Automated Environmental Monitoring

"The solution for the next decade"

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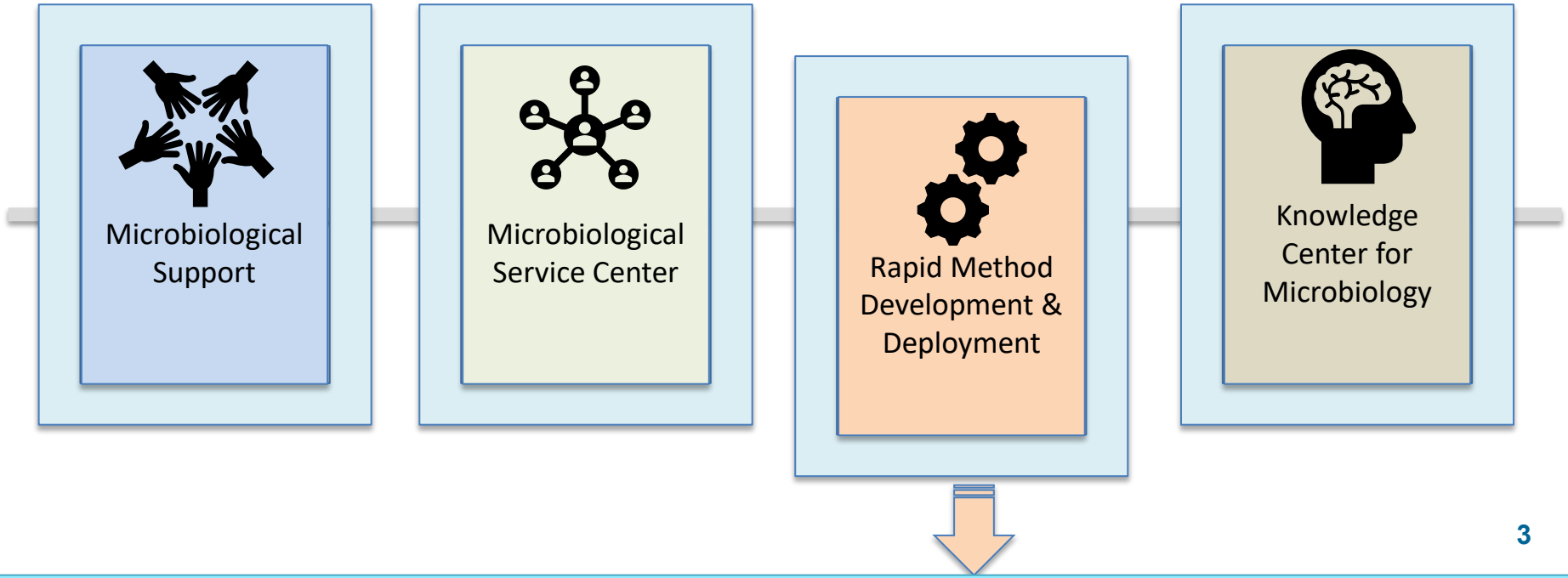
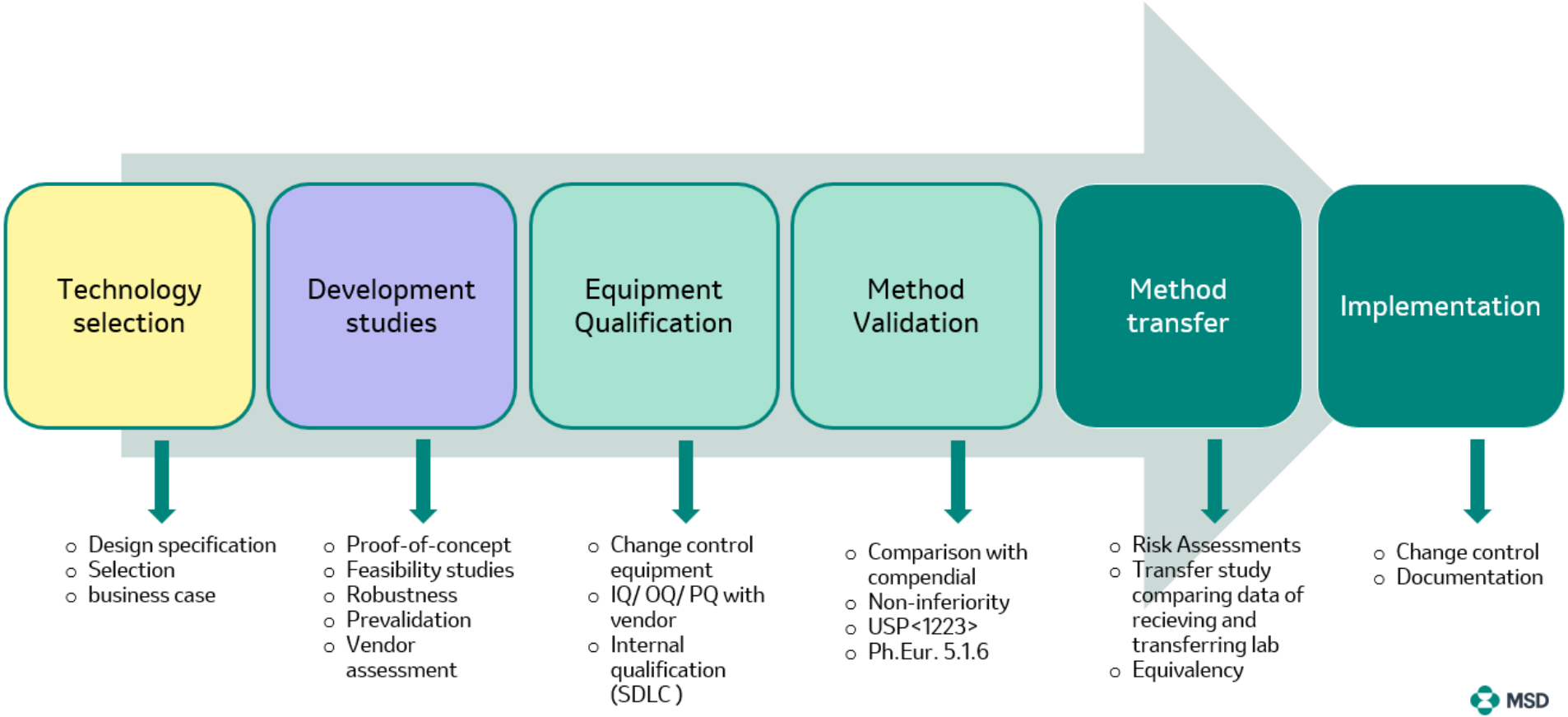

