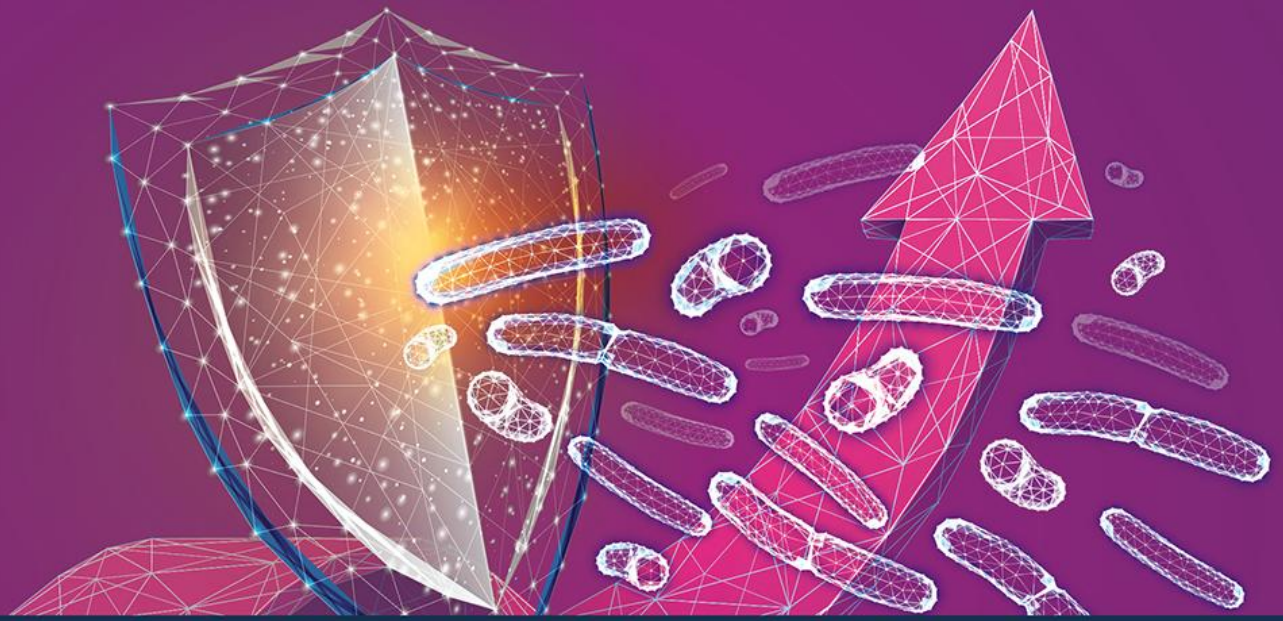

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A Global Approach to New Technology Introduction and Validation: Automated Colony Counting

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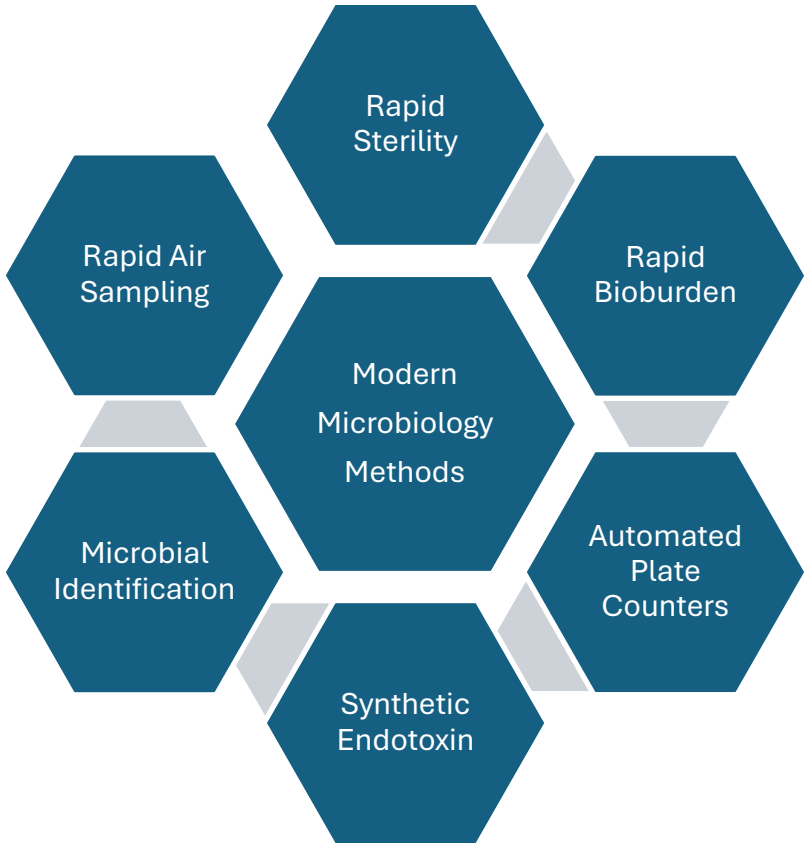
Senior Director, Global QC

AstraZeneca

So much technology, so little time



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	Local 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

