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White Paper

April 30, 2021

Keywords or phrases:
Microbial ingress, Single-use Systems, Assemblies, PUPSIT, *Brevundimonas diminuta*, Integrity

Sterility Assurance for Single-Use Systems, Such as Filter Transfer Set Final Filling by Microbial Ingress Test

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Abstract

Biopharmaceutical manufacturers increasingly rely on modular single-use systems (SUS), which are versatile and flexible solutions, and an increased use of disposable components in pharma | biotech production in upstream processing (USP), downstream processing (DSP), and fill and finish (F&F) can be observed. SUSs, such as filter transfer set final filling assemblies, are preassembled, presterilized, and fully prequalified assemblies that are ready to use for connection and integration with single-use processing solutions. SUSs are comprised of several fully disposable components, including the entire fluid pathway and filters. The assemblies used in biopharmaceutical manufacturing must maintain sterility and product quality of the fluid inside; therefore, such systems should be validated as providing an effective barrier against microbial ingress. The aim of microbial ingress testing of sterile SUSs used in biopharmaceutical manufacturing is to evaluate the ability of a system's fluid path to remain sterile after the system has been challenged by microbial exposure. A microbial ingress test by liquid immersion is the most accepted method, where microbial exposure is achieved by directly placing a SUS into a container of microbial challenge solution and evaluating its ability to remain sterile after seven days.

In this article, we demonstrate the integrity of Sartorius filter transfer set final filling assemblies by performing a microbial ingress test in accordance with ASTM standard E3251 – "Test Method for Microbial Ingress Testing on Single-Use Systems."[1]

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Introduction

Sterility Assurance for Single-Use Systems, Such as Filter Transfer Set Final Filling Assemblies by Microbial Ingress Test

Over the years, single-use technologies have evolved to become a mainstream approach for achieving productivity. Single-use workflows and biomanufacturing facilities are in high demand. The reason for this demand, and a significant driver for adopting single-use technologies, is the continuous improvements and redesigns of the devices, making disposables more useable, more effective (fewer contamination risks), more economical (lower cleaning and validation costs), and more acceptable to regulators (better leachable and extractable data and profiles).

Single-use systems (SUS), such as filter transfer set final filling assemblies, are preassembled, presterilized, and fully prequalified assemblies ready to use for connection and integration with single-use processing solutions[2,3]. Such systems consist of a variety of components (filters, tubing, connectors) that are connected by appropriate joints, and they must therefore act as an effective barrier against microbial ingress. An overview of a pre-use, post-sterilization integrity test (PUPSIT) assembly is provided in Figure 2.

The aim of microbial ingress testing of sterile SUSs used in biopharmaceutical manufacturing is to evaluate the ability of a SUS fluid path to remain sterile after the system has been challenged by microbial exposure. This is achieved by either placing a SUS directly into a container of microbial challenge solution, or by placing it inside a test chamber designed to generate and deliver an aerosolized microbial challenge. The choice of the test challenge organism should be justified based on a risk assessment of the SUS and conditions of use.

Microbial ingress test by immersion exposure is the most widely accepted method, where the SUS filled with sterile media is immersed in a challenge solution and incubated for seven days. Any defect or leak in the system would lead to contamination inside the SUS during incubation. This procedure is explained in ASTM standard E3251 – "20 Standard Test Methods for Microbial Ingress Testing on Single-Use Systems."[4]

Figure 1: Stainless steel holder with filter transfer set final filling assembly.

