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Validating the Negatives

A practical guide to validate EMMA's FNR for reliable environmental monitoring in pharmaceutical QC

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Reliable automated environmental monitoring in pharmaceutical QC: validating EMMA's FNR

This whitepaper introduces a scientifically robust validation protocol focused on determining EMMA's false negative rate (FNR), a critical performance metric for regulatory compliance. The proposed method applies binomial statistics to confirm, with 95% confidence, that the FNR remains below 0.1%, reflecting a significant improvement over conventional manual readings. This approach supports the integration of validated automation into microbiological quality control processes while aligning with USP <1223>, Ph. Eur. 5.1.6, and EU GMP Annex 1.

Environmental monitoring in GMP-regulated pharmaceutical QC

Environmental monitoring is a crucial aspect of ensuring the safety and quality of pharmaceutical products manufactured according to Good Manufacturing Practices (GMP). A controlled and monitored environment is essential to protect both the production process and the final product from microbial contamination, which could pose a significant risk to patient health and safety.

A key component of environmental monitoring is the regular analysis of air, surface, and water samples collected in cleanrooms and production facilities. These samples are evaluated to detect potential microorganisms such as bacteria, yeasts and molds.

Traditional environmental monitoring methods involve the culturing of these samples on agar plates in Petri dishes, followed by manual colony counting and identification. While widely used and accepted, these conventional methods that rely on human analysis are time-consuming and susceptible to human error. False negative rates are highly depending on the individual performing the analysis as their training and experience vary. The industry reports risks of 5-10%. The four-eyes principle is recommended to mitigate the risk. It ensures data integrity and accuracy as the readings are performed by two qualified microbiologists who independently assess and verify the presence or absence of microbiological contaminations, microbial counts and colony morphology. This dual-review process minimizes errors and enhances compliance with regulatory standards such as EU GMP Annex 1, USP <1116> and the FDA's Aseptic Processing Guidance. It also strengthens the reliability of microbial monitoring results.

Key take aways

- This whitepaper, along with its accompanying protocol, aims to provide a step-by-step guide to a practical, time-efficient and scientifically sound validation process, focusing on reporting the false negative rate.
- To ensure the effectiveness of the EMMA system in GMP regulated pharmaceutical QC, it is crucial to validate its performance as recommended in Eur Ph 5.1.6 and USP <1223> on validation of alternative microbiological methods.
- Regulatory compliance, reliable microbial detection and operational efficiency are ensured by focusing on false negative validation.
- By prioritizing specificity, EMMA supports strict GMP environmental monitoring standards while reducing manual workload and improving operational efficiency. EMMA's workflow also enables the enhancement of overall data integrity.

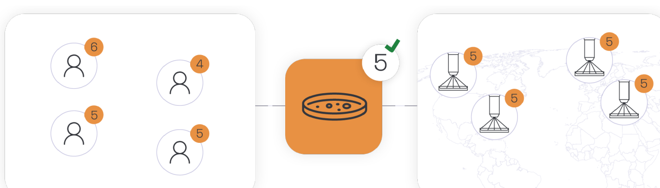


Figure 1: Imaging and vision AI allow for reproducible analysis and enumeration.

The value of automated readings and AI based analysis in pharmaceutical QC

	Manual reading	EMMA
Time per sample	5-10 min	<1 min
Error rate (FNR)	5-10%	<0.1%
Subjectivity	High	None
Scalability	Low	High
Data Integrity	Manual logs	Digital, tracable

Table 1: comparison between manual and digital readings.

GMP Annex 1 (2022 revision) guidelines encourage the adoption of automated methods to enhance the reproducibility, accuracy, and reliability of environmental monitoring processes. Integrating automated sample evaluation and AI driven analysis will improve organisations' QC processes. By minimizing the risks associated with non-conformities they ensure to continue to meet regulatory standards and maintain production integrity.

Recent advancements in artificial intelligence (AI) and computer vision technology offer a solution to the challenges that come with manual readings. These systems, such as the EMMA system, can 1) automate and digitize Petri dish reading and 2) automate the image analysis and triage. A shift to automated reading and analysis significantly reduces human error and increases throughput, making the testing process more reliable and efficient (Table 1).

