Microbial Contamination and Control Conference

A Global Approach to New Technology Introduction and Validation: Automated Colony Counting

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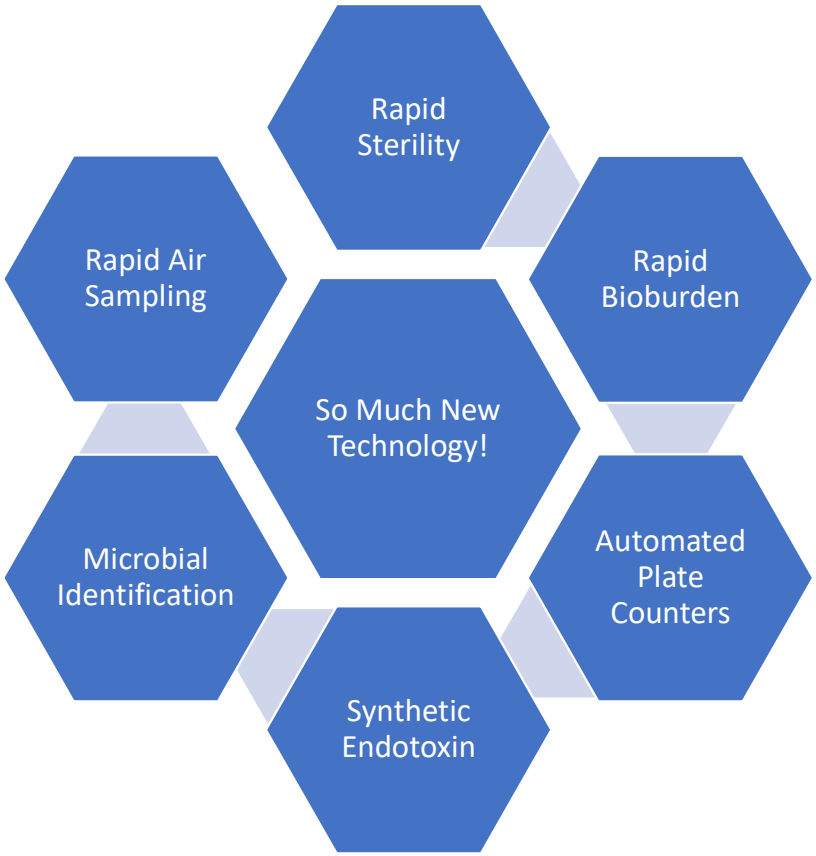
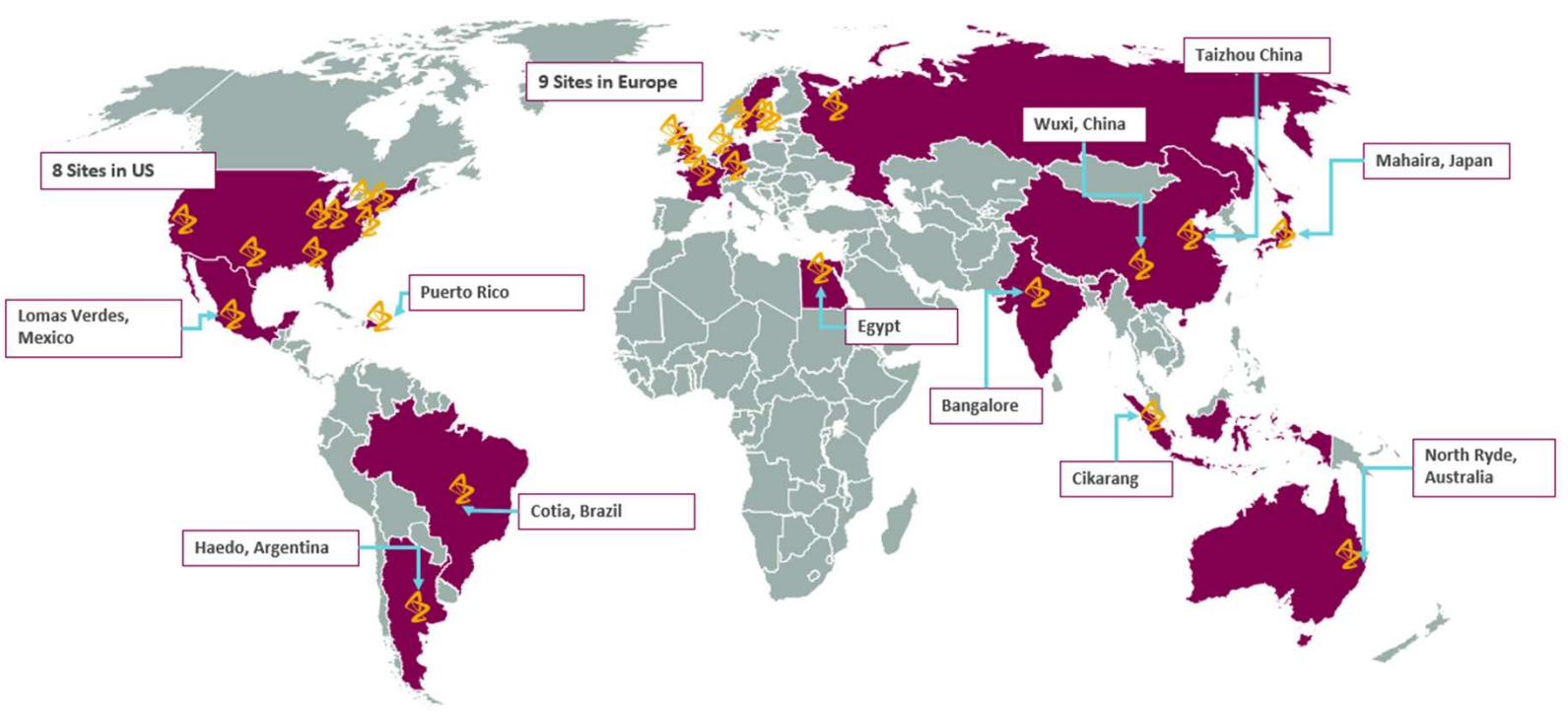
Reasons for the Global Introduction of Technology: Lessons Learned Through Experience