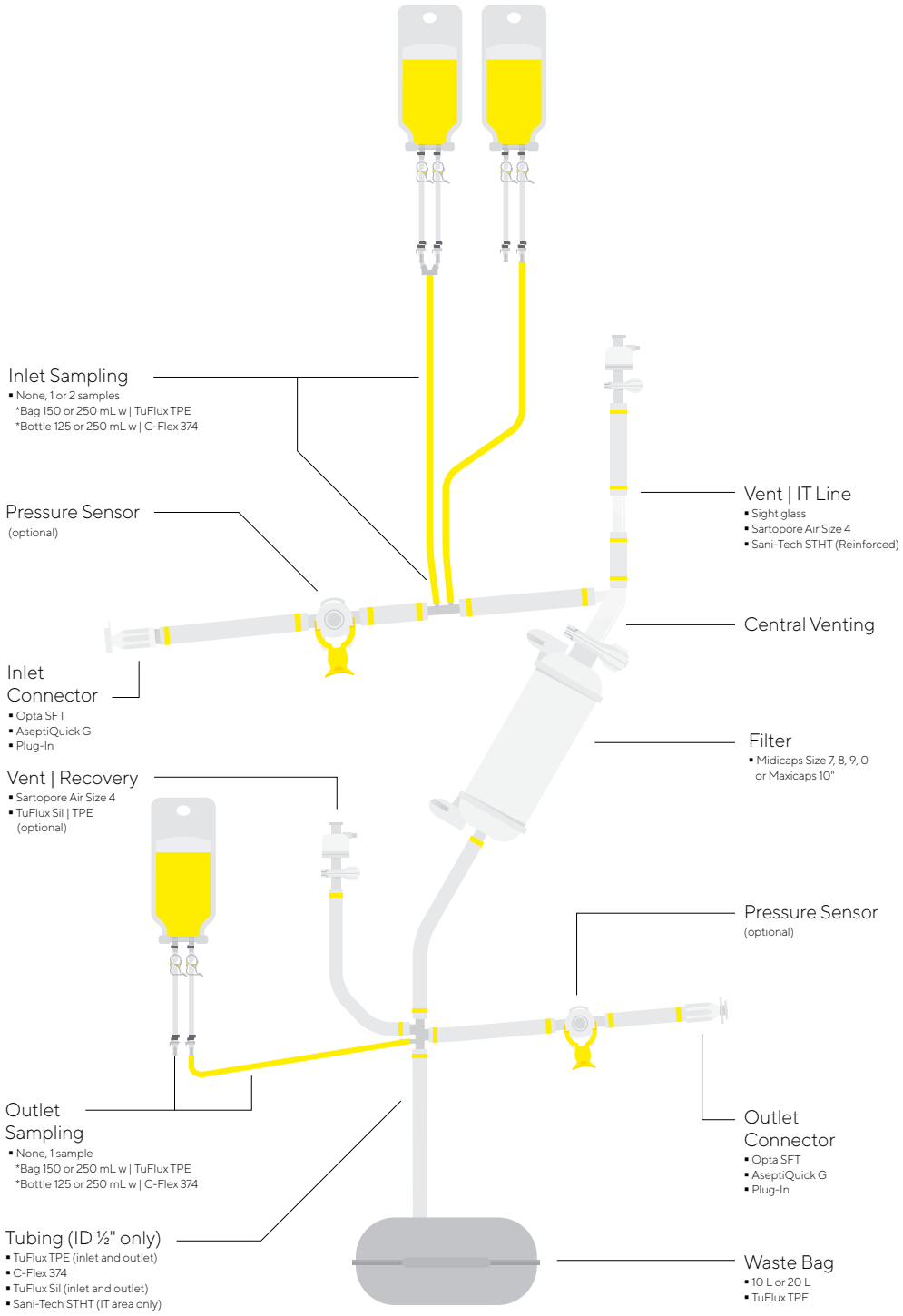


Figure 2: Overview of filter transfer set final filling.

