Automated Reading of Agar Plates using AI

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Reliable environmental monitoring plate reading powered by A.I.

APAS PharmaQC

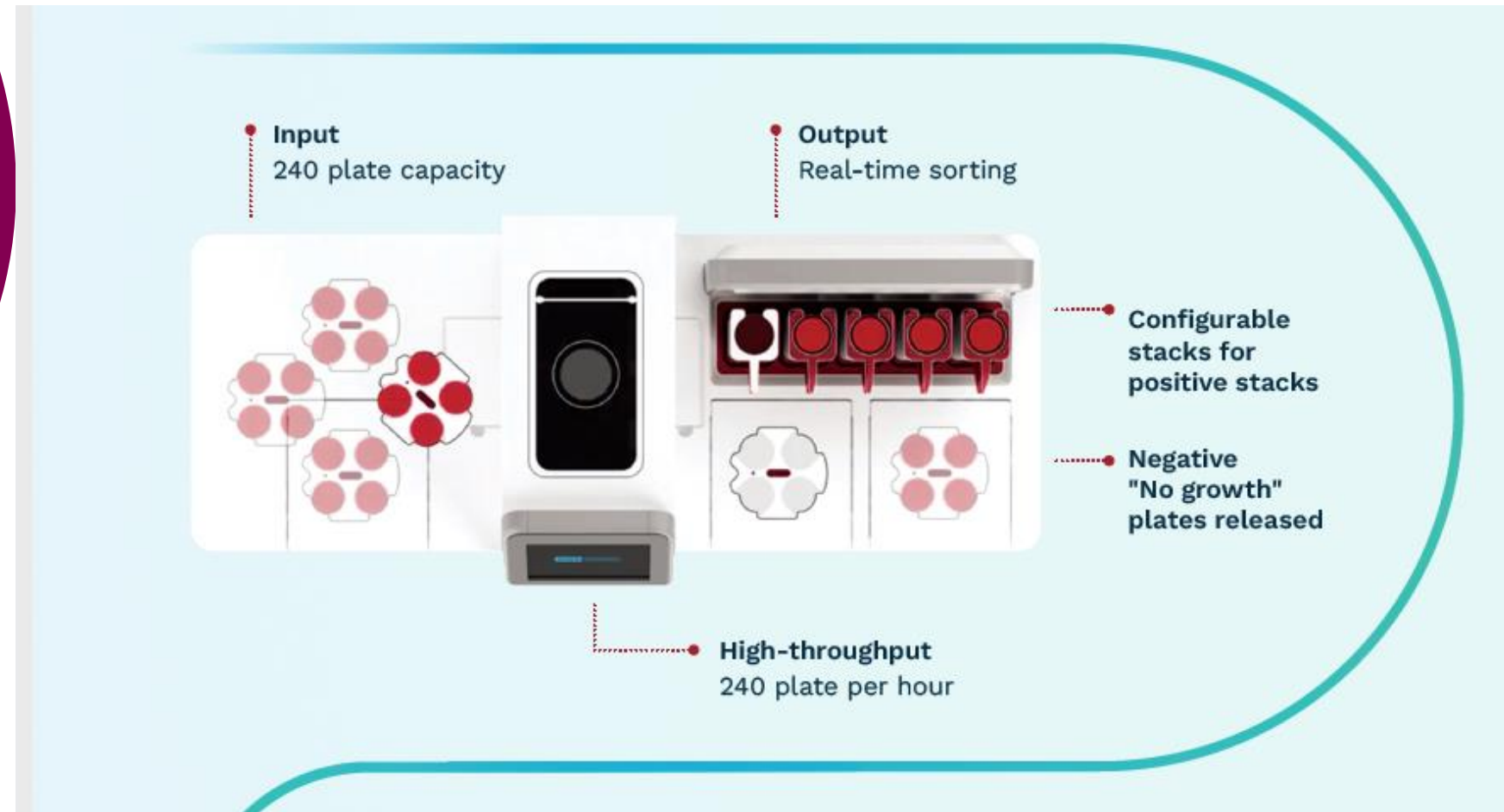Never review a negative plate again.

Introduction to APAS

APAS (Automated Plate Assessment System) Independence, by Clever Culture Systems (Adelaide, Australia), is an automated plate reader that uses a camera system and machine learning model to count and sort plates.

Topographical view

Input
240 plate capacity

Output
Real-time sorting

Configurable stacks for positive stacks

Negative "No growth" plates released

High-throughput
240 plate per hour

Why are AstraZeneca interested?

- Up to 30,000 EM agar plates are read manually and verified every month at large AZ sites

- Annual EM data from aseptic manufacturing facilities shows that >98% of plates are negative

- Occasionally humans make mistakes

- Resolves data integrity challenges

Benefits of this technology

- APAS processes ~200-240 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
- Current process plates destroyed on day of reading – All images stored in APAS for 45 days
- Not media supplier restricted and different incubation practices can be accommodated

Benefits of using AI for this purpose

Imaging Plate Reader Challenges	AI solution
• Colony variability • Agar Supplier differences • Plate labelling • Rim colonies • Condensation • Plate issues and sampling faults	• Machine learning trained by microbiologists allows all this variability to be managed • Use AI and machine learning to improve the classifier

Data needed to develop the Machine Learning

Data Collection	Colony variability	Plate variability	Count variability
• >8000 plates read by the reader. • Duplicate read in normal way. • Images analysed and algorithm developed	• Fungal isolates • Coloured isolates • Multi coloured isolates • Swarming colonies • Bacillus species	• Different media suppliers. • Different labelling • Different bar-coding methods	• Inherent variability in manual counting

Pilot Primary Validation Study (ongoing)

Developed validation strategy to assess performance of APAS PharmaQC aligned with compendial requirements as outlined in USP<1223> and Ph.Eur 5.1. Testing protocols include detection of colonies deposited on the perimeter of plates and ability of APAS to detect a single colony only.

