CHALLENGES AND SOLUTION FOR PERFORMANCE VALIDATION OF EM AUTOMATION

Laura Bailac, Lucas Pires, Lisa Mallam and Laurent Leblanc • bioMérieux SA • 376 Chemin de l'Orme 69280 Marcy l'Étoile France

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INTRODUCTION

Environmental Monitoring (EM) is crucial to assure patient safety. The visual end point reading of petri dishes has been used for decades without a true performance assessment. Automatization technologies are more present and improve EM practices.

3P® STATION is a colony counter system based on automatic timely plate reading incubation. It is part of the global 3P® ENTERPRISE solution for EM. This instrument intends to automatize the current traditional method for monitoring microbial levels in pharmaceutical production areas, preserving its analytical performances. How to validate those analytical performances?

There are many existing guidelines for microbiological method validation. But those guidelines are product testing oriented and do not address the subject of EM. Concerning EM methods, there are very few harmonized guidelines covering the analytical methods and even less covering their validation. The first step was to define a relevant validation strategy using new adapted tools/metrics to the 3P STATION intended use.

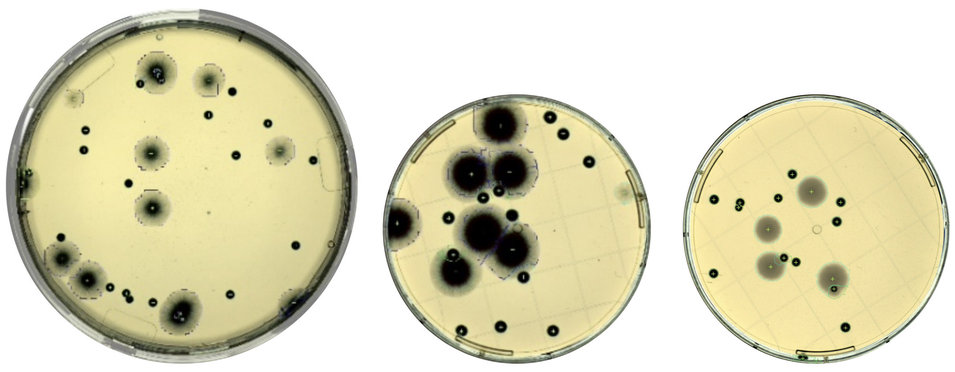
EQUIPMENT

AUTOMATED PLATE COUNTER 3P STATION

The 3P STATION is an automated Petri dish Incubator/counter. This brand-new system can incubate plates in a range of temperature between 20 and 35°C and can follow the growth of the microorganisms that are present on the surface of the dishes. Thanks to a high-resolution camera and an advance telecentric optical lens, the 3P STATION can take a high-quality picture every hour and combine them to create a movie that traces the entire growth evolution of the microorganisms. In this study, the inoculated plates were incubated in the machine to generate the growth images of the entire incubation time. In the case of swarming colonies that could hide or make the enumeration difficult, the system allowed users to rewind the growth movie until a time where the enumeration is possible.

Picture 1: bioMérieux 3P STATION

EXEMPLE OF GENERATED COMBINED AND MARKED RAW IMAGE



90mm plate *CT plate*

VALIDATION STRATEGY

3P STATION notably uses the exact same plates and sampling methodology, with same incubation sequence as current method used on the customer site and truly automatizes the counting step. Based on a deviation analysis between the traditional method and the 3P STATION, **we propose an approach focused on counts comparison between original and automated method** with rational and counting attributes illustrated in Fig. 1.