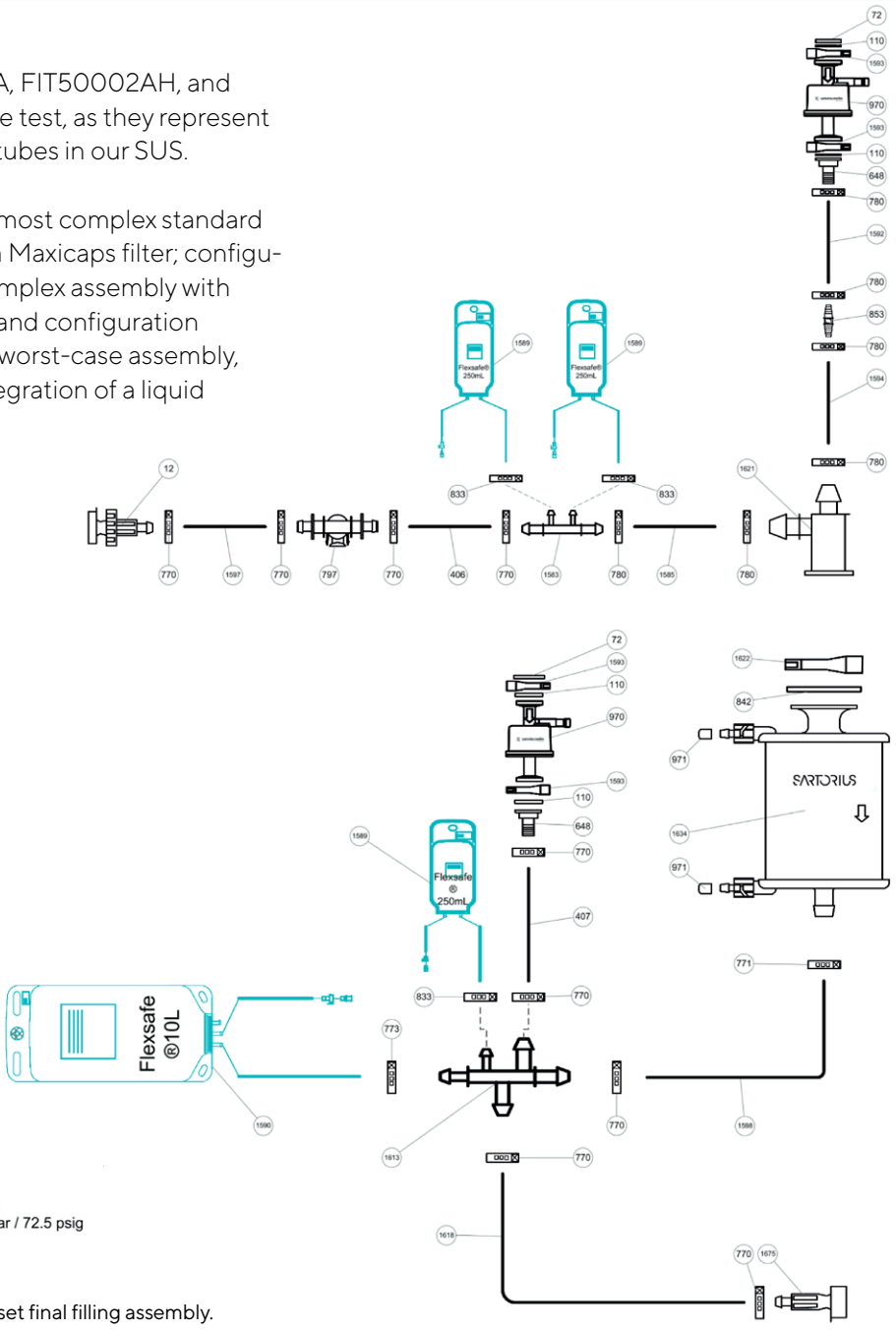
Filter Transfer Set Final Filling Assembly Preparation

Representative filter transfer set final filling assemblies were defined for the investigation and manufactured in product development in accordance with standard manufacturing instructions. Figure 3 provides an example of a schematic drawing of the manufactured assembly. The assemblies were gamma irradiated at 50 kGy by an external service provider. The sterilized assemblies were visually inspected for any defects, such as scratches, cuts, or any particles, before using it for the test.

Three configurations (FIT50002AA, FIT50002AH, and FIT50002AU) were selected for the test, as they represent the complicated connections and tubes in our SUS.

Configuration FIT50002AA is the most complex standard assembly with Sartopore® Platinum Maxicaps filter; configuration FIT50002AH is the most complex assembly with Sartopore® 2 XLM Midicaps® filter; and configuration FIT50002AU is the most complex worst-case assembly, with aseptic connectors for the integration of a liquid sterile filter.

By "most complex," we mean that the selected assemblies have the maximum possible number of connection points. We did not include sampling bags | bottles or waste bags for the study – all connections have a sealed tubing end.



Max. operating pressure filter upstream: 1 bar / 14,5 psig
Max. operating pressure filter downstream: 1 bar / 14,5 psig
Max. operating pressure redundant: 1 bar / 14,5 psig
Max. operating pressure integrity test: for gamma : 5 bar / 72.5 psig

Figure 3: Drawing of standard filter transfer set final filling assembly.