- Linearity
- Precision
- Specificity
- Accuracy
- Robustness
- Ruggedness
- Operational range
- Limit of Detection
- Limit of Quantification
- Repeatability

Ruggedness and Precision

Table 3. Ruggedness and Precision for Day 3

Org Day 3	Growth level CFU per plate	Replicate	APAS1		APAS2		APAS3		All APAS	
			Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
E. coli	10-100 CFU	1	48.7	4.3	51.8	5.7	48.3	6.2	49.6	6.2
		2	43.9	6.5	46.1	8.1	45.3	6.5	45.1	7.2
		3	66.7	3.6	63.5	5.5	74.7	8.4	68.3	9.4
		4	64.1	4.8	67.2	3.4	69.9	3.7	67	5.3
		5	68.9	5.2	68.3	6.3	73.2	6.1	70.2	6.5
S. aureus	10-100 CFU	1	81.7	2.3	81.2	2.4	80.5	1.7	81.1	2.2
		2	80.7	1.3	81.1	1.4	80.7	1.4	80.8	1.3
		3	69.2	2.1	69.6	1.3	69.8	2.1	69.5	1.9
		4	81.8	1.2	82.2	1.1	81.9	1.7	82	1.4
		5	97.1	1.5	96.1	1.7	95.7	1.5	96.3	1.6
P. aeruginosa	10-100 CFU	1	80.7	4.1	71.1	5.1	71.8	5.3	74.5	7.6
		2	65	6	58	13.2	64	7.9	62.3	10.3
		3	86.7	6.8	81.6	5.5	84.4	10.6	84.2	8.2
		4	81.9	9.3	78.9	8.5	74.5	8.4	78.4	9.4
		5	89	5.4	82.9	5.7	80	6.2	84	7.2
B. spizizenii	10-100 CFU	1	27.5	14.7	24.3	12.8	30.6	19.4	27.5	18.6
		2	19.3	14.7	16.5	8.8	17.1	9.3	17.7	13.3
		3	52.3	12	60.3	11.4	45.1	17.1	52.6	17.7
		4	34.9	7	35.1	8.6	37.6	7.7	35.9	8.3
		5	45.7	13.2	36.5	24.5	44.3	17.9	42.2	20.4

This test measures the consistency of the APAS result within the same instrument and across multiple instruments

- 5 replicates for each organism
- 5 results readings taken at 3 different rotations per instrument
- Performed at Day 3 and Day 5 (not shown)
- Results compared across 3 APAS instruments
- Mean result and coefficient of variation (CV) calculated for each plate
- Results within CV range of compendial expectation (combined Ph.Eur and USP)

All APAS PharmaQC values are within the combined compendial limits (bar one ruggedness CV for *B.spizizenii* on Day 5)

Linearity and Accuracy

Table 5. Summary data for Linearity and Accuracy

Organism	1-10				1-50				1 - 100				Overall			
	Cases	r^2	Slope	Intercept	Cases	r^2	Slope	Intercept	Cases	r^2	Slope	Intercept	Cases	r^2	Slope	Intercept
A. brasiliensis	9	0.3971	1.19	1.44	17	0.7891	0.41	3.11	26	0.5116	0.22	5.85	36	0.4626	0.16	6.59
B. spizizenii	6	0.3405	0.67	5.59	10	0.9291	1.4	2.25	17	0.7601	0.79	11.83	36	0.7639	0.64	17.78
C. albicans	8	1	1	0	24	0.9898	1.03	-0.43	32	0.984	0.99	0.3	36	0.9861	0.99	0.23
M. luteus	3	1	1.17	-0.17	12	0.9879	0.9	1.42	20	0.8997	1.04	-1.69	36	0.9416	0.91	4.42
S. aureus	6	0.8276	1	0.17	8	0.9932	0.98	0.2	18	0.9922	0.94	0.53	36	0.9877	0.83	4.44
S. epidermidis	3	1	1	0	18	0.9816	0.9	1.42	24	0.9698	0.84	2.67	36	0.9644	0.85	1.99
M. osloensis	8	0.2196	1.15	2.09	16	0.9522	0.98	2.59	22	0.9834	0.97	2.66	36	0.9962	0.96	2.62

This tests APAS ability to detect and count colonies over a range of counts. Counts were compared to that made by a trained microbiologist.

- A high level of agreement was observed for all bacterial organisms in the 1-50 range (r^2 values of 0.92-0.99)
- Intercepts were close to zero and slopes close to 1, indicating a high level of accuracy
- Counting difficulties observed with *A.brasiliensis* and *B.spizizenii* especially at the higher counts. This is due to colony morphology and overlapping colonies.

Proposed Secondary Validation Study

A two-stage approach:

1. Establish expected performance

   - The number of positive plates chosen to deliver the confidence intervals from the statistical table below assuming a 1.5% positivity rate.

   - Positive plates would be 'contrived' by exposing plates in general labs and interspersed with large enough number of negative plates to keep the humans 'reading' in representative manner.

   - Statistically driven study; number of plates likely to be in the region of 3-5k by the use of 'contrived' plates.

PPA Target	Lower 95% Confidence Interval Target	True APAS PPA	Required Positive Plates	Approximate Total Plates (Rounded up to nearest 1000)
98.0%	96.0%	99.0%	310	21, 000
98.0%	96.0%	99.5%	150	10, 000
98.5%	97.0%	99.5%	250	17, 000
99.0%	98.0%	99.5%	510	34, 000

Proposed Secondary Validation Study

2. Establish in-use 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
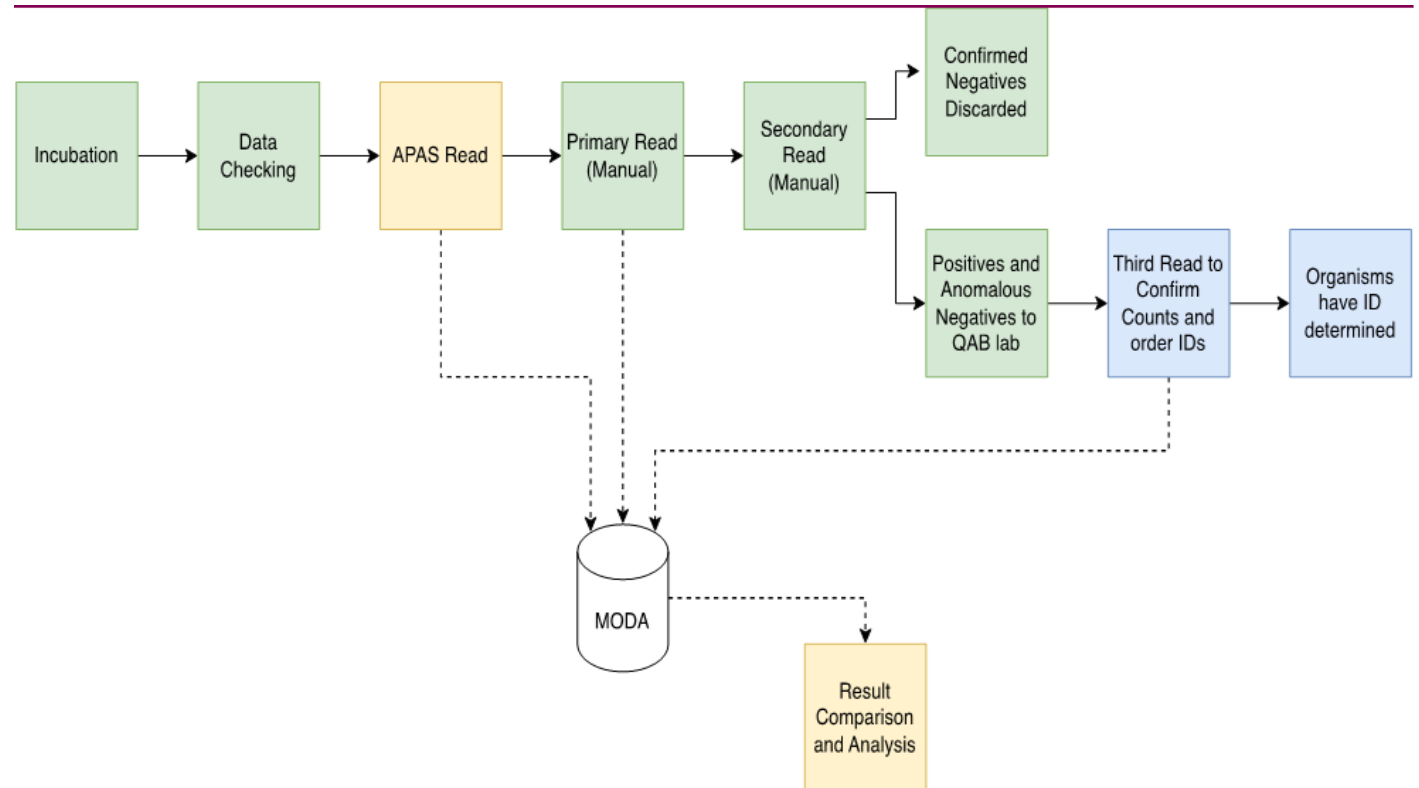
Key Learning Points

