Validation Summary

The Milliflex Oasis® system for bioburden and pharmaceutical water testing
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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

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

1.2.3 Vacuum Pump

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

<table>
<thead>
<tr>
<th>Description</th>
<th>Milliflex Oasis® Cat. No.</th>
<th>Milliflex® Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Filtration Unit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mL funnels, 0.45 µm white gridded, cellulose esters, 24 units per pack</td>
<td>MMHAWG124</td>
<td>MXHAWG124</td>
</tr>
<tr>
<td>100 mL funnels, 0.22 µm white gridded, cellulose esters, 24 units per pack</td>
<td>MMGSWG124</td>
<td>MXGSWG124</td>
</tr>
<tr>
<td>100 mL funnels, 0.45 µm black gridded, cellulose esters, 24 units per pack</td>
<td>MMHABG124</td>
<td>MXHABG124</td>
</tr>
<tr>
<td>250 mL funnels, 0.45 µm white gridded, cellulose esters, 24 units per pack</td>
<td>MMHAWG224</td>
<td>MXHAWG224</td>
</tr>
<tr>
<td><strong>PVDF Membrane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack</td>
<td>MMHVMFX24</td>
<td>RMHVMFX24</td>
</tr>
<tr>
<td>250 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack</td>
<td>MMHVMFX24</td>
<td>RMHVMFX24</td>
</tr>
<tr>
<td>Rapid Microbiology 100 mL funnels, 0.45 µm white no gridding, PVDF Durapore®, 24 units per pack</td>
<td>MMHVMFX24</td>
<td>RMHVMFX24</td>
</tr>
</tbody>
</table>

1.3 Technical specifications

1.3.1 Media Plate

<table>
<thead>
<tr>
<th>Description</th>
<th>Milliflex Oasis®</th>
<th>Milliflex®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Construction material</strong></td>
<td>Body, bottom, and lid</td>
<td>Polystyrene (PS)</td>
</tr>
<tr>
<td><strong>Dimension</strong></td>
<td>Maximal diameter 66.4 mm (2.6 in.)</td>
<td>Height 21.35 mm</td>
</tr>
<tr>
<td><strong>Minimal cassette weight</strong></td>
<td>26.5 g</td>
<td></td>
</tr>
<tr>
<td><strong>Agar quantity</strong></td>
<td>At least 10 mL</td>
<td></td>
</tr>
<tr>
<td><strong>Color of unit</strong></td>
<td>R2A Blue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSA Green</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDA Pink</td>
<td></td>
</tr>
<tr>
<td><strong>Sterilization method</strong></td>
<td>Irradiation of empty units followed by aseptic filling</td>
<td></td>
</tr>
<tr>
<td><strong>Storage temperature</strong></td>
<td>2–8 °C</td>
<td></td>
</tr>
<tr>
<td><strong>Shelf life</strong></td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td><strong>2D code identification</strong></td>
<td>Data matrix, can be read with standard 2D reader</td>
<td></td>
</tr>
</tbody>
</table>
### 1.3.2 Filtration Unit

<table>
<thead>
<tr>
<th>Construction material</th>
<th>Lid, funnel and base</th>
<th>Styrene butadiene copolymer (SBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td>Mixed cellulose esters (MCE) or polyvinylidene difluoride (PVDF)</td>
<td></td>
</tr>
<tr>
<td>Membrane ring material</td>
<td>Polyethylene (PE)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Height</th>
<th>100 mL: 57 mm (2.2 in.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>250 mL: 113 mm (5.5 in.)</td>
</tr>
<tr>
<td>Maximal diameter</td>
<td>82 mm</td>
<td>(3.2 in.)</td>
</tr>
<tr>
<td>Membrane diameter</td>
<td>49 mm</td>
<td>(1.9 in.)</td>
</tr>
<tr>
<td>Sterilization method</td>
<td>Irradiation</td>
<td></td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>60 °C</td>
<td></td>
</tr>
<tr>
<td>(of filtered liquid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shelf life</td>
<td>1 year (2 years after complete qualification)</td>
<td></td>
</tr>
</tbody>
</table>

