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Application Note

Microbial Integrity of NovaSeptum® Sampling Systems upon Performance of Multiple Sampling Actuations

Introduction

The NovaSeptum® and NovaSeptum® GO sampling systems are a family of products designed for single-use sterile sampling throughout the biomanufacturing process. However, there are certain unique conditions that may require more than one actuation performed with the same sampling needle to collect a number of critical and non-critical samples. Performing multiple actuations beyond standard single-use applications can stress the materials of construction and possibly generate defects. Any defects have the potential to compromise the sterility of the sample collection process and the sample source, such as a bioreactor. This study focuses on understanding the sterility of the sample source and the collection process after multiple actuations of the NovaSeptum® system.

Materials and Methods

Figure 1 shows the experimental test system for microbial ingress testing, the red arrow indicating flow direction. The NovaSeptum® device assembly includes NovAseptic® Tri-clamp connector (ANS5/190x160-312) carrying the 5-port NovaSeptum® Holder (AT51/5-3D0) that is equipped with NovaSeptum® sampling system (or devices). This NovaSeptum® device assembly is sterilized using steam-in-place (SIP) procedure at ~124 °C and 17 psi for 30 min. The remaining parts shown are sterilized using a validated cycle in a validated autoclave. All sterilized parts are transferred aseptically to an ISO® Class 7 laminar flow laboratory, where the assembly and microbial ingress testing is performed.

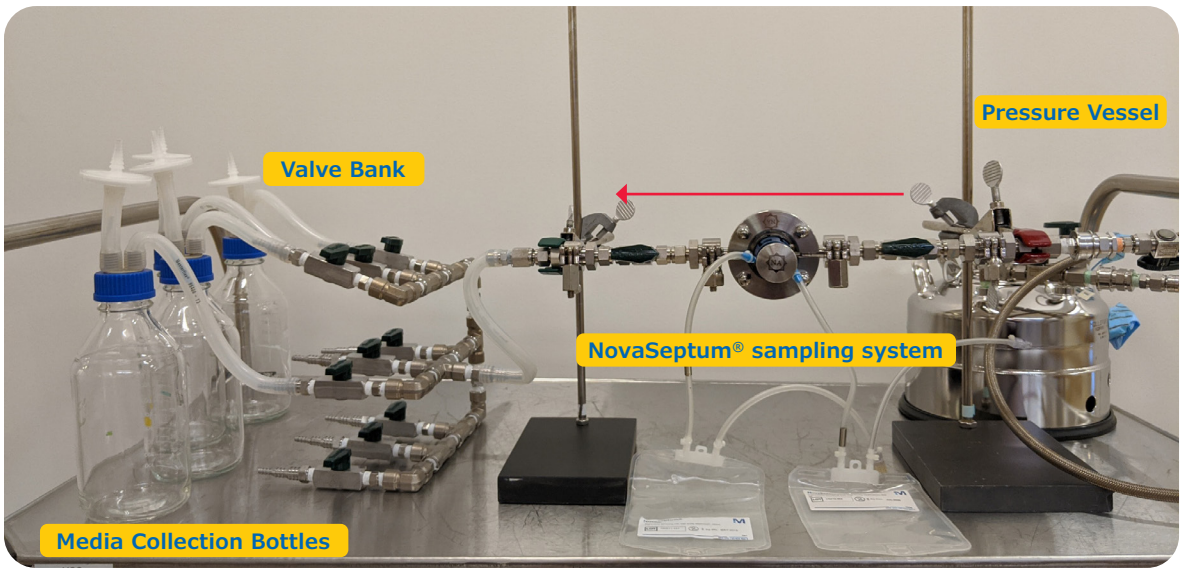

Figure 1. Experimental test system for microbial ingress testing with NovaSeptum® sampling devices