- Current practices using tape caused mechanical issues

- Data suggests that there is a 0% false negative rate and a 15.8% false positive

- Tape also caused false positives. Excluding tape from the results, false positive rate = 7.3% *Latest data 5.9%*
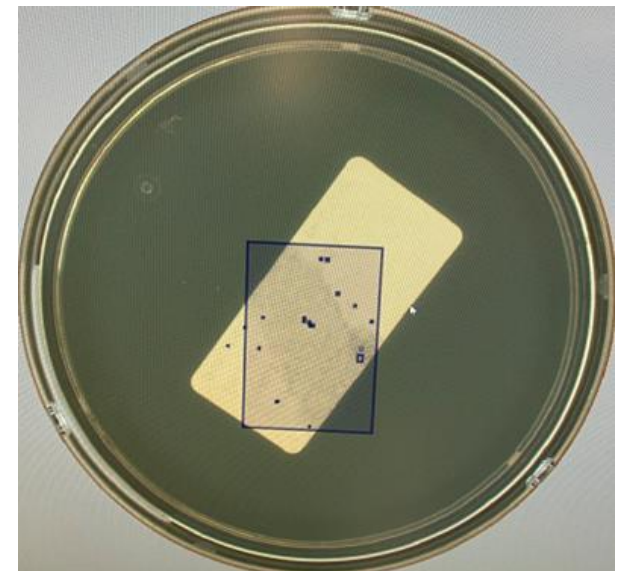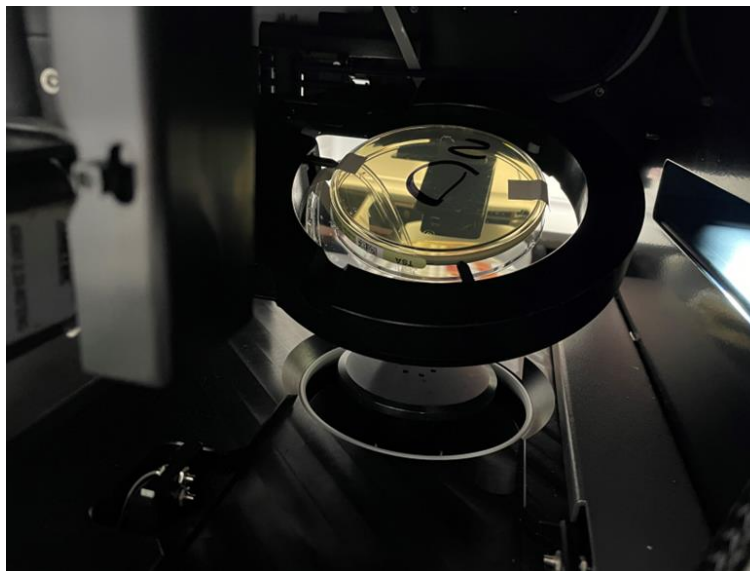
Key Learning Points

