Validation Summary

The Milliflex Oasis® system for bioburden and pharmaceutical water testing

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Millipore®

Preparation, Separation, Filtration & Monitoring Products

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1. Milliflex Oasis® Product Summary

1.1 Purpose of this summary

This guide is designed to provide a basic understanding of the methods used to qualify the new Milliflex Oasis® platform.

Section 1 of this guide provides an introduction, a product description, a set of catalogue numbers, internal documentation, comparison of Milliflex® & Milliflex Oasis® material and our Quality Standards.

Section 2 of this guide provides a summary of test methods and test results used to qualify the Milliflex Oasis® platform.

This validation summary shows that the performance of the Milliflex Oasis® platform is in accordance with the specified acceptance criteria and offers equivalent or better performance to the Milliflex® system on all the tested parameters.

1.2 Product description

1.2.1 Media plate

Media plate cover

Body

Bottom

- Tighter closure for longer storage
- Easy opening to avoid agar contact

- Color coded units to differentiate agar type
- Traceability thanks to unique 2d barcode

- Assembled/locked function to stack units together
- Medium name engraved for easy identification

Three culture media types are available in the Milliflex Oasis® format:

The Milliflex Oasis® R2A media plate is a low nutrient agar for water microbiological controls. This low nutrient agar is used for the recovery of stressed heterotrophic bacteria found in various types of waters, especially high purity water.

The Milliflex Oasis® Tryptic Soy media plate is for the detection of aerobic and anaerobic bacteria in water.

The Milliflex Oasis® Sabouraud Dextrose media plate is for the detection of yeast & molds.

1.2.2 Filtration Unit

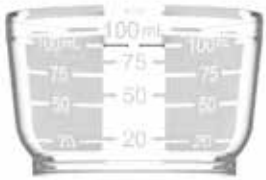
The materials of which the Milliflex Oasis® filtration unit is made optimize and secure the laboratory workflow and, at the same time, allow convenient and ergonomic handling by the laboratory user. The chosen material (styrene butadiene copolymer) improves our funnels in several ways:

- Effortless liquid level control, thanks to the fully transparent material
- Limited liquid residues after filtration, thanks to the ultra hydrophobic property of the material
- Effortless funnel and membrane separation
- Reduced risk of secondary contamination, thanks to the new hinged lid which allows the device to be closed during filtration

Cover

- 3 positions, detachable lid

Funnel



- High transparency and liquid level visibility
- Ultra-hydrophobicity to avoid liquid residues after filtration
- Flexibility for easy funnel removal

Sealed membrane

- Overall design ensures user independent transfer of the membrane
- No-touch membrane transfer

Membrane support

- Patented design to ensure fast filtration

1.2.3 Vaccum Pump

The Milliflex Oasis® filtration pump has 2 filtration stands for higher throughout.

1.2.4 Catalog Numbers

	Description	Milliflex Oasis® Cat. No.	Milliflex® Cat. No.
	Filtration Unit		
MCE Membrane	100 mL funnels, 0.45 µm white gridded, cellulose esters, 24 units per pack	**MMHAWG124**	**MXHAWG124**
MCE Membrane	100 mL funnels, 0.22 µm white gridded, cellulose esters, 24 units per pack	**MMGSWG124**	**MXGSWG124**
MCE Membrane	100 mL funnels, 0.45 µm black gridded, cellulose esters, 24 units per pack	**MMHABG124**	**MXHABG124**
MCE Membrane	250 mL funnels, 0.45 µm white gridded, cellulose esters, 24 units per pack	**MMHAWG224**	**MXHAWG224**
PVDF Membrane	100 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack	**MMHVWP124**	**MXHVWP124**
PVDF Membrane	250 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack	**MMHVWP224**	**MXHVWP224**
PVDF Membrane	Rapid Microbiology 100 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack	**MMHVMFX24**	**RMHVMFX24**
	Media Plates		
	R2A media plates, 48 units per pack	**MMSMCRA48**	**MXSMCRA48**
	Tryptic Soy media plates, 48 units per pack	**MMSMCTS48**	**MXSMCTS48**
	Sabouraud Dextrose media plates, 48 units per pack	**MMSMCSD48**	**MXSMCSD48**
	Hardware		
	Pump	**MMSYSTVAC** **MMSYSTMM1**	**MXPPLUS--**

1.3 Technical specifications

1.3.1 Media Plate

Construction material	Body, bottom, and lid	Polystyrene (PS)
Dimension	Maximal diameter	66.4 mm (2.6 in.)
	Height	21.35 mm
	Internal diameter	49,8 mm
Minimal cassette weight	26.5 g	
Agar quantity	At least 10 mL	
Color of unit	R2A	Blue
	TSA	Green
	SDA	Pink
Sterilization method	Irradiation of empty units followed by aseptic filling	
Storage temperature	2–8 °C	
Shelf life	12 months	
2D code identification	Data matrix, can be read with standard 2D reader	

1.3.2 Filtration Unit

Construction material	Lid, funnel and base	Styrene butadiene copolymer (SBC)
	Membrane	Mixed cellulose esters (MCE) or polyvinylidene difluoride (PVDF)
	Membrane ring material	Polyethylene (PE)
Dimensions	Height	100 mL: 57 mm (2.2 in.) 250 mL: 113 mm (5.5 in.)
	Maximal diameter	82 mm (3.2 in.)
Membrane diameter	49 mm (1.9 in.)	
Sterilization method	Irradiation	
Maximum temperature (of filtered liquid)	60 °C	
Shelf life	1 year (2 years after complete qualification)	