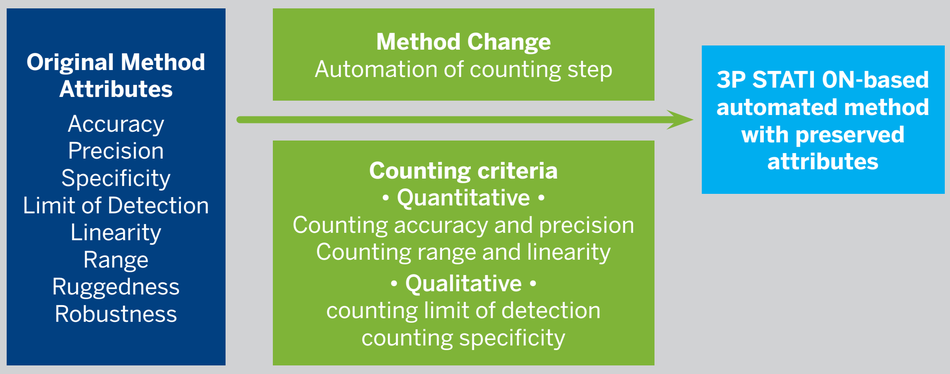


Fig. 1: Validation Approach and Selected Attribute

This comparison approach led us to define a standardized CFU count of the traditional method, in order to have a robust and unbiased comparison, called the **"Reference Traditional Count".**

Among different operators, a different count can be given for the exact same plate according to site rules interpretation and operator performances.

A standardized CFU count must be defined to evaluate the current methodology performance. To obtain this gold standard, we developed a methodology to correct the actual traditional count to what we defined as the **"Reference Traditional Count".**

Each plate recovered from 3P STATION, after incubation, is counted by several operators, independently, following the traditional counting method. The operators are not influenced by the data of the 3P STATION (both pictures or counts). Each operator records their count on independent sheets.

In a second step, after the plate has been read by the traditional method, the physical plate is analyzed a second time with help of the results of 3P STATION. By replaying the video, zooming on specific plate areas, the operators can directly tick the colonies on the final image of the plate, giving as a result, a corrected count, which is referred to as the **"Reference Traditional Count".**

This value is intended to represent an "individual-free" result of the traditional method.

The three main performances attributes are validated following the experimental plan and acceptance criteria detailed in Table 1.

Table 1: Performances attributes: metrics and associated acceptance criteria

Performance attribute	Metric		Target	Test details
Accuracy *(vs reference count)*	Linear regression	R^2	> 0,95	Absolute comparison
		Slope	[0,9 ; 1,1]	Absolute comparison
		Intercept	[-1 ; 1]	Absolute comparison
	Linear regression		[90%-110%]	Absolute comparison
Limit of detection *(vs traditional method)*	False negative rate @ colony level		Equivalent to traditional method	Z-test @ 95% confidence level
	False negative rate @ plate level		0	Absolute comparison
Specificity *(vs reference count)*	Recovery rate per strains		> 90%	Absolute comparison
	False positive rate @ plate level		Upper bound of CI < 10%	95% confidence level

In addition, a ruggedness study was performed using three different equipment to demonstrate equivalency of the global variability compared to the traditional method.

MATERIALS AND METHOD

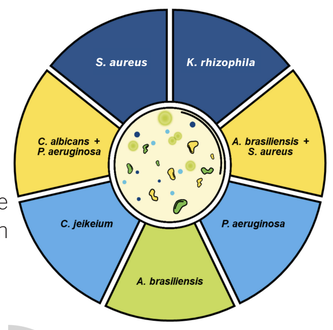
ACCURACY, PRECISION & LIMIT OF DETECTION

Inoculation range and levels: covering the CFU range from grade A to grade D

90mm [5-10-25-50-100-200] CFUs

CT [5-10-25-50-75-100] CFUs

Strains and mixture selected: 5 pharmacopeia strains + one mold + two bacteria + one mixture mold/bacteria + one mixture yeast/bacteria representative of pharmaceutical production environment.