Microbiological Support
Microbiological Service Center
Rapid Method Development & Deployment
Knowledge Center for Microbiology

Our vision Rapid methods roadmap



MSD

Our vision Rapid methods roadmap II

Methods in development

- **Rapid Mycoplasma**
  - Film-array
- **Rapid Bioburden testing**
  - Vision-based
  - Autofluorescence of colonies
- **Water bioburden testing**
  - Online water bioanalyzing (OWBA)
- **Air monitoring**
  - Biofluorescent particle counting (BFPC)
- **Absence of contaminants testing (AVA, Mycoplasma)**
  - NGS
- **Endotoxin testing**
  - Fluorescent probe (non-LAL)

Methods in validation

- **Rapid MVM test**
  - qPCR
- **Automated EM**
  - High Resolution Optics

Methods in deployment

- **Rapid Sterility testing**
  - CO2 detection
- **Rapid Bioburden testing**
  - Fluorescent staining of colonies
- **Rapid water testing**
  - Fluorescent staining of colonies
- **Automated Environmental Monitoring**
  - Autofluorescence of colonies
- **Rapid Identification**
  - 16S rDNA
  - MaldiTOF
- **Rapid Mycoplasma test**
  - qPCR detection after enrichment
- **CHO residual DNA**
  - qPCR detection

Automated EM methods Problem & Solution

Current State → *Future State*

- High touch times
- Labour intensive
- Multiple handovers

Innovation is key to business survival

- Increase efficiency by automated colony counting
- Decreasing manual handovers

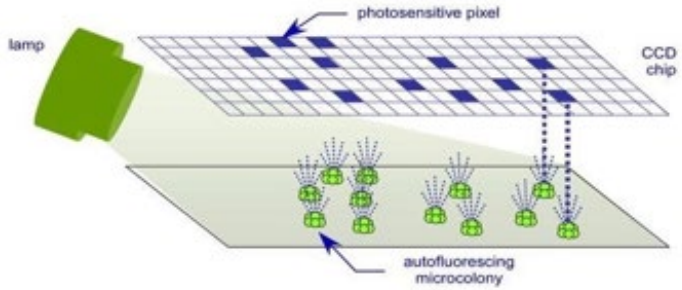
- Data Integrity improvements
- Creating paperless workflow

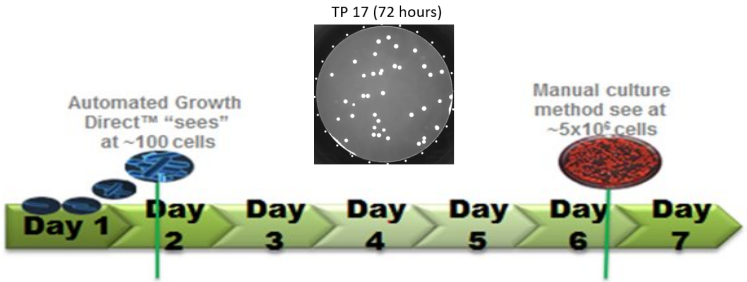
- Decrease time to results

- Costs savings & avoidance

Automated EM methods *New Technologies…*

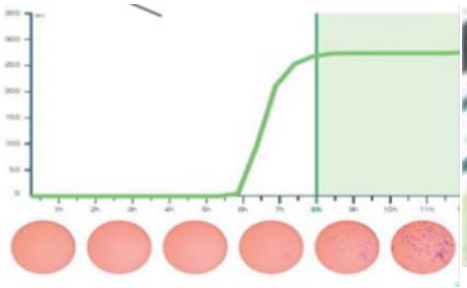
System 1





Method Validated Q2 2020

System 2



Development testing completed, starting validation Q3 2020

Automated EM methods *High level differences*

Parameter	System 1	System 2
Maturity	Established	New
Detection principle	Autofluorescence	Vision-based
Type of consumable	Media overlaid by black membrane	Petri dishes
Format Type	60 mm	Contact plates and 90/100 mm
Capacity	>600 Cassettes	300 plates
Incubation	Two incubators	One incubator
Frequency of reading	Every 4 hours	Every hour
Image availability	Not available	Images and video available for review
Access to system	Completely locked down	More open
Treatment of Negative Plates	Negative plates are automatically discarded	Negatives removed manually

Automated EM methods *Path Forward*

USP<1116> Microbiological Control:

"Typically, for general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20°C–35°C have been used with an incubation time of not less than 72 hours."