1.3.2.1 Membrane

Catalogue number codification	HAW	HAB	GS	HV
Construction material	Mixed cellulose ester (MCE)	Mixed cellulose ester (MCE)	Mixed cellulose ester (MCE)	Polyvinylidene fluoride (PVDF)
Membrane pore size	0.45 µm	0.45 µm	0.22 µm	0.45 µm
Membrane color & gridding	White gridded	Black gridded	White gridded	White plain

1.3.3 Pump

Pump dimension	Height	90 mm (3.5 in.)
	Width	230 mm (9.0 in.)
	Depth	310 mm (12.2 in.)
	Filtration support height	70 mm (2.8 in.)
	Weight with filtration heads	3.4 kg (7.5 lb)
	Power supply	24–30 V DC, 25 W - One set can be used for up to three pumps
	Surface decontaminants	Quaternary ammonium, isopropyl alcohol (70%), ethanol (70%), bleach (250 ppm), solution of peracetic acid (<1%)
Operating conditions	Storage temperature	0 °C to 40 °C
	Operation temperature	15 °C to 40 °C
	Relative humidity (RH)	80% at temperatures up to 31 °C, then linear decrease to 50% RH at 40 °C
Constituting materials	Frame	Blend of acrylonitrile styrene acrylate and polycarbonate
	Head	Stainless steel AISI 316L
	Check valve	Silicone
	Seals	Silicone
	Tubings	Low density polyethylene
	Fittings	Polypropylene

1.4 Material certification

1.4.1 Media plate material certification

The media plates are made of polystyrene (PS), of which Petri dishes are typically produced. The material does not contain any of the substances of very high concern (SVHC) listed on the candidate list as published on the ECHA website on January 15, 2018 (http://echa.europa.eu/web/guest/candidate-list-table) in concentrations above the reportable limits (0.1% wt). The material is in compliance with the requirements of Article 4.1 of the EU Directive 2011/65/EU.

1.4.2 Funnel material certification

Candidate list of SVHC substances according to Art 59.

Regulation (EC) No 1907/2006 REACH as amended on June 27, 2018.

None of the substances of very high concern (SVHC) on the candidate list as published on the ECHA website on June 27, 2018 (http://echa.europa.eu/web/guest/candidate-list-table) are used as additives or ingredients in concentrations above 0.1% (w/w) in production.

European Pharmacopoeia (EP)

Currently there is no monograph in the EU Pharmacopoeia containing requirements for styrenic polymers. The 9th Edition of the European Pharmacopoeia was reviewed in regard to Chapter 3.2.2 “Plastic Containers and Closures for Pharmaceutical Use”. Although there is no specific information on styrenic polymers in these chapters, we can state that the material meets the general stipulations where applicable for raw materials.

Compliance status with the United States Pharmacopoeia (USP) and ISO 10993 biocompatibility standards

The material meets the requirements of the USP Plastic Class VI and complies with ISO 10993 regulations regarding biological evaluation tests as specified below.

US Pharmacopoeia

Biological Reactivity Tests, USP Plastic Class VI (USP VI)

ISO 10993-4

Biological Evaluation of Medical Devices Part 4: *In vitro*: Hemolysis

ISO 10993-5

Biological Evaluation of Medical Devices Part 5: Test for Cytotoxicity

1.5 Sterilization conditions

The sterilization of Milliflex Oasis® filtration units is performed by irradiation. E-beam is the mode used for the final sterilization of the assembled and packaged product.

The Milliflex Oasis® media plates are not sterilized: although the empty media plates are sterilized by irradiation, the media is poured under aseptic filling conditions.

1.6 The differences to Milliflex® system: Summary

1.6.1 Changes in materials and design

The Milliflex Oasis® system is designed, manufactured, and packaged to optimize microbial recovery and minimize cross-contamination, while setting new standards for ease-of-use. To this end, the design has been changed and the materials of several components replaced for improved performance.

Table: Summary of changes in materials and design

Unit component	Milliflex®	Milliflex Oasis®	Material change	Design change
Filtration device	Milliflex® MXH---	Milliflex Oasis® MMH---		
Lid	SAN (polystyrene acrylonitrile)	Styrene butadiene copolymer (SBC)	√	√
Funnel	Polyethylene	SBC	√	√
Ring	Polyethylene	Polyethylene, new supplier	No	√
Membrane	MCE or PVDF	MCE or PVDF	No	No
Support	Polyethylene	SBC	√	√
Filtration diameter	49 mm (1.9 in.)	49 mm (1.9 in.)	N/A*	N/A*
Media plate	Milliflex® cassette	Milliflex Oasis® media plate		
Bottom	Styrene acrylonitrile (SAN)	Polystyrene (PS)	√	√
Body	Styrene acrylonitrile (SAN)	Polystyrene (PS)	√	√
Cover	Polypropylene (PP)	Polystyrene (PS)	√	√
TSA formulation	EP/USP/JP formulation	EP/USP/JP formulation	No	N/A*
SDA formulation	EP/USP/JP formulation	EP/USP/JP formulation	No	N/A*
R2A formulation	EP/USP/JP formulation	EP/USP/JP formulation, new supplier	No	N/A*

*N/A: Not Applicable

1.7 Standard for quality assurance & environment

The Milliflex Oasis® system is manufactured in our facility at Molsheim (France), which is approved by an accredited registering body to ISO 9001 Quality Systems Standards.

1.7.1 Quality Assurance

The Milliflex Oasis® system's quality program and manufacturing process follow cGMP requirements. We have determined an effective and efficient process that leads to a consistently high quality for each batch of product.