Microbial Ingress Test Setup

Microbial ingress tests by immersion exposure were performed for three types of standard Filter Transfer Set Final Filling assemblies in accordance with ASTM standard E3251 – "20 Standard Test Method for Microbial Ingress Testing on Single-Use Systems."

Filter transfer set final filling assemblies, after gamma irradiation, were filled with approximately 1 L of sterile tryptic soy broth (TSB) media (aseptically under biosafety cabinet) and all open ends were sealed using a Biosealer®. The assemblies filled with media were then immersed in 25 L of a challenge solution containing the test organism *Brevundimonas diminuta* in a concentration of 1×10^7 CFU/mL. A mixing bag in a mixing tank was used as a container for the challenge solution (Figure 4).

Figure 4: Filter transfer set final filling assembly immersed in the challenge solution.

Positive control is used to ensure that the microorganism can pass through a defect and can be detected by the test method. Exposed negative control confirms the correct preparation and assembly of the test article. Defect-based positive control, with defect and exposed negative control without any defect (Figure 4), are incubated along with the test assembly in the mixing bag for seven days at 30 °C.

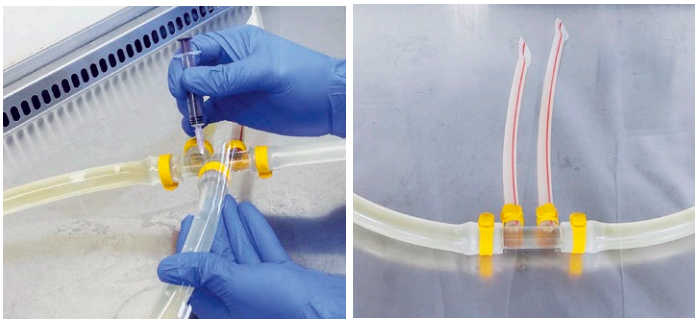


Figure 5: Defect-based positive control and exposed negative control.

To ensure the challenge concentration is 1×10^7 CFU/mL, a growth promotion test for the challenge solution was performed. The turbidity and optical density (OD) of the challenge solution, as well as positive and negative control solutions, were measured before and after incubation.

Test setup is incubated for seven days at 30 °C. The results are analyzed after incubation by checking the turbidity and OD of the media inside the assembly. The result sample was spread plated on tryptic soya agar (TSA) media, and 1 mL was inoculated into 10 mL tryptic soya broth (TSB) and incubated for five to seven days to check for any microbial growth. Sterile results indicate there was no microbial ingress into the test system.

Microbial Ingress Test Result

The microbial ingress test results are measured by the turbidity and OD of microbial cultures after incubation. Turbidity measurement is a widely used method for determining cell density of growing microorganisms in a culture. A Turbidimeter measures cloudiness or haziness caused by microbial growth in the liquid media in nephelometric turbidity units (NTU). Optical density measures the absorbance value of a liquid microbial culture in a photometer at 600 nm (A600 nm).

Turbidity and OD were measured for positive control, negative control, challenge solution, and result sample inside the filter transfer set final filling assembly after seven days of incubation at 30 °C. Positive control and challenge solution will show high turbidity due to microbial growth, whereas negative control and result sample should measure low turbidity (if we have sterile results). Any microbial ingress during the incubation would cause microbial growth inside the filter transfer set final filling assembly, and we can see turbidity visually and measure it.

The Turbidity results are given in Table 1 and Table 2 below. We can see the turbidity values of our result samples are like negative control, indicating no growth observed inside the PUPSIT assembly.

The bacterial load in the inoculum and challenge solution was determined by a growth promotion test by membrane filtration and spread plating. We observed a bacterial load of 4×10^6 CFU/mL on day 0, and 3×10^7 CFU/mL on day 1 of incubation in the challenge solution and approximately 1x109 CFU/mL in the inoculum. The growth test result of the inoculum and challenge solution can be seen in Figure 6.

Figure 6: Inoculum and challenge solution concentration.