Local Validation teams often want additional validation	Local QA often want additional requirements	Local IT have additional requirements	Health & Safety requirements vary globally
Equipment that never gets unpacked	Equipment that is unable to be validated	Equipment tagged out of service on benches taking up space (still being calibrated)	New technology doesn't replace the existing, both are kept


Rapid Sterility
Rapid Air Sampling
Rapid Bioburden
So Much New Technology!
Microbial Identification
Automated Plate Counters
Synthetic Endotoxin


9 Sites in Europe
Taizhou China
Wuxi, China
Mahaira, Japan
8 Sites in US
Puerto Rico
Lomas Verdes, Mexico
Egypt
Bangalore
Cikarang
North Ryde, Australia
Cotia, Brazil
Haedo, Argentina

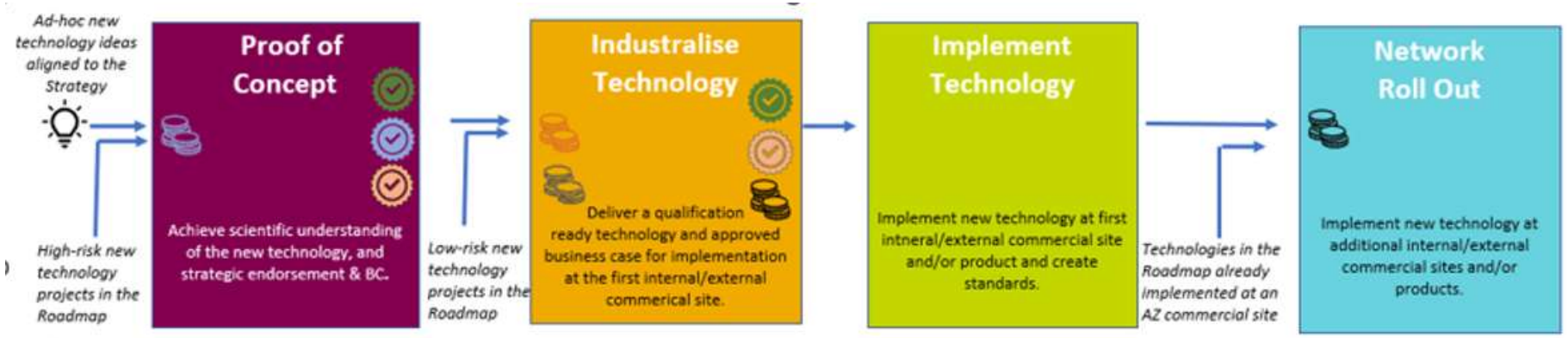
LICENSE TO OPERATE

DIGITAL

EFFICIENCY

GREAT PLACE TO WORK

A cohesive enterprise level approach aimed at optimising and accelerating the integration of innovative technologies.



Case Study

Automated Colony Counting for Environmental Monitoring

Why Are We Interested In This Technology?

- ~ 30,000 EM agar plates are read and verified manually each month at large AZ sites
- Aseptic facilities produce ~ 98% plates that are negative (0 CFU)
- Humans are not perfect. Many factors influence the quality of their work.
- Potential benefits to the right solution:
  - Resolve data integrity challenges
  - Great place to work for microbiologists
  - Consistent quality of result

APAS Independence Clever Culture Systems

Technology Selection Drivers:

Capacity

Flexibility of media manufacturer

Cost (both CAPEX and Ongoing)

