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INTRODUCTION

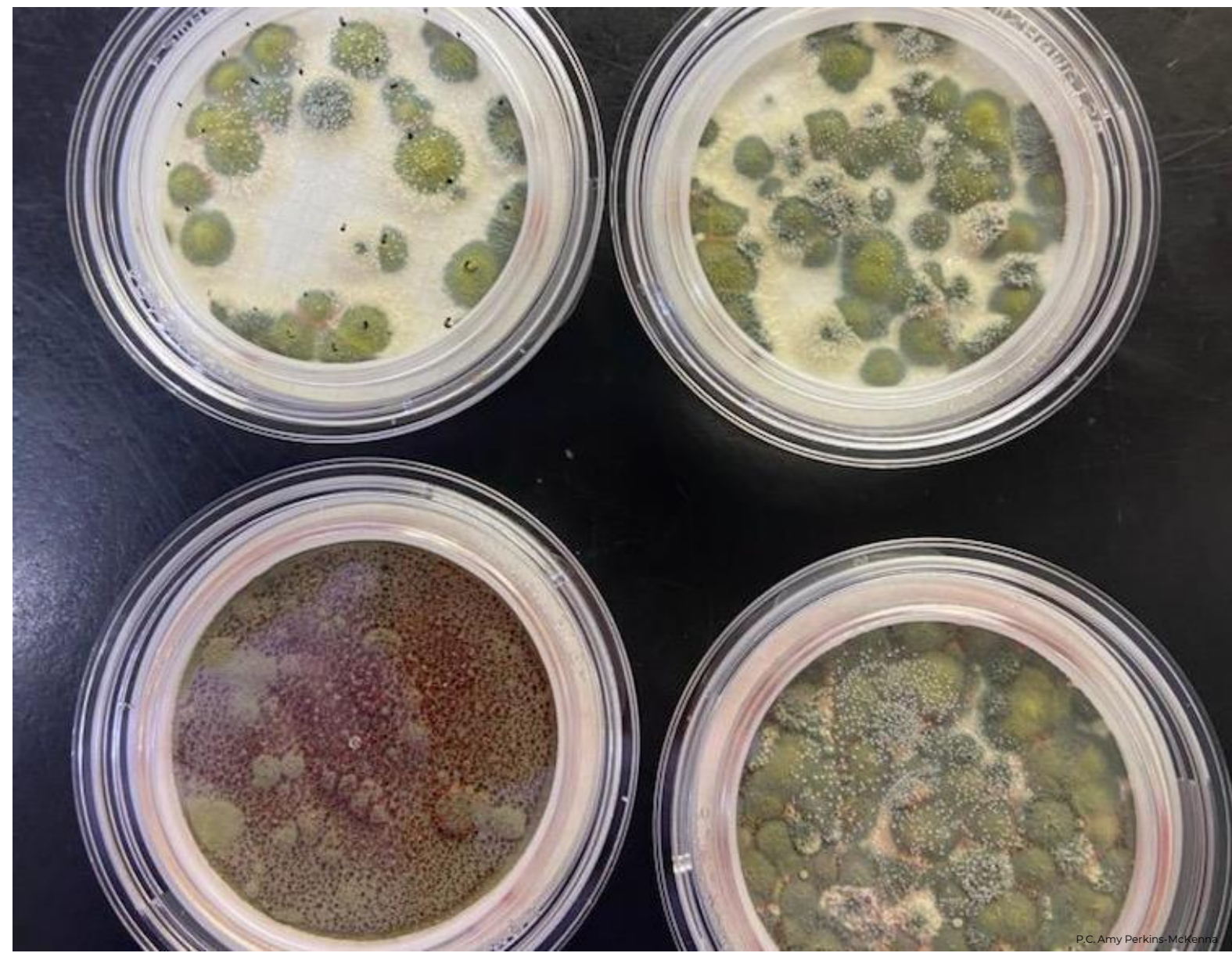
Microbial identification is a critical component for a facility's contamination control strategy and contamination response. Knowing your contaminant genus and species allows a contamination response team to specifically target the problem organism, treat it quickly, and maintain control of the manufacturing process.

