Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Tech Note

Milliflex® Rapid System 2.0 Performance Assessment in comparison with Milliflex® Rapid System (previous generation)

1 Introduction

The purpose of this document is to summarize an assessment of how the Milliflex® Rapid System 2.0 and the historical Milliflex® Rapid System compare with respect to performance. The two systems comprise the following components:

1.Milliflex® Rapid System 2.0 test platform:

- Milliflex® Rapid 2.0 AutoSpray Station (**MXRP2SPRKT**)
- Milliflex® Rapid 2.0 Detection Tower (**MXRDP2DT00**)

2.Historical Milliflex® Rapid System:

- Milliflex® Rapid AutoSpray Station (**MXRPSPRKT**)
- Milliflex® Rapid Detection Tower (**MXRPKT110**).

The validation of the alternative microbiological test method for microbiological performance is documented in the validation summary “Bioburden Test Method Validation Using Milliflex® Rapid System 2.0”, document ID VS11812EN.

2 Test Method with Milliflex® Rapid System 2.0

2.1 Milliflex® Rapid System 2.0 description

The Milliflex® Rapid System 2.0 is an automated solution for the rapid detection, imaging, and quantification of viable microbial contaminants in filterable samples throughout the manufacturing process.

The system's results help to improve process control, product yield, and the timely release of final products. In case of contamination, corrective action can be taken earlier, avoiding loss of time, money, and production capacity.

Based on highly sensitive adenosine triphosphate (ATP) bioluminescence technology, the Milliflex® Rapid System 2.0 delivers faster test results than traditional microbial contamination detection methods. Particularly when used in combination with Milliflex Oasis® sample preparation and filtration devices, the Milliflex® Rapid System 2.0 ensures consistent and reliable results.

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Proven technologies

The Milliflex® Rapid System 2.0 is based on three proven technologies, which makes validation easier. These are membrane filtration, ATP bioluminescence and image analysis.

1. Membrane filtration

As today's standard for preparing samples to detect and enumerate microorganisms, membrane filtration allows a large volume of product to be processed, while most inhibitory substances are easily rinsed away. The method optimizes the subsequent enumeration of microorganisms by filtering fast and ensuring reliable results.

2. ATP bioluminescence

ATP is an excellent indicator of cell viability. Unlike other methods that detect both living and dead cells, the ATP-based method is highly reliable and consistent with the detection rates of current compendial testing methods.

3. Image analysis for enumeration of microcolonies

Unlike traditional methods that require visual evaluation, the Milliflex® Rapid System 2.0 uses a complementary metal oxide semiconductor (CMOS) camera to detect and enumerate any microcolonies present. The ATP concentration required for recognition is equivalent to one yeast or mold cell or approximately 100 bacterial cells, depending on their metabolic state. The camera's sensitivity, combined with state-of-the-art image analysis and optimized reagents, requires only a short incubation period to detect enough ATP for the recognition and enumeration of microcolonies. The system intensifies the bioluminescence it detects from each microcolony and records the results. The software enumerates the microcolonies and displays results as familiar colony forming units (CFUs).

2.2 Test method with Milliflex Rapid System 2.0

The Milliflex® Rapid System 2.0 workflow is performed in three steps:

Step 1: Sample preparation

Filter the desired sample volume through a pre-sterilized, ready-to-use disposable Milliflex Oasis® filtration unit according to your standard operating procedure. Microorganisms contained in the sample are captured on a membrane filter, which is then transferred onto a media plate and incubated for growth.

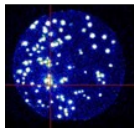
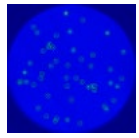
Step 2: Apply reagents

After incubation, remove the membrane filter from the Milliflex Oasis® prefilled agar plate using the membrane removal tool and place the membrane filter on the Milliflex® Rapid 2.0 AutoSpray Station to apply the reagents. Both reagents are automatically sprayed across the membrane filter in quick succession, to enable an enzymatic reaction that leads to bioluminescence where microcolonies are present.