False negative rate

Must handle 90mm and 55mm plates

Sustainability

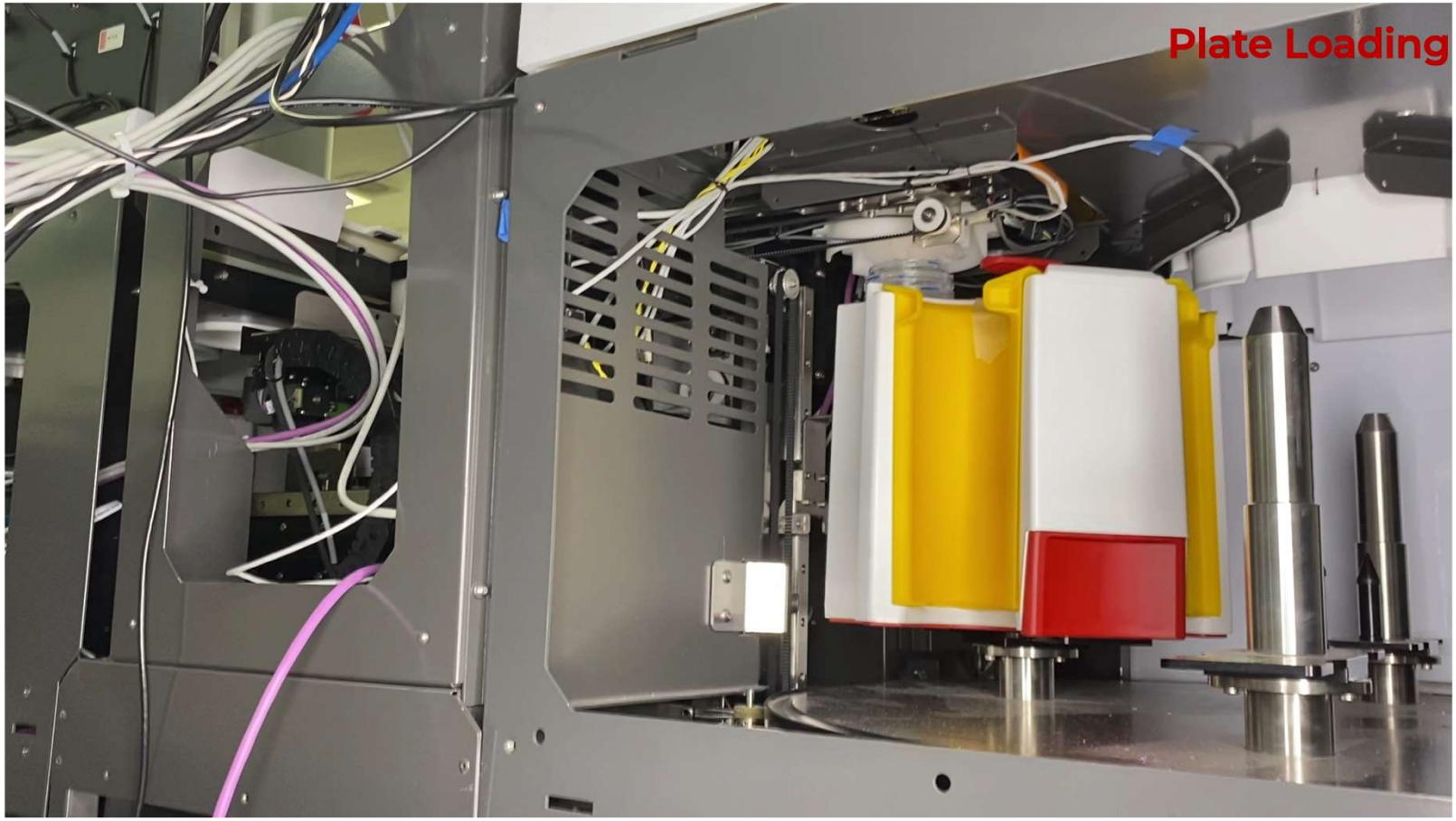


Plate Loading

APAS Independence

APAS processes ~200 plates/hour and sorts them into categories

Only plates with growth or processing errors are second checked- vastly reducing technician time

Data automatically transferred to LIMS system – manual transcription and chance of error removed

Currently process plates destroyed on day of reading – All images stored in APAS for a minimum of 45 days

Not media supplier restricted, and different incubation practices can be accommodated

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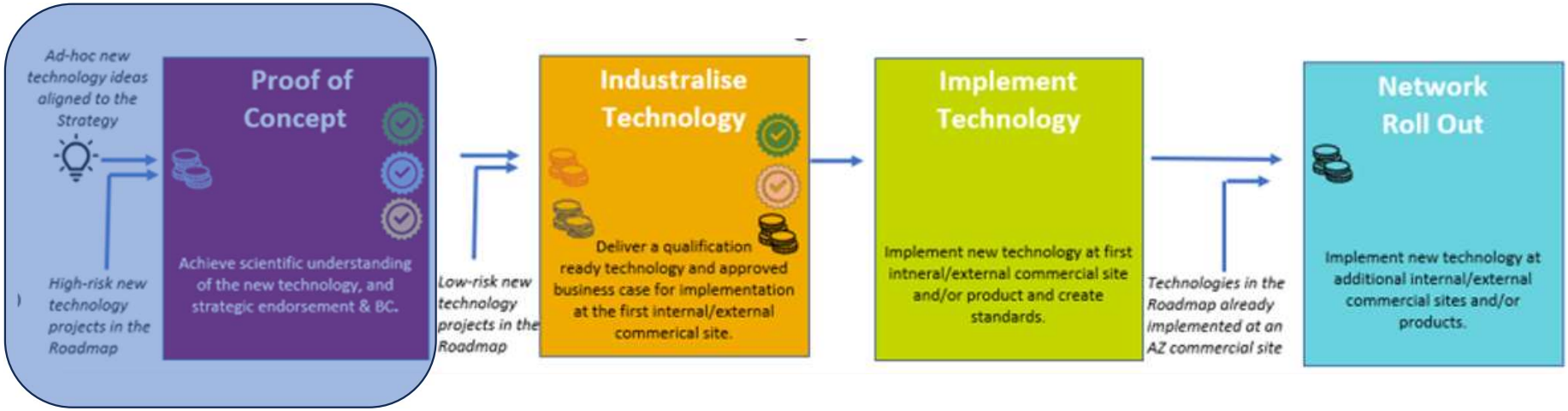


Key Learnings Proof of Concept

Counting is impacted by areas of growth confluence where it is difficult to accurately count and can also be subjective between technicians

Here APAS counts 44 CFU vs actual 5 CFU

Key Learnings from Proof of Concept

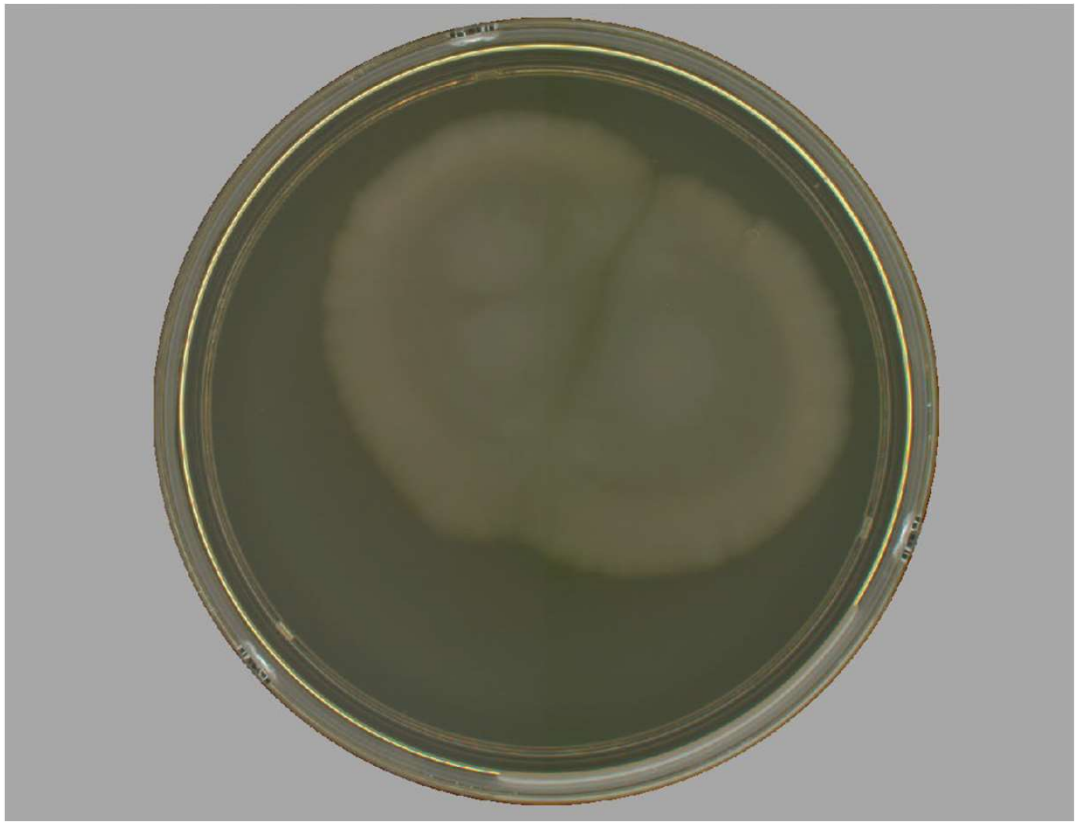
For some species, larger and older colonies have significant morphological textural features that influence APAS counting algorithms e.g. *Bacillus* & *Aspergillus*