FDA guidance: ***The microbiological culture media used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and molds) as well as bacteria and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35oC for 48 to 72 hours. Total combined yeast and mold count can generally be obtained by incubating at 20 to 25oC for 5 to 7 days.***

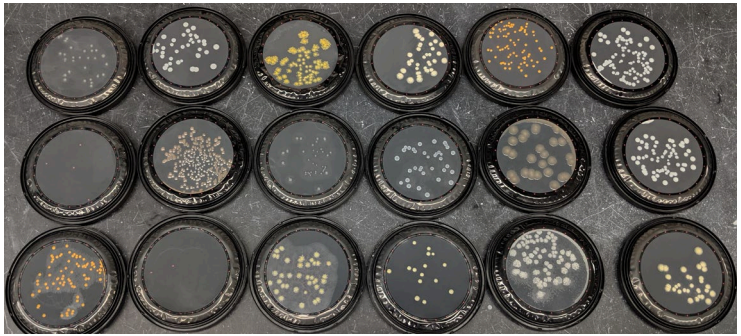
Ph. Eur: ***No guidance on temperature regimes and incubation times***

Establishing Multiple EM methods in the Merck RMM portfolio, while using a Single Temperature regime.

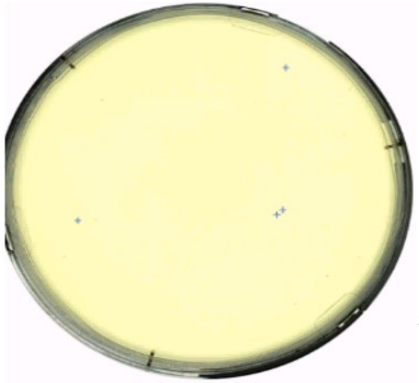
Results Time to Results (TTR) at Single Temperature 28°C for both systems

STUDY AIM:

- Three incubation regimes assessed in the same system.
- Both systems assessed
- Temperature regimes:
  - **Single temperature: 28˚C** for 7 days.
  - **Low to High (L2H)** : 20-25˚C for 72 h followed by 30-35˚C for 72 h
  - **High to Low (H2L)**: 30-35˚C for 72 h followed by 20-25˚C for 72 h
- Large panel of 30 microorganisms of which ~20% fungi

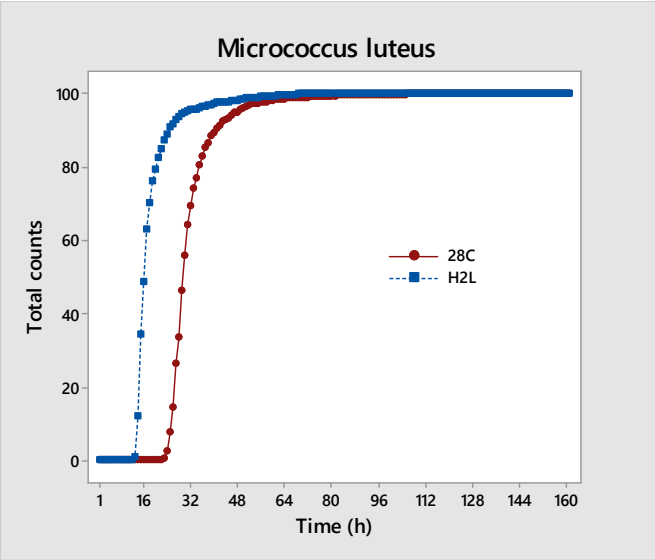


Above: a Selection of varied bacterial colony morphologies by using method 1.

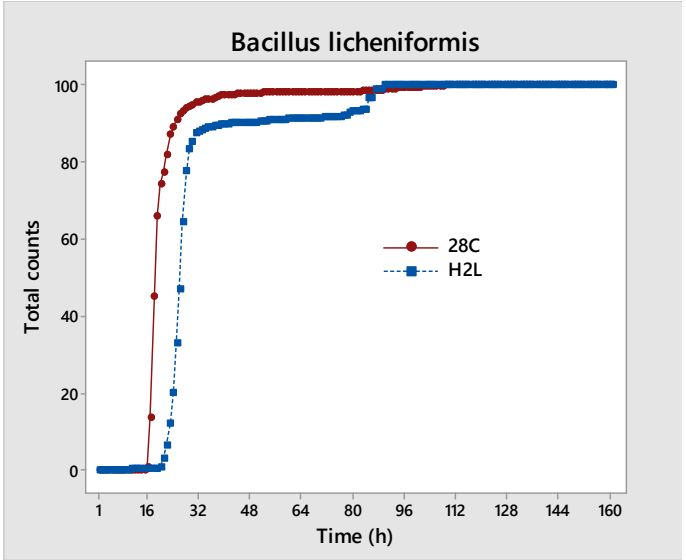


Left: Vision based colony counting in early stage of incubation by using method 2

Comparing TTR in one system using different incubation regimes (single Temp at 28°C and H2L)

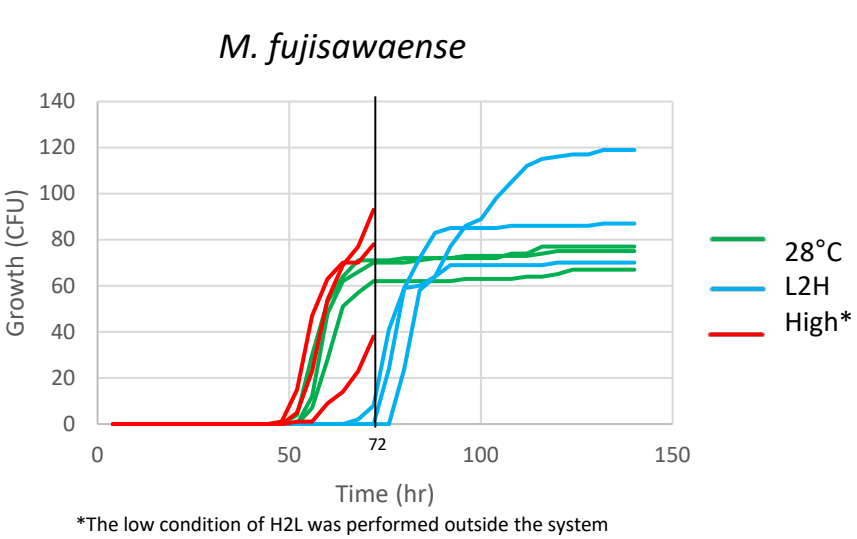


Example of strain that grows faster at high temperature



Example of strain that grows faster at low temperature

Comparing TTR in one system using three different incubation regimes (single at 28°C, H2L and L2H)