Standard operating and testing procedures are precisely defined to closely monitor the process and to ensure the product's compliance with the specifications.

We follow the recommendations of the United States, European and Japanese pharmacopeias to ensure the consistent performance of the product.

1.7.2 Traceability

Batch records are generated and saved during each filtration unit and culture media manufacturing run. Traceability is ensured for all the raw materials used in production. The records demonstrate that all the steps are followed as defined in the internal procedures. It is verified that the defined controls are performed accurately to ensure that the products comply with the specifications.

The products can be identified by reference, lot number and expiration date. Batch records are reviewed for compliance and released by Quality Assurance.

1.7.3 Process change control

Any change to the manufacturing process or product is evaluated in order to avoid any impact on product quality, legal requirements and user safety. Changes are validated as necessary and appropriately communicated to customers according to our internal policy.

1.7.4 Environmental

The filtration units and the culture media are manufactured in an environment that is controlled according to internal specifications, including microbiological and particulates monitoring.

Furthermore, internal procedures describe the general rules, restrictions and cleaning instructions to be followed by all staff working in the manufacturing area.

2. Validation Tests: Summary

2.1 Validation purpose

2.1.1 Objective

The validation objective was to ascertain whether the performance of the Milliflex Oasis® system meets internal requirements and external regulations.

Relevant tests were performed to compare the Milliflex Oasis® system and the Milliflex® system. These tests have demonstrated that the Milliflex Oasis® system equals or outperforms the Milliflex® system.

2.1.2 Test microorganisms

Table of the microorganisms used for the tests

Microorganism	Collection	Source
Aspergillus brasiliensis	ATCC 16404	Certificate of quality
Bacillus subtilis	ATCC 6633	
Candida albicans	ATCC 10231	
Escherichia coli	ATCC 8739	
Methylobacterium extorquens	NBRC 15911	
Staphylococcus aureus	ATCC 6538	
Burkholderia cepacia	ATCC 25416	Pharmaceutical industry environments
Geobacillus stearothermophilus	DSM 22	
Kocuria rhizophila	ATCC 9341	
Methylobacterium aminovorans	ATCC 51358	
Methylobacterium fujisawaense	ATCC 43884	
Methylobacterium radiotolerans	ATCC 27329	
Micrococcus luteus	ATCC 4698	
Propioniobacterium acnes	ATCC 6919	
Pseudomonas aeruginosa	ATCC 9027	
Pseudomonas fluorescens	ATCC 17386	
Ralstonia pickettii	ATCC 27511	
Staphylococcus epidermidis	ATCC 12228	
Stenotrophomonas maltophilia	ATCC 13637	

2.1.3 Guide for Milliflex® system users

Component altered	Description of the changes	Validation test performed	Section	Performance result of Milliflex Oasis® vs Milliflex®
Media	Changes to media plate material, design, sterilization method No change to media formulation	Sterility	2.3.1.1	Pass
		Microbial performance: growth promotion test by spreading	2.3.1.2	
		Microbial performance: growth promotion test by filtration	2.3.1.3	
		Shelf life study including opened package hold time	2.3.1.4	
		Shipment study including stress test	2.3.1.5	
Filtration unit	Funnel and membrane support: change to design, material and sterilization method Ring & membrane: no change of material	Sterility	2.3.2.1	Pass
		Funnel material	2.3.2.2	
		Microbial performance	2.3.2.3	
		Shelf life study	2.3.2.4	
		Shipment study	2.3.2.5	
Filtration unit and pump	Funnel: change to design	Filtration time performance	2.3.3.2	Pass
	Pump: changes to design, material			
Pump	Pump: changes to material, design & cleaning method	Cleaning/sanitization of the pump	2.3.3.1	Pass
Filtration unit, media, pump	All changes described above	Microbial performance comparison of Milliflex Oasis® and Milliflex® system	2.3.4	Pass

2.2 Validation tests

2.2.1 Media validation

2.2.1.1 Sterility

Test summary

Sterile empty plates were poured with agar under aseptic conditions. The purpose of this test was to verify that the proportion of sterile media plates relative to the total number of plates produced in a batch is within manufacturing specifications.

Test objective

The objective was to test for the absence of viable microorganisms on Milliflex Oasis® media plates.

Test sampling

3% of each media lot of plates according to AQL 0.4% level S4

The test was performed using the following items:

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Incubation conditions:

7 days at 20–25 °C and 7 days at 30–35 °C.

Acceptance criteria

Test 1: Microbial growth < 1% after 7 days at 20–25 °C and 30–35 °C

Test 2: Milliflex Oasis® media plate performance must be equal or higher than current Milliflex® cassette performance.

Results

Incubation conditions	20–25 °C			30–35 °C		
Media type	**R2A**	**SDA**	**TSA**	**R2A**	**SDA**	**TSA**
Test 1 Milliflex Oasis® system	Pass	Pass	Pass	Pass	Pass	Pass
Test 2 Milliflex Oasis® vs. Milliflex® system	Pass	Pass	Pass	Pass	Pass	Pass

Pass: accepted

All the results were found to be within the specifications.

Conclusion

The tested lots of Milliflex Oasis® media plates with R2A/TSA/SDA met the tests' acceptance criteria and equaled or outperformed Milliflex® cassettes with R2A/TSA/SDA.

2.2.1.2 Microbial performance: Growth promotion test by spreading

Test summary

The purpose of this test was to determine the microbiological recovery performance of the Milliflex Oasis® media plates with R2A/TSA/SDA by plate spreading. A panel of microorganisms as defined by the EU/US/JP pharmacopeias was used as well as microorganisms representative of the pharmaceutical industry environment.