Technology Drivers

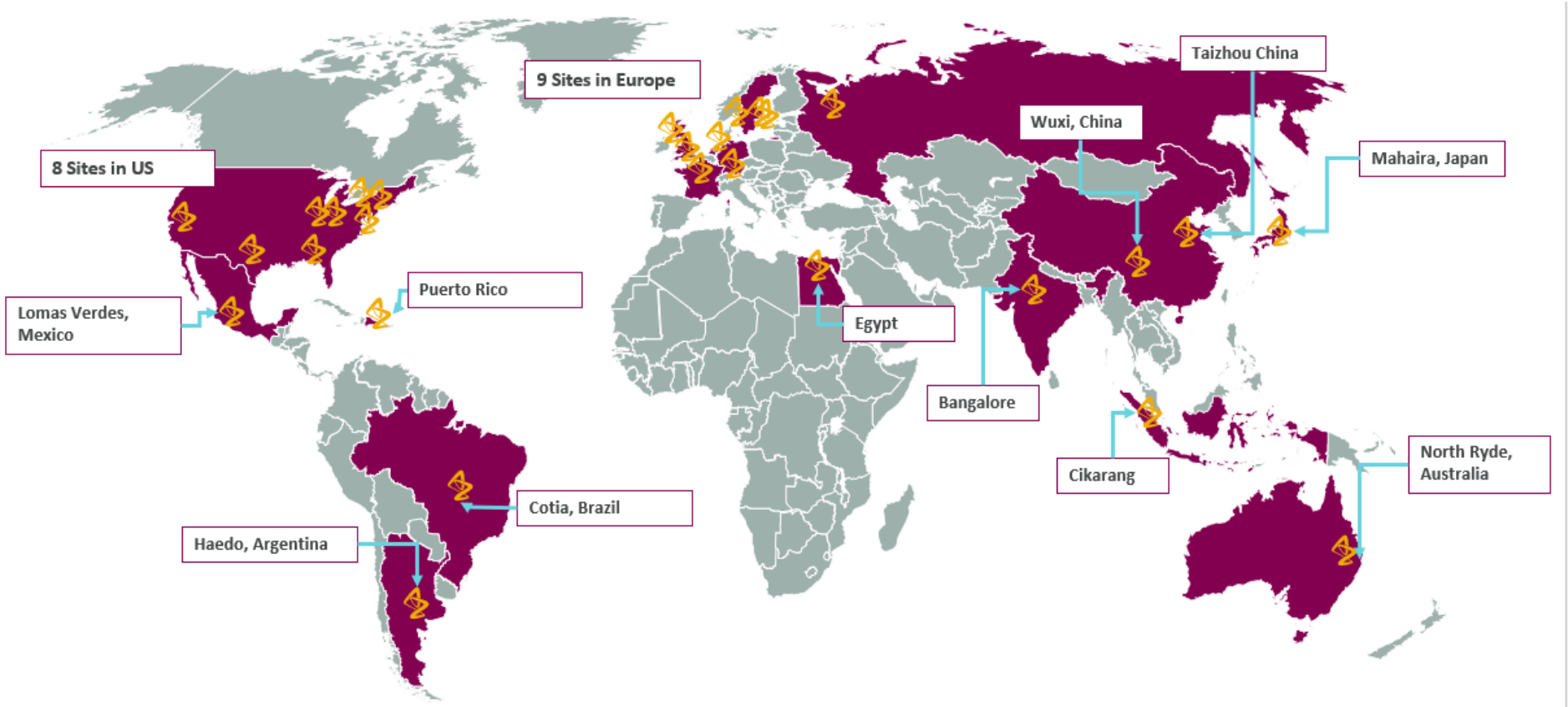
LICENSE TO OPERATE

DIGITAL

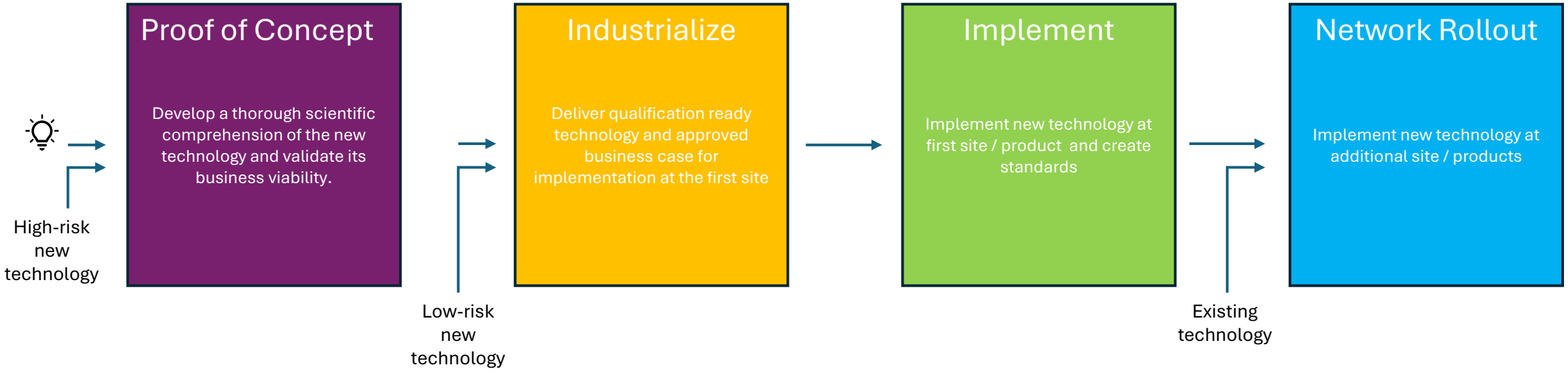
EFFICIENCY

GREAT PLACE TO WORK

AZ Microbiome



A **cohesive enterprise** level approach aimed at **optimizing** and **accelerating** the integration of innovative technologies



Case Study

Automated Colony Counting for Environmental Monitoring

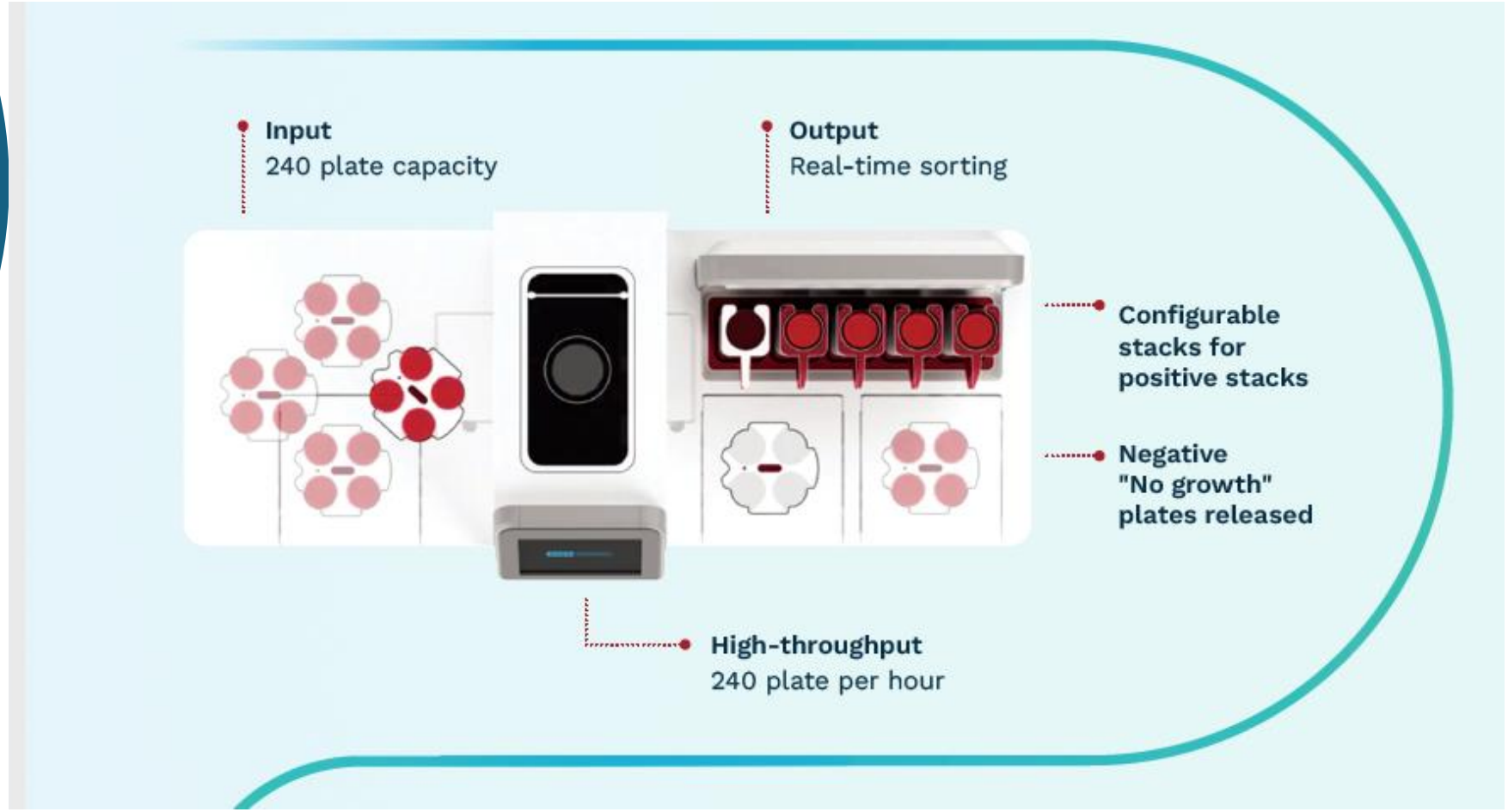
Why are AstraZeneca interested?