Day 3

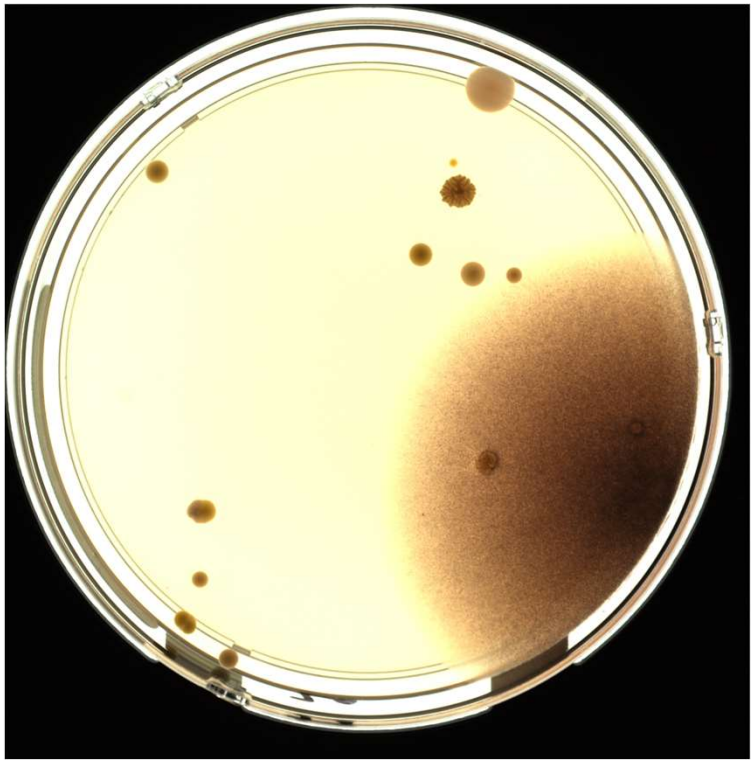
APAS counts 3 CFU vs actual 3 CFU

Day 5

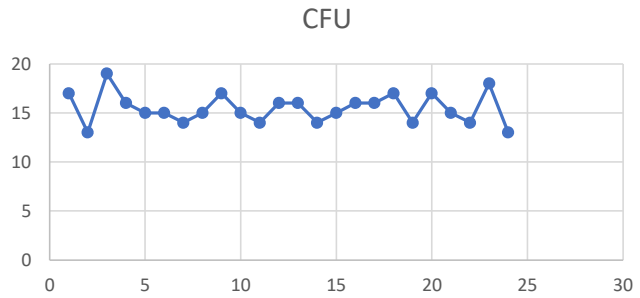
APAS counts 26 CFU vs actual 3 CFU



How many CFU on this plate?

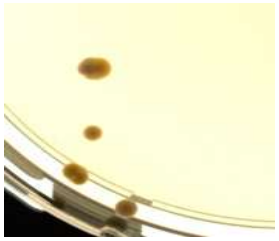


Is there a "right" answer?



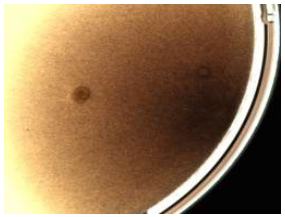
Range of reported results 13-19CFU

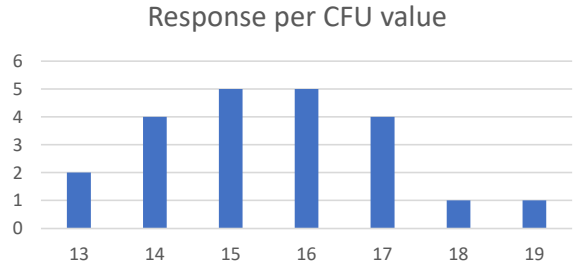
7 different CFU counts



Points for ambiguity

- Are the ones in the bottom left 1 or 2CFU?
- Responses with "ish"
- Offering a range in the response
- Differentiation of fungi and other CFUs





Average result reported 15.5CFU (0.5CFU does not exist, so this reports up to 16CFU)