There are several strategies for microbial identification with varying degrees of accuracy and speed. At AGC Biologics, we examined our process turn around before and after implementation of a

Bruker MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry paired with the Axcis Organism Database from Accugenix and Charles River.

Time from sample to result was examined for both routine lot disposition work as well as between high urgency cases of contamination events.

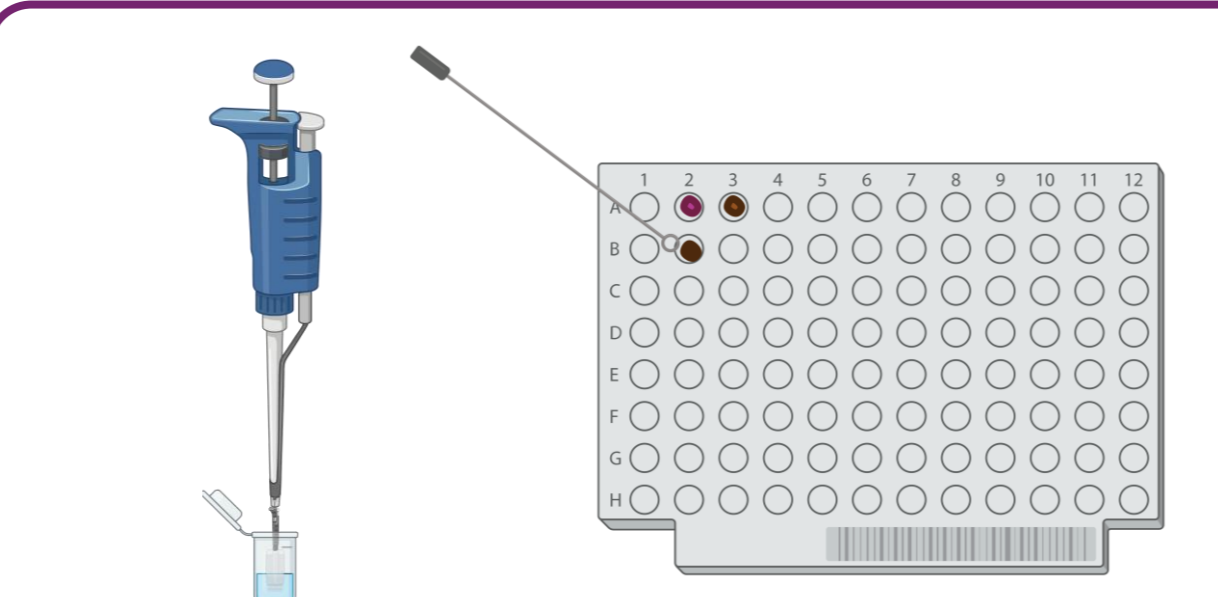
IF YOU SAW THIS...



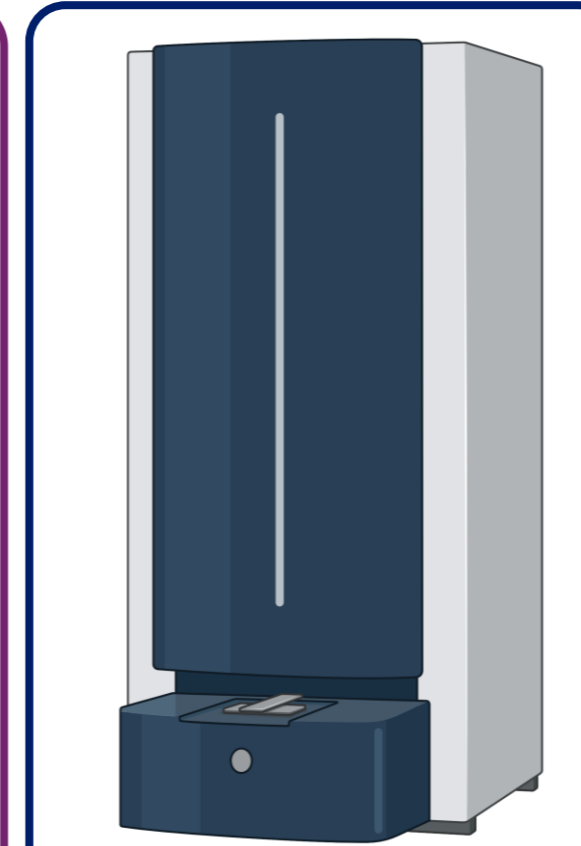
Unique isolates are selected from the primary sample and restreaked for isolation.