Step 3: Enumerate microorganisms

Transfer the membrane filter to the Milliflex® Rapid System 2.0 Detection Tower for enumeration of microcolonies. Using the computer, enter sample information and run the test to count the microcolonies (reported in CFUs). The emitted light allows microcolonies on the membrane filter to be automatically detected and counted by the Milliflex® Rapid System 2.0 Detection Tower and its software. An image of the membrane filter and its colonies is automatically recorded, displayed, and archived.

2.3 Comparison with the previous Milliflex® Rapid System generation

		Milliflex® Rapid System		Milliflex® Rapid System 2.0	
Sample preparation (filtration)	Pump	Milliflex® PLUS Pump		Milliflex Oasis® Filtration pump	
	Funnels	Milliflex® Rapid Funnel with 0.45 µm PVDF membrane		Milliflex Oasis® Rapid Funnel with 0.45 µm PVDF membrane	
Reduced incubation time		Milliflex® Culture Media Cassettes		Milliflex Oasis® Media Plates	
Automated reagent dispensing		AutoSpray Station		AutoSpray Station updated	
Signal detection		Milliflex® Rapid Detection Tower		Milliflex® Rapid 2.0 Detection Tower	
Data acquisition		Software algorithm		Software algorithm	

3 References

Reference	Title
FRPPI032-VQ1	Validation Master Plan for the Milliflex® Rapid 2.0 System
FRPPI032 VQ2	Qualification report for Detection Tower 2.0 and calibration source
FRPPI032 TMVP1	Qualification protocol for bioburden application method validation using Milliflex® Rapid 2.0
FRPPI032-TMVR1	Qualification report for bioburden application method validation using Milliflex® Rapid 2.0
VS11812EN	Validation Summary: Bioburden Test Method Validation Using Milliflex® Rapid System 2.0

4 Test Methods and Materials

4.1 Methods

Optical performance (sensitivity)

The optical performance of the new Milliflex® Rapid 2.0 Detection Tower (**MXRDP2DT00**) and the legacy Milliflex® Rapid Detection Tower (**MXRPKT110**) is determined and compared.

Drops of ATP (adenosine triphosphate) solutions at different concentrations are deposited on Milliflex® membranes using a high precision dispenser. After spraying the bioluminescence reagent (“Reagent 2”) using the Milliflex® Rapid AutoSpray station, the membrane filters are read by both detection towers.

Comparative testing using microorganisms

Comparative testing compares the CFU counts of the Milliflex® Rapid 2.0 System with the ones obtained using the legacy Milliflex® Rapid System during the qualification of microbial performance (quantitative method).

4.2 Materials

Equipment

Description	Reference or Cat. No	Serial or lot number
Milliflex® Rapid 2.0 Detection Tower	**MXRDP2DT00**	SP2H21013B SP2H21013C SP2H21013D SP2H21013E SP2H21013F SP2H21013G
Milliflex® Rapid Detection Tower (legacy)	**MXRPKT110**	444279 694019 66
Milliflex® Rapid AutoSpray Station (legacy)	**MXRPSPRKT**	48 / 52 / 214
BioFluidix SiJet Picodispenser	**SJ-10105 E**	P9S1 2021-26-1601

Reagents

Description	Reference or Cat. No	Supplier
ATP 1 mg	FLAAS	Sigma-Aldrich®
Endotoxin-free water 125 mL	PF17467	
Cartridge for dispenser	SJ-30200 type 20/20	BioFluidix

5 Validation Methods

5.1 Optical performance (sensitivity)

Applying ATP to the Milliflex® membrane filters is done with a microfluidic dispenser by dropping a precise amount of ATP solution onto the top of the membrane, to simulate a microcolony. The AutoSpray station then sprays “Reagent 2” onto the membrane to generate a bioluminescence reaction which is detected using a Milliflex® Rapid Detection Tower.