Test objectives

These tests had the following objectives:

Test 1: To verify the growth promotion performance of Milliflex Oasis® media plates using a panel of microorganisms by the spreading method.

Test 2: To compare the results when using the Milliflex Oasis® media plates with those when using the current Milliflex® cassettes.

Test sampling

Sampling was performed using each test microorganism on each media type and lot.

10 units of Milliflex Oasis® media plates and 10 Milliflex® agar cassettes were used per testing condition. Controls were performed on 90 mm Petri plates by spreading.

The test was performed using the following items:

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Incubation conditions:

R2A, 30-35°C ≤ 3 days TSA, 30-35°C ≤ 3 days SDA, 20-25°C ≤ 5 days

Acceptance criteria

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50%–200%).

Test 2: Milliflex Oasis® media plates must equal or outperform the current Milliflex® cassettes.

Results

Microorganism	R2A		SDA		TSA	
	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs. Milliflex®	Test 1	Test 2	Test 1	Test 2
Aspergillus brasiliensis **ATCC 16404**	Pass	Pass	Pass	Pass	Pass	Pass
Bacillus subtilis **ATCC 6633**	Pass	Pass	N/A	N/A	Pass	Pass
Candida albicans **ATCC 10231**	Pass	Pass	Pass	Pass	Pass	Pass
Clostridium sporogenes **ATCC 19404**	N/A	N/A	N/A	N/A	Pass	Pass
Escherichia coli **ATCC 8739**	Pass	Pass	N/A	N/A	Pass	Pass
Staphylococcus aureus **ATCC 6538**	Pass	Pass	N/A	N/A	Pass	Pass
Geobacillus stearothermophilus **DSM 22**	Pass	Pass	N/A	N/A	Pass	Pass
Methylobacterium aminovorans **ATCC 51358**	Pass	Pass	N/A	N/A	N/A	N/A
Methylobacterium extorquens **NBRC 15911**	Pass	Pass	N/A	N/A	N/A	N/A
Methylobacterium fujisawaense **ATCC 43884**	Pass	Pass	N/A	N/A	N/A	N/A
Methylobacterium radiotolerans **ATCC 27329**	Pass	Pass	N/A	N/A	N/A	N/A
Micrococcus luteus **ATCC 4698**	Pass	Pass	N/A	N/A	N/A	N/A
Pseudomonas aeruginosa **ATCC 9027**	Pass	Pass	N/A	N/A	Pass	Pass
Pseudomonas fluorescens **ATCC 17386**	Pass	Pass	N/A	N/A	N/A	N/A
Ralstonia pickettii **ATCC 27511**	Pass	Pass	N/A	N/A	N/A	N/A
Staphylococcus epidermidis **ATCC 12228**	N/A	N/A	N/A	N/A	Pass	Pass

Pass: Accepted N/A: Not applicable

All the results were found to be within the specifications.

Conclusion

The tested lots of Milliflex Oasis® media plates with R2A/TSA/SDA met the tests' acceptance criteria and equaled the performance of Milliflex® cassettes with R2A/TSA/SDA.

2.2.1.3 Microbial performance: Growth promotion test by filtration

Test summary

The purpose of this test was to determine the microbiological recovery performance of the Milliflex Oasis® media plates with R2A/TSA/SDA by filtration. A panel of microorganisms defined by the EU/US/JP pharmacopeias was used as well as microorganisms representative of the pharmaceutical industry environment.

Test objectives

The tests had the following objectives:

Test 1: To verify the growth promotion performance of Milliflex Oasis® media plate using a panel of microorganisms by the filtration method.

Test 2: To compare the Milliflex Oasis® media plates' performance to that of the current Milliflex® cassettes.

Test sampling

Sampling was performed using each test microorganism on each media type and lot.10 units of Milliflex Oasis® media plates and 10 Milliflex® agar cassettes were used per testing condition. Controls were done on 90 mm Petri plates by spreading.

The test was performed using the following items:

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Acceptance criteria

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50%–200%).

Test 2: Milliflex Oasis® media plates must equal or outperform current Milliflex® cassettes.

Results

Microorganism		Incubation condition	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs Milliflex®
Aspergillus brasiliensis	ATCC 16404	SDA, 20–25 °C ≤5 days	Pass	Pass
Candida albicans	ATCC 10231		Pass	Pass
Aspergillus brasiliensis	ATCC 16404	TSA, 30–35 °C ≤3 days	Pass	Pass
Bacillus subtilis	ATCC 6633		Pass	Pass
Candida albicans	ATCC 10231		Pass	Pass
Clostridium sporogenes	ATCC 19404		Pass	Pass
Escherichia coli	ATCC 8739		Pass	Pass
Micrococcus luteus	ATCC 4698		Pass	Pass
Pseudomonas aeruginosa	ATCC 9027		Pass	Pass
Staphylococcus aureus	ATCC 6538		Pass	Pass
Staphylococcus epidermidis	ATCC 12228		Pass	Pass
Bacillus subtilis	ATCC 6633	R2A, 30–35 °C ≤3 days	Pass	Pass
Pseudomonas aeruginosa	ATCC 9027		Pass	Pass
Ralstonia pickettii	ATCC 27511		Pass	Pass
Methylobacterium extorquens	NBRC 15911	R2A, 20–25 °C for 4-7 days	Pass	Pass
Pseudomonas fluorescens	ATCC 17387		Pass	Pass

Pass: Accepted

All the results were found to be within the specifications.