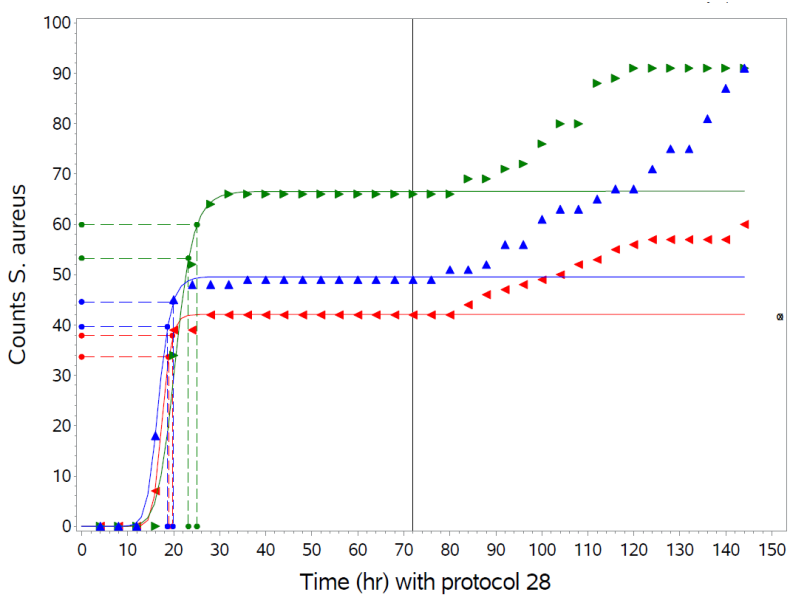
*The low condition of H2L was performed outside the system

When incubation moves from "low" condition to "high" condition, growth of *Methylobacterium fujisawaense* begins to show.

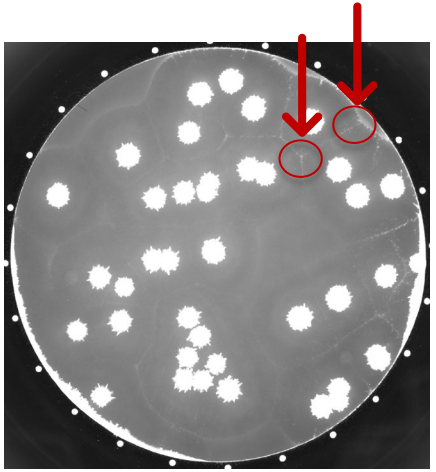
Overall conclusions:

- All strains were well recovered using 28°C.
- Temperature sensitivity of strains was observed.
- In general H2L had the lowest TTR except for some strains that didn't grow at high temperature e.g. *Epicoccum nigrum.*
- Some strains didn't grow well at L2H e.g. *Staphylococcus hominis* and *Methylobacterium fujisawaense.*

- Extended incubation may result in false positive signals after reaching the plateau (final count)



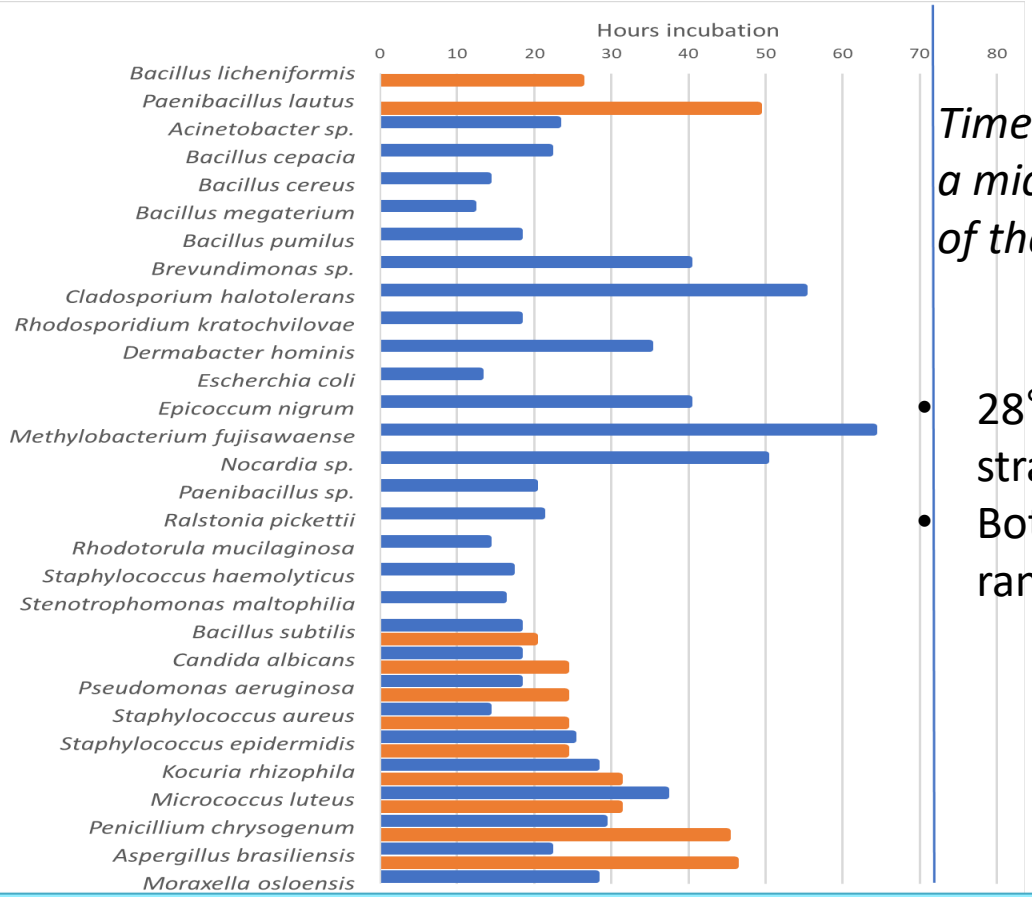
S. aureus metabolizes lecithin and the metabolic byproducts autofluoresce.