In this particular case, automation does not replace human expertise but optimizes the workflow by facilitating for example the triage of positive (= plates with microbial contamination) versus negative plates (=plates without microbial contamination). Negative plates, which constitute the majority (>99%) of samples in Grade A and B cleanrooms (as per EU GMP Annex 1) can be efficiently processed by automation, allowing qualified microbiologists to focus on confirming and analyzing positive plates. This ensures that the four-eyes principle remains in place, with AI assisting in pre-screening while the microbiologist perform the final evaluation and confirmation.

GMP guidelines encourage the adoption of automation to improve consistency, reduce human error, and enhance traceability, as described in EU GMP Annex 1. In addition, the implementation of automation in Petri dish analysis offers benefits such as an increased efficiency thanks to the automated and high throughput analysis. Thousands of samples can be evaluated in a fraction of the time required for manual inspection. In addition, the concept of automated plate reading shows improved accuracy and excludes subjectivity compared to manual plate reading and analysis. Lastly, thanks to digitization, the results are archived and securely stored, enabling long-term traceability, retrospective analysis, regulatory compliance and audit-readiness.

The automated workflow (shown in Figure 2) maintains regulatory compliant as it facilitates the four-eye principle by automated image generation of the Petri dishes that need to be evaluated. Once validated, the algorithm can classify samples as positive or negative without human input. It offers the possibility to double check the plates by one or two trained microbiologists. While the AI system independently detects microbial contaminations, human verification remains key, ensuring regulatory alignment and data integrity.

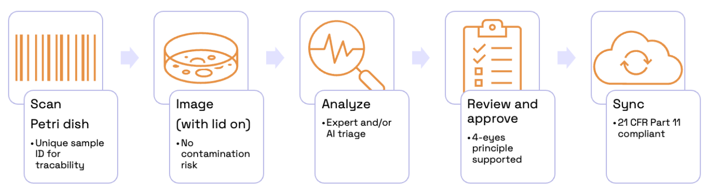


Figure 2: From sample to report. EMMA provides a fully digitized and compliant process. Step 1: the integrated barcode scanner reads the side barcodes enabling logging of each unique sample with time and user stamp. Step 2: time-stamped, high resolution image acquisition with lid on, 100% Petri dish imaging including lateral walls. Step 3 and 4: AI assisted and/or manual analysis for positive/negative triage. Step 4: the 4-eyes principle enforces supervisor review of plates flagged as positive. Step 6: Export the results in .pdf or .xml formats.

Bringing EMMA into your lab - The validation process

To ensure the effectiveness of the EMMA system in a regulated environment, it is crucial to validate its performance against traditional Petri dish analysis methods as recommended in Eur Ph 5.1.6 and USP <1223> on validation of alternative microbiological methods, where a new method should perform at least as good as the current standard practice. By validating the system, organizations can ensure its compliance with regulatory standards and its ability to support quality assurance in GMP facilities.

In this whitepaper, there is a focus on one specific part of the validation process, being the evaluation and validation of the false negative rate (FNR), as part of the performance validation of the system. Since EMMA's automated workflow separates the positive from the negative plates, the critical quality attribute of the validation process lies in the confirmation of the FNR, as it demonstrates EMMA's ability to correctly identify true negatives, ensuring that plates without contamination are accurately classified. Therefore, the protocol outlined in this whitepaper focuses on determining EMMA's FNR. It offers a guideline to demonstrate EMMA's ability to accurately classify negative plates as true negatives, ensuring that no false negatives occur. The strategy proposed in this whitepaper is tailored to the positive-to-negative plate ratios typically observed in Grade A/B and Grade C/D environments.

Why focus on FNR

Regulatory bodies like the FDA and EU GMP consider FNR as the **most critical risk** in environmental monitoring in pharmaceutical QC.

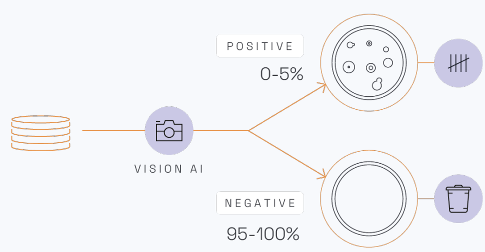


Figure 3: 95-100% of the Petri dishes that need to be evaluated are negative. Once validated, there is no need to review these plates manually, hereby significantly improving throughput. Therefore, it is important to validate the FNR.

