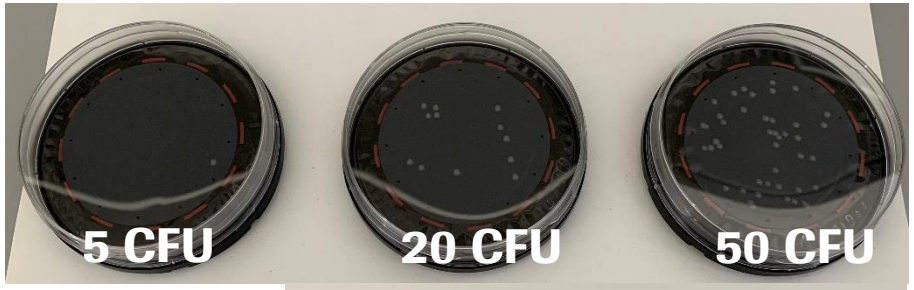
Equipment Qualification / Method Validation

Supportive Studies: Range

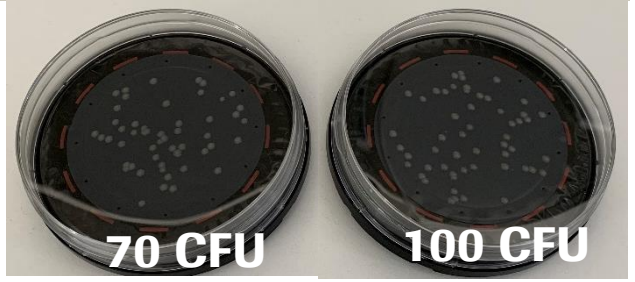


P. aeruginosa (Ps)





R. pickettii (Rp)



Sample	Counted CFU/Platte	
	Growth Direct after 36 h (Mean)	Visual Count after 3 days (Mean)
Ps_100KBE	120	106
Ps_70KBE	72	69
Ps_50KBE	59	56
Ps_20KBE	23	23
Ps_5KBE	6	6
Rp_100KBE	57	56
Rp_70KBE	50	49
Rp_50KBE	33	35
Rp_20KBE	11	11
Rp_5KBE	1	1

Numbers underneath the plates are inoculated CFU/plate

Equipment Qualification / Method Validation

Method Suitability Test for Drug Substance

Results of the Method Suitability Test (example: 3rd PPQ batch):

PPQ 3	Growth Direct			Visual		
Release sample	Reference	after 36h	Recovery %	Reference	MW visual	Recovery %
Staph. aureus	23	27	117.39	23	27	117.39
P. aeruginosa	26	26	100.00	26	26	100.00
B. subtilis	25	22	88.00	25	22	88.00
A. brasiliensis	23	14	60.87	23	14	60.87
C. albicans	33	27	81.82	33	27	81.82
R. pickettii	41	29	70.73	43	29	67.44
Staph. hominis	79	56	70.89	83	57	68.67