Isolated colonies are picked from the streak plate and transferred to the MALDI plate.



Matrix and Acid is added on top of the directly transferred bacterial colony. These chemicals assist in the ionization of peptides during laser ablation. An extraction method may also be used for yeasts, molds and difficult bacteria.



Generated spectra is compared against the library and a result is generated in seconds.

THE MALDI-TOF PROCESS

...HOW QUICKLY CAN YOU RESPOND?

Microorganisms won't always cooperate on a timeline necessary in biologics manufacturing. When shipping organisms out to a contract laboratory for identification, small delays in growth can add up when time is of the essence.

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immediately compared to the microbial database, Axcis, and a report is generated within seconds, providing immediate feedback and results.

Time from sample to result was examined for both routine lot disposition work as well as between high urgency cases of contamination events. During routine preventative maintenance, delays in instrument release highlighted the cost and time savings felt by the laboratory, manufacturing and clients as we returned to shipping critical samples out for delivery. Even with a requested 0-day turnaround, identification could take 4 to 5 days.

CASE STUDY A: FOREIGN BODY in DAY 11 N-STAGE

Friday	Filamentous foreign bodies detected on Vicell imaging. Day 11 retain sample placed on test for microbial analysis
Saturday	
Sunday	Observed contaminant restreaked for isolation by QC Microbiology
Monday	Samples shipped to Accugenix
Tuesday	
Wednesday	Results received from Charles River

In each of these cases, immediate response and action was critical. In Case A, the organism was a filamentous fungi and required both restreaking and waiting for the primary work week to ship (a Monday). With the shortest requested turnaround, due to the timing of the contamination event, identification results took 6 days from the time the contamination event was noticed.

While the event occurred in a single-use system, the room remained quarantined until appropriate remediations could be made as the ingress and root cause required the identification to proceed.

Critical Contamination Turnaround

The most critical samples, those recovered from in-process manufacturing samples, require the quickest turnaround for identification within the bounds of organism performance. Evaluation for objectionable organisms is critical for appropriately triaging risk to the process and patient.