5.2 Comparative testing with microorganisms

For microbial performance qualification, a panel of culture collection microorganisms and environmental microorganisms (bacteria, a yeast and a mold) was selected, covering a wide range of microorganisms with different growth rates. The incubation conditions depended on the specific microorganism.

Microorganism / Culture collection	Culture medium	Incubation conditions
Aspergillus brasiliensis NCPF 2275 / ATCC 16404	SDA	20 to 25 °C, aerobic
Bacillus spizizenii NCTC 10400 / ATCC 6633	TSA	30 to 35 °C, aerobic
Candida albicans NCPF 3179 / ATCC 10231	SDA	20 to 25 °C, aerobic
Clostridium sporogenes NCTC 12935 / ATCC 19404	TSA	30 to 35 °C, anaerobic
Cutibacterium acnes DSM 1897 / ATCC 6919	RSTM	30 to 35 °C, anaerobic
Escherichia coli NCTC 12923 / ATCC 8739	TSA	30 to 35 °C, aerobic
Methylobacterium extorquens NRBC 15911	R2A	20 to 25 °C, aerobic
Pseudomonas aeruginosa NCTC 12924 / ATCC 9027	TSA	30 to 35 °C, aerobic
Staphylococcus aureus NCTC 10788 / ATCC 6538	TSA	30 to 35 °C, aerobic

5.3 Statistical Methods

5.3.1 Percent Recovery

Percentage of recovery is defined as the colonies detected by the Rapid test method, as a percentage of those detected by the Compendial method:

$$\% \text{ Recovery} = \frac{\text{Average Milliflex® Rapid counts}}{\text{Average Compendial Milliflex® counts}} \text{ X } 100$$

Bayesian analyses (simulations by Markov chain Monte Carlo methods) are an alternative to the frequently used hypothesis test methods as well as to the use of the p-values. Bayesian methods make it possible to obtain the value of a parameter and its credibility interval (also known as confidence interval). This approach is used for additional comparative testing to assess accuracy. Analyses are done using R project for Statistical Computing.

6 Qualification results

6.1 Optical performance (sensitivity)

Test design

Microdroplets of ATP (adenosine triphosphate) solutions of different concentrations are deposited on Milliflex® membranes using a high precision dispenser. The number of droplets is increased until a signal can be detected with a Milliflex® Rapid system. The amount of ATP required for luminescence detection of a microcolony is about 200 attomoles, which is equivalent to the detectable ATP content of one yeast or mold cell or approximately 100 bacterial cells, depending on their metabolic state. Both generations of Milliflex® Rapid were used to read the membranes after spraying with bioluminescence reagent.

The objectives were to:

- Verify the signal detection sensitivity in attomoles (= $1x10^{-18}$ moles) of the Detection Towers
- Verify the homogeneity of detection (number of spots detected across the membrane)
- For robustness purposes several systems were tested:
- Milliflex® Rapid 2.0 Detection Tower (MXRDP2DT00): 6 systems
- Legacy Milliflex® Rapid Detection Tower (MXRPKT110): 3 systems

Acceptance criteria

- Approx. 200 attomoles can be detected
- The correct number of spots (eight) are counted

Test Results

Detection Tower	Number of systems tested	ATP detection of 200 attomoles	8 spots are counted
Milliflex® Rapid 2.0 Detection Tower (**MXRDP2DT00**)	6	Pass	Pass
Legacy Milliflex® Rapid Detection Tower (**MXRPKT110**)	3	Pass	Pass

Conclusion

The Milliflex® Rapid 2.0 Detection Tower achieves the same sensitivity of detection as the historical Milliflex® Rapid Detection Tower.

6.2 Comparative Testing

Test design

In the validation to assess the performance of microbial detection, accuracy with both the Milliflex® Rapid 2.0 and the legacy Milliflex® Rapid methods were tested in parallel, at different cell concentration levels. The comparison of both methods across the range of microorganisms used Relative Risk (RR), calculated on the basis of the coefficients in the generalized linear Poisson regression model, after a simulation using Bayesian analyses.