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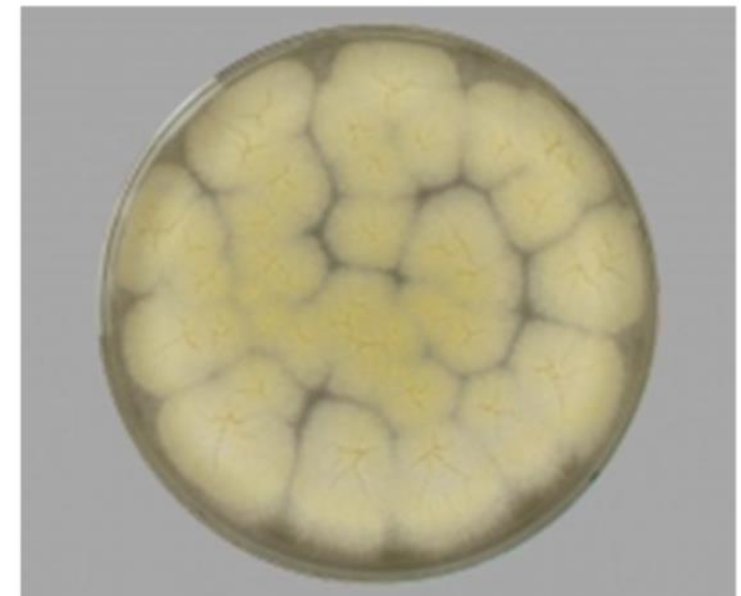
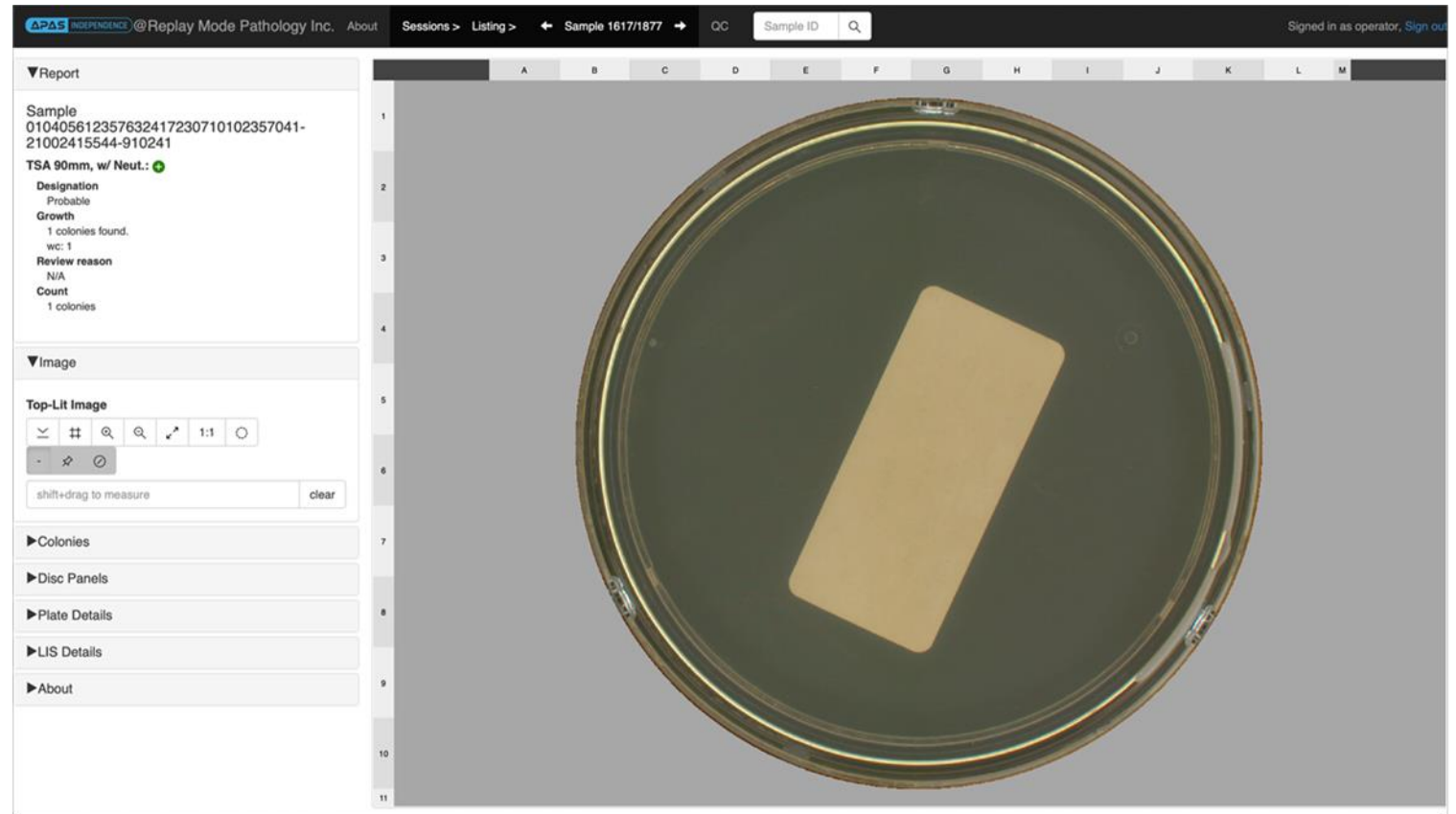Figure 4. Example of A. brasiliensis growth changes over time demonstrating counting challenges

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.