1. Up to 30,000 EM agar plates are read manually and verified every month at large AZ sites
2. Annual EM data from aseptic manufacturing facilities shows that >98% of plates are negative
3. Occasionally humans make mistakes
4. Resolves data integrity challenges

Benefits of this technology

1. APAS processes ~200 plates/hour and sorts them into categories
2. Only plates with growth or processing errors are second checked- vastly reducing technician time
3. Data automatically transferred to LIMS system – manual transcription and chance of error removed
4. Current process plates destroyed on day of reading – All images stored in APAS for 45 days
5. Not media supplier restricted, and different incubation practices can be accommodated

Topographical view



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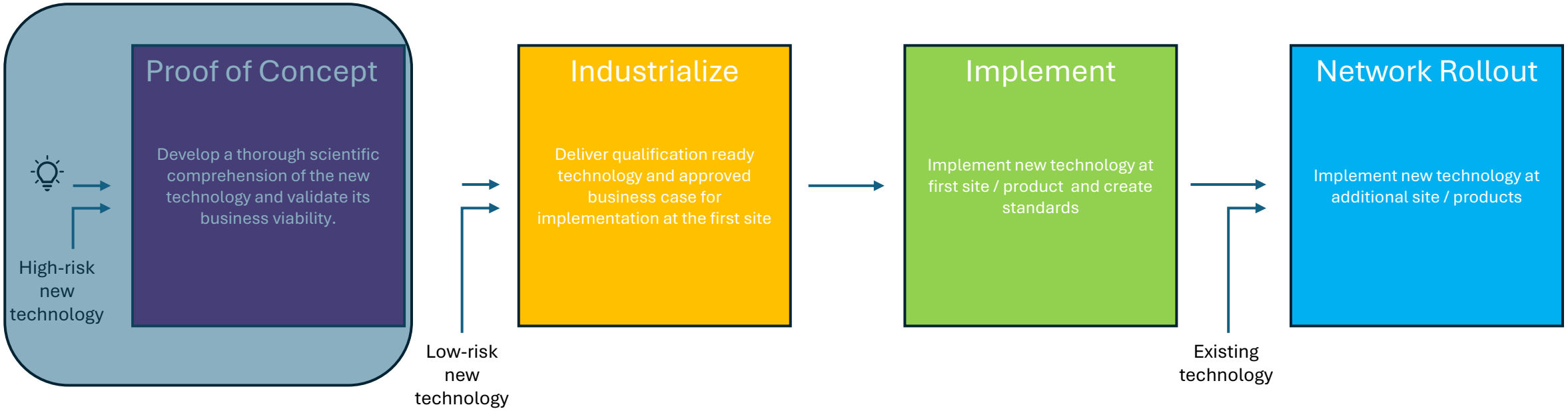
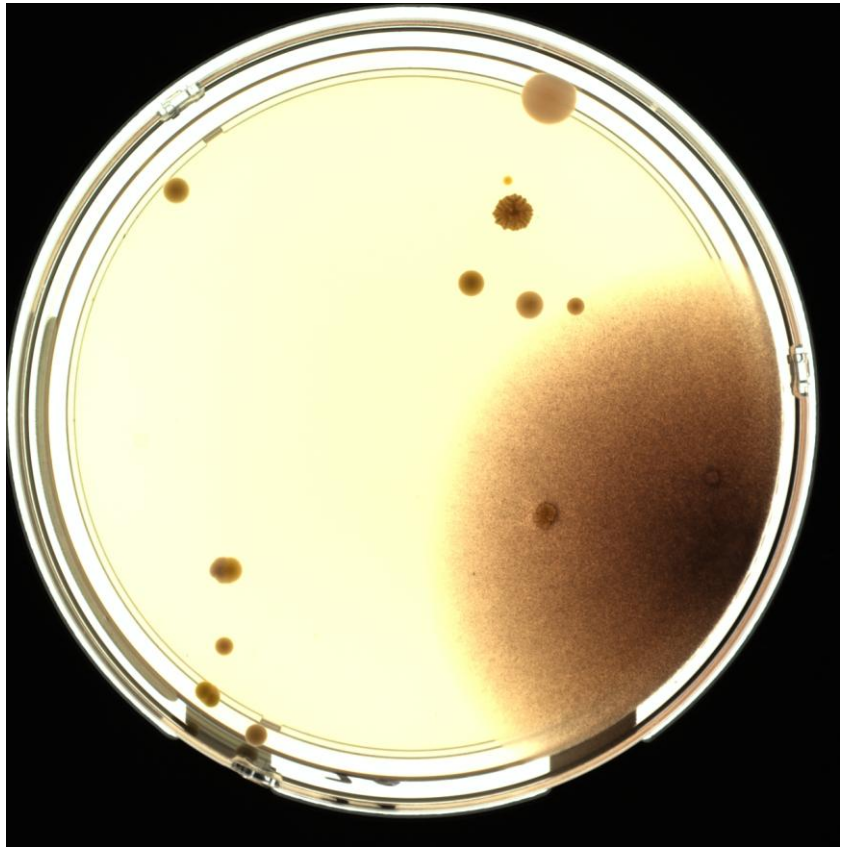


How many CFU on this plate?