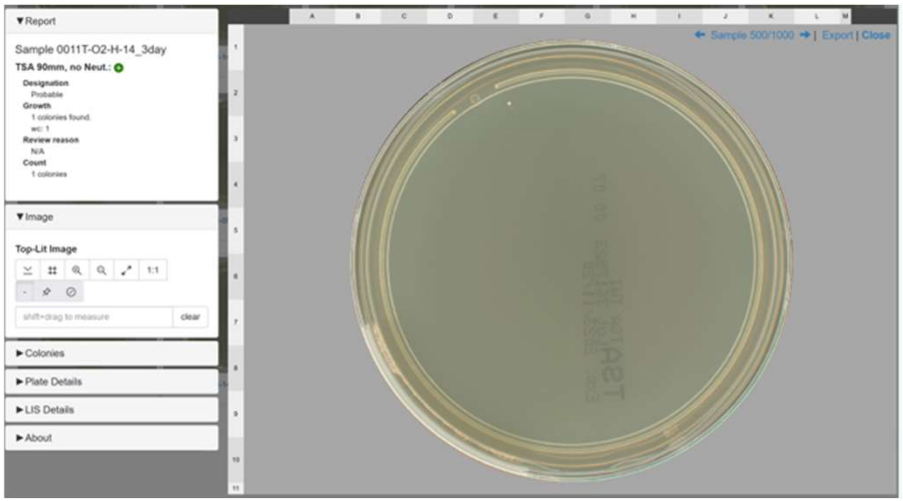
16CFU

Does it matter?

Whilst some differences may not matter, **ALERT LIMIT BREACHES MATTER (or missing them)**

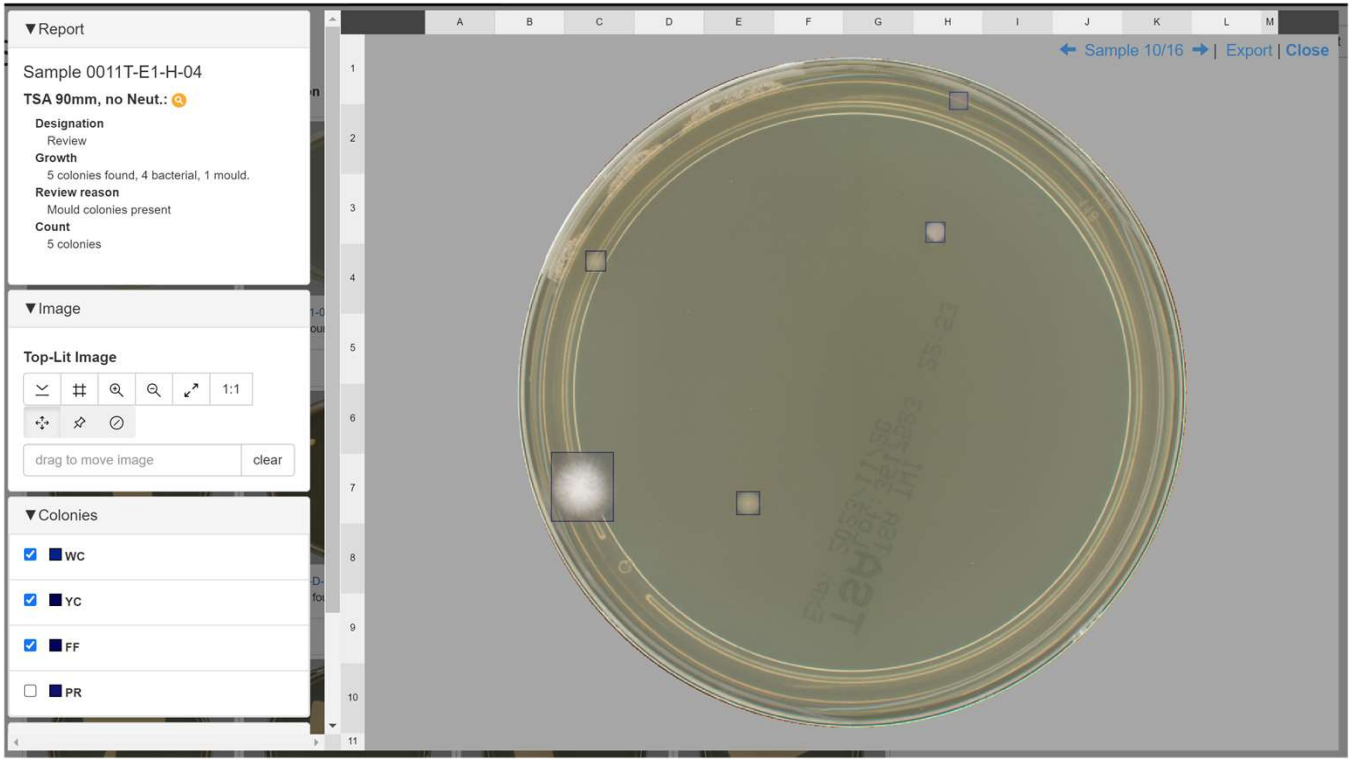
With 98% of plates from cleanrooms being 0 CFU the most important differentiation is between growth and no growth.