Why focus on the FNR and what plates are evaluated?

The FNR is thus a critical metric for assessing the primary objective of environmental monitoring: the accurate detection of microbial contamination. False negatives pose a significant risk, as contamination can remain undetected within a controlled environment, undermining both safety and compliance. Regulatory standards, such as GMP EU Annex 1, USP <1116>, and Eur Ph 5.1.6, stress the necessity of reliable and robust environmental monitoring systems to mitigate these risks. Furthermore, addressing the FNR directly aligns with risk management principles, as false negatives are considered the most critical error in environmental monitoring workflows.

Environmental monitoring in grade A/B cleanrooms is typically characterized by contamination rates below 1% (Figure 3). In this context, the current protocol proposes the use of a positive-to-negative plate ratio of 5%, with a predominance of negative plates, to accurately represent the typical conditions found in these environments. This balance ensures a realistic representation of contamination patterns, where the majority of plates are negative, consistent with cleanroom operational standards.

In terms of microbial contamination detection, the proposed protocol suggests the use of real-world positive samples, which more accurately reflect contamination patterns encountered in the cleanroom. This is in contrast to the use of artificially contaminated plates. Positive plates sourced directly from the cleanroom, for example from lower grade areas, are preferable. However, the accumulation of large quantities of positive plates from the cleanroom may require extensive time. In contrast, negative plates, which are commercially available and considered as true negatives, can be obtained with ease. Therefore, prioritizing negative plates (95%) in this FNR validation is both scientifically justified and operationally feasible.

The Vision AI algorithm utilized in this protocol has been trained predominantly on negative plates. This dataset offers high consistency in visual data, providing an optimal foundation for training AI models to achieve accurate and reliable results.

In conclusion, focusing on false negative validation ensures EMMA's alignment with regulatory requirements and the preservation of microbial detection integrity. By prioritizing specificity (ensuring that negative plates are correctly identified as negative) EMMA facilitates compliance with GMP environmental monitoring standards. This approach enhances operational efficiency by reducing manual intervention, streamlining the validation process, and improving overall data integrity. The proposed protocol represents a scientifically justified, time-efficient solution for environmental monitoring validation.

Managing false positives: ensuring accurate and reliable results

While the primary validation focus is on the FNR as explained in the previous section, it is also important to acknowledge the role of false positives in automated microbial detection. False positives, which occur when a clean plate is mistakenly flagged as positive, are inherently more acceptable in QC workflows than false negatives, as they do not pose a risk of undetected contamination.

EMMA's AI-based triage is designed for high specificity, but in case a plate is flagged as positive, it undergoes manual review by trained personnel. This ensures that any false positive is correctly reclassified as a true negative, maintaining the integrity of environmental monitoring data. This workflow, which aligns with the four-eyes principle, ensures that only true negatives and true positives are retained in the final dataset, eliminating false negatives while effectively managing false positives.

The protocol in a nutshell

The goal of the protocol is to validate the FNR of the EMMA system, which is designed to detect microbial contamination on Petri dishes in environmental monitoring and pharmaceutical QC. The protocol aims to demonstrate that the EMMA system has an FNR below 0.1% at a 95% confidence interval. The system has a unique optical architecture combined with AI-driven image analysis to differentiate positive from negative plates. The system automatically triages plates, requiring a secondary confirmation of positive plates through manual review by a microbiologist, hereby adhering to the four-eyes principle. This validation protocol focuses on determining how accurate EMMA can identify negative plates.

Scope and methodology

The protocol provides a clear framework for validating the FNR. It sets specific acceptance criteria based on contamination risk profiles for Grade A/B and Grade C/D environments, as outlined in EU GMP Annex 1. Table 2 shows the risk profile and validation goal for each category.