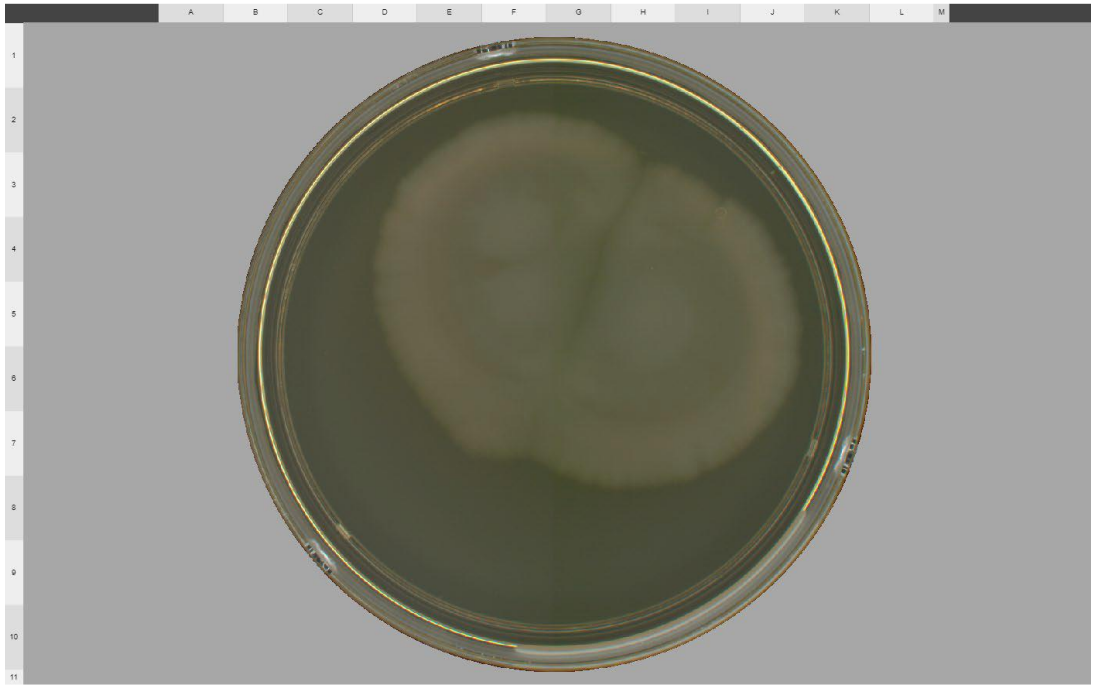


Key Learnings Proof of Concept

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*

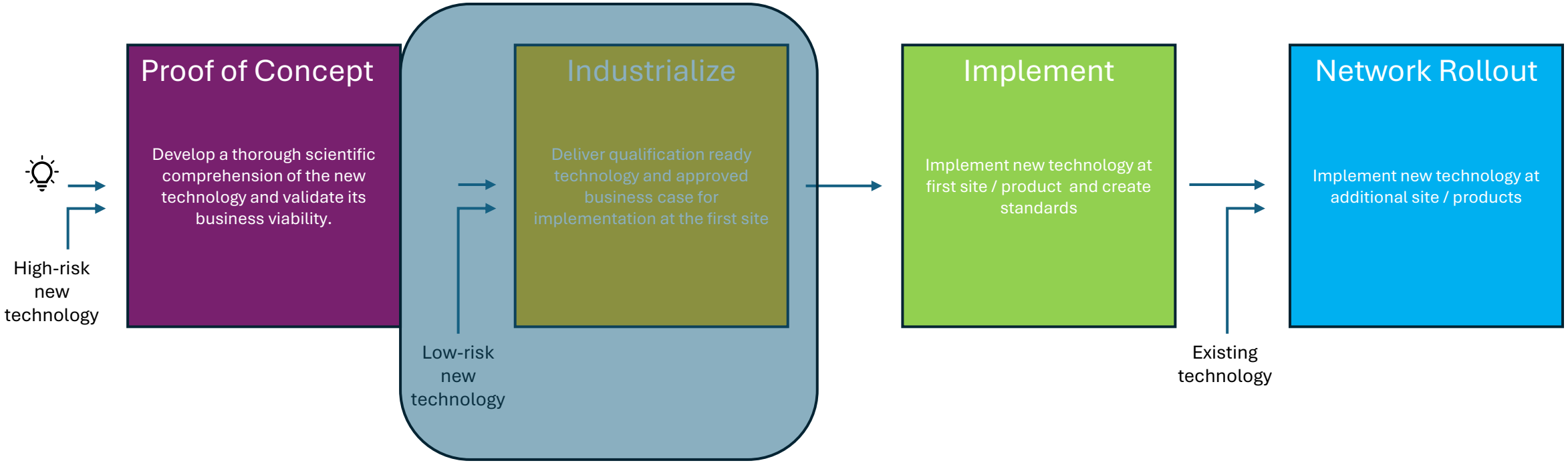
Day 3

APAS counts 3 CFU vs actual 3 CFU

Day 5

APAS counts 26 CFU vs actual 3 CFU

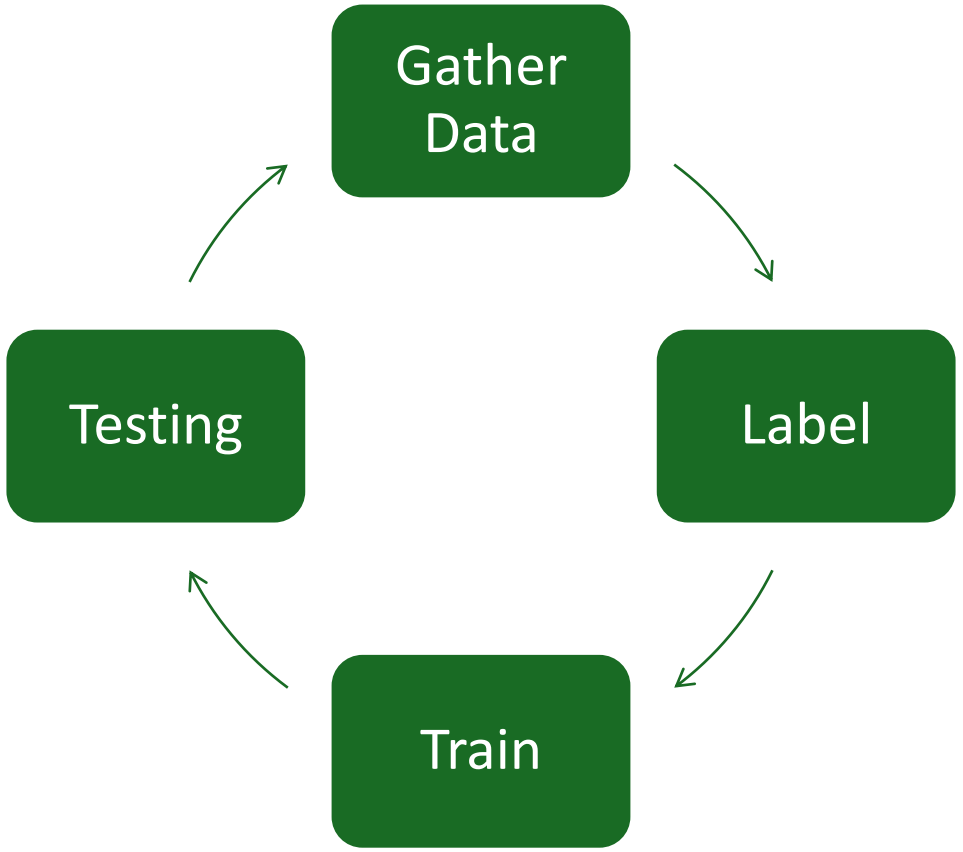
A **cohesive enterprise** level approach aimed at **optimizing** and **accelerating** the integration of innovative technologies



