Case Studies Applying Enhanced Automation and Rapid Detection of Mold from Bacteria

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Introduction

The use of environmental monitoring (EM) testing is crucial for contamination control in cleanrooms and production areas. Mold contamination is considered a loss of control over the environment according to Annex 1. Due to the significant impact of mold contamination in a final product, detecting the presence of mold quickly is essential for patient safety. Rapid Micro Biosystems (RMB) has developed sophisticated vision algorithms combined with automated colony counting with the Growth Direct® System can confirm the qualitative presence of mold in less than 24 hours and has shown to detect a panel of sixteen common environmental mold isolates with ≥ 95% accuracy. In software verification studies, automated mold detection was shown to detect mold when present across those sixteen species within 12 – 24 hours, with a consistent overall count within validated testing parameters, in this case: 72 hours.

The following case studies have demonstrated that automated mold detection enhances EM testing workflow and eliminates the need for additional operator assessment. This leads to faster remediation using validated cleaning procedures to return facilities to a decontaminated state. This presentation will explore validation approaches for this rapid microbial method, including deviation investigations and reporting for mold contamination when it has been detected.

Methods

Routine-Use Simulation (n = 200)
- Sampling conducted over 4 days, 5 different non-classified zones
- 100 total surface samples
- 100 total active air samples

Time and Savings Analysis
- Evaluated trending data of Class A/B, C/D, and compared with data from routine-use simulation and manual/visual methods. Modeled impact of mold on overall detection remediation following guidance with a zero-tolerance mold policy.

Technology

The Growth Direct® System is an automated, rapid microbial detection and enumeration platform suitable for in-process product testing, environmental, and water monitoring that integrates digital imaging, robotic cassette handling, incubation, and software control. When paired with RMBNucleus™ Mold Alarm algorithm enhancement software, currently available for environmental monitoring applications, the system automatically detects the presence or absence of mold in addition to providing the total count in 50% of the time as traditional manual methods.

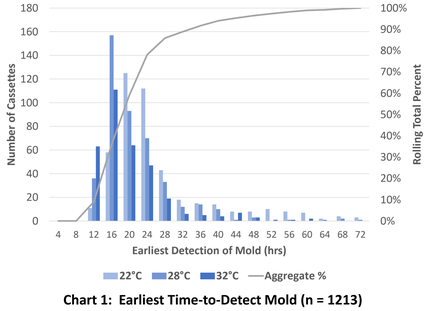
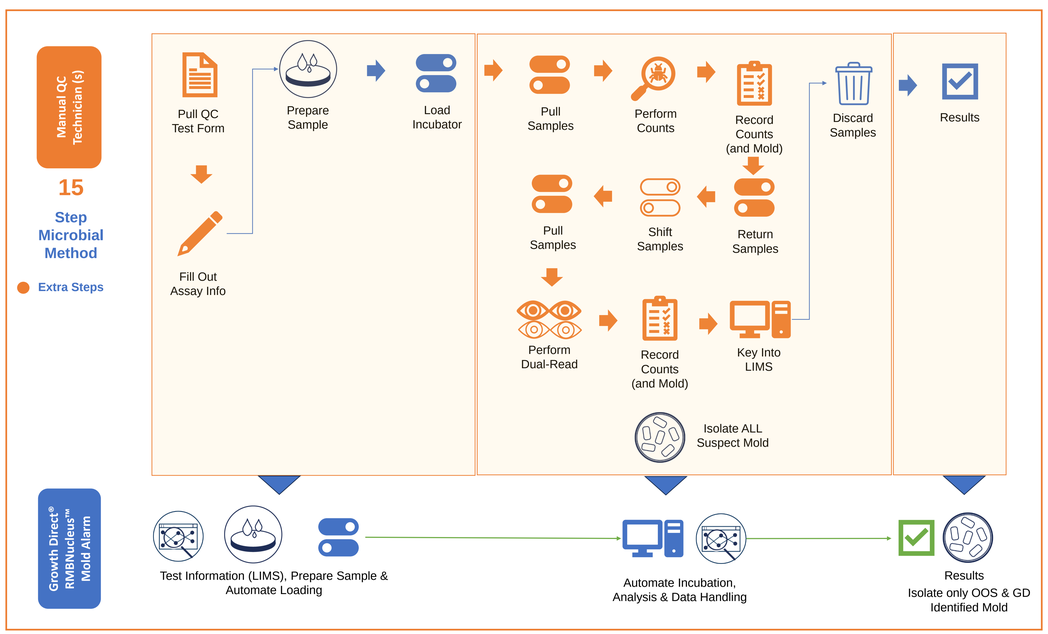


Chart 1: Earliest Time-to-Detect Mold (n = 1213)

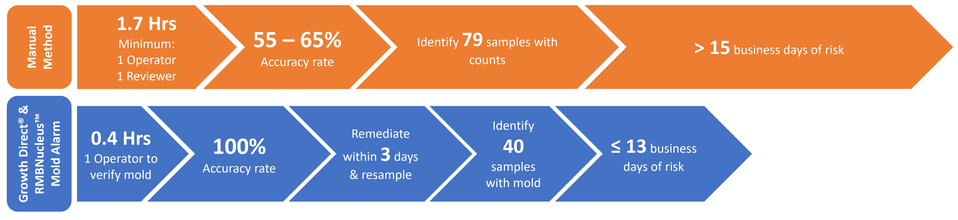
Scan the QR code to gain direct access to our technology page for additional details.