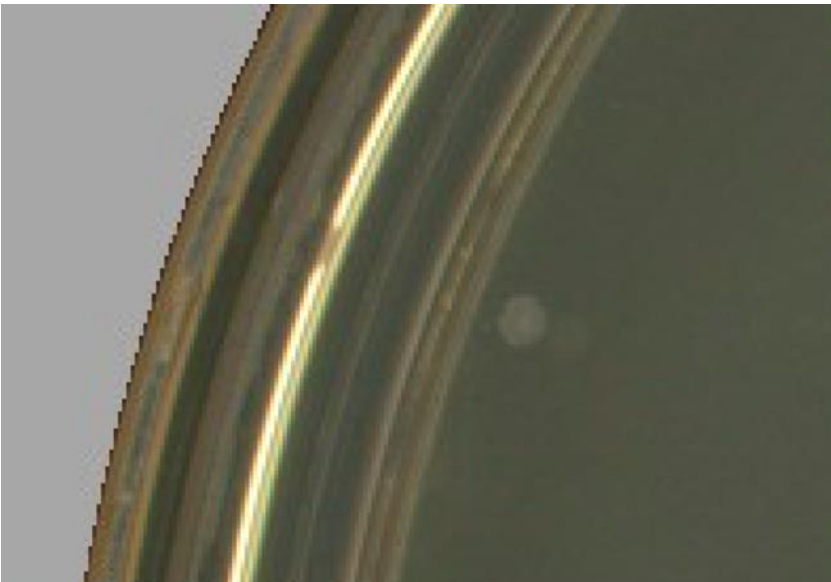- Single colony detection the most important factor.
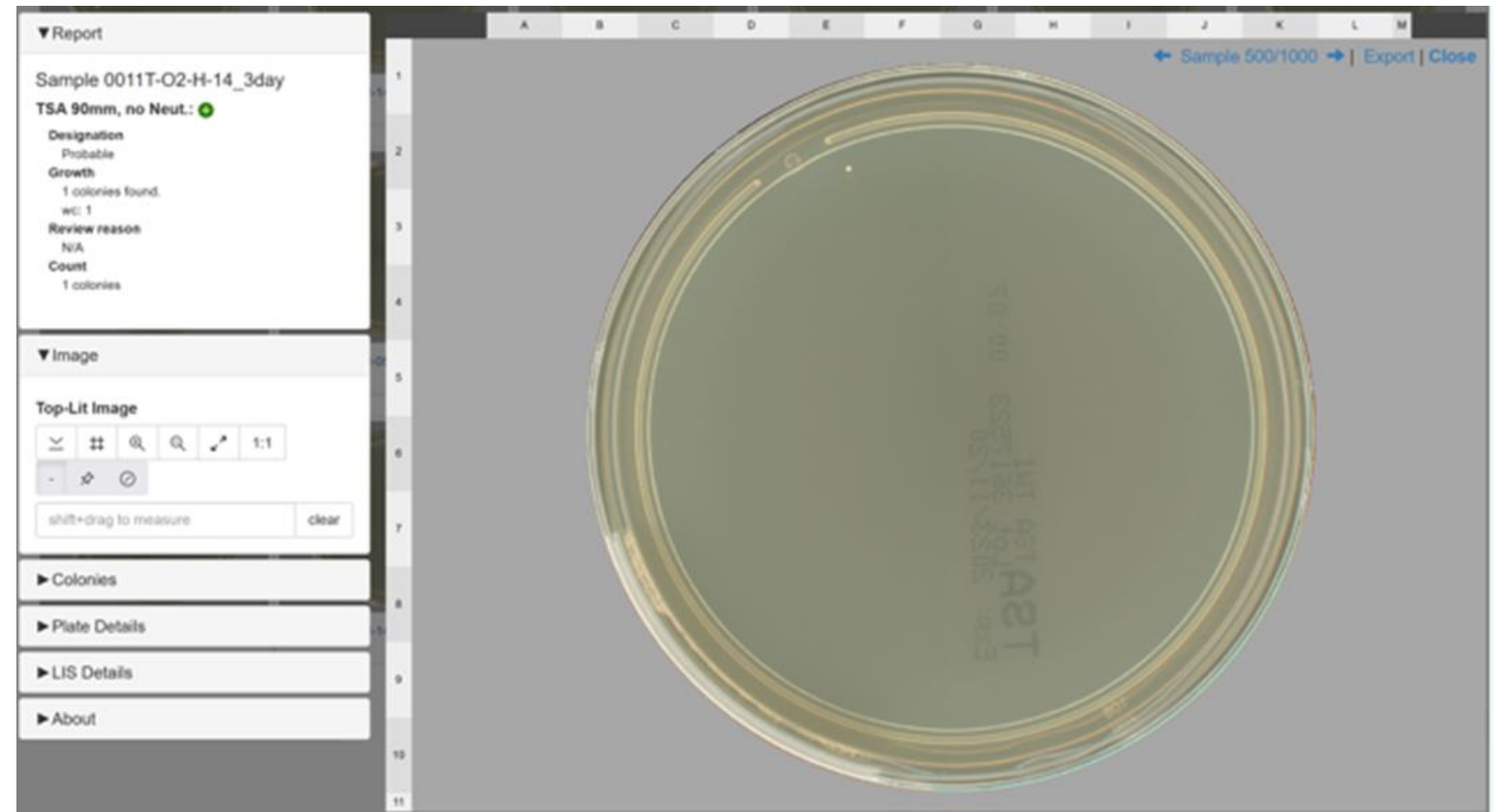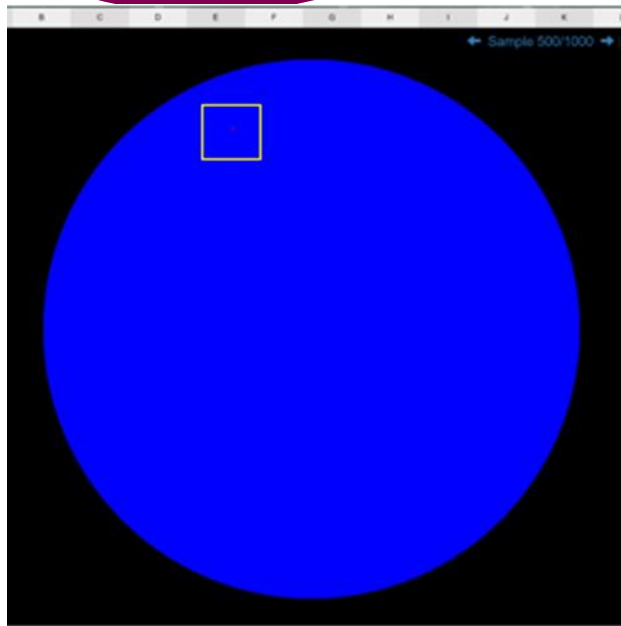
Key Learning Points

- Value of APAS proven during data collection
- Single colony missed by humans, detected by APAS
- Most important acceptance criteria are that it never misses a positive plate, and doesn't give too many false positives

▼ Report

Sample 0017T-31-09

TSA 90mm, no Neut.: ⊕

Designation
  Probable
Growth
  2 colonies found.
Review reason
  N/A
Count
  2 colonies

▼ Image

Top-Lit Image

drag to move image | clear

▼ Colonies

☐ ■ WC

▶ Plate Details

▶ LIS Details

▶ About

▼ Report

Sample 0017T-31-09

TSA 90mm, no Neut.: ⊕

Designation
Probable
Growth
2 colonies found.
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2 colonies

▼ Image

Top-Lit Image

[⌣ | ♯ | ⊕ | ⊖ | ↗ | 1:1]

[↔ | 📌 | ⊘]

drag to move image   clear

▼ Colonies

☑ ■ wc

▶ Plate Details

▶ LIS Details

▶ About

▼Report

Sample 0011T-E1-H-04

TSA 90mm, no Neut.: 🔍

Designation
Review
Growth
5 colonies found, 4 bacterial, 1 mould.
Review reason
Mould colonies present
Count
5 colonies

▼Image

Top-Lit Image

drag to move image | clear

▼Colonies

☐ ▪WC

☐ ▪YC

☐ ▪FF

☐ ▪PR

▼Report

Sample 0011T-E1-H-04

TSA 90mm, no Neut.: 🔍

Designation
Review
Growth
5 colonies found, 4 bacterial, 1 mould.
Review reason
Mould colonies present
Count
5 colonies