### 1.3.2.1 Membrane

<table>
<thead>
<tr>
<th>Catalogue number codification</th>
<th>HAW</th>
<th>HAB</th>
<th>GS</th>
<th>HV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Construction material</td>
<td>Mixed cellulose ester (MCE)</td>
<td>Mixed cellulose ester (MCE)</td>
<td>Mixed cellulose ester (MCE)</td>
<td>Polyvinylidene fluoride (PVDF)</td>
</tr>
<tr>
<td>Membrane pore size</td>
<td>0.45 µm</td>
<td>0.45 µm</td>
<td>0.22 µm</td>
<td>0.45 µm</td>
</tr>
<tr>
<td>Membrane color &amp; gridding</td>
<td>White gridded</td>
<td>Black gridded</td>
<td>White gridded</td>
<td>White plain</td>
</tr>
</tbody>
</table>

### 1.3.3 Pump

<table>
<thead>
<tr>
<th>Pump dimension</th>
<th>Height</th>
<th>90 mm (3.5 in.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Width</td>
<td>230 mm (9.0 in.)</td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>310 mm (12.2 in.)</td>
</tr>
<tr>
<td>Filtration support height</td>
<td>70 mm</td>
<td>(2.8 in.)</td>
</tr>
<tr>
<td>Weight with filtration heads</td>
<td>3.4 kg</td>
<td>(7.5 lb)</td>
</tr>
<tr>
<td>Power supply</td>
<td>24–30 V DC, 25 W - One set can be used for up to three pumps</td>
<td></td>
</tr>
<tr>
<td>Surface decontaminants</td>
<td>Quaternary ammonium, isopropyl alcohol (70%), ethanol (70%), bleach (250 ppm), solution of peracetic acid (&lt;1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>Storage temperature</th>
<th>0 °C to 40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operation temperature</td>
<td>15 °C to 40 °C</td>
</tr>
<tr>
<td>Relative humidity (RH)</td>
<td>80% at temperatures up to 31 °C, then linear decrease to 50% RH at 40 °C</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Constituting materials</th>
<th>Frame</th>
<th>Blend of acrylonitrile styrene acrylate and polycarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Stainless steel AISI 316L</td>
</tr>
<tr>
<td>Check valve</td>
<td>Silicone</td>
<td></td>
</tr>
<tr>
<td>Seals</td>
<td>Silicone</td>
<td></td>
</tr>
<tr>
<td>Tubings</td>
<td>Low density polyethylene</td>
<td></td>
</tr>
<tr>
<td>Fittings</td>
<td>Polypropylene</td>
<td></td>
</tr>
</tbody>
</table>
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.


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

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

*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.
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>
<thead>
<tr>
<th>Microorganism</th>
<th>Collection</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus brasiliensis</td>
<td>ATCC 16404</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC 6633</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC 10231</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC 8739</td>
<td></td>
</tr>
<tr>
<td>Methylobacterium extorquens</td>
<td>NBRC 15911</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 6538</td>
<td></td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>ATCC 25416</td>
<td></td>
</tr>
<tr>
<td>Geobacillus stearothermophilus</td>
<td>DSM 22</td>
<td></td>
</tr>
<tr>
<td>Kocuria rhizophila</td>
<td>ATCC 9341</td>
<td></td>
</tr>
<tr>
<td>Methylobacterium aminovorans</td>
<td>ATCC 51358</td>
<td></td>
</tr>
<tr>
<td>Methylobacterium fujisawaense</td>
<td>ATCC 43884</td>
<td></td>
</tr>
<tr>
<td>Methylobacterium radiotolerans</td>
<td>ATCC 27329</td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>ATCC 4698</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>ATCC 6919</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 9027</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>ATCC 17386</td>
<td></td>
</tr>
<tr>
<td>Ralstonia pickettii</td>
<td>ATCC 27511</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>ATCC 12228</td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>ATCC 13637</td>
<td></td>
</tr>
</tbody>
</table>

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.
2.2 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

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>20–25 °C</th>
<th>30–35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media type</strong></td>
<td><strong>R2A</strong></td>
<td><strong>SDA</strong></td>
</tr>
<tr>
<td>Test 1 Milliflex Oasis® system</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>Test 2 Milliflex Oasis® vs. Milliflex® system</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