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

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

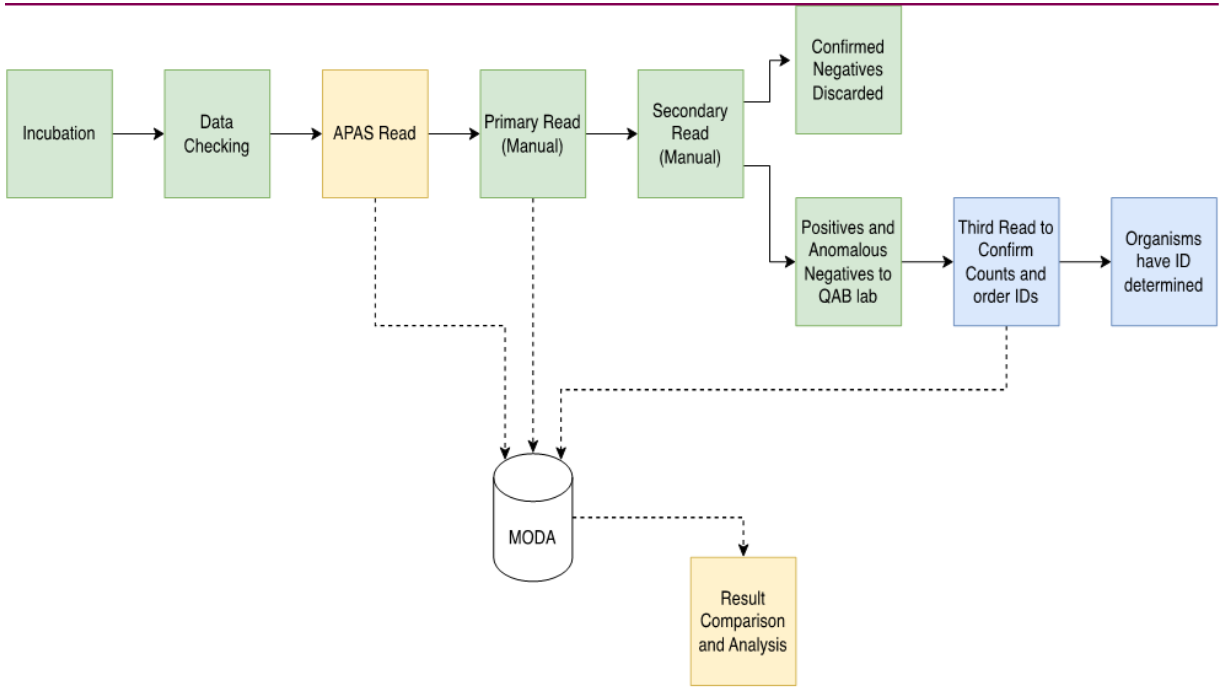
- Actual plates from production environmental monitoring compared to APAS result with the current manual method (two people reading each plate)

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

Secondary Validation Study

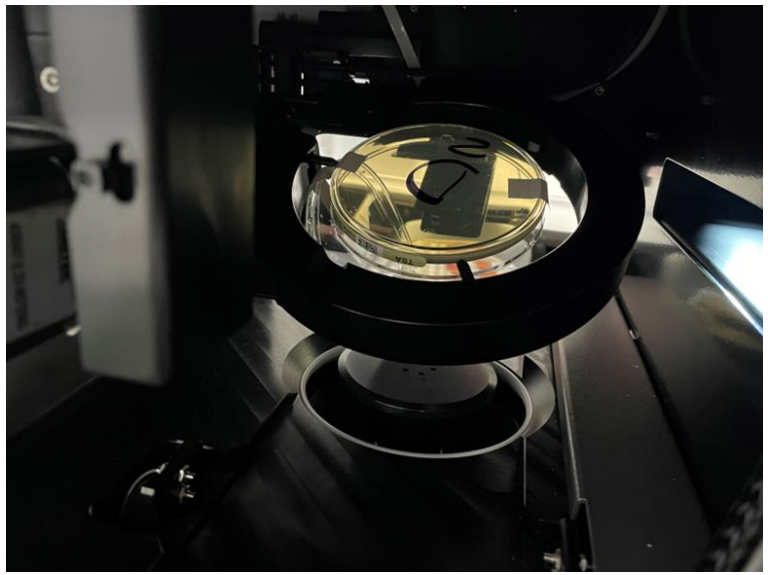
Establish Actual Performance

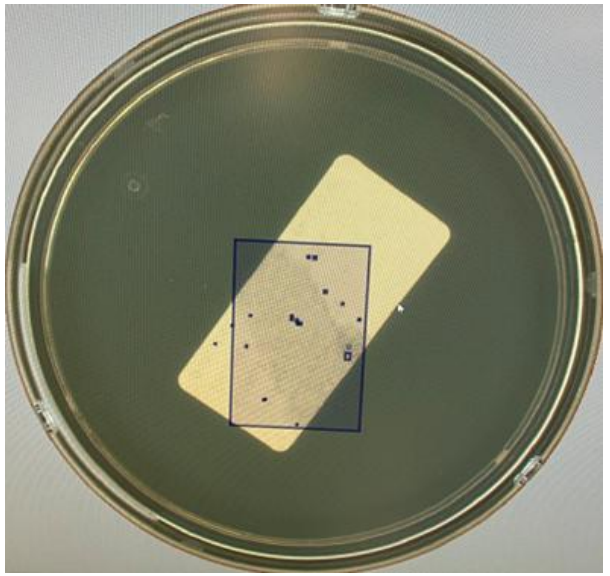
- APAS instrument used as primary reader for real EM plates.
- ALL plates checked by humans and results corrected where necessary
- Rate of corrections tracked and used to form acceptance criteria for AZ validation



Key Learning Points

Current practices using tape caused mechanical issues driving higher false positive rate





Key Learning Points

Simple solution to utilise clip and bags used at other AZ sites introduced via change control.

Key Learning Points

- Pilot primary validation study has shown difficulties in counting accurately at higher end of the count range especially with some organisms.
- However, this is also seen in humans, where differences of 20-40 colonies have been observed.
- IS THIS IMPORTANT?
- APAS would sort these plates as requiring human review.

Update : Latest software is much improved in this aspect, however in the real world all positive plates are flagged for review.

Figure 3. Example of B. spizizenii growth demonstrating variable morphology, size, and confluence