Figure 2: Bacterial inoculation location

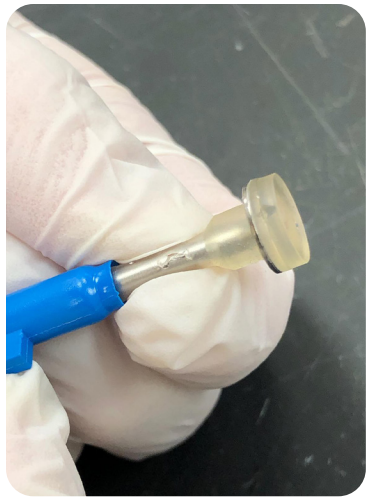

Figure 3: Damaged needle sheath

Ingress testing is performed by first filling the test system with Trypticase Soy Broth (TSB) media using a medium containing pot pressurized to 5 psi. As a negative control, a 500 mL medium flow through is collected to demonstrate absence of the challenge organism prior to challenge. Next, a 1.0 mL aliquot of high titer bacterial culture (>10^8 CFU/mL) is applied to the NovaSeptum® device on the sampling needle sheath exterior to supply the microbial challenge, as shown in **Figure 2**. The device actuation is then performed, which causes the needle to pierce through the septum into the fluid path adjacent to the septum. Finally, medium is flowed through the system again and collected, to recover any challenge organisms that may have ingressed with actuation.

Each device was challenged with *Brevundimonas diminuta* (ATCC® 19146™) that was prepared using a two-step culture method based on the ASTM® F838 standardized methodology.[1] *Brevundomonas diminuta* was selected as a challenge organism owing to its small size, motility, and well established culture conditions.

Table 1: Experimental plan for microbial ingress testing of NovaSeptum® devices of different sizes and presterilization methods

NovaSeptum® Size	Presterilization Method	Experiments
1 mm NovaSeptum®	Gamma-irradiated	Actuation Series
	Beta-irradiated	Pre-actuated Samples
2 mm NovaSeptum®	Gamma-irradiated	Pre-actuated Samples
	Beta-irradiated	Actuation Series
2 mm NovaSeptum® GO	Beta-irradiated	Actuation Series

The testing was performed on both the 1 mm and 2 mm NovaSeptum® devices, and 2 mm NovaSeptum® GO devices. Every device is presterilized prior to shipment by beta or gamma radiation. The multiple piercings (also referred to as actuations) of the devices and the testing is performed by two different methods. In one method, the actuations are performed on the test system as shown in **Figure 1**, and bacterial challenge samples are collected after desired number of actuations intermittently up to 50 actuations. This method is referred to as actuation series testing. In another method, the NovaSeptum® devices are actuated 50 times prior to SIP and subsequent assembly in the laminar flow laboratory. The bacterial challenge sample is collected after additional piercing. This method is referred to as pre-actuated sample testing. **Table 1** shows the experimental plan for testing NovaSeptum® devices with different size needles and presterilization method.

Each test is performed using both a negative and a positive control test. The negative control confirms the absence of the challenge organism in the test system prior to conducting the challenge. In this test, the growth medium is passed through the assembly before any inoculation and actuation of NovaSeptum® device, and sample is collected in the collection bottle. The positive control test is performed last on the experimental system. In this test, a NovaSeptum® device is deliberately damaged by making an incision in the sheath protecting the needle. **Figure 3** shows an example of this incision. As a result of this incision, the inoculum is in direct contact with the needle inside the sheath. Upon actuation of the NovaSeptum® device, the inoculum will travel into the fluid space of the NovAseptic® connector, which simulates the sample source of the actual application. This contaminated growth medium is collected in the sample bottle.

In all cases, when actuations are performed, the NovaSeptum® devices are held in the actuated position for a duration of 2 minutes.

Criteria for Test Success/Failure

The negative control is successful if the challenge organism is absent in the initial media flow-through. The positive control is considered successful if the challenge organism is present in the media flow-through after challenge of the damaged device and actuation. The test results are invalidated if either of the controls does not meet the success criteria. Presence/absence of microbial growth is assessed by visual inspection of turbidity and confirmation by plating and phenotypic identification.