$RR = \frac{\lambda \text{ Rapid}}{\lambda \text{ Reference}}$ in which λ is the mean in the Poisson model.

RR values come with a bilateral 95% credibility interval and a probability (Prob RR) that the credibility interval is within the tolerance of 0.7 to 1.3 (or 70 to 130%).

Acceptance criterion

Prob® is significant at a threshold of 95%.

Test Results

Strain	RR	R% Detection Tower 2.0 vs legacy	95% Credibility Interval for RR	Prob(RR)	Prob(RR) is significant at threshold 95%
Aspergillus brasiliensis	1.067	106.7%	[1.003-1.135]	1	Yes
Bacillus spizizenii	1.093	109.3%	[1.002-1.190]	1	Yes
Candida albicans	1.114	111.4%	[1.024-1.211]	1	Yes
Clostridium sporogenes	1.356	135.6%	[1.233-1.490]	0.196	No
Cutibacterium acnes	0.966	96.6%	[0.883-1.055]	1	Yes
Escherichia coli	1.049	104.9%	[0.985-1.115]	1	Yes
Methylobacterium extorquens	1.102	110.2%	[1.004-1.207]	1	Yes
Pseudomonas aeruginosa	1.083	108.3%	[1.003-1.167]	1	Yes
Staphylococcus aureus	1.122	112.2%	[1.031-1.219]	1	Yes

Conclusion

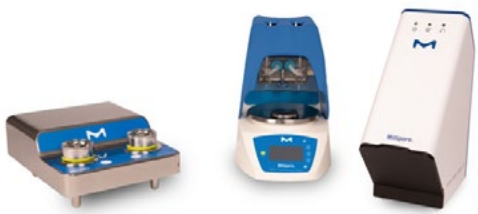
For all the microorganisms tested, except *C. sporogenes*, and across the concentration range, the CFU counts obtained with the Milliflex® Rapid 2.0 Detection Tower were within 70 to 130% of counts using the legacy Milliflex® Rapid Detection Tower.

For *C. sporogenes* the counts obtained with the new system were significantly higher, thus the statistical test failed. Our hypothesis to explain this result is that the Detection Tower 2.0 discriminates merged colonies at an earlier growth stage, leading to a higher count.

7 Conclusion of the Equivalency Assessment

The physical performance (sensitivity) of the Milliflex® Rapid 2.0 Detection Tower is equivalent to the historical Milliflex® Rapid Detection Tower.

The determined microbiological detection performance of the Milliflex® Rapid System 2.0 was within the 70 to 130% range of the historical system for eight of the nine tested microorganisms. The only exception was *C. sporogenes*, for which counts where higher when using the new Detection Tower.



The Milliflex® Rapid System 2.0 equals or outperforms the historical Milliflex® Rapid platform with regards to all the tested parameters.

8 Glossary

Abbreviation	Definition
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
CBS	Central Bureau of Yeast Culture
CFU	Colony forming unit
CI	Credibility Interval
DSM	German Collection of Microorganisms
MFR	Milliflex® Rapid method
MFX	Compendial Milliflex® Oasis method
mg	Milligrams
MSC	Microbiological safety cabinet
N/A	Not applicable
NCPF	National Collection of Pathogenic Fungi (UK)
NCTC	National Collection of Type Cultures (UK)
NRBC	NITE Biological Resource Center
PVDF	Polyvinylidene difluoride
R%	Recovery percentage
RR	Relative risk
R2A	Reasoner's 2A agar
RSTM	Rapid sterility test medium
sCMOS	Scientific Complementary Metal–Oxide–Semiconductor
SDA	Sabouraud Dextrose Agar
TSA	Tryptic Soy Agar
USP	United States Pharmacopeia
VMP	Validation Master Plan

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