Conclusion

The tested lots of Milliflex Oasis® media plates with R2A/TSA/SDA met the testing acceptance criteria and equaled the performance of Milliflex® cassettes with R2A/TSA/SDA.

2.2.1.4 Shelf life study including opened package hold time

Test summary

Shelf life: The purpose of this test was to ensure that the Milliflex Oasis® media plates perform within the specifications until their defined expiry date.
Opened package hold time: One Milliflex Oasis® media plate sleeve (primary package) contains 8 plates. The purpose of this test was to ascertain that an opened sleeve can be stored at room temperature for 7 days without affecting the microbiological performance of the media. Evaluation was performed at different time points until the defined expiry date.

Test objectives

The tests had the following objectives:

Test 1: To verify the growth promotion performance of Milliflex Oasis® media plates using a panel of microorganisms by the spreading method.

Test 2: To compare the performance of Milliflex Oasis® media plates with that of the current Milliflex® cassettes.

Test sampling

The following parameters were challenged:

Visual inspection of media properties, 100% of plates used for this test were checked.

pH measurement: 3 Milliflex Oasis® media plates per lot.

Recovery test by spreading method: 3 media plates per microorganism, per media type and per lot, controls performed on 90 mm Petri plates.

The test was performed on the following items:

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

The incubation conditions were in accordance with the applicable regulations

Acceptance criteria

Test 1:

Visual inspection of media properties: Usual attributes should be observed
pH measurement: Same criterion used for routine release test of the current TSA, SDA and R2A media.
Obtained growth must not differ by a factor greater than 2 from the control (50%–200%).

Test 2:

Milliflex Oasis® media plates must equal or outperform the current Milliflex® cassettes.

Results

			Test 1			Test 2
Microorganisms	Source	Media	Visual inspection	pH	Recovery test: Spreading	Milliflex Oasis® vs. Milliflex® system
Aspergillus brasiliensis	ATCC 16404	R2A, SDA, TSA	Pass	Pass	Pass	Pass
Candida albicans	ATCC 10231	R2A, SDA, TSA	Pass	Pass	Pass	Pass
Bacillus subtilis	ATCC 6633	R2A, TSA	Pass	Pass	Pass	Pass
Escherichia coli	ATCC 8739	R2A, TSA	Pass	Pass	Pass	Pass
Staphylococcus aureus	ATCC 6538	R2A, TSA	Pass	Pass	Pass	Pass
Pseudomonas aeruginosa	ATCC 9027	R2A, TSA	Pass	Pass	Pass	Pass
Methylobacterium extorquens	NBRC 15911	R2A, TSA	Pass	Pass	Pass	Pass
Pseudomonas fluorescens	ATCC 9027	R2A, TSA	Pass	Pass	Pass	Pass
Methylobacterium aminovorans	ATCC 51358	R2A	Pass	Pass	Pass	Pass
Methylobacterium fujisawaense	ATCC 43884	R2A	Pass	Pass	Pass	Pass
Methylobacterium radiotolerans	ATCC 27329	R2A	Pass	Pass	Pass	Pass
Geobacillus stearothermophilus	DSM 22	R2A, TSA	Pass	Pass	Pass	Pass
Ralstonia pickettii	ATCC 27511	R2A	Pass	Pass	Pass	Pass
Burkholderia cepacia	ATCC 25416	R2A, TSA	Pass	Pass	Pass	Pass
Staphylococcus epidermidis	ATCC 12228	R2A, TSA	Pass	Pass	Pass	Pass
Micrococcus luteus	ATCC 4698	R2A, TSA	Pass	Pass	Pass	Pass
Clostridium sporogenes	ATCC 11437	R2A, TSA	Pass	Pass	Pass	Pass
Propionibacterium acnes	ATCC 6919	TSA	Pass	Pass	Pass	Pass

All results were found to be within specifications.

Conclusion

The tested lots of Milliflex Oasis® media plates with R2A/TSA/SDA met the tests' acceptance criteria and equaled the performance of Milliflex® cassettes with R2A/TSA/SDA**.**

2.2.1.5 Shipment study including stress test (stability study)

Test summary

The purpose of this test was to challenge the capability of the packaging to withstand transportation hazards.

Test objectives

This test was carried out by subjecting packaged product to a sequence of representative, anticipated hazards: shock/drop, compression, vibration, low pressure exposure, and temperature variations up to 40 °C.
The distribution simulation test according to the ISTA 7E standard and ASTM D4169 DC13 is performed to release high performance packaging system designs for pharmaceutical, medical device and consumer product manufacturers. ISTA 7E (heating stress) simulates shipping under worst case conditions. Temperature peak cycles up to 40 °C and subsequent storage at 2–8 °C until the expiration date was applied.
ASTM D4169, 2016. Distribution cycle: 13 - Air (intercity) and motor freight (local, single package up to 150 lb (61.8 kg)). Criticality level: level II.

Test sampling

Up to 5 media plates per microorganism, per media type and per lot. The recovery test was performed by filtration on a HAWG membrane. Controls were performed on 90mm Petri plates.

The test was performed on the following items:

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Milliflex Oasis® 250 mL funnel – **MMHAWG224**

Acceptance criteria

ISTA 7E

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50% to 200%).
Test 2: Milliflex Oasis® media plates must equal or outperform current Milliflex® cassettes.

ASTM D4169 DC13 standard
No damage affecting the items must be found to have occurred. Evaluation includes visual inspection, use and performance.