Results and Discussion

Table 2: Microbial ingress testing of NovaSeptum® devices with 1 mm needle

Test Article Description		Presence (+)/Absence (-) of Challenge Organism						
Presterilization	**Testing Method**	**Sample #**	**Negative Control**	**1st Actuation**	**20th Actuation**	**40th Actuation**	**50th Actuation**	**Positive Control**
Gamma Irradiation	Actuation Series	1	-	-	-	-	-	+
		2	-	-	-	-	-	+
Beta Irradiation	Pre-actuated	1	-				-	+
		2	-		NA		-	+
		3	-				-	+

Table 3: Microbial ingress testing of NovaSeptum® devices with 2 mm needle

Test Article Description		Presence (+)/Absence (-) of Challenge Organism						
Presterilization	**Testing Method**	**Sample #**	**Negative Control**	**1st Actuation**	**20th Actuation**	**40th Actuation**	**50th Actuation**	**Positive Control**
Beta Irradiation	Actuation Series	1	-	-	-	-	-	+
		2	-	-	-	-	-	+
Gamma Irradiation	Pre-actuated	1	-				-	+
		2	-		NA		-	+
		3	-				-	+

Table 4: Microbial ingress testing of NovaSeptum® GO devices with 2 mm needle

Test Article Description		Presence (+)/Absence (-) of Challenge Organism						
Presterilization	**Testing Method**	**Sample #**	**Negative Control**	**1st Actuation**	**20th Actuation**	**40th Actuation**	**50th Actuation**	**Positive Control**
Beta Irradiation	Actuation Series	1	-	-	-	-	-	+
		2	-	-	-	-	-	+

Tables 2-4 show the results of the microbial ingress studies performed with NovaSeptum® and NovaSeptum® GO devices that are presterilized with beta and gamma-radiation. As discussed, the NovaSeptum® devices were tested in two formats. One set of experiments included devices that were pre-actuated 50 times in a biosafety cabinet prior to assembling in the experimental system. The second set of experiments included integral devices that were actuated. Bacterial growth medium flow-through samples were collected intermittently up to 50 actuations. Due to the similarity in the design of the trigger and septum between the NovaSeptum® and NovaSeptum® GO devices, only actuation series testing was performed with the NovaSeptum® GO devices as it simulates the actual application more closely than the pre-actuated series testing. As shown in the **Tables 2-4**, the negative control test indicates absence of the challenge organism. The challenge organism was absent in all the actuations tested up to 50 actuations. The absence of the challenge organism proves that there was no ingress of the challenge bacteria after 50 actuations. The positive control indicates a deliberately damaged septum can allow ingress and the test system was sensitive enough to detect ingress. Based on these results, we have demonstrated that the NovaSeptum® sampling systems remain integral to microbial ingress, as long as the needle sheath material is not damaged, even when operated in a multiple actuation mode.

Conclusion

The decision to perform multiple actuations must be assessed independently depending upon the nature of the fluid being sampled (or is in contact with the face of the NovaSeptum® being actuated) and the critical nature of the application. This study demonstrates that the NovaSeptum® and NovaSeptum® GO devices can be actuated up to 50 times without introducing an externally applied bacterial challenge organism to a sterile flow path. The results of this study suggest that NovaSeptum® can be actuated up to 50 times to collect multiple samples without the risk of contaminating the sterile fluid being sampled. Prior to a decision to perform multiple actuations for a sampling application, a thorough risk assessment should be conducted to justify need and ensure minimal risk to the process fluid or sample integrity.

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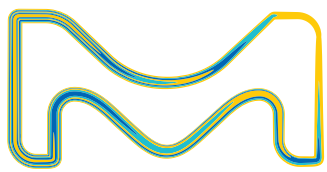

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