Differences in reporting could result in different actions

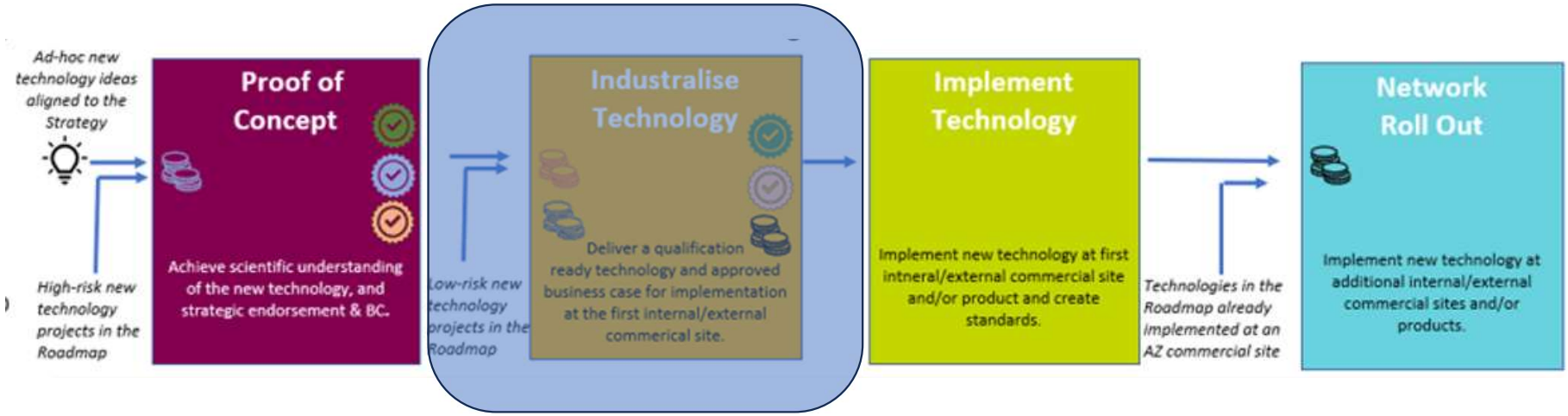


- APAS most important feature is to sort 'Growth' from 'No Growth'
- 98% of plates are 0 CFU (AZ facility)
- The difference between 0 and 1 in Grade A is critical, but the difference between 17 and 25 is less important.
- False negative results are the most important factor

Example APAS Outputs



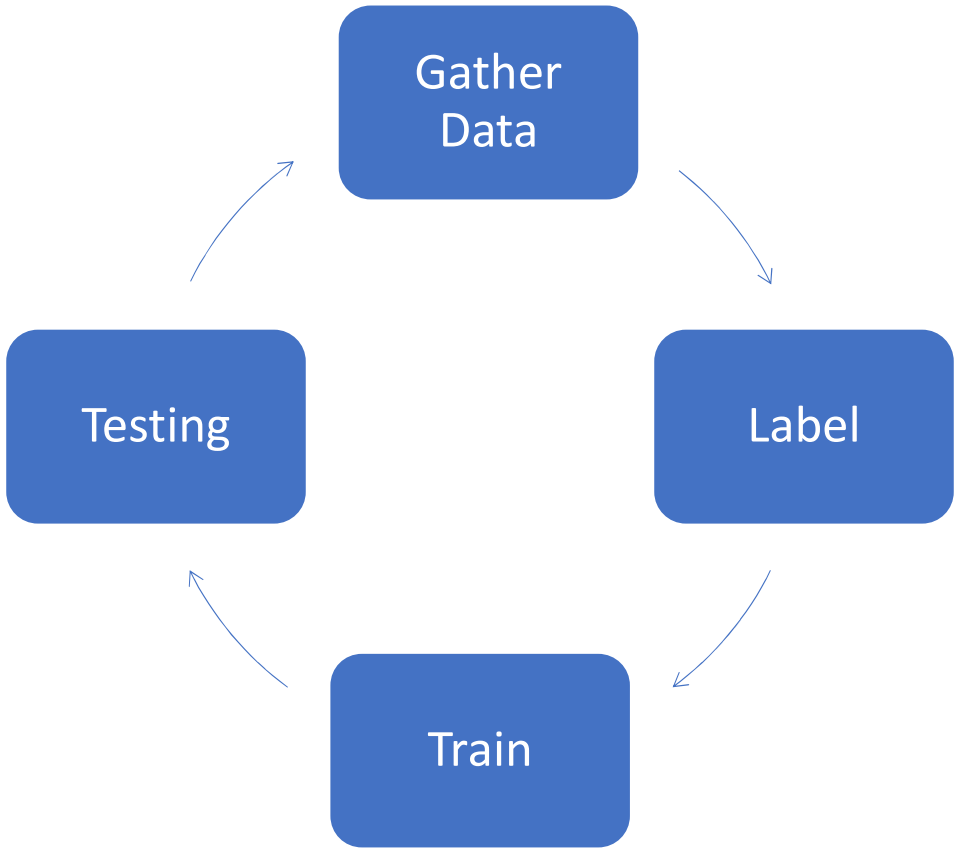
A cohesive enterprise level approach aimed at optimising and accelerating the integration of innovative technologies.



Industrializing the technology using CCS AI & ML

Challenges to plate reading

- Colony variability
- Agar supplier differences
- Plate labelling by technicians
- Colonies located on the edge or rim
- Condensation
- Plate issues and sampling faults



Industrializing the technology using CCS AI & ML

- Data Collection >8000 plates read from AZ, images analyzed and algorithm developed
- Colony Variability: Different morphologies, colony colors, swarming, molds, different Bacillus sp, ‘wild‘ and ATCC strains
- Agar Plate Variability: Multiple batches of different media suppliers, to accommodate batch to batch variability different plate labelling, different barcodes
- Count Variability: Range of counts from 0 CFU – 250 CFU

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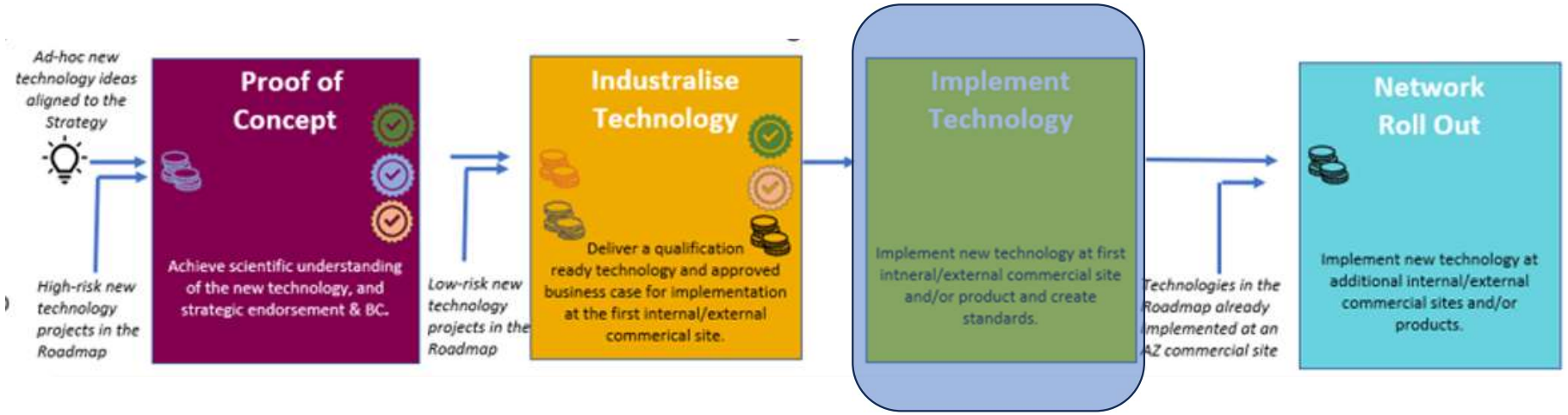