Results

ISTA 7E: Recovery test – Filtration method

		Performance	
Microorganism	Incubation conditions	**Test 1 Milliflex Oasis®**	**Test 2 Milliflex Oasis® vs Milliflex®**
***Bacillus subtilis* ATCC 6633**	TSA, 30–35 °C ≤3 days	Pass	Pass
***Aspergillus brasiliensis* ATCC 16404**	SDA, 20–25 °C ≤5 days	Pass	Pass
***Ralstonia pickettii* ATCC 27511**	R2A, 30–35 °C ≤3 days	Pass	Pass

ASTM D4169 DC13 standards

Parameter	**Test 1 Milliflex Oasis®**	**Test 2 Milliflex Oasis® vs Milliflex®**
Visual	Pass	Pass
Use	Pass	Pass
Performance	Pass	Pass

All results were found to be within specifications.

Conclusion

The tested lots of Milliflex Oasis® media plates with R2A/TSA/SDA met the testing acceptance criteria.

2.2.2 Filtration Unit

2.2.2.1 Sterility

Test summary

The Milliflex Oasis® filtration unit is sterilized by radiation. The purpose of this test was to qualify the sterilization process following the ISO 11137-1, -2, and -3 standards.

Test objective

The Sterility Assurance Level (SAL) of a product is defined as the probability that a product unit remains non-sterile after the applied sterilization procedure. The objective of this test is to verify that the sterilization dose SAL 10-6 sterilizes the Milliflex Oasis® filtration unit as expected and has no impact on microbial growth.

Test 1: To verify that the dose SAL 10-6 sterilizes the Milliflex Oasis® filtration unit as expected.

Test 2: To verify that the sterilization dose has no impact on Milliflex Oasis® filtration unit performance.

Test 3: To compare the performance of Milliflex Oasis® filtration unit media plates to that of current Milliflex® cassettes.

Test sampling

Test 1:

100 Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Test 2 & 3:

10 Units per microorganism and per lot. Controls were performed on 90 mm Petri plates.

Incubation conditions:

TSA, 30–35 °C ≤3 days SDA, 20–25 °C ≤5 days

Acceptance criteria

Test 1: After 14 days of incubation at 30 ±2 °C. On TSA Milliflex Oasis® media plate, the number of positive rate on units must be ≤2.

Test 2: Obtained growth must not differ by a factor greater than 2 from the control (50%–200%).

Test 3: Milliflex Oasis® media plates must equal or outperform current Milliflex® cassettes.

Results

Sterility test	Test 1
Milliflex Oasis® 100 mL filtration unit	Pass

Microbial performance

Microorganisms	Incubation conditions	Test 2 Milliflex Oasis®	Test 3 Milliflex Oasis® vs Milliflex®
***Aspergillus brasiliensis* ATCC 16404**	SDA	Pass	Pass
***Candida albicans* ATCC 10231**		Pass	Pass
***Bacillus subtilis* ATCC 6633**	TSA	Pass	Pass
***Escherichia coli* ATCC 8739**		Pass	Pass
***Pseudomonas aeruginosa* ATCC 9027**		Pass	Pass
***Staphylococcus aureus* ATCC 6538**		Pass	Pass

All results were found to be within specifications.

Conclusion

The tested Milliflex Oasis® filtration units met the tests' acceptance criteria. The sterilization process has been qualified according to the ISO 11137-1, -2 and -3 standards. There is no impact of the sterilization process on the microbial growth of the tested microorganisms.

2.2.2.2 Milliflex Oasis® filtration unit material

Test summary

The purpose of this test was to ascertain that the new filtration unit material (SBC resin) and the new irradiation sterilization process have no impact on microbial growth.

Test objective

The aim of this test was to determine the amount of carbon in the flush water after filtration using the Milliflex Oasis® filtration unit. The samples were analyzed by total organic carbon (TOC) determination. Elemental composition of the membrane surface was analyzed by X-ray photoelectroscopy (XPS).

Test sampling

2 Milliflex Oasis® funnel, non-sterile, **MMHAWG124**

2 Milliflex Oasis® funnel, sterilization performed with the routine dose, **MMHAWG124**

2 Milliflex® funnel, sterilization performed with the routine dose, **MXHAWG124**

The test was performed at the following water temperatures:

20 °C

60 °C

Acceptance criteria

Milliflex Oasis® filtration unit must yield comparable results to those of current Milliflex® funnel.

Results

Flush water temperature	20 °C		60 °C	
Parameter	Non-sterile Milliflex Oasis® over Milliflex®	Sterile Milliflex Oasis® over Milliflex®	Non-sterile Milliflex Oasis® over Milliflex®	Sterile Milliflex Oasis® over Milliflex®
TOC	Pass	Pass	Pass	Pass
XPS	Pass	Pass	Pass	Pass

Conclusion

The surface composition of the Milliflex Oasis® filtration unit and Milliflex® filtration unit did not differ significantly for both the sterile and the non-sterile units. Therefore, the new filtration unit material (SBC resin) and the new E-beam sterilization process have no impact on the microbial growth.

2.2.2.3 Milliflex Oasis® filtration unit microbial performance

Test summary

The purpose of this test is to verify that the microbial growth promotion test using Milliflex Oasis® filtration units was within the specification range and met expected performance.

Test objective

The test is conducted according to the EU, US and JP pharmacopeia recommendations and specifications: EP 2.6.12 & 2.6.13; USP <61> & <62>; JP 4.05; EP Water, highly purified; JP Pharmacopeia G8 chapter.

The microorganisms used for this qualification are those listed in the table “Preparation and use of test microorganisms” in the chapter “Microbiological examination of non-sterile products” (EP 2.6.12, USP <61>).