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 - MMSMCR48
- Milliflex Oasis® Tryptic Soy media plates - MMSMT48
- Milliflex Oasis® Sabouraud Dextrose media plates - MMSMCD48
- 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Test 1 Milliflex Oasis®</th>
<th>Test 2 Milliflex Oasis® vs. Milliflex®</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 1</th>
<th>Test 2</th>
</tr>
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<td>Pass</td>
<td>Pass</td>
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<td>Pass</td>
</tr>
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<td>ATCC 16404</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacillus subtilis</td>
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<td>Pass</td>
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<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
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<tr>
<td>ATCC 6633</td>
<td></td>
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</tr>
<tr>
<td>Candida albicans</td>
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<td>Pass</td>
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<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>ATCC 10231</td>
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<tr>
<td>Clostridium sporogenes</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>ATCC 19404</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
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<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>ATCC 8739</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
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<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
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<td>ATCC 6538</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Geobacillus stearothermophilus</td>
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<td>Pass</td>
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<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
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</tr>
<tr>
<td>Methylobacterium aminovorans</td>
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<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ATCC 51358</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Methylobacterium extorquens</td>
<td>Pass</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>NBRC 15911</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>N/A</td>
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</tr>
<tr>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ATCC 27329</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>Pass</td>
<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ATCC 4698</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pass</td>
<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>ATCC 9027</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Pass</td>
<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>ATCC 17386</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ralstonia pickettii</td>
<td>Pass</td>
<td>Pass</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ATCC 27511</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>ATCC 12228</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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 - MMSMCR4A8
Milliflex Oasis® Tryptic Soy media plates - MMSMCTS48
Milliflex Oasis® Sabouraud Dextrose media plates - MMSMCS48

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

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

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 - MSMCSD48
- 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Incubation conditions</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Milliflex Oasis® vs Milliflex®</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>TSA, 30–35 °C ≤3 days</td>
<td>Pass</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>ATCC 6633</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus brasiliensis</em></td>
<td>SDA, 20–25 °C ≤5 days</td>
<td>Pass</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>ATCC 16404</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ralstonia pickettii</em></td>
<td>R2A, 30–35 °C ≤3 days</td>
<td>Pass</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>ATCC 27511</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ASTM D4169 DC13 standards

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Milliflex Oasis® vs Milliflex®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>Pass</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>Use</td>
<td>Pass</td>
<td>Pass</td>
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</tr>
<tr>
<td>Performance</td>
<td>Pass</td>
<td>Pass</td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Sterility test</th>
<th>Test 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milliflex Oasis® 100 mL filtration unit</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Microbial performance**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Incubation conditions</th>
<th>Test 2 Milliflex Oasis®</th>
<th>Test 3 Milliflex Oasis® vs Milliflex®</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus brasiliensis</em> ATCC 16404</td>
<td>SDA</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 10231</td>
<td></td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633</td>
<td>TSA</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td></td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 9027</td>
<td></td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td></td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>20 °C</th>
<th>60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>XPS</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

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 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

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

*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

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

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.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>ATCC number</th>
<th>Source</th>
<th>Incubation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus brasiliensis</td>
<td>16404</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td>TSA, 30–35 °C ≤3 days</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>6633</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Kocuria rhizophila</td>
<td>9341</td>
<td>BioReliance US bank</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9027</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Ralstonia pickettii</td>
<td>27511</td>
<td>BioReliance US bank</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6538</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>12228</td>
<td>Remel Quanti-Cult&lt;sup&gt;™&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>13637</td>
<td>BioReliance US bank</td>
<td></td>
</tr>
</tbody>
</table>

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 funnel, 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

<table>
<thead>
<tr>
<th></th>
<th>MMHAWG124</th>
<th>MMHABG124</th>
<th>MMGSWG124</th>
<th>MMHVWP124</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milliflex Oasis® vs. Milliflex® system</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

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

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

*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.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Incubation conditions</th>
<th>Test 1 Milliflex Oasis®</th>
<th>Test 2 Milliflex Oasis® vs Milliflex®</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>ATCC 17387</td>
<td>R2A, 20–25 °C for 4–7 days*</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Ralstonia pickettii</em></td>
<td>ATCC 27511</td>
<td>R2A</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 6538</td>
<td>TSA</td>
<td>Pass</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>ATCC 12228</td>
<td>TSA</td>
<td>Pass</td>
</tr>
</tbody>
</table>

*Incubation conditions according to JP pharmacopeia requirements.