Halo forming around colonies after 72 hours

Two graphs of the TTR study in system 1 showing increased counts after reaching the plateau and after 72 hours (vertical line). Green, blue and red are replicate samples (triplicates).

Results Time to Results (TTR) at Single Temperature 28°C for both systems (V)



Time To Result is defined as the time that a micro-organism will need to reach 90 % of the plateau

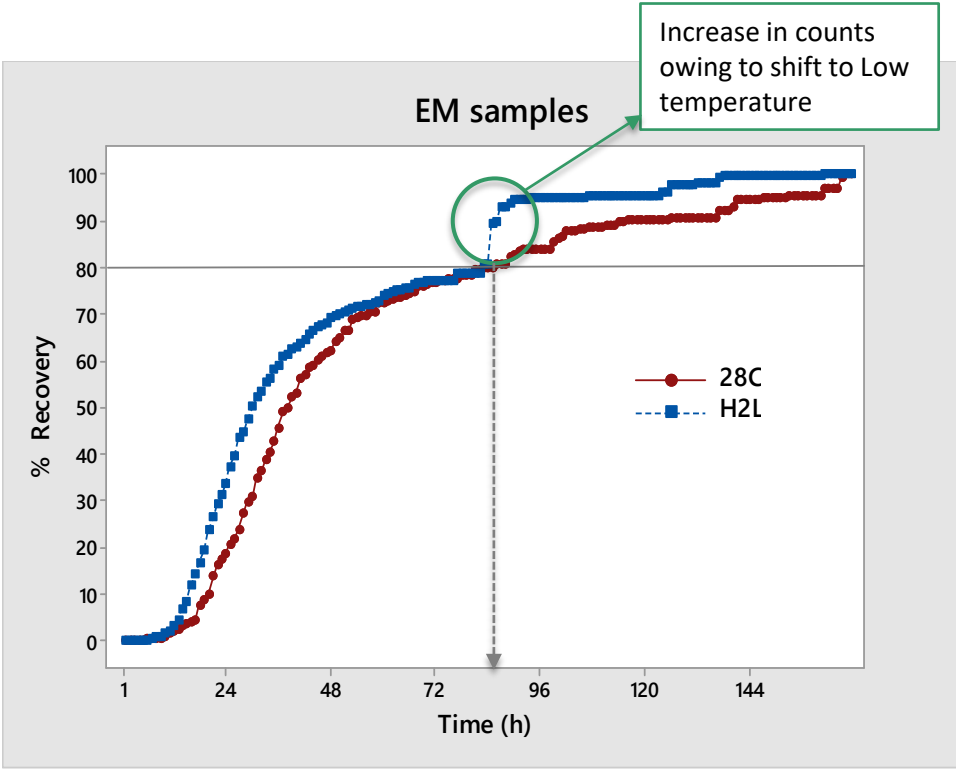
- 28°C Incubation reaches TTR of tested strains <72hr
- Both systems are able to detect a wide range of micro-organisms.

Legend

Blue bars = System 1
Orange bars = System 2
Solid blue line = 72 hours

In total 150 ***routine*** environmental monitoring samples were tested at two temperature regimes in system 2

- Results show that H2L has a shorter TTR than single temperature at 28C
- At 80 hours approx. 80% is recovered at both H2L and at single temperature regime.
- Environmental strains have a delayed growth due to environmental stress



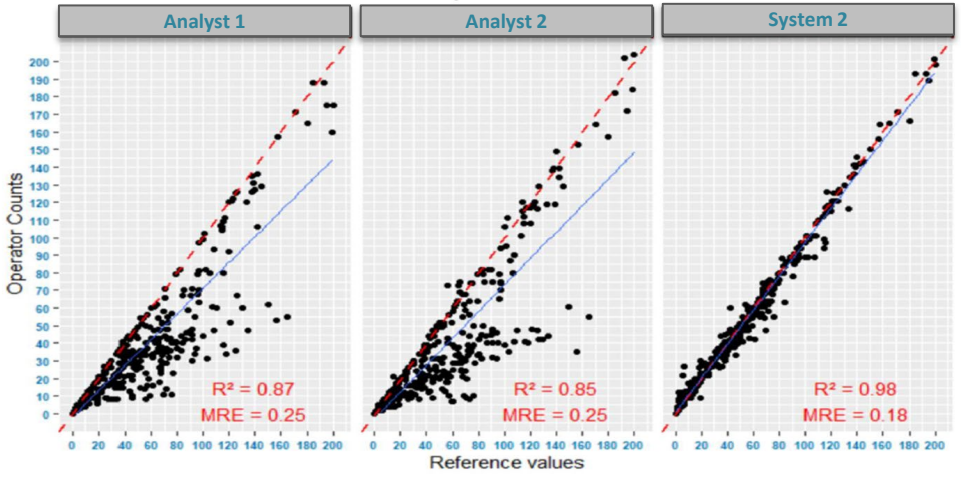
Results Counting performance comparison (system 2 vs human eye)

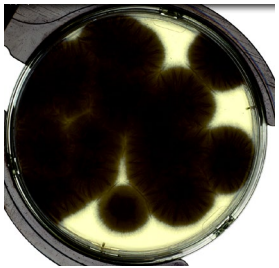
A reference value was used to define the counting performance of the readers (analysts & system).