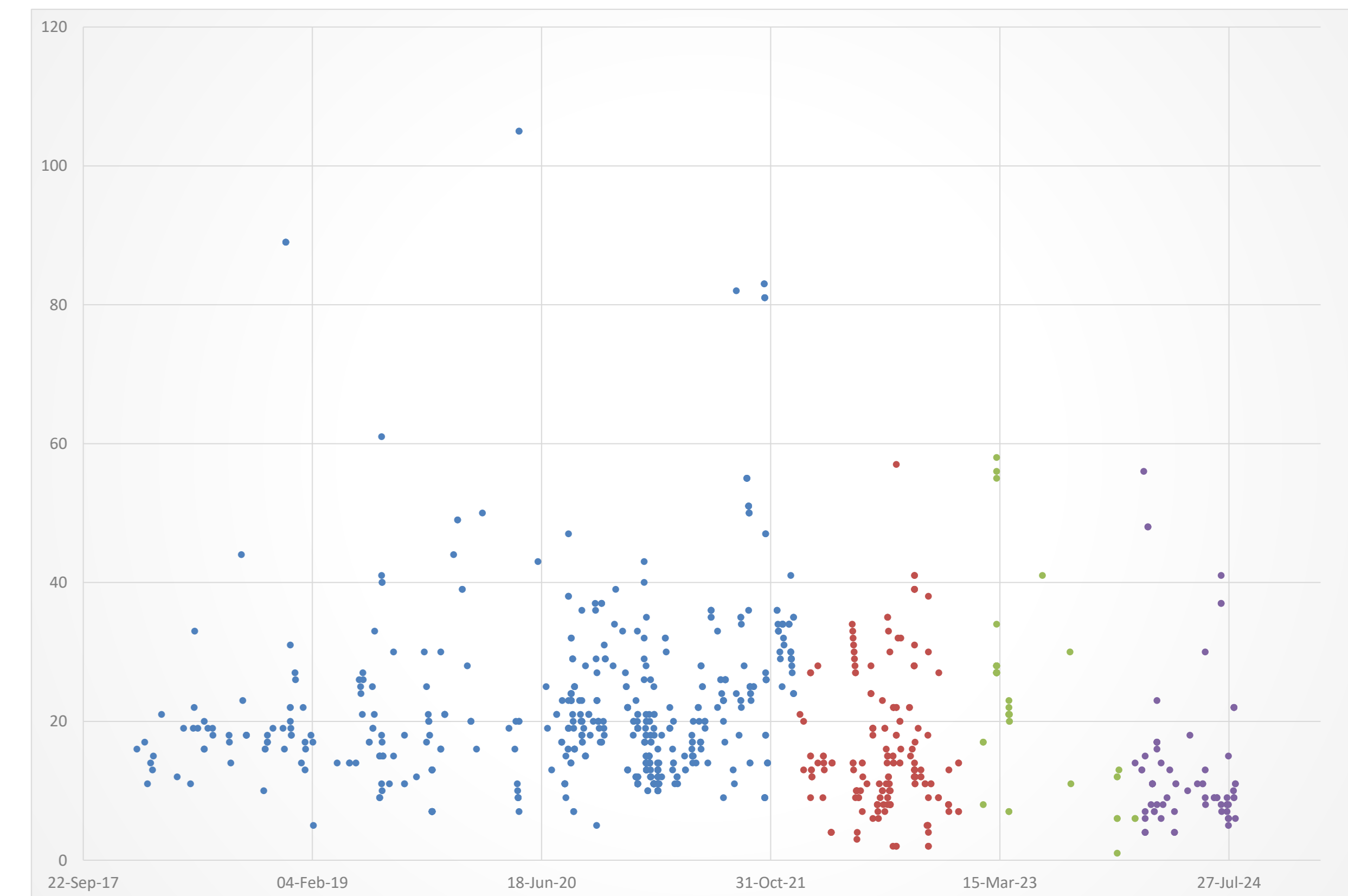
The graph to the right establishes time from the sample being generated to known identification being reported. From 2018 through mid 2022, all microbial identification was shipped to Accugenix. From 2022 onward, turnaround time clusters closer toward the 10 day mark.

Outliers are still present, notably for samples which presented difficulty in achieving valid results in house, which then still required shipping for further processing at Accugenix. Additional spikes in time to result occurred during preventative maintenance when the MALDI-TOF was out of service for a software update and change control.