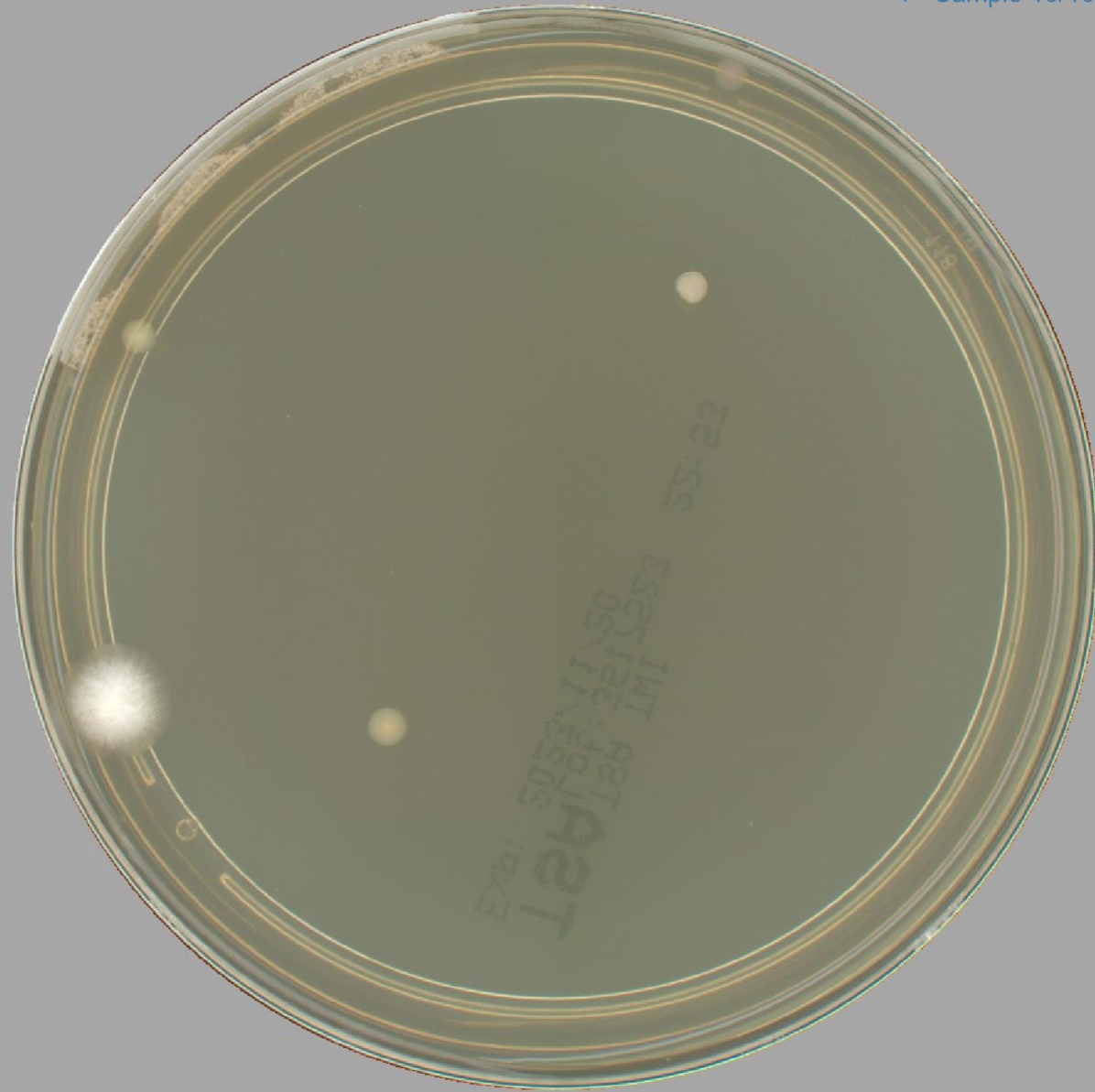# ▼Image

Top-Lit Image

drag to move image | clear

▼Colonies

☑ ■ WC

☑ ■ YC
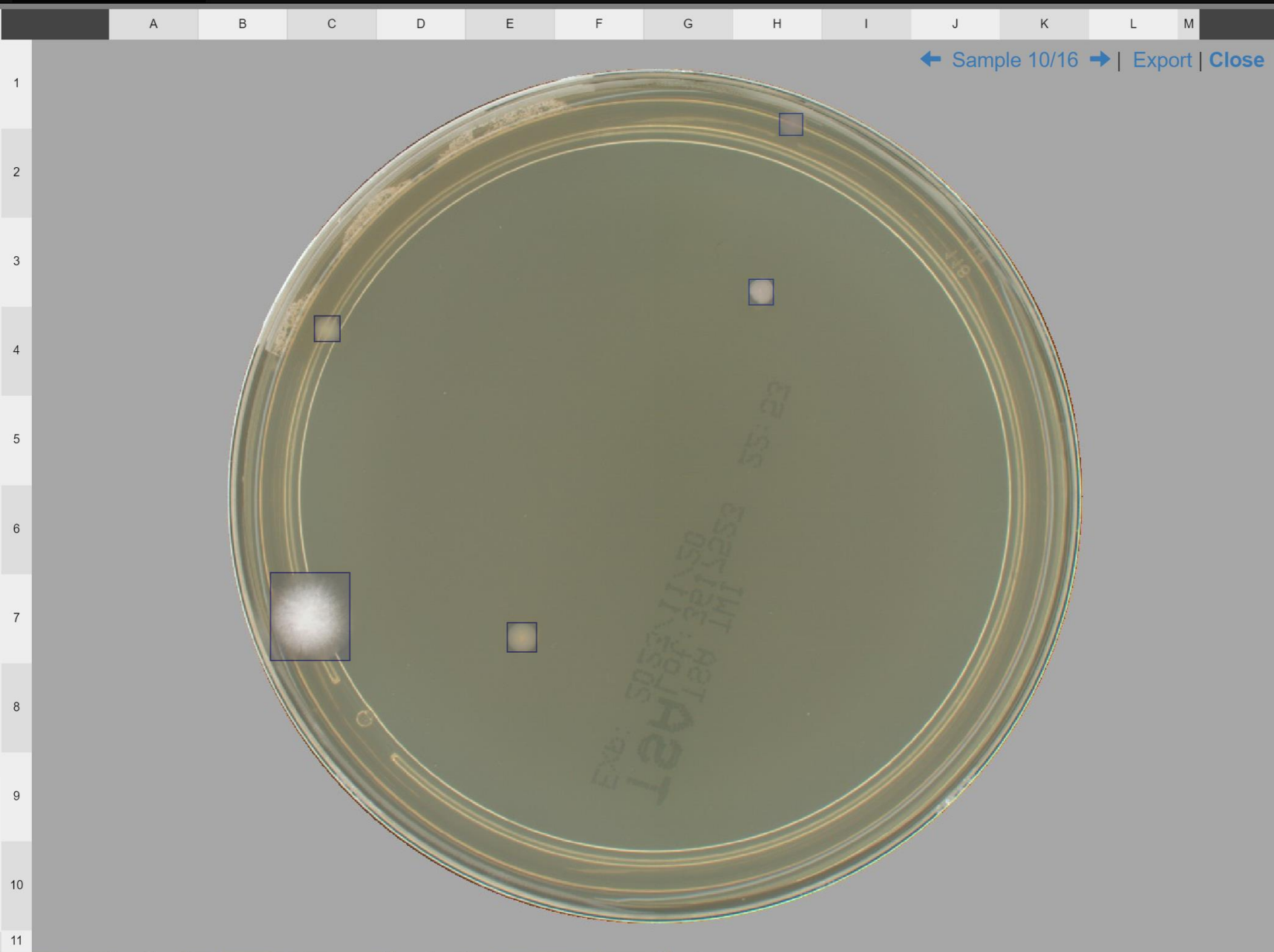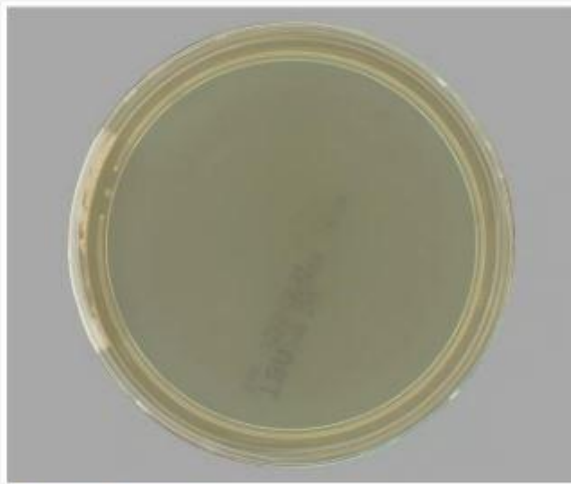
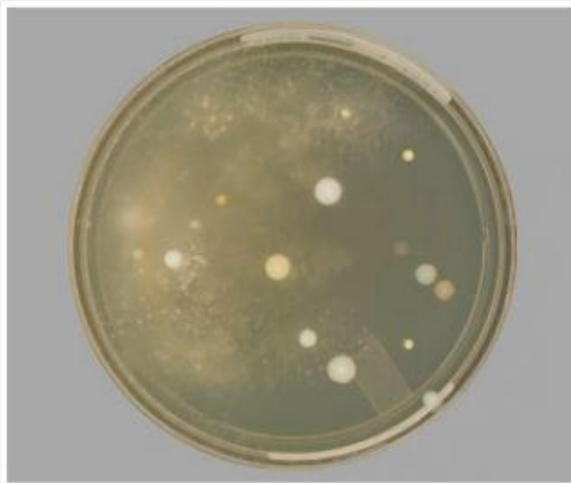☑ ■ FF