	Risk profile	Validation goal
Grade A/B	Contamination rates typically <1% (positive-to-negative ratio of ~1:100)	Demonstrate with 95% confidence that the FNR is <0.1%
Grade C/D	Contamination rates can go up to 50% (positive-to-negative ratio of 1:1)	Demonstrate with 95% confidence that the FNR is <1%

Table 2: Risk profile and validation goal for Grade A/B and Grade C/D environments.

The protocol provides guidelines for calculating the sample size using the binomial confidence interval method. This method is ideal for analyzing scenarios where you have a set number of trials (e.g. Petri dish readout), where each trial will result in one of the two outcomes (e.g. positive or negative plate). The approach allows to estimate the probability for a specific outcome (e.g. FNR), within a certain level of confidence (e.g. 95%). In addition, the method estimates the sample size needed to achieve a desired level of precision, which is critical for validating the performance of the EMMA system.

The validation process begins with an initial sample size that was calculated using the binomial confidence interval method (Clopper-Pearson exact binomial confidence interval method), based on 0 false negatives. If more false negatives are observed during the initial run, a recalculation of the sample size is done, taking into account the number of false negatives observed. As the primary validation demonstrated 0 false negatives in the initial sample size, an increase of sample size is not expected.

Execution steps

The scheme depicted in Figure 4 provides an overview of the protocol. For a detailed description of the protocol, scan the QR code on the next page.

Acceptance criteria

For Grade A/B environments, if the upper bound of the confidence interval is ≤ 0.1%, the validation is successful. For Grade C/D environments, the acceptable upper bound is ≤ 1%. If these thresholds are not met, the failure needs to be investigated to determine the root cause. Based on the investigation, corrective and/or preventive actions are taken, and the qualification is repeated using a predefined and justified sample size. In conclusion, this protocol provides a rigorous and flexible framework to validate the EMMA system's performance in detecting microbial contamination, ensuring its reliability and compliance with regulatory standards in pharmaceutical quality control workflows.

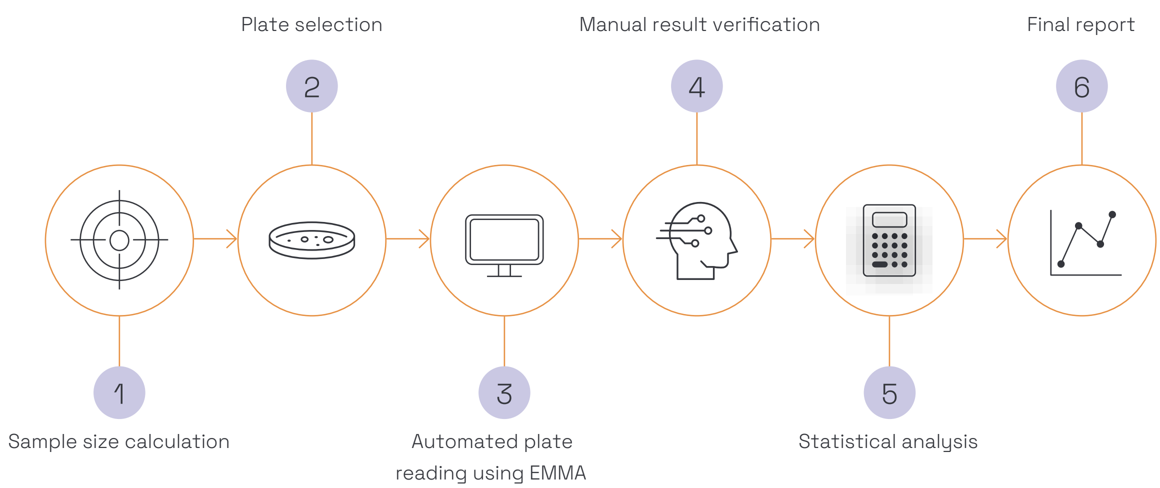
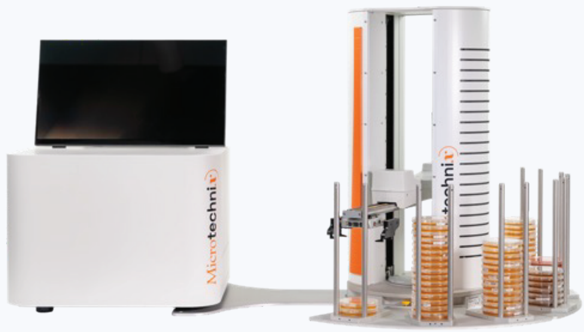
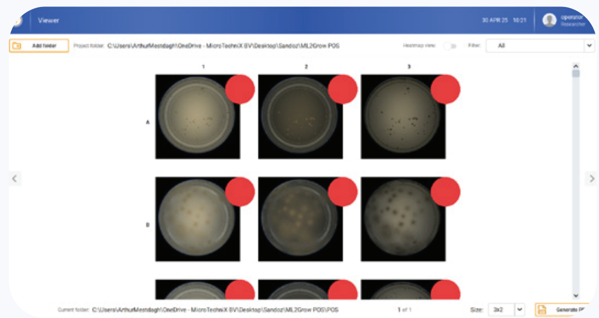