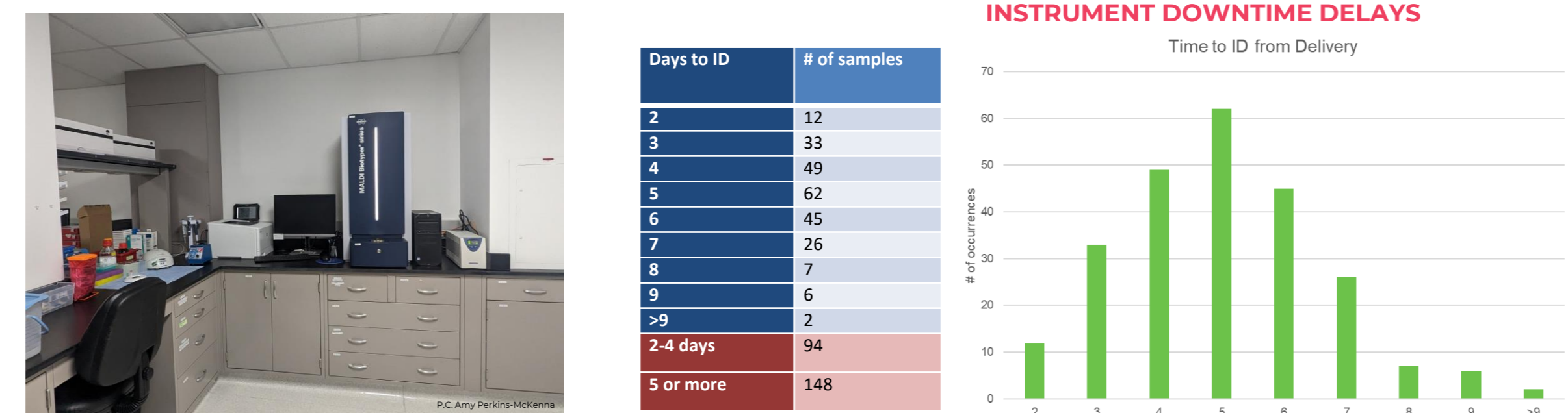
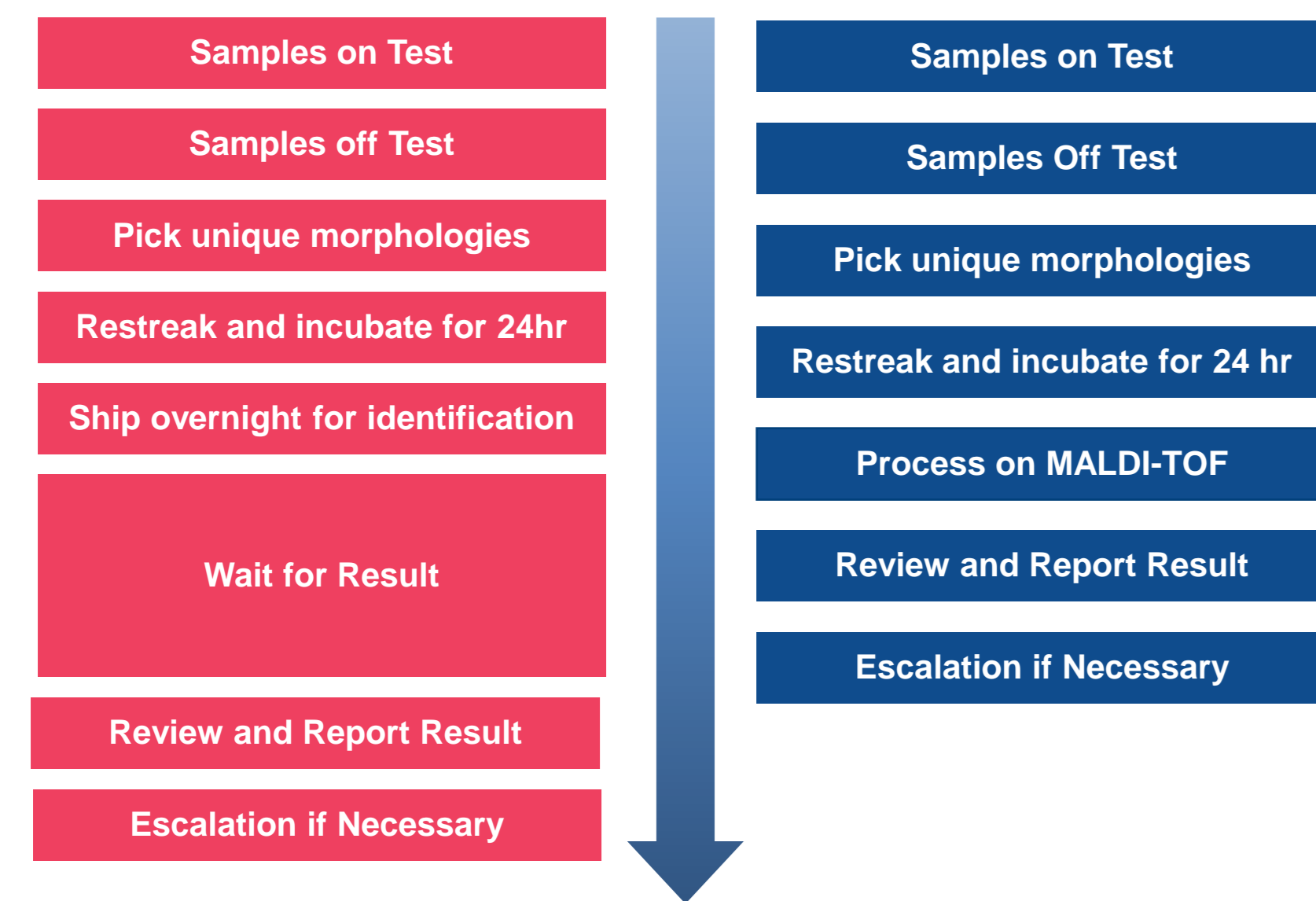
CASE STUDY B: BIOREACTOR CONTAMINATION