☐ ■ PR

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

0017T-31-09
2 colonies found.

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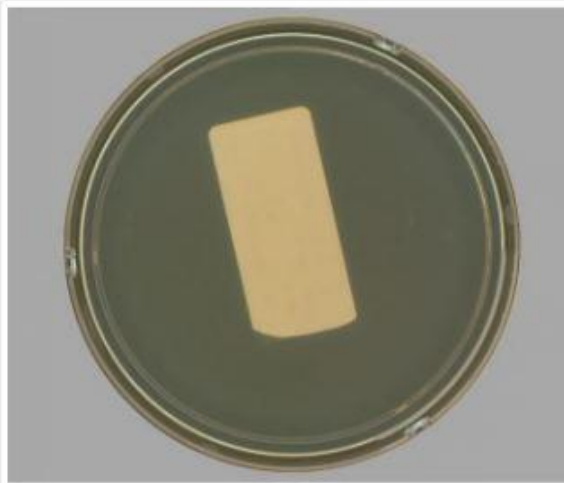50 colonies found, 38 bacterial, 12 mould.

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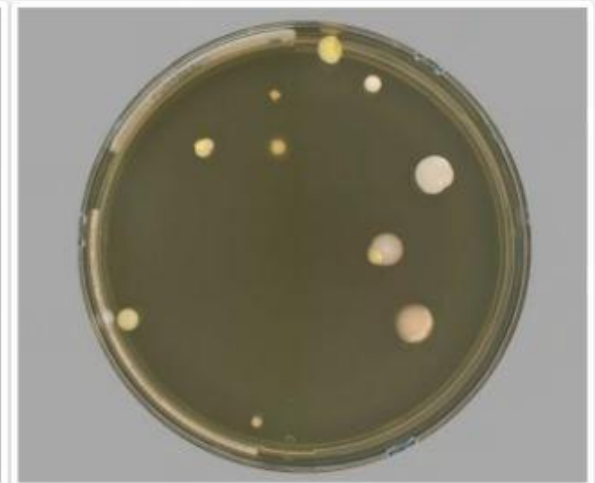30 colonies found, 28 bacterial, 2 mould.

0006TN-D-U4-1
239 colonies found, 214 bacterial, 25 mould.
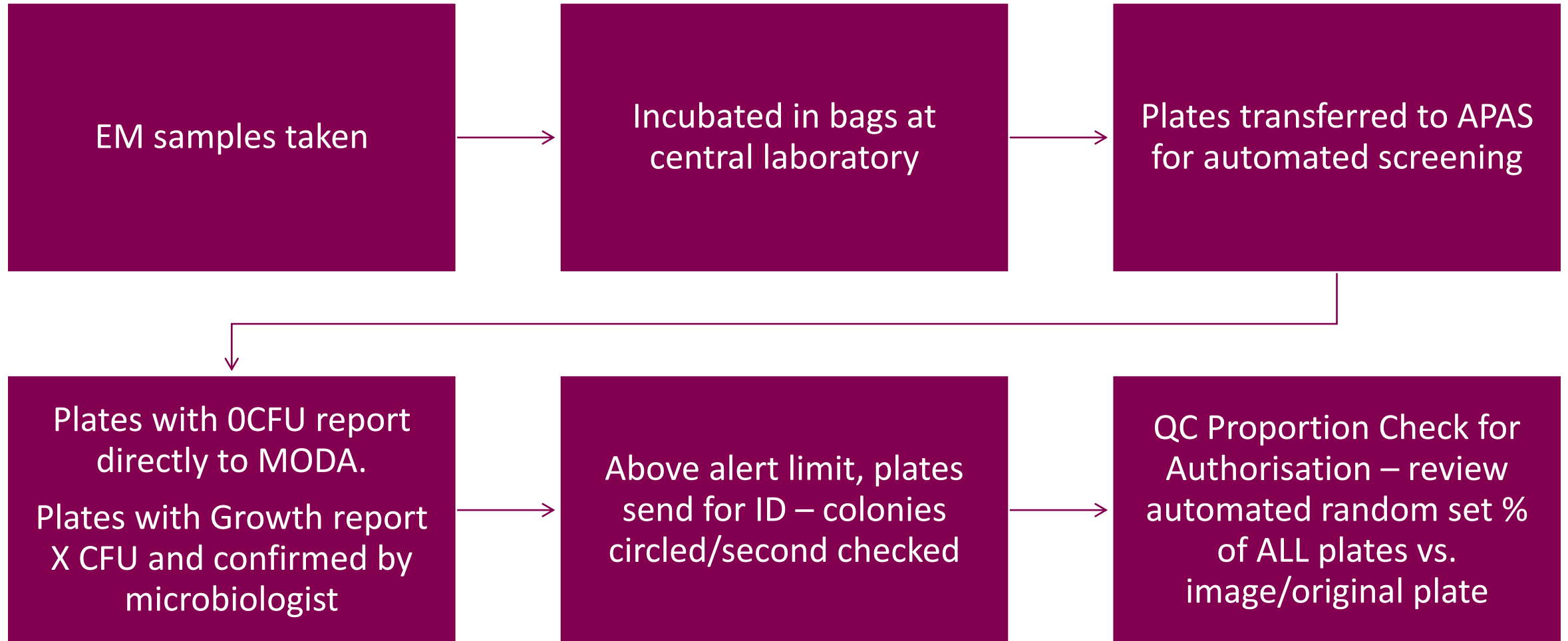
21003134863-910313
0 colonies found.