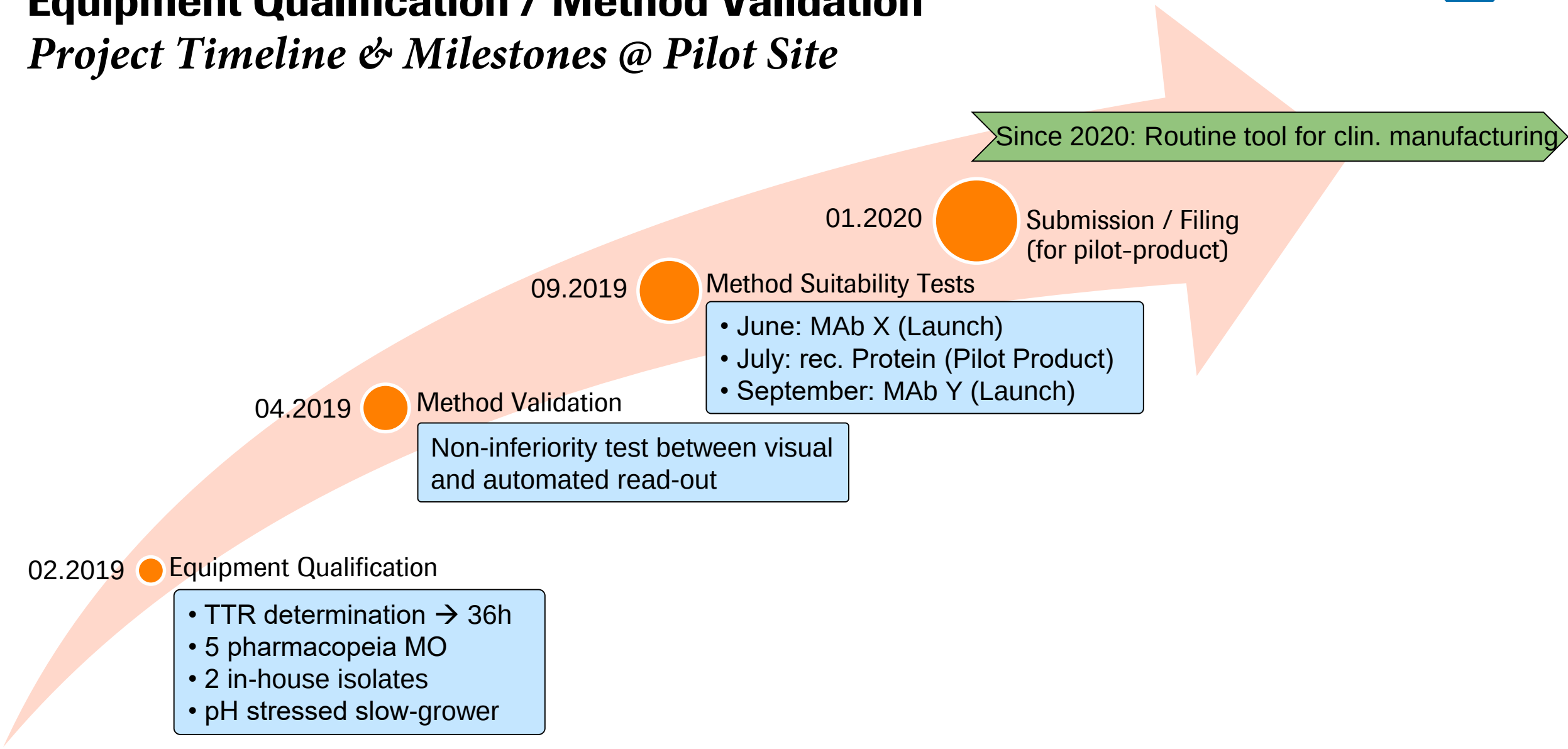
Note: Numbers are cfu per membrane

Summary:

- the Drug Substance samples of all three PPQ-batches passed the method suitability test

Equipment Qualification / Method Validation

Project Timeline & Milestones @ Pilot Site



Acknowledgements

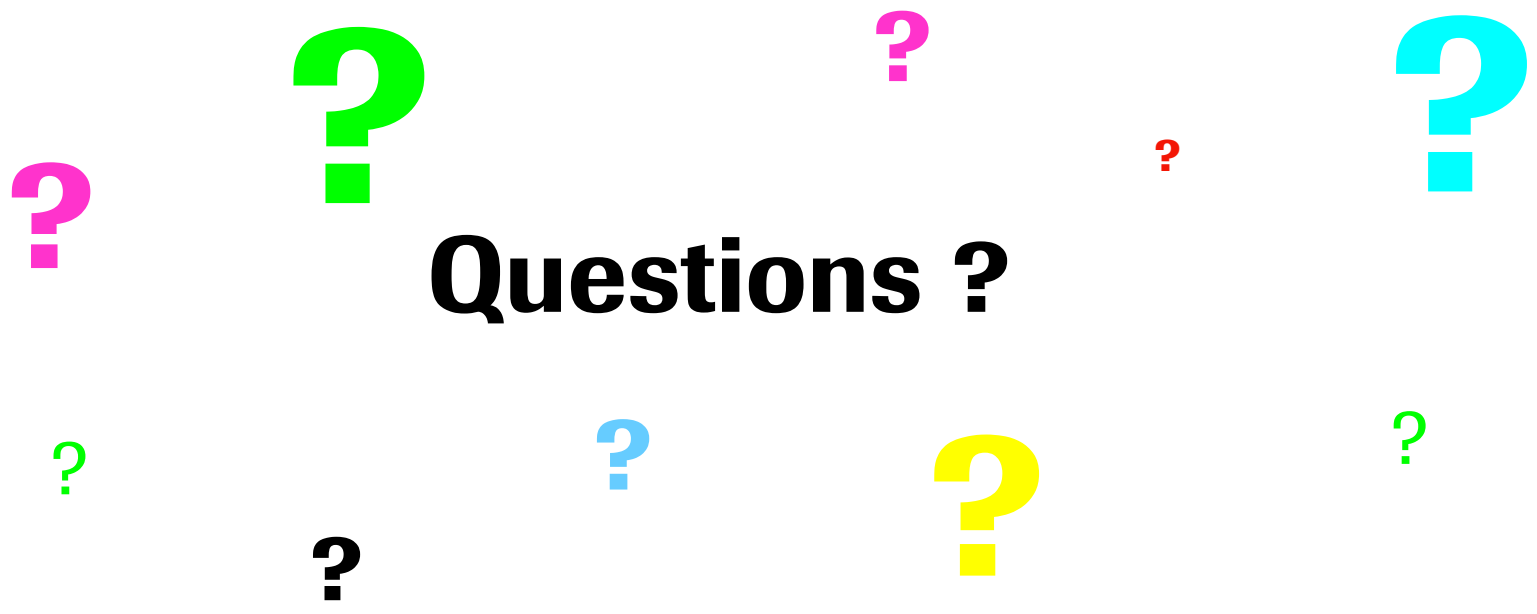
Microbiology

Microbiology Dept. QC Penzberg (Germany)

Statistical Support

Dr. Viviane Grunert da Fonseca

Thank you for your attendance !