The reference value is defined by comparing individual readings of the two analysts and the system and verifying the data with the images and video.

Example	Counts	Reference Value	FN	FP
Analyst 1	10	10	0	0
Analyst 2	9		1	0
System 2	11		0	1

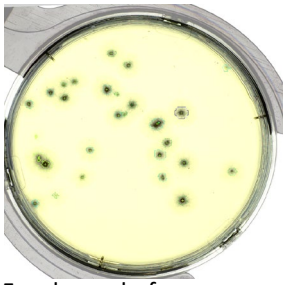
Inoculated plates (n=240) & EM plates (n=150)





Strains diversity and encorachment

Going back in time



Few hours before

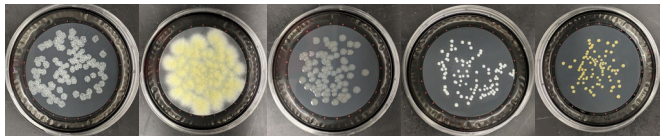
Conclusion:

- System performs better in the colony counting, because analysts tend to miss colonies during counting.

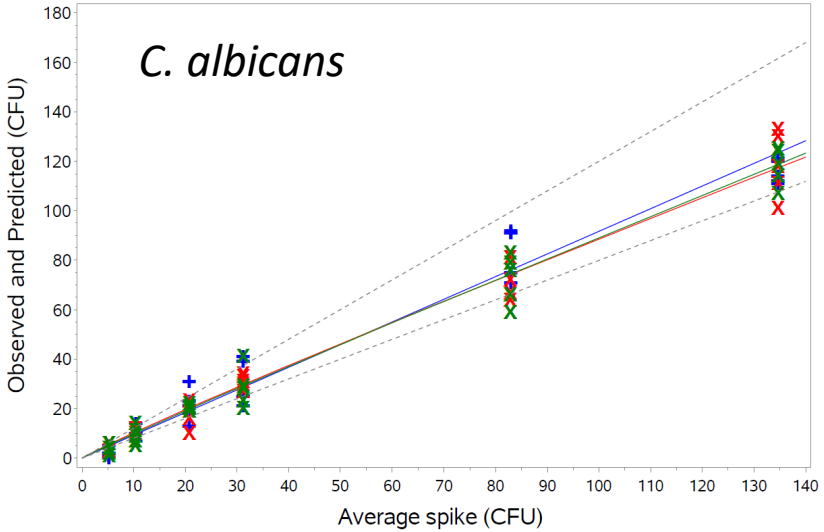
Rapid Method
72 hours at 28°C with automated colony counting

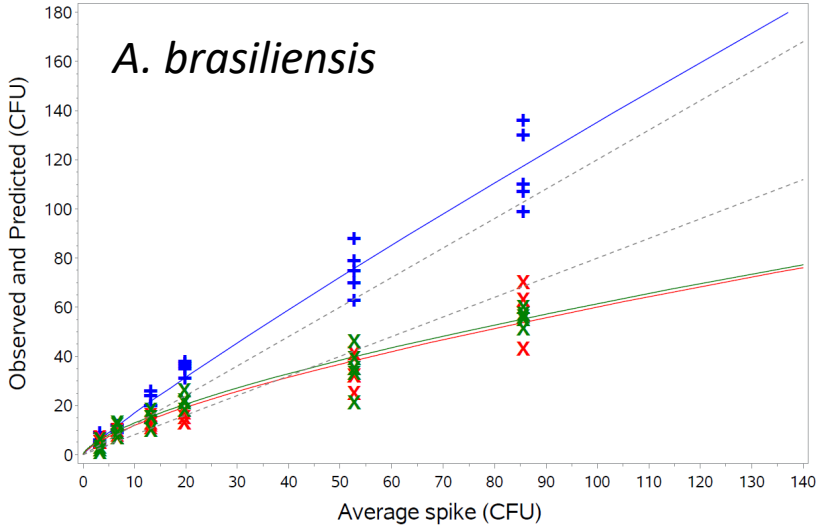
Traditional Methods
"High-to-low" and "Low to High" with manual reads

Overview of Validation of Automated EM

Parameter	Experiment	Result
Specificity	11 EM strains selected from TTR studies	Pass ✓
Precision • Repeatability • Ruggedness	8 runs, 2 strains, 2 analysts, 2 spike levels, 3 media lots of each	Pass ✓
Accuracy	5 standard ATCC strains tested at 6 spike levels (5-130 CFU) 	Pass ✓
Linearity		Pass ✓
Range		Pass ✓
LOQ and LOD		Pass ✓
Non-Inferiority		Pass ✓