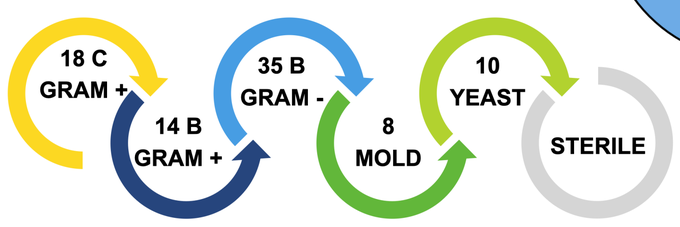
SPECIFICITY

Strains and inoculation level:

85 strains + sterile plates

[10-100] CFUs for bacteria

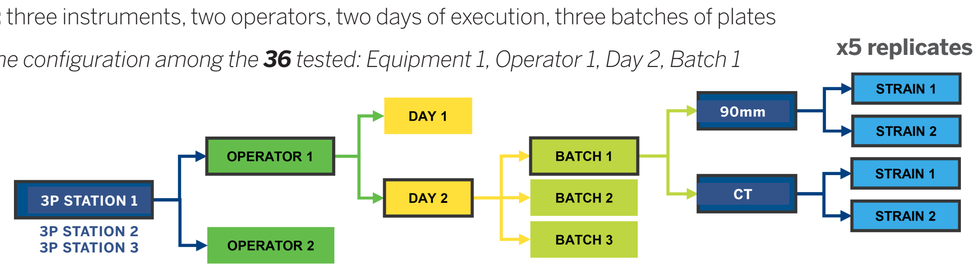
[10-35] CFUs for fungi



RUGGEDNESS

Parameters: three instruments, two operators, two days of execution, three batches of plates

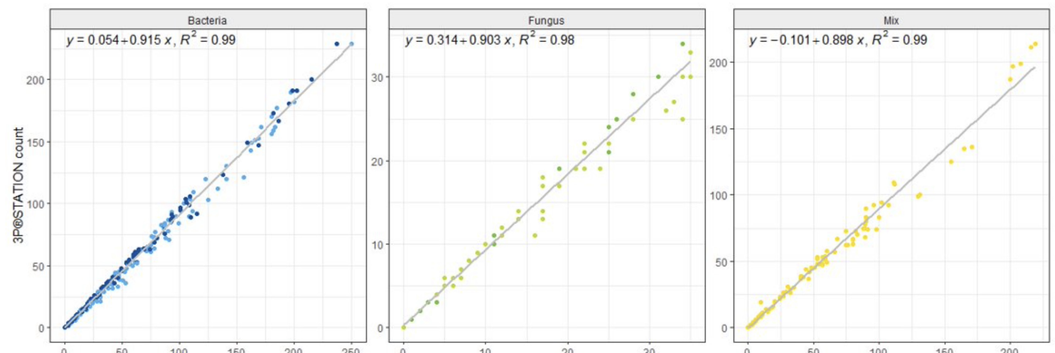
*Example of one configuration among the **36** tested: Equipment 1, Operator 1, Day 2, Batch 1*



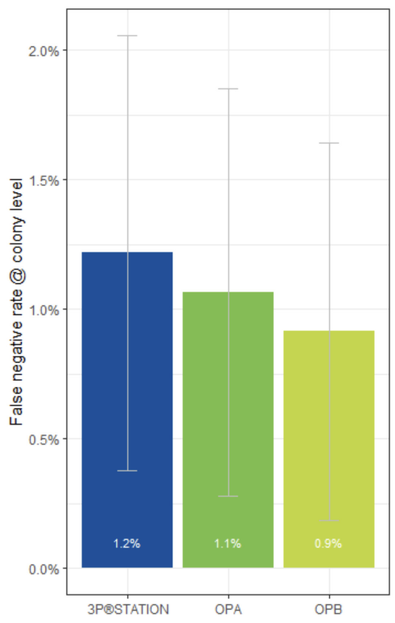
Strains and inoculation level : A. brasiliensis and S. aureus inoculated at 25 CFUs