Clever Culture Systems
QE-041730

- Primary Validation of 90mm TSA/TSA + Neutraliser Analysis Module
  - REP-0414490 BD
  - REP-0416042 Merck
  - REP-0416032 bioMerieux
- Primary Validation of 55mm TSA + Neutraliser Analysis Module
  - Currently in development, validation to be performed

AZ Macclesfield Global Validation
QE-080821

- Secondary validation of APAS 90mm Analysis modules
- Computer Systems Validation
  - User setup, Audit trails, CFR21 part 11, LDAP user login, User interface.
  - Validation of APAS/MODA interface and data transfer
- Secondary Validation of APAS 55mm Analysis Modules
  - Currently in development, validation to be performed

All sites where APAS is installed
Change Controls will be raised for the deployment at sites

- Installation Qualification
- Operational Qualification
- Functional Acceptance Qualification

CCS

- AZ Performance Qualification - If deemed necessary to qualify any additional local site requirements from Global Validation

Supplier Primary Validation

- Primary Validation as per the principles of USP <1223> and Ph.Eur 5.1.6, but isn‘t really an alternative method per se; it is a different way of reading the results from a traditional method
- Linearity, Precision, Specificity, Accuracy, Robustness, Ruggedness, Operational range, Limit of Detection, Limit of Quantification, Repeatability
- CCS completed Primary Validation March 2024

Secondary Validation at AZ

Stage 1 Establish Expected Performance

- Positive plates 'contrived' by exposing plates in general labs and containing enough negative plates to keep the humans 'reading' in representative manner.

Stage 2 Establish Actual Performance

- Actual plates from production environmental monitoring compared to APAS result with the current manual method (two people reading each plate)

- The desired target is non-inferiority to manual read (zero false negatives).

Table 1 – Summary of microbial growth detection rates

	Number of 90mm plates analysed	Number of plates with growth	Number of plates **Manual** detected Growth	Number of plates **APAS** detected Growth	Accuracy at growth detection **Manual**	Accuracy at growth detection **APAS**	**Manual** False Negative Rate	**APAS** False Negative Rate
Overall	11104	2414	2324	2344	96.3%	97.1%	3.7%	2.9%

Table 2 – Comparison of microbial growth detection for Manual and APAS plate reads when the Median of the 3 manual reads (Stage 1) or the MODA manual read(Stage 2) is determined as the 'Truth State'.

	Number of 90mm plates analysed	Number of plates with growth[a]	Number of plates where **APAS** also detected growth	Accuracy at growth detection **APAS**	**APAS** False Negative Rate
Stage 1	2556	1902	1872	98.4%	1.6%
Stage 2	8548	475	465	97.9%	2.1%
Overall	11104	2377	2337	98.3%	1.7%

[a] – Growth determined by the Median of 3 manual reads for Stage 1 or the entered MODA result for Stage 2.(MODA result determined by 2 person manual read and check)

Table 3 – Comparison of microbial growth detection for Manual and APAS plate reads when APAS image and review of agar plates is used as the 'Truth state'.

	Number of 90mm plates analysed	Number of plates with growth[a]	Number of plates with growth detected by **Manual**	Number of plates with growth detected by **APAS**	Accuracy at growth detection **Manual**	Accuracy at growth detection **APAS**	**Manual** False Negative Rate	**APAS** False Negative Rate
Stage 1	2556	1924	1851[b]	1872	96.2%	97.3%	3.8%	2.7%
Stage 2	8548	492	475[c]	474	96.5%	96.3%	3.5%	3.7%
Overall	11104	2416	2326	2346	96.3%	97.1%	3.7%	2.9%

[a] – Presence of growth determined by analysis of each APAS image (top lit and bottom lit images) and the agar plates.

[b] - In Stage 1 there were 3 separate and independent manual counts. If any of the 3 manual readers missed growth on a plate, it was counted as a miss/non-detect event. For APAS a miss/non-detect event was when APAS did not 'box' and detect microbial growth which was visible on the image/plate.

[c] – In Stage 2 the Manual count was defined by the current plate reading process which is the agreement of 2 separate reads e.g. read and checked result entered in to MODA. A miss/non-detect for manual readers in this instance was when there was microbial growth on the image/agar plate, but the result in MODA was recorded as 0 cfu. For APAS a miss/non-detect event was when APAS had not 'boxed' and detected microbial growth which was visible on the image.