APPENDIX

Appendix 1: Regulatory Guidance
Equipment Qualification / Method Validation Parameter (1)

Ph. Eur. and USP Requirements:

- **USP <61>,** "Microbiological Examination of Non-Sterile Products: Microbial Enumeration Methods", and **EP 2.6.12** "Microbial Examination of Non-Sterile Products: Microbial Enumeration Tests" both state that ***"Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeia method has been demonstrated."***
- **EP 5.1.6 (01/2008)** "Alternative Methods for the Control of Microbiological Quality" states: "*It is critical to the validation effort to identify the portion of the test addressed by the alternative method. For example, there are a variety of methods available to detect the presence of viable cells. These methods may have applications in a variety of tests (e.g. bioburden, sterility tests) but may not, in fact, replace critical aspects of the test entirely.* [...] ***Validation of this application would, therefore, require validation of the recovery system employed rather than the entire test."***
- **USP40/NF35 <1223>** "Validation of Alternative Microbiological Methods" 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**

Appendix 1: Regulatory Guidance
Equipment Qualification / Method Validation Parameter (2)

Specific USP Requirements

- **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.
  - **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"*

Appendix 1: Regulatory Guidance
Equipment Qualification / Method Validation Parameter (3)

PDA Technical Report 33 Requirements:

- The **PDA Technical Report TR33** "*Evaluation and Validation of New Microbiological Test Methods*" (Sept. 2013) contains the following text: "***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. A risk assessment should be performed to determine the required testing that would support the validation of the alternative or rapid technology.*"
- The two key parameters to evaluate during the equipment qualification* are thus defined as **Accuracy and Precision**. Both **parameters apply** to the automated count of a specific sample compared to the human count for the **same sample**.

* note: although the methodology is unchanged some companies define this step as primary method validation rather than equipment qualification

Appendix 2: Statistical Model
Accuracy: Non-Inferiority Testing & Acceptance Criterion

Statistical Model:

The validation parameter "accuracy" is studied per organism with the aim of verifying whether the (geometric) mean ratio of the GD counts to the REF counts (visual counting of colonies by abalysts) is greater than 0.85 (H_1 = non-inferiority hypothesis). The non-inferiority test is carried out with log-transformed data, where, in log-scale, the mean difference is considered with respect to the non-inferiority margin $\Delta = -\log(0.85) = 0.1625$.

The acceptance criterion for accuracy is passed, if the non-inferiority test can reject the null hypothesis H_0 for a significance level $\alpha = 0.05$. This is the case, when, based on the log-transformed data, the lower limit (LCL) of the one-sided 95% confidence interval for the difference of means $[X ; \infty)$ is greater than $-\Delta = -0{,}1625$.

Statistical Procedure:

- Fit **mixed effects repeated measures model** to log-transformed GD/REF count data to account for dependencies in the data
- Statistical hypothesis test with non-inferiority hypothesis (H_1): *GD accuracy **equivalent** with or **much better** than REF accuracy*
- Hypothesis test considers **difference of means of (natural) log-transformed data**

  → Non-inferiority means difference $> -0.1625 = -log(0.85)$

  For original data this corresponds to ratio of (geometric) means

  → Non-inferiority means ratio > 0.85

Acceptance Criterion:

➢ **Non-inferiority is accepted** if lower bound one-sided 95% confidence interval for the difference of means is > -0.1625

Appendix 2: Statistical Model

Precision (Intermediate): Non-Inferiority Testing & Acceptance Criterion

Statistical Model:

The validation parameter "precision" was studied per organism with the aim of verifying whether the intermediate precision in terms of RSD is smaller than 0.7 (H_1 = non-inferiority hypothesis). The non-inferiority test was carried out with log-transformed data, where in log-scale, the standard deviation (SD) is compared against the value 0.7. Given that the count data is negative-binomial distributed, for small values, SD of the log-transformed data corresponds to RSD of the original data. For larger values it holds that $SD_{log\text{-}data} > RSD_{original\text{-}data}$. If the non-inferiority test decides with statistical significance, that $SD_{log\text{-}data} < 0.7$, then, as a consequence, one can also conclude that $RSD_{original\text{-}GD} < 0.7$.

The acceptance criterion for precision was passed, if the non-inferiority test can reject the null hypothesis H_0 for a significance level $\alpha = 0.05$. This is the case, when, based on the log-transformed data, the upper limit (UCL) of the one-sided 95% confidence interval for the variance component "total" (0 ; X] is smaller than 0.7 squared (= 0.49).

Statistical Procedure:

- Fit **repeated measures random effects model** to log-transformed GD count data to account for dependencies in the data
- Statistical hypothesis test with non-inferiority hypothesis (H_1):*GD precision **equivalent** with or **much better** than (fixed) reference precision*
- Hypothesis test considers **Standard deviation (SD) of (natural) log-transformed data**

  → Non-inferiority means SD < 0.7

  For original data this (appr.) corresponds to relative standard deviation (RSD)

  → Non-inferiority means RSD < 0.7

Acceptance Criterion:

➢ **Non-inferiority is accepted** if upper bound one-sided 95% confidence interval for the variance component „Total" is $< 0.7^2$

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