21005290102-910529
0 colonies found.

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

Envisaged Future State

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┌─────────────────────┐       ┌─────────────────────┐       ┌─────────────────────┐
│                     │       │                     │       │                     │
│  EM samples taken   │  ──▶  │  Incubated in bags  │  ──▶  │ Plates transferred  │
│                     │       │  at central         │       │ to APAS for         │
│                     │       │  laboratory         │       │ automated screening │
└─────────────────────┘       └─────────────────────┘       └─────────────────────┘
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┌─────────────────────┐       ┌─────────────────────┐       ┌─────────────────────┐
│ Plates with 0CFU    │       │ Above alert limit,  │       │ QC Proportion Check │
│ report directly to  │       │ plates send for ID  │       │ for Authorisation – │
│ MODA.               │  ──▶  │ – colonies          │  ──▶  │ review automated    │
│ Plates with Growth  │       │ circled/second      │       │ random set % of ALL │
│ report X CFU and    │       │ checked             │       │ plates vs.          │
│ confirmed by        │       │                     │       │ image/original      │
│ microbiologist      │       │                     │       │ plate               │
└─────────────────────┘       └─────────────────────┘       └─────────────────────┘
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Key Points for Regulatory Opinion

- Image Storage
  - Manual process plates are discarded, and the raw data is the count.
  - Other plate readers approved for use have no image storage capability.
  - Sustainability and software speed challenges with storing 30,000 a month.
  - Proposal is to store validation images.
  - In process images until authorisation of results in MODA.

- Guidance on the need to second check the negative plates.

- Once the model is "locked" and no longer learning. Follow normal laboratory change control GMP processes.
  - Software updates could either be compared against the original validation images or a set of plates with counts prepared and read before software update and then immediately after and results compared.
  - Are there specific expectations for validation for the AI algorithm even though it will be locked down?

Key Points for Regulatory Opinion

- Once validated, and because there is a secure audit trail and traceable data transfer from APAS to MODA, there will be enough evidence to minimise any requirement or expectation for second checks and /or verification of negative counts?

- What is the specific minimum expectation to define equivalent or better since there is some subjectivity in counting by humans?
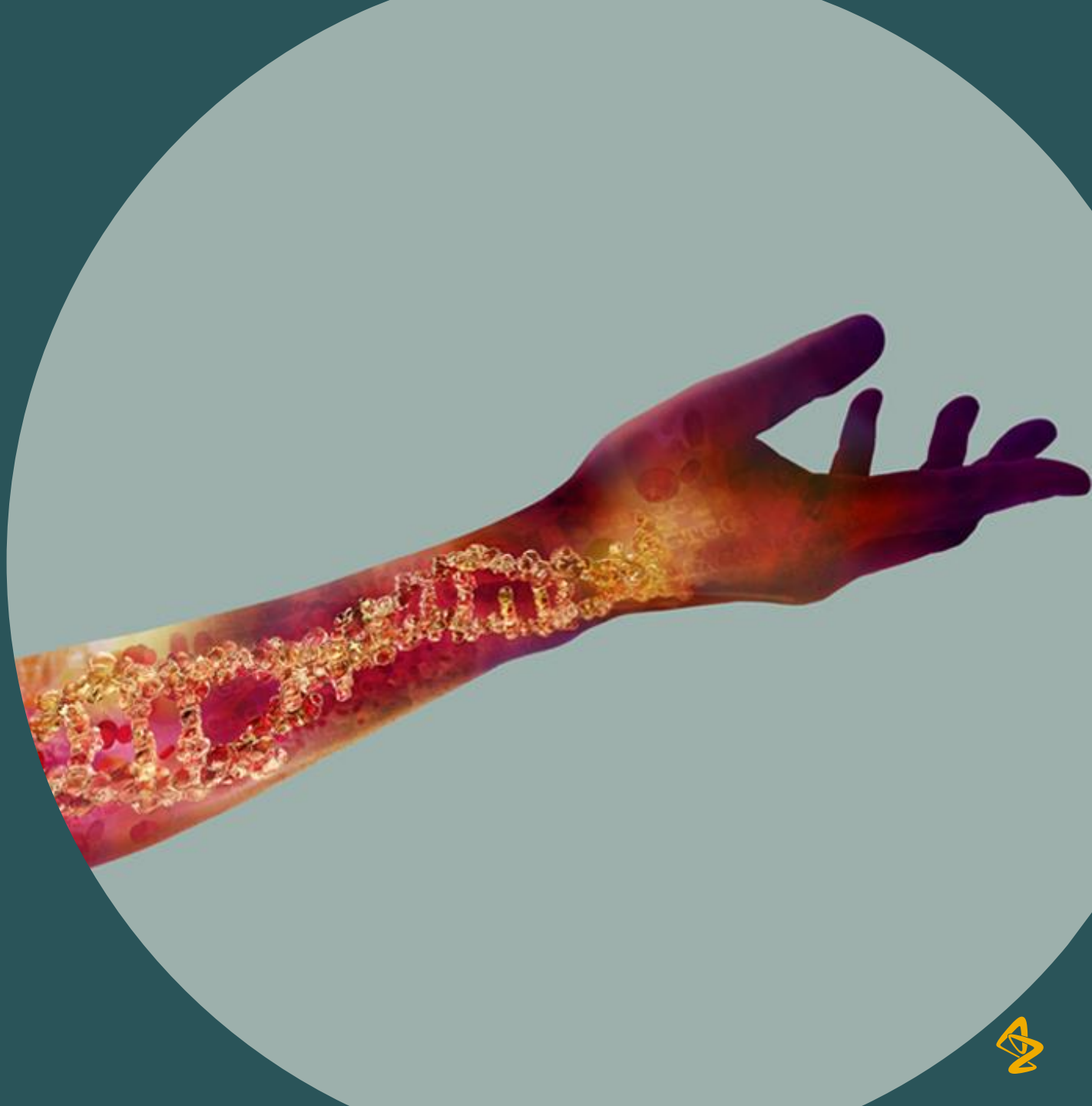
Potential Risks and future considerations

- Acceptance by regulators?
- Considerations for image retention
- Flawless interface with MODA
- Requirement to expand to 55mm contact plates
- Number of false positives needs to be acceptable
- On-going Performance Monitoring of APAS
- Consideration for number of 'checks' – percentage of negatives reviewed?
- 'Reading' ability of humans needs to be retained

- We see all these as important but solvable and the benefits far exceed the risks.

Questions & Discussion

Confidentiality Notice