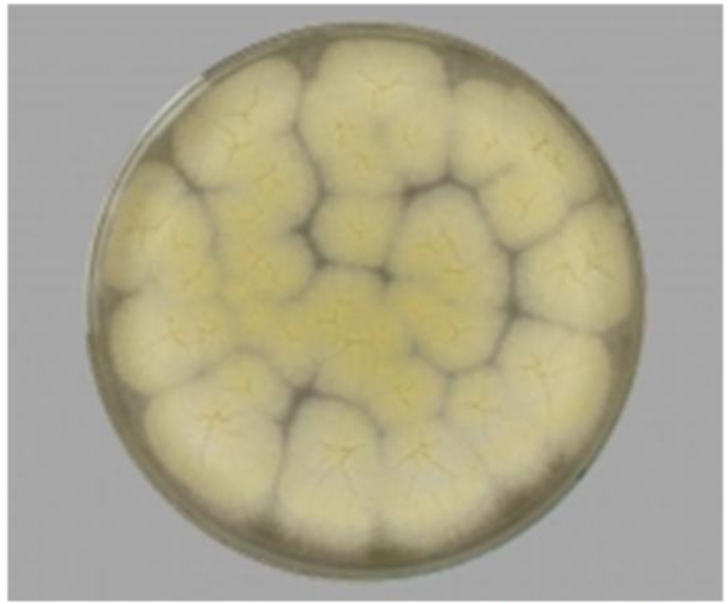
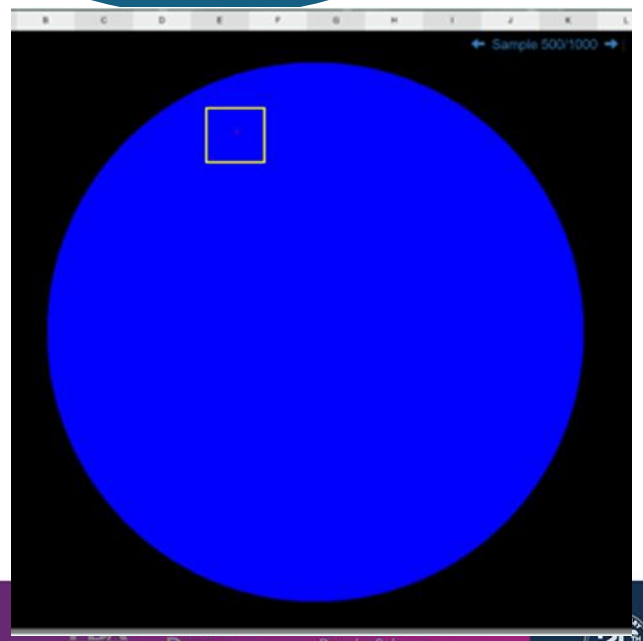
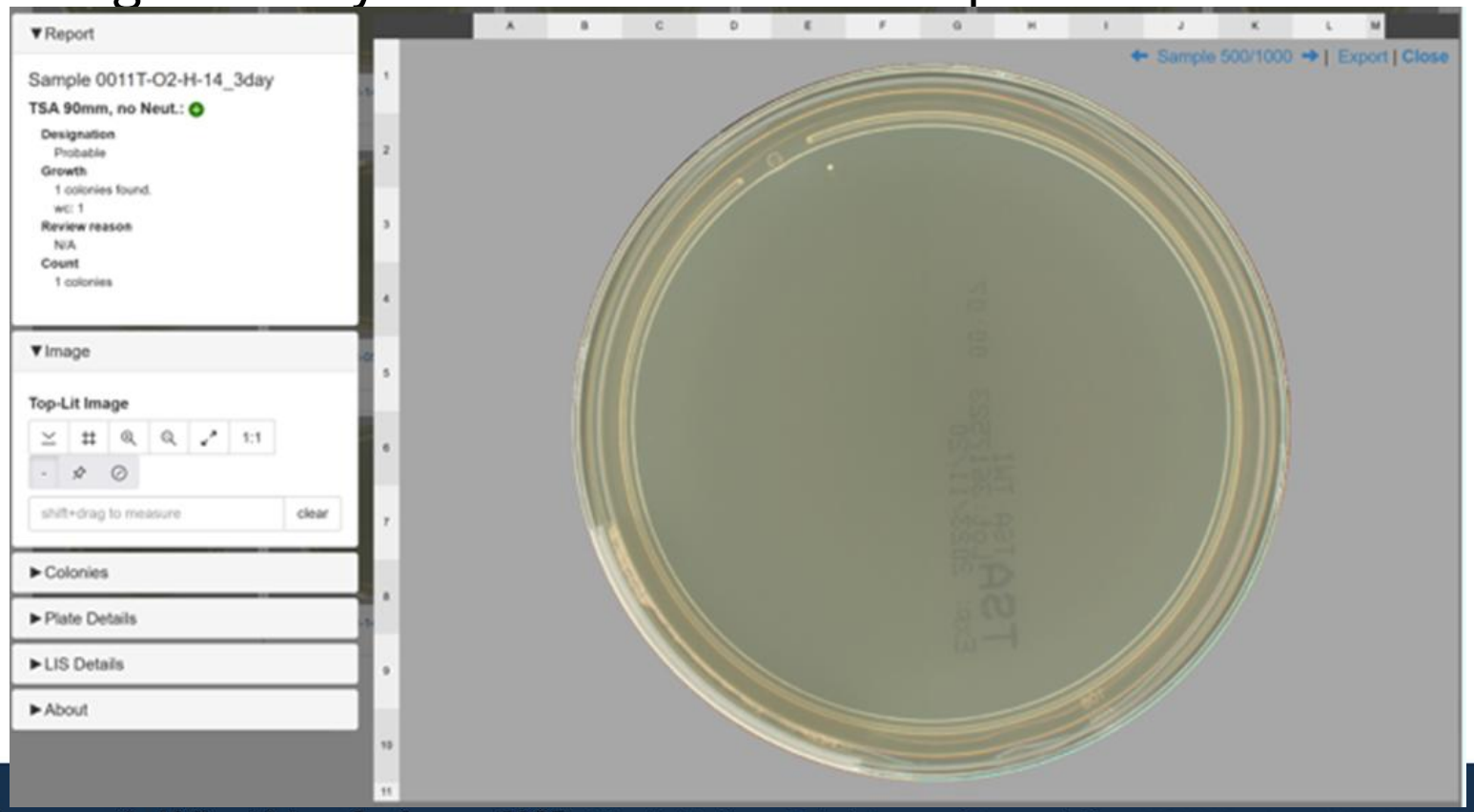


Figure 4. Example of A. brasiliensis growth changes over time demonstrating counting challenges

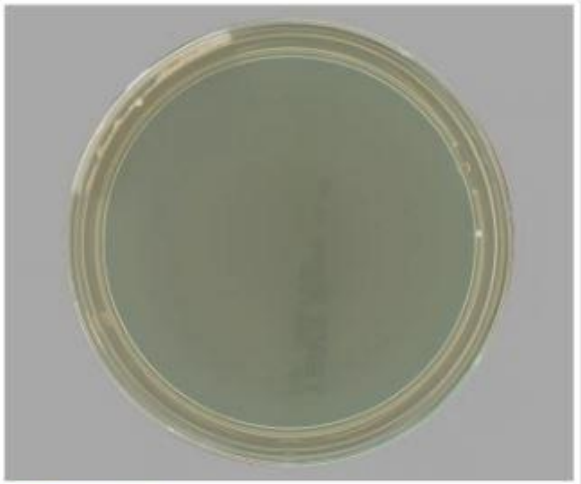
Key Learning Points

- APAS primary function is to sort ‘Growth’ from ‘No Growth’
- Remember, over 98% of plates are zero cfu (AZ facility)
- The difference between 0 and 1 is massive in Grade A, the difference between 15 and 19 is negligible.
- Single colony detection the most important factor.



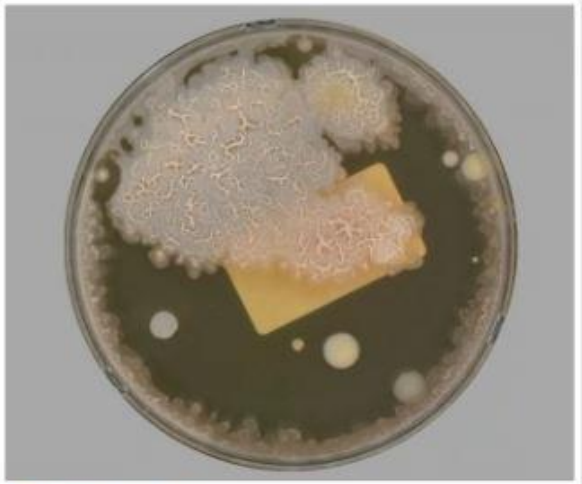


0012-SA-33-0.5-1
0 colonies found.



0017T-31-09
2 colonies found.