This test is also intended to demonstrate that Milliflex Oasis® system performs similarly to the Milliflex® filtration units. An extended panel of 7 strains representative of the pharmaceutical industry environment was also used with the Milliflex Oasis® system.

Test sampling

10 product units per strain and per Milliflex Oasis® filtration unit lot were tested. Controls were performed on 90 mm Petri plates.

The test was performed using the following items

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Milliflex Oasis® Tryptic Soy media plates - **MMSMCTS48**

Milliflex Oasis® Sabouraud Dextrose media plates - **MMSMCSD48**

Incubation conditions:

R2A, 30–35 °C ≤3 days TSA, 30–35 °C ≤3 days SDA, 20–25 °C ≤5 days

Acceptance criteria

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50%–200%).

Test 2: Milliflex Oasis® media plates must equal or outperform the current Milliflex® cassettes.

Results

Microorganism		Incubation conditions	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs Milliflex®
Aspergillus brasiliensis	**ATCC 16404**	SDA	Pass	Pass
		TSA	Pass	Pass
Bacillus subtilis	**ATCC 6633**	TSA	Pass	Pass
		R2A	Pass	Pass
Candida albicans	**ATCC 10231**	TSA	Pass	Pass
		SDA	Pass	Pass
Clostridium sporogenes	**ATCC 19404**	TSA	Pass	Pass
Escherichia coli	**ATCC 8739**	TSA	Pass	Pass
Methylobacterium extorquens	**NBRC 15911**	R2A, 20-25 °C for 4-7 days*	Pass	Pass
Micrococcus luteus	**ATCC 4698**	TSA	Pass	Pass
Pseudomonas aeruginosa	**ATCC 9027**	TSA	Pass	Pass
		R2A	Pass	Pass
Pseudomonas fluorescens	**ATCC 17387**	R2A, 20-25 °C for 4-7 days*	Pass	Pass
Ralstonia pickettii	**ATCC 27511**	R2A	Pass	Pass
Staphylococcus aureus	**ATCC 6538**	TSA	Pass	Pass
Staphylococcus epidermidis	**ATCC 12228**	TSA	Pass	Pass

*Incubation conditions according to JP pharmacopeia requirements.

All results were found to be within specifications.

Conclusion

The tested Milliflex Oasis® filtration units met the testing acceptance criteria and therefore offer equivalent performance levels to Milliflex® filtration units.

2.2.2.4 Shelf life study

Test summary

The purpose of this test is to ensure that the filtration units performance stays within the specifications until the defined expiry date.

Test objective

The tests had the following objectives:

Test 1: To verify the growth promotion performance of Milliflex Oasis® filtration units using a panel of microorganisms by filtration method.

Test 2: To compare the performance of the Milliflex Oasis® filtration units with that of the current Milliflex® filtration units.

Test sampling

Sampling was performed using each test microorganism on each media type and lot. 5 units of Milliflex Oasis® filtration units and 5 Milliflex® filtration units were used per testing condition. Controls were performed on 90 mm Petri plates.

Acceptance criteria

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50%–200%)

Test 2: Milliflex Oasis® filtration units must equal or outperform current Milliflex® filtration units.

Results

Microbial performance

Microorganisms	Incubation conditions	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs Milliflex®
Aspergillus brasiliensis **ATCC 16404**	SDA, 20–25 °C ≤5 days	Pass	Pass
Candida albicans **ATCC 10231**		Pass	Pass
Bacillus subtilis **ATCC 6633**	TSA, 30–35 °C ≤3 days	Pass	Pass
Escherichia coli **ATCC 8739**		Pass	Pass
Pseudomonas aeruginosa **ATCC 9027**		Pass	Pass
Staphylococcus aureus **ATCC 6538**		Pass	Pass

All results were found to be within specifications.

Conclusion

The tested lots of Milliflex Oasis® filtration units met the tests' acceptance criteria and therefore offer equivalent performance levels to Milliflex® filtration units.

2.2.2.5 Shipment study

Test summary

The purpose of this test was to challenge the capability of the packaging to withstand transportation hazards. This test was performed in the course of the shipment study of the Milliflex Oasis® media plates as described in the section "2.3.1.5 Shipment study including stress test (stability study)". Please refer to that section for details.

Conclusion

The tested lots of Milliflex Oasis® filtration units met the tests' acceptance criteria and offer equivalent performance levels to Milliflex® filtration unit.

2.2.3 Milliflex Oasis® filtration pump validation

2.2.3.1 Pump cleaning/decontamination study

Test summary

The purpose of this study was to check that the cleaning and decontamination recommendations stated in the user guide are adequate to control the risk of secondary sample contamination.

Test objective

The Milliflex Oasis® filtration pump was deliberately contaminated with nine challenge organisms (see table 1, panel of challenge organisms). Different critical areas on the Milliflex Oasis® pump were contaminated with levels of contamination from 100 to 200 CFU per mL. The subsequent cleaning processes were performed according to the Milliflex Oasis® system user guide's recommendations. Then microbiological tests were conducted to monitor the biological load.

Microorganism	ATCC number	Source	Incubation conditions
Aspergillus brasiliensis	16404	Remel Quanti-Cult^{Plus}	TSA, 30–35 °C ≤3 days
Bacillus subtilis	6633	Remel Quanti-Cult^{Plus}	
Candida albicans	10231	Remel Quanti-Cult^{Plus}	
Kocuria rhizophila	9341	BioReliance US bank	
Pseudomonas aeruginosa	9027	Remel Quanti-Cult^{Plus}	
Ralstonia pickettii	27511	BioReliance US bank	
Staphylococcus aureus	6538	Remel Quanti-Cult^{Plus}	
Staphylococcus epidermidis	12228	Remel Quanti-Cult^{Plus}	
Stenotrophomonas maltophilia	13637	BioReliance US bank	

Test sampling

3 Milliflex Oasis® filtration pumps - **MMSYSTMM1**.

Acceptance criteria

No cross-contamination must be observed during the filtration process.