Sunday	Low dissolved oxygen value in the bioreactor. Contamination observed on ViCell imaging. Samples placed on test for microbial analysis as part of contamination response.
Monday	Contaminant restreak by QC Microbiology for isolated colonies
Tuesday	Isolated colonies growth is sufficient. Analysed same day on MALDI-TOF, results immediately shared with Deviation investigation team.

For Case B, the organism was robust and grew into a healthy lawn in less than 24 hours. Restreaking for isolation was required to guarantee a valid result on MALDI. Results were available to the Deviation investigation team within three days, allowing the team to bring in an appropriate contractor to remediate the stainless-steel bioreactor and have it ready for the next scheduled batch of product.



PROCESS FLOW CHART BEFORE AND AFTER

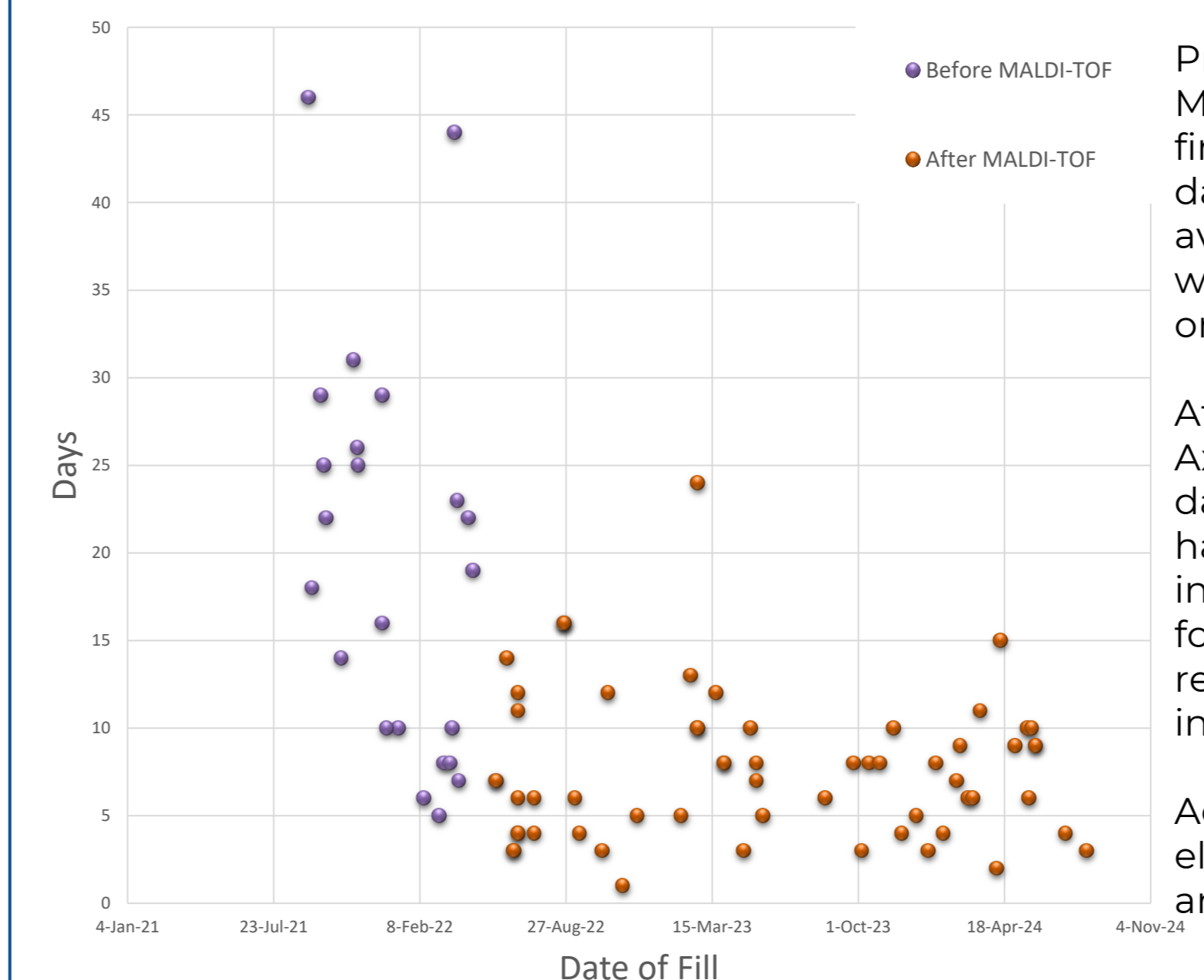


IMPACTS FROM DELAYS

- BUSINESS RISK**
 - Extended deviation closure time negatively impacts site KPIs
 - Delay in lot disposition for client lots means a delay in payment
 - Increased price per sample to ship out for testing vs test in-house.
- QUALITY and PATIENT SAFETY RISK**
 - Root cause analysis (RCA) is more successful the closer to the deviating event. RCA requires organism identification
 - Organism identification better enables risk assessment in contamination events
 - Corrective and Preventative Action (CAPA) may not address the root cause if implemented without knowing the ID

OVERALL PROCESS PRODUCTIVITY

Process Specific Monitoring: From Final Fill to Identification



Prior to completed onboarding of the MALDI, the average time from the date of final fill to organism identification was 28 days. Subtracting incubation time, the average was 19 days, and almost three weeks before investigation could input organism ID into root cause analysis.

After onboarding of the MALDI-TOF and Axcis libraries, the average time from date of final fill to organism identification has dropped to 16 days. Subtracting incubation time, brings the average wait for identification to 7 days, over a 50% reduction in wait, allowing deviation investigations to proceed quickly.

Additionally, extreme outliers have been eliminated. Averages continue to drop as analysts increase efficiency.

CONCLUSIONS

Implementation of a Bruker MALDI-TOF microbial identification system paired with Accugenix Axcis data base improved turnaround times for situations where microbial identification gated forward progress. Deviation closure was no longer delayed, waiting for identity, and removed ID as a gating item to lot disposition.

Agility improved when responding to high criticality contaminations in both upstream and downstream processes. System remediation could be initiated and completed prior to the next in-flight batch, preventing batch loss. Per sample cost is decreased when testing internally compared to shipment to offsite vendors.

Additional impact is seen in cost per sample to process and achieve identification. Costs increased 3.5 fold during instrument downtime for a software upgrade and re-validation to process and ship samples externally.

ACKNOWLEDGEMENTS

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