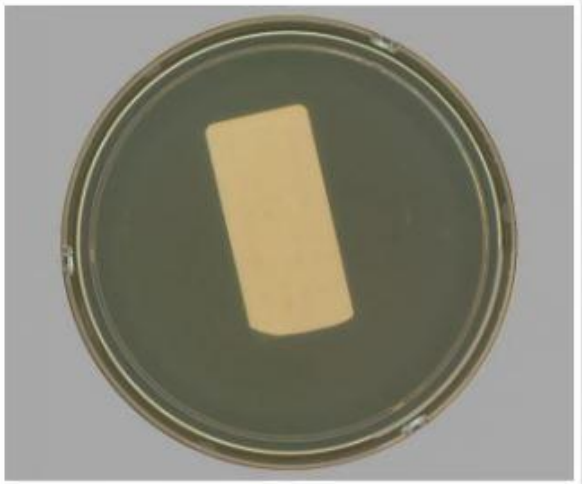
0006BN-A1-U4-2
50 colonies found, 38 bacterial, 12 mould.



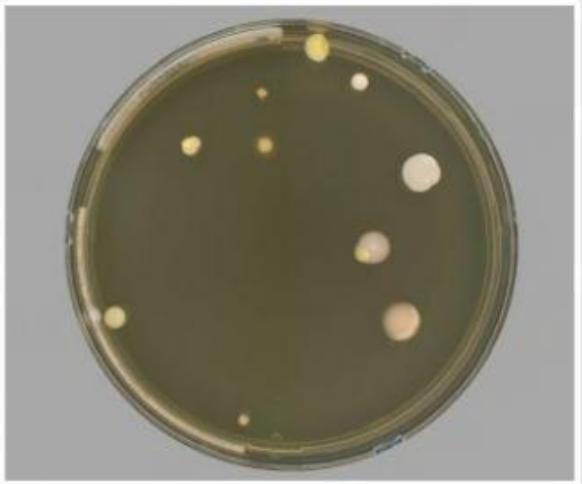
0006BN-A1-U2-1
30 colonies found, 28 bacterial, 2 mould.

0006TN-D-U4-1
239 colonies found, 214 bacterial, 25 mould.

21003134863-910313
0 colonies found.



21005290102-910529
0 colonies found.



0006BN-D-U3-2
11 colonies found.

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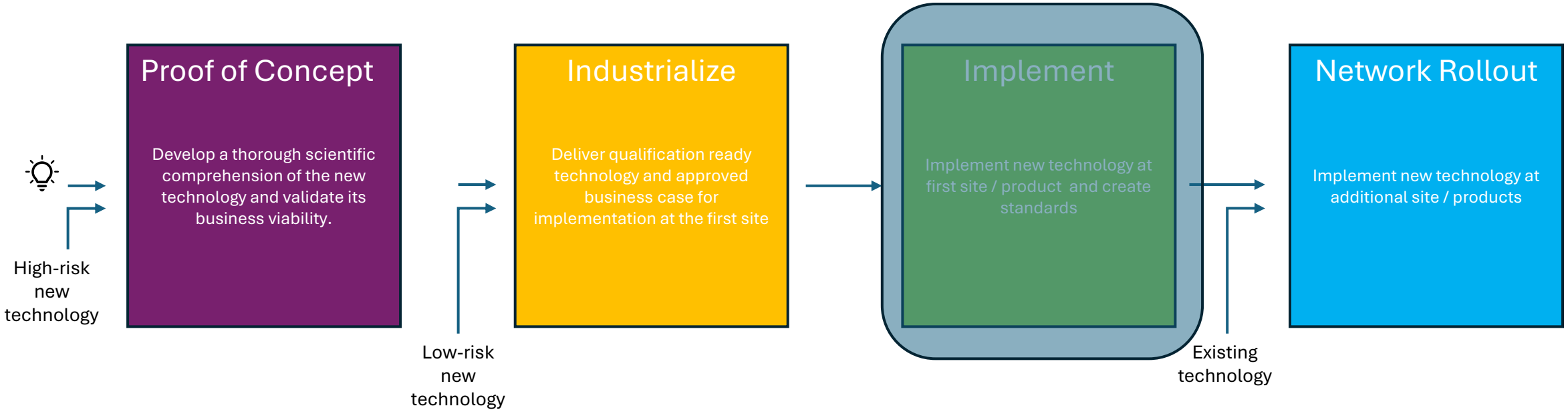


Comparison of Growth Detection for Manual and APAS

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'.

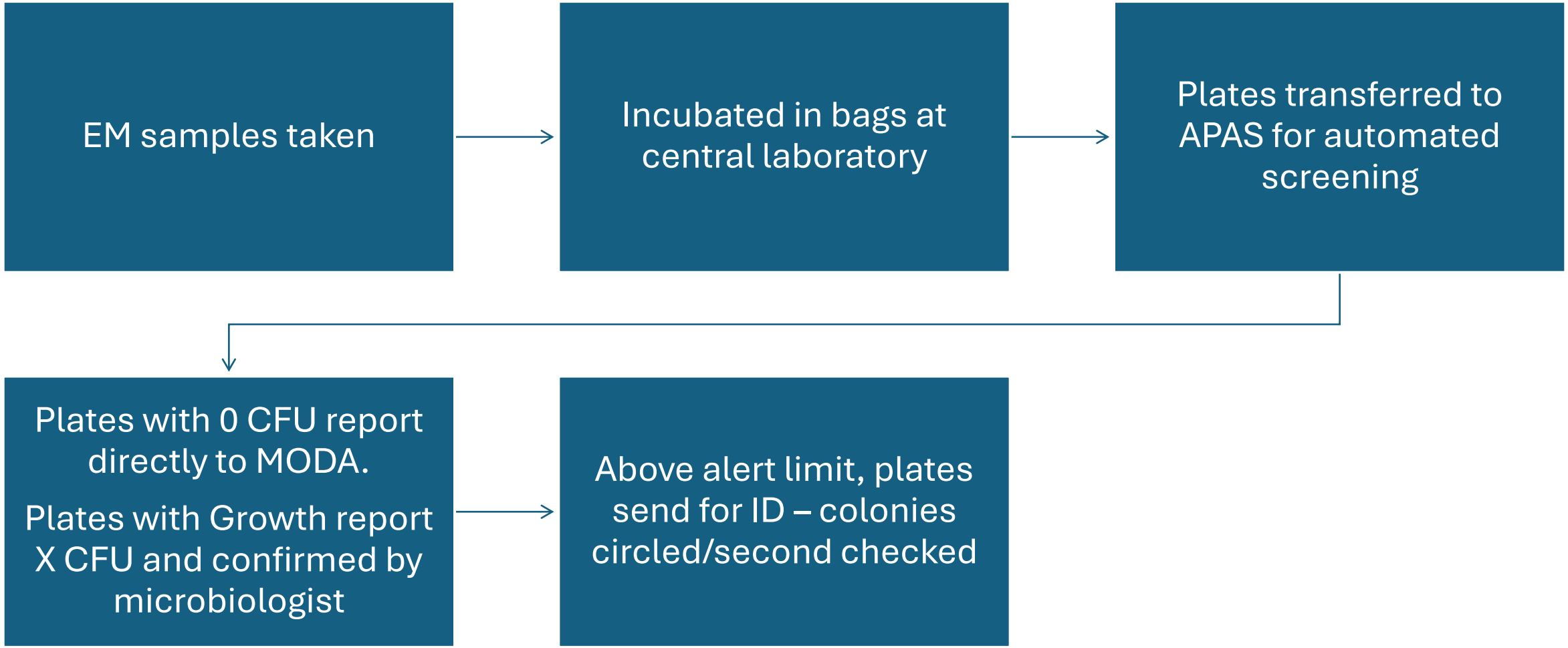
	Number of 90mm plates analysed	Number of plates with growth[a]	Number of plates with growth detected by **Manual**	Number of plates with growth detected by **APAS**	Accuracy at growth detection **Manual**	Accuracy at growth detection **APAS**	**Manual** False Negative Rate	**APAS** False Negative Rate
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.