Last year at PDA Micro, RMB presented accuracy data from a 1000+ cassette verification study (see Chart 1). The data indicated RMBNucleus™ Mold Alarm algorithm performance has ≥ 95% accuracy within 72 hours for the detection and differentiation of mold. Initial time to detection (TTD) was shown to occur for 80% of the mold panel within 24 hours or in "as little as a day." Additionally, the technology allows earliest consistent detection within 12 hours for 18% of the organism panel – these case studies presented continue to verify those results with natural bioburden.



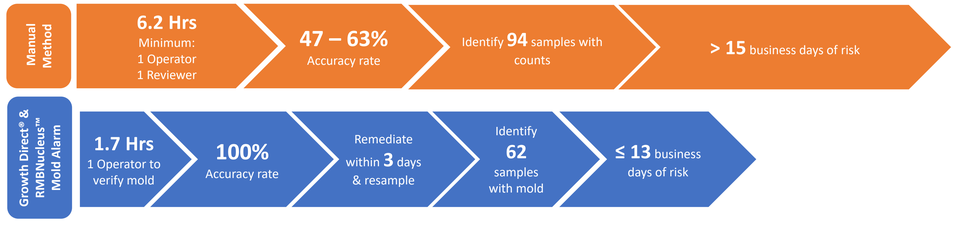
Case Study: Class A/B Comparison and Savings

Work-flow examples for Class A/B rooms using human accuracy for visual mold detection. In this example, Class B is defined as < 10 CFU max expected counts and Action limit policy is < 6 CFU.



Case Study: Class C/D Comparison and Savings

Work-flow examples for Class C/D rooms using human accuracy for visual mold detection. Class C/D in this example is defined as < 51 CFU max expected counts and Action limit policy is < 10 CFU.



Acceptance Criteria & Simulated Routine Results

- ☑ Equivalent or better total count: Growth Direct® vs. Operators
- ☑ Reduced TTR (time to result): 3 days from 5 days
- ☑ Reduced TTD (time to detect): 12 hours
- ☑ Mold detection capability – 173 samples had mold
  - RMBNucleus™ Mold Alarm false negative mold: **0%**
  - RMBNucleus™ Mold Alarm false positive mold (4): **2%**

Human Operator Performance

- ☒ Human mold detection capability – 123 samples detected
  - All operators agree (90): **52%**
  - At least 1 of 3 operators (33): **71%**
- ☒ False negative mold:
  - All operators agree: **31%**
  - At least 1 of 3 operators: **50%**

Impact

In aggregate of A/B and C/D environments, 80% of the mold species were detected within the first 24 hours and 94% within the first 36 hours (See Chart 2), due to the accelerated detection capability, cleaning remediation can begin immediately. In parallel, identifying the 40 mold containing samples for records. It is possible to receive results of successful remediation through cleaning, training, and re-sampling within the time it may take to receive identification results.

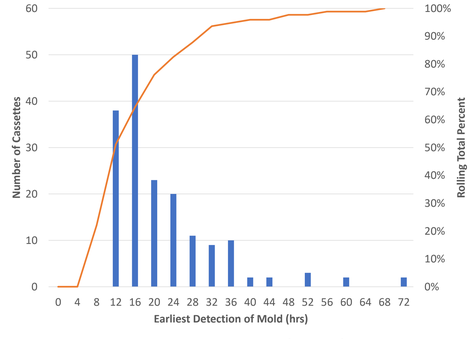


Chart 2: Aggregate Time to Detection in A/B and C/D Environments

Conclusion

These case studies confirm that RMBNucleus™ Mold Alarm for the Growth Direct® System delivers 76% faster time to detection in Class A/B and 73% faster time to detection in Class C/D than the manual method detection, with 100% accuracy compared to 47 – 65% accuracy in initial detection by human operators. These enhancements improve the micro-QC experience and reduce risk compared to reliance on human operators. Remediation and re-sampling can occur within a day, reducing the risk of additional excursions during investigation. While zero-tolerance policies do require identification of molds for trending, those identifications can occur in parallel rather than gating other remediation activities.

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References

1. Anders, Hans Joachim, et al. "Multisite qualification of an automated incubator and colony counter for environmental and bioburden applications in pharmaceutical microbiology." *PDA Journal of Pharmaceutical Science and Technology*, vol. 77, no. 3, 2022, pp. 236 – 247, https://doi.org/10.5731/pdajpst.2022.012742
2. USP <1223> Validation of Alternative Microbiological Methods
3. Annex 1: Manufacture of Sterile Products