Results System 1 Linearity



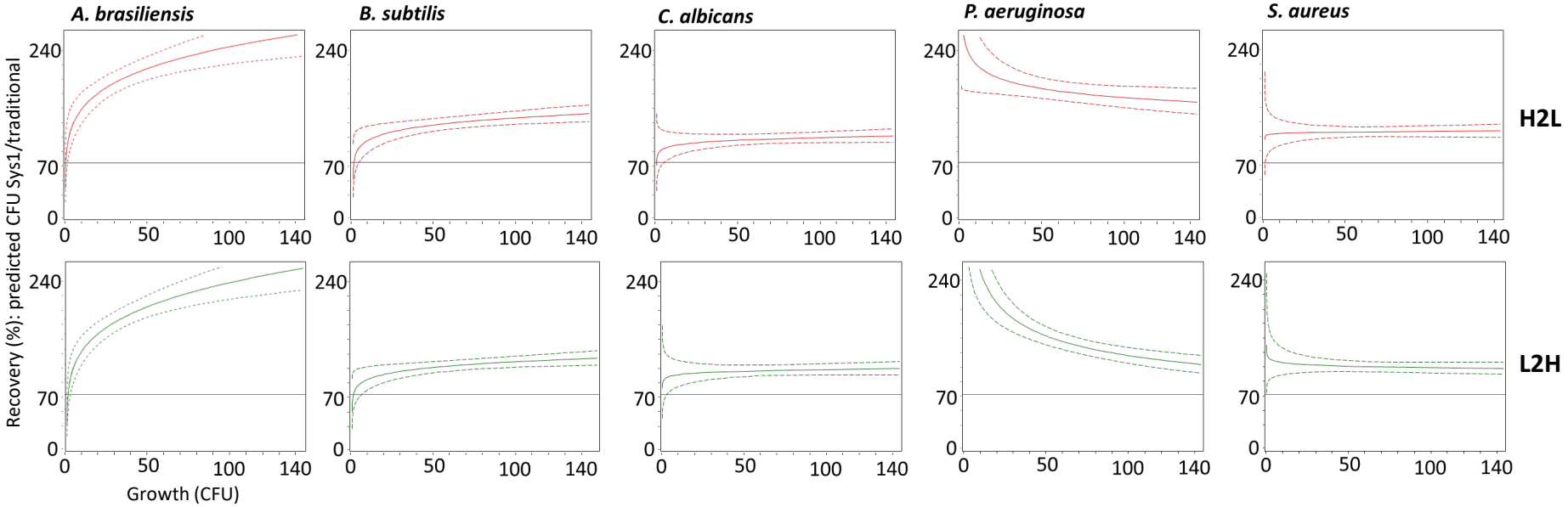


- System 1 out-performs at higher spike levels, especially in *A. brasiliensis*
- **Linearity**: Approximate linearity versus the average spike level holds for a method in the range where the solid line is between the grey lines. Counts are close to (e.g. *C. albicans*) or greater than (e.g., *A. brasiliensis*) the average spike levels, based on the inoculum controls, acceptable linearity.

Legend

- Blue line = System 1 results
- Red line = H2L results
- Green line = L2H results
- **x** and **+** = individual counts
- Dashed grey lines = 80% average spike, 120% average spike

Results System 1 Accuracy, Non-Inferiority



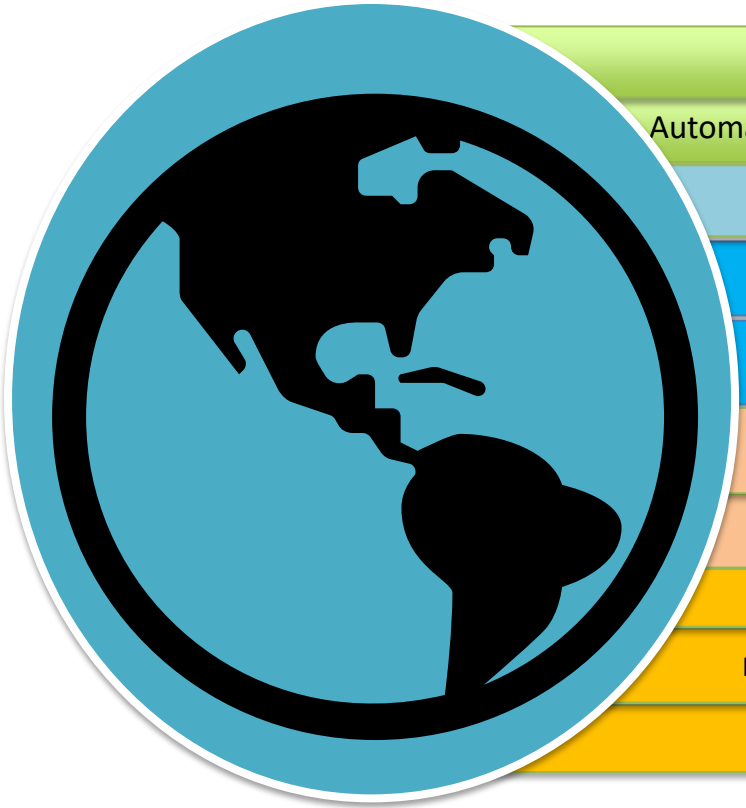
Accuracy: 90% CIs are above 70% throughout the tested range.