OD ($A_{600\,nm}$)	Challenge Solution (Day 7)	Positive Control (Day 7)	Negative Control (Day 7)	Result Sample (Day 7)	Result
FIT50002AA	1.3	0.7	0.04	0.04	Pass
FIT50002AH	1.5	0.6	0.04	0.05	Pass
FIT50002AU	1.2	0.4	0.04	0.05	Pass

Table 1: OD values.

Turbidity (NTU)	Challenge Solution (Day 7)	Positive Control (Day 7)	Negative Control (Day 7)	Result Sample (Day 7)	Result
FIT50002AA	215.0	180.0	0.65	0.66	Pass
FIT50002AH	279.0	230.0	0.62	0.61	Pass
FIT50002AU	284.0	214.0	0.59	0.77	Pass

Table 1: Turbidity in nephelometric turbidity units.

Figure 7: Positive and negative control after incubation.

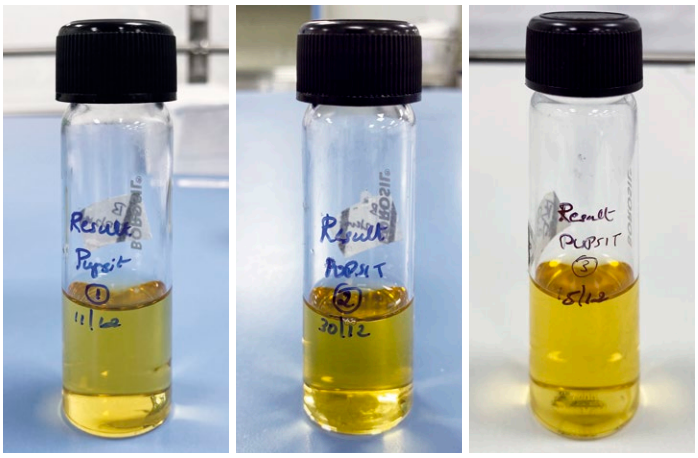


Figure 9: Sterile result in TSB.

Positive and negative controls ensure the correct preparation of a test system. Turbid positive control and clear negative control results after incubation are shown in Figure 7.

Test results are evaluated after the incubation period. The test assembly removed from the challenge solution was cleaned with 70% v/v isopropanol. It was placed inside a biosafety cabinet and observed for any visible turbidity, and the result samples were extracted from the assembly and evaluated. Microbial evaluation results of all three configurations confirm that the test system did not allow any bacteria to intrude during incubation.

Result samples were evaluated by spread plating 100 µL of the result sample on a TSA plate and 1 mL of result sample was inoculated to sterile TSB media. These were incubated for three to five days at 30 °C. Results are shown in the examples in Figure 8 and Figure 9.

No colonies were observed on spread plates and no turbidity was observed in the liquid media after five days of incubation, indicating sterile results inside the assembly in all the assemblies tested.

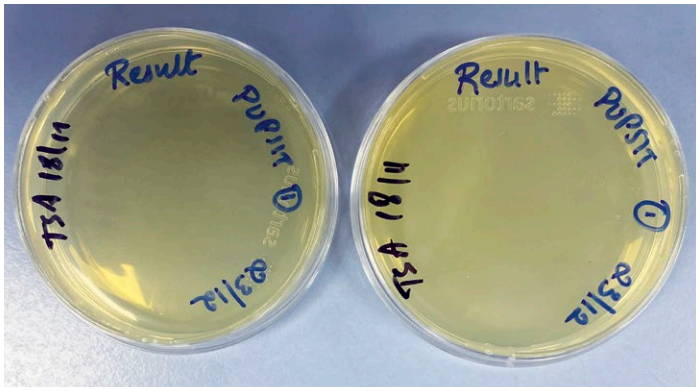


Figure 8: Sterile result on TSA media.

Conclusion

Three filter transfer set final filling configurations were tested for microbial ingress test using a liquid immersion method. The assemblies were defect free and sterile results were observed in the microbial ingress test. There was no bacterial ingress during seven days of incubation in challenged solution containing 10^7 CFU/mL of *Brevundimonas diminuta*, which proves that filter transfer set final filling assemblies provide sterile conditions, even in worst-case scenarios of microbial exposure.

References

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Status: 12 | 01 | 2021