Automated Colony Counting Workflow



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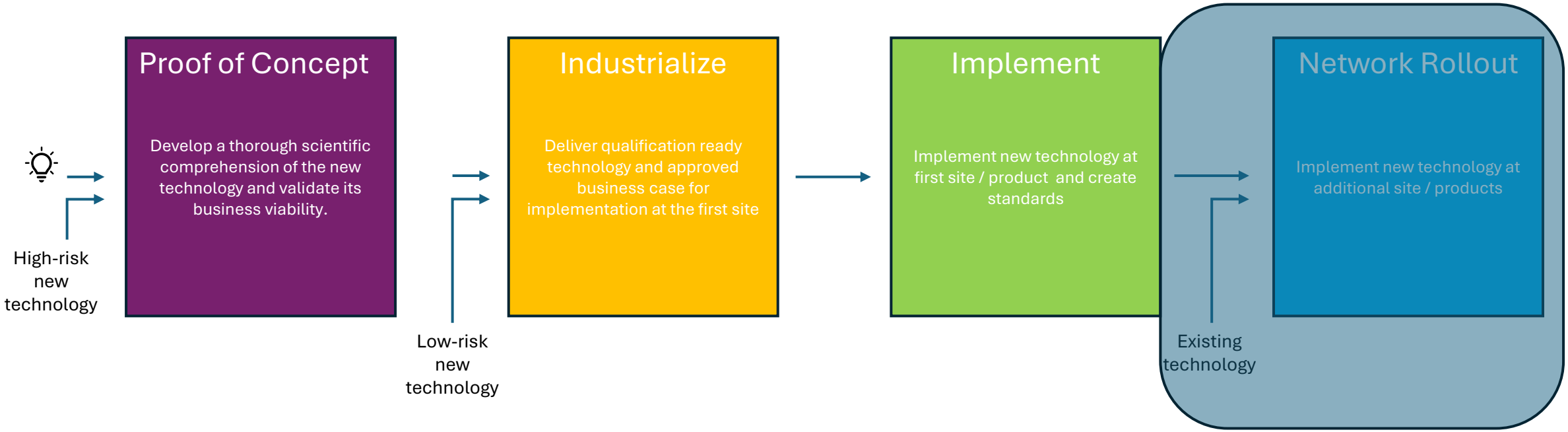


Network Rollout Validation Strategy

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

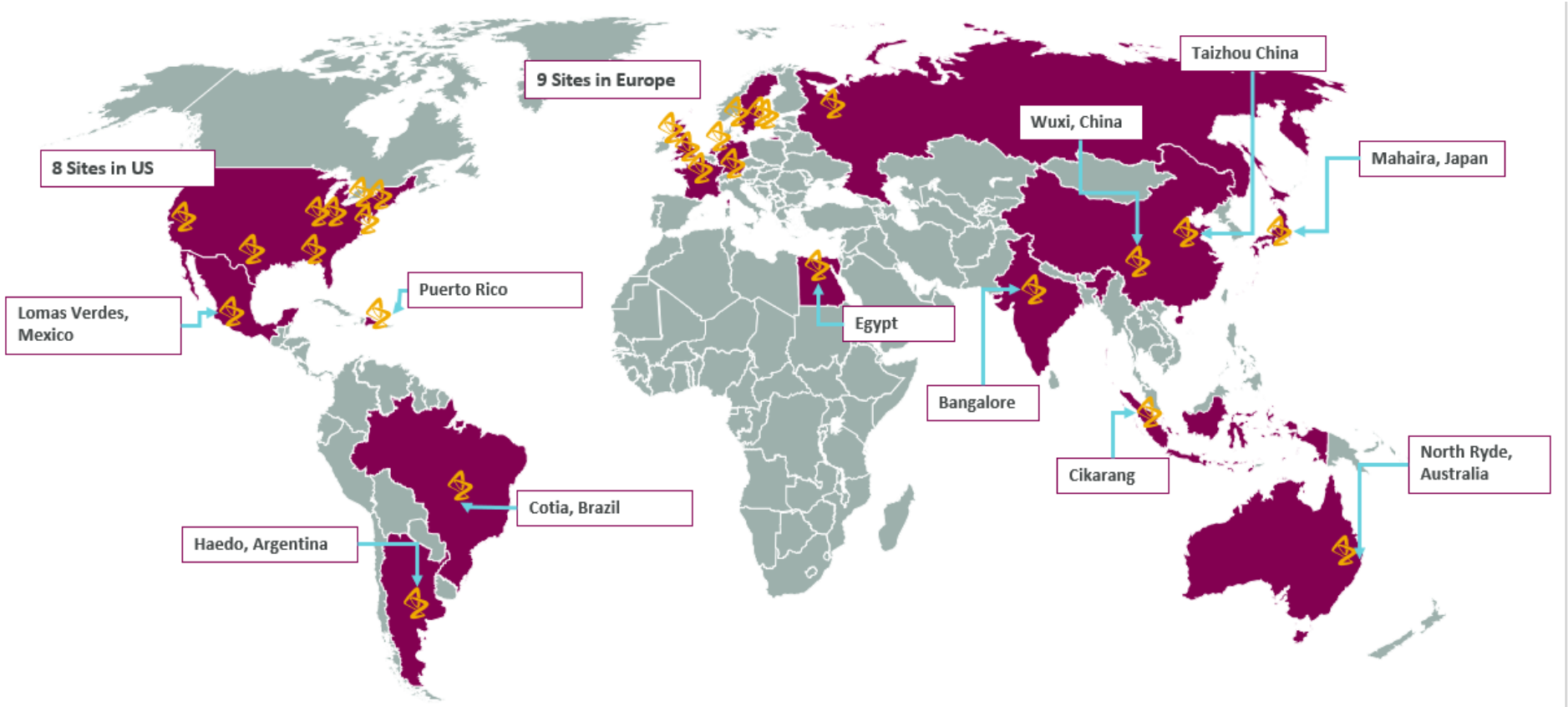
All sites where APAS is installed
Change Controls will be raised for the deployment at sites

- Installation Qualification
- Operational Qualification
- Functional Acceptance Qualification

CCS

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

AZ Microbiome



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.

Acknowledgements

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Steve Giglio – Scientific Director, Clever Culture Systems

Miriam Guest – Former Principal Scientist, AstraZeneca (now Charles River)