Non-inferiority is satisfied at a certain average spike level if the 90% CI is entirely above or equal to 70%, i.e. where the lower dashed line is above the reference line at 70%

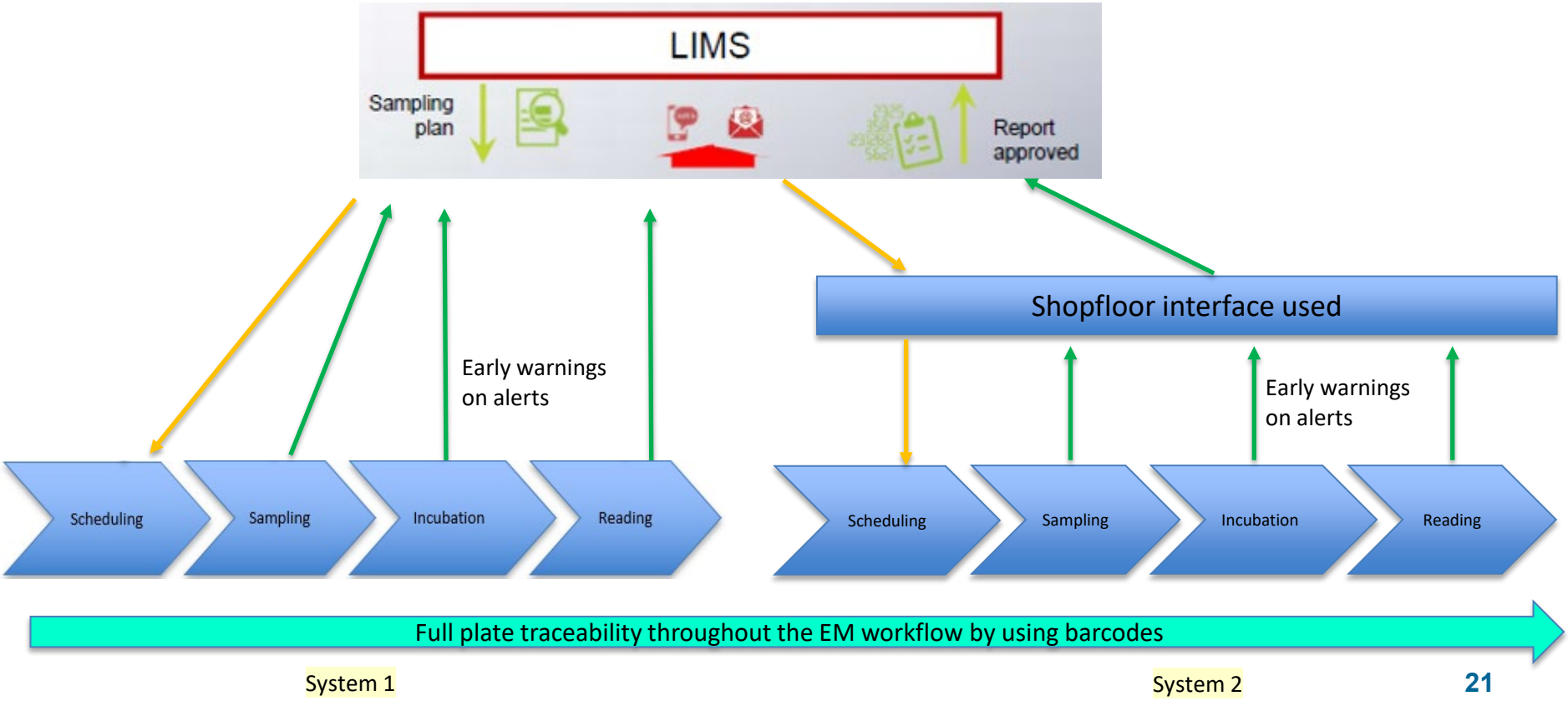
Legend

- Green Dotted line = 90% CI based on L2H
- Red Dotted line = 90% CI based on H2L
- Solid red/green line = Recovery (%): predicted CFU ratio System1/traditional
- Solid gray line = 70% recovery threshold (reference line)

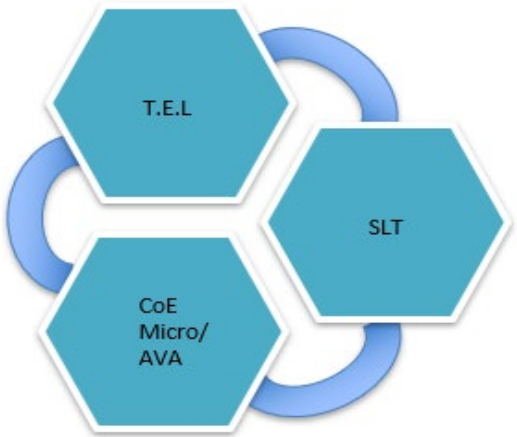
Implementation Strategy Starting a business case….



- Microbial counts by the system are more accurate than the human eye
- Automated Env. Monitoring avoids invalid results due to confluent growth on plates
- A single temperature regime avoids transfers of plates between incubators
- 3 day incubation regime results in faster release of EM campaigns
- Early indication of non compliant situations
- Improved data integrity level by implementing a fully digitalized workflow
- Instant status updates for plate counts
- Automated EM leads to cost savings in FTEs
- Instruments do not requiere expensive lab space to reduce costs for new designed facilities
- Qualified automated counting avoids a 4-eye principle for plate reading


LIMS
Sampling plan
Report approved
Shopfloor interface used
Early warnings on alerts
Early warnings on alerts
Scheduling
Sampling
Incubation
Reading
Scheduling
Sampling
Incubation
Reading
Full plate traceability throughout the EM workflow by using barcodes
System 1
System 2

- 1. Starting Assessment on system selection
- 2. Starting collaboration
- 3. Breaking down the complete workflow, to calculate the costs&avoidance step by step
- 4. Summarize Investment, Avoidance and Savings translating into a ROI
- 5. Assess system benefits on Quality, Complaince, DI & Safety aspects
- 6. Decision taking



Thank you, for your attendance

MSD CoE Microbiology / AVA


Niels Visschers


Marja Claassen


Jessica Long


Regina Flores


Mark Nabuurs


Pieta IJzerman - Boon

In collaboration with:

- Analytical sciences MRL Microbiology
- Center for Mathematical scienses
- Sterile Microbial Quality Assurance