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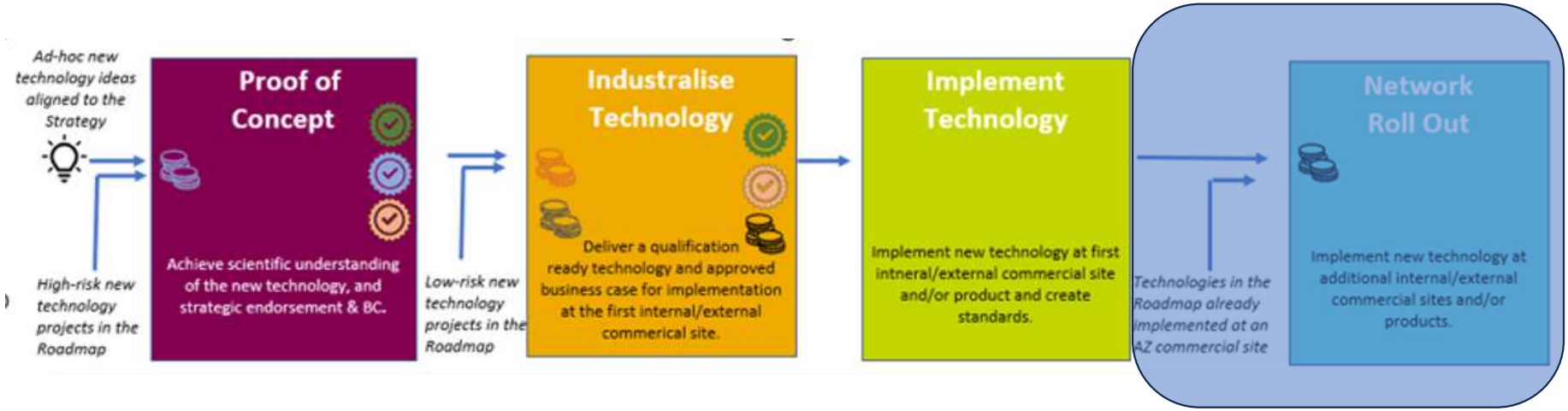

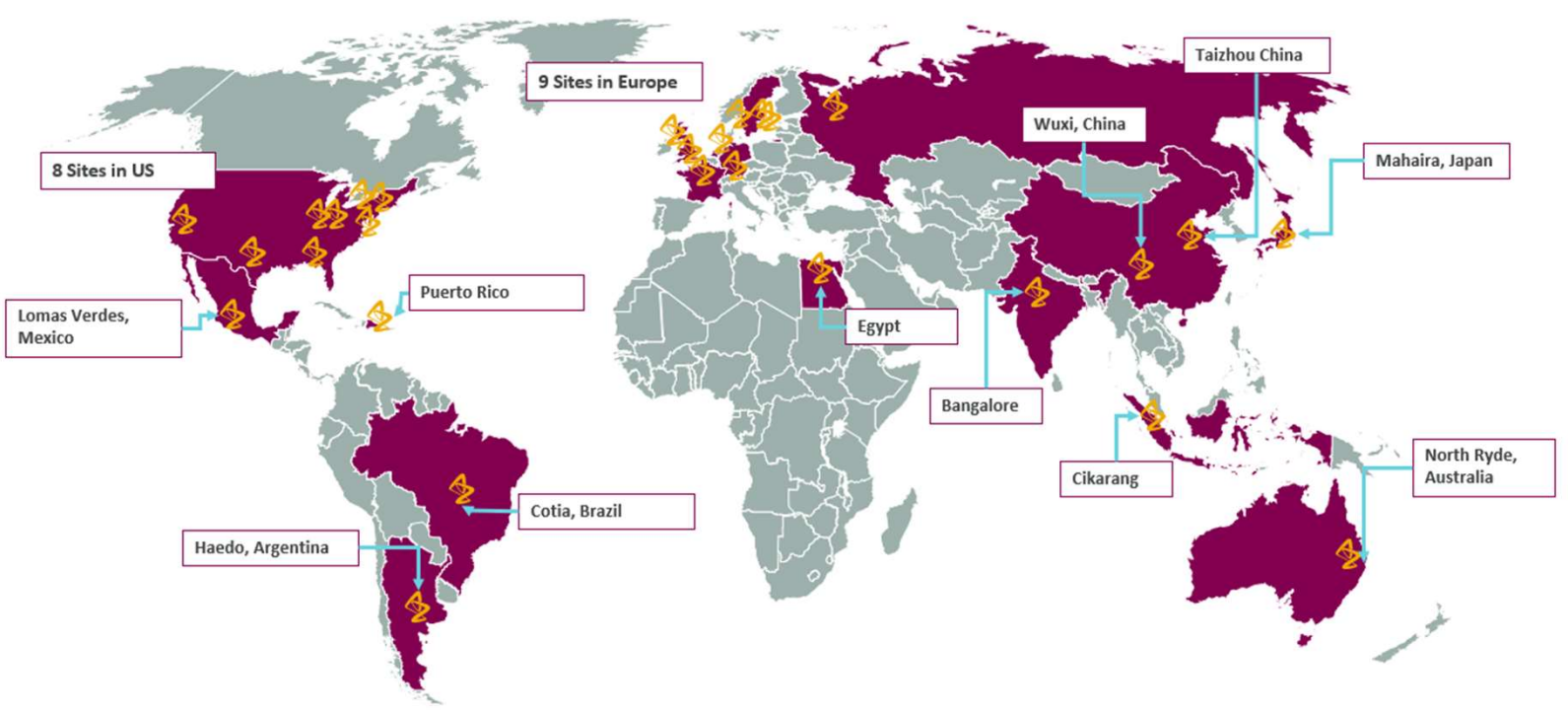
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North Ryde, Australia
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Future Considerations

- Will APAS and our validation approach be accepted by regulators?
- Will images be stored?
- Expansion to include 55mm contact plates is underway and must be successful
- Number of false positives needs to be acceptable (circa 10%)
- Define on-going performance monitoring of APAS
- Plate reading ability of laboratory personnel needs to be retained (Business continuity)
- What testing/revalidation is required following an update to the counting algorithm?
- Smaller system using same counting algorithm would benefit smaller labs in the AZ network

Conclusions

- Accurate colony counting is impacted by areas of growth confluence. The "correct" colony count is also subjective between technicians
- APAS estimates CFU for plates with growth and sorts them as 'requiring human review'. Therefore, the false negative rate is more important
- APAS performance met our acceptance criteria and is equivalent and non-inferior to the manual method
- Using APAS technology brings additional benefits such as data integrity improvements,
- elimination of human error, standardization, job role enhancement for microbiologists and the potential that with advancements and improvements, the technology will become even better.

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