Figure 4: Protocol overview. 1: Calculate the sample size using binomial confidence interval method; 2: Select real world plates for validation; 3: Document and analyze plates using EMMA workflow; 4: Human review of results in EMdi viewer based on 4-eyes principle; 5: Statistical analysis based on Clopper-Pearson method; 6: Generate final report for performance qualification and regulatory discussion.

Transforming pharmaceutical microbiology QC: MEET EMMA





EMMA is designed to digitize and automate pharmaceutical microbiology QC. The patent-pending optical system ensures 100% acquisition of the Petri dish surface, including lateral walls, while maintaining the lid in place. Equipped with an integrated barcode reader and a 21 CFR Part 11 compliant software platform, EMMA delivers enhanced data integrity, driving digitization and streamlining workflows to meet modern QC standards.

The unique optical architecture allows EMMA to capture clear, reproducible images that are perfectly suited for analysis by operators or vision AI models that are designed to differentiate negative (non-contaminated) plates from positive (contaminated) plates. This innovation sets a new benchmark in microbiology QC, combining precision and reproducibility with ease of use. EMMA simplifies and enhances your processes, offering scalable solutions for any throughput while seamlessly integrating with your current workflows to ensure precision, efficiency, and compliance.

EMMA:

- 21CFR part 11 compliant
- ALCOA+ principles
- 100% of the Petri dish surface imaged Imaging with lid on
- Benchtop system
- Barcode reader integrated
- Software designed around four-eyes principle

Request access to full validation protocol as a the step-by-step guide

References

1. European Medicines Agency (EMA). (2022). EudraLex Volume 4, Annex 1: Manufacture of Sterile Medicinal Products.European Commission.
2. United States Pharmacopeia (USP). Chapter <1116>: Microbiological Control and Monitoring of Aseptic Processing Environments. United States Pharmacopeial Convention; 2023.
3. United States Pharmacopeia (USP). Chapter <1223>: Validation of alternative microbiological methods. United States Pharmacopeial Convention; 2023.
4. European Directorate for the Quality of Medicines & HealthCare (EDQM). (2023). Eur. Ph. 5.1.6: Alternative Methods for Control of Microbiological Quality. European Pharmacopoeia 11th Edition.
5. U.S. Food and Drug Administration (FDA). (2004). Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice.
6. Sutton, S. Validating alternative microbiological methods: A guide to USP <1223> and validation of rapid methods. Am Pharm Rev [internet]. 2022.
7. Heuser E, Becker K, Idelevich EA. Evaluation of an automated system for the counting of microbial colonies. Microbiol Spectrum 2023;11(4).
8. Ijzerman-Boon PC, van den Heuvel ER. Validation of qualitative microbiological test methods. Pharm Stat. 2015;14(2):120-8.
9. Anders HJ, Männle D, Carpenter W, Eder W, Heckel I, Götzen T, et al. Multisite qualification of an automated incubator and colony counter for environmental and bioburden applications in pharmaceutical microbiology. PDA J Pharm Sci Technol. 2023;77(3):236-247.
10. Sutton S. Accuracy of plate counts. J Valid Technol. 2011;17(3):42-46.
11. Bailac L, Mallam L, Leblanc L. New standardized methodology to evaluate environmental monitoring plates inspection using an automated system. Biomérieux, 2024.

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