RESULTS AND DISCUSSION

ACCURACY

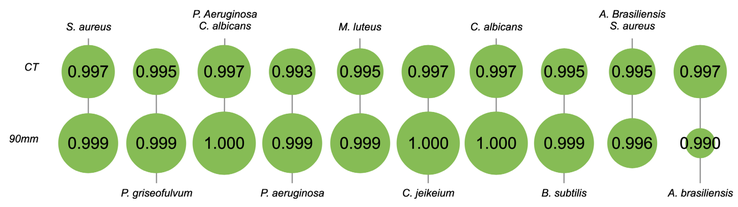


LIMIT OF DETECTION

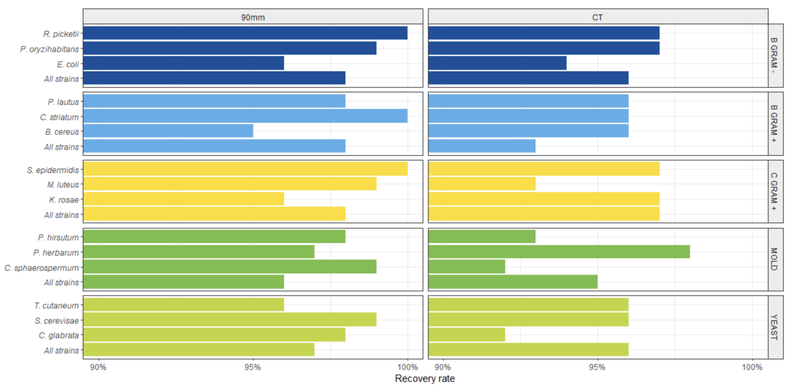


0% **False negative @ plate level**

CORRELATION (COMPLEMENTARY INFORMATION)

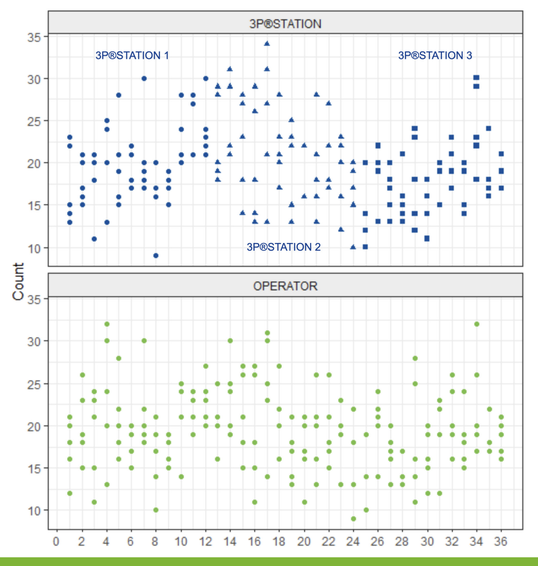


SPECIFICITY

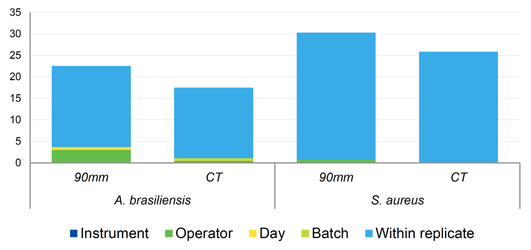


RUGGEDNESS

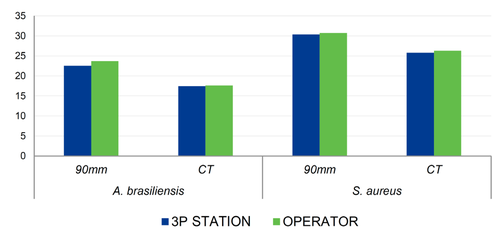
Plot pattern for *A. brasiliensis* on 90mm plates for the 36 tested configurations



Contribution to total variance for 3P STATION



Total variance 3P STATION vs Operator



CONCLUSION

Automated methods can be implemented as part of Environmental Monitoring control of pharmaceutical production areas and replace the traditional method of visual end point reading. Indeed, the 3P STATION has reached equivalency in counting performances, demonstrated on a large panel of strains representative of pharmaceutical environment using adequate metrics and strict acceptance criteria. In addition, the automated kinetic reading provides early results all along the incubation time. This time gain is crucial for new therapeutic applications.