Results

All results were found to be within the acceptance criteria.

No cross-contamination was observed during the filtration process when following the instructions given in the cleaning & maintenance section of the user guide.

Conclusion

The instructions given in the cleaning & maintenance section of the user guide allow for optimal cleaning of the Milliflex Oasis® pump and minimize the risks of secondary contamination to the sample caused by the pieces of equipment. Therefore, there is no need to autoclave the Milliflex Oasis® filtration heads.

2.2.3.2 Filtration time performance

Test summary

The purpose of this test is to compare the filtration time when using the Milliflex Oasis® system, with water as the sample.

Test objective

100 mL of water were used as the sample to compare the Milliflex Oasis® filtration pump and the Milliflex® Plus pump with regards to filtration time.

The tests were performed using the following devices:

Milliflex Oasis® 100 mL funnel, **MMHAWG124**

Milliflex Oasis® 100 mL funnel, **MMHABG124**

Milliflex Oasis® 100 mL funnelt, **MMGSWG124**

Milliflex Oasis® 100 mL funnel, **MMHVWP124**

Acceptance criteria

The filtration time using the Milliflex Oasis® filtration pump must be equal or shorter than when using the Milliflex® Plus pump.

Results

	MMHAWG124	MMHABG124	MMGSWG124	MMHVWP124
Milliflex Oasis® vs. Milliflex® system	Pass	Pass	Pass	Pass

Thanks to the shorter dry-out step (5 s instead of 14 s), the Milliflex Oasis® system filters a 100 mL sample of water 50% faster.

Conclusion

Filtration of a 100 mL water sample using the Milliflex Oasis® system (pump & filtration unit) is faster than when using the Milliflex® system.

2.2.4 Microbial recovery performance of the Milliflex Oasis® system compared to the Milliflex® system

Test summary

The purpose of this test was to determine the microbiological recovery performance of the Milliflex Oasis® system relative to that of the Milliflex® system. A panel of microorganisms listed in the EU/US/JP pharmacopeias was used as well as microorganisms representative of the pharmaceutical industry environment.

Test objective

The test was conducted according to the EU, US and JP pharmacopeia recommendations and specifications: EP 2.6.12 & 2.6.13; USP <61> & <62>; JP 4.05; EP Water, highly purified; JP Pharmacopeia G8 chapter.

The microorganisms used for this qualification are those listed in the table “Preparation and use of test microorganisms” in the chapter “Microbiological examination of non-sterile products” (EP 2.6.12, USP <61>).

This test was also intended to demonstrate that the Milliflex Oasis® system offers similar performance to the Milliflex® system.

An extended panel of 7 strains representative of the pharmaceutical industry environment was also used for tests with the Milliflex Oasis® system.

Test Sampling

10 product units per strain and per Milliflex Oasis® media plate type were tested. Controls were performed on 90 mm Petri plates.

The test was performed using the following items:

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Milliflex Oasis® 100 mL funnel - **MMHAWG124**

Milliflex Oasis® R2A media plates - **MMSMCRA48**

Incubation conditions:

R2A, 30–35 °C ≤3 days TSA, 30–35 °C ≤3 days SDA, 20–25 °C ≤5 days

Accepting criteria

Test 1: Obtained growth must not differ by a factor greater than 2 from the control (50% – 200%)

Test 2: Milliflex Oasis® media plates must equal or outperform current Milliflex® cassettes.

Results

Microorganism		Incubation conditions	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs Milliflex®
Aspergillus brasiliensis	**ATCC 16404**	SDA	Pass	Pass
		TSA	Pass	Pass
Bacillus subtilis	**ATCC 6633**	TSA	Pass	Pass
		R2A	Pass	Pass
Candida albicans	**ATCC 10231**	TSA	Pass	Pass
		SDA	Pass	Pass
Clostridium sporogenes	**ATCC 19404**	TSA	Pass	Pass
Escherichia coli	**ATCC 8739**	TSA	Pass	Pass
Methylobacterium extorquens	**NBRC 15911**	R2A, 20–25 °C for 4–7 days*	Pass	Pass
Micrococcus luteus	**ATCC 4698**	TSA	Pass	Pass
Pseudomonas aeruginosa	**ATCC 9027**	TSA	Pass	Pass
		R2A	Pass	Pass

Microorganism		Incubation conditions	Test 1 Milliflex Oasis®	Test 2 Milliflex Oasis® vs Milliflex®
Pseudomonas fluorescens	**ATCC 17387**	R2A, 20–25 °C for 4–7 days*	Pass	Pass
Ralstonia pickettii	**ATCC 27511**	R2A	Pass	Pass
Staphylococcus aureus	**ATCC 6538**	TSA	Pass	Pass
Staphylococcus epidermidis	**ATCC 12228**	TSA	Pass	Pass

*Incubation conditions according to JP pharmacopeia requirements.

All results were found to be within specifications.

Conclusion
The tested Milliflex Oasis® system met the tests' acceptance criteria and offers equivalent performance levels to the Milliflex® system.

3. General conclusion

The tested Milliflex Oasis® system met the acceptance criteria of all tests and equaled or outperformed the Milliflex® system with regards to all the tested parameters.

Notes

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