BioTrak Real-Time Viable Particle Counter — Gelatin Filter Handling Guide

Application Note CC-134 (US)

Introduction

EU GMP Annex 1: Manufacture of Sterile Medicinal Products (2022) states the following:

> *9.31 Microorganisms detected in the grade A and grade B areas should be identified to species level and the potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Consideration should also be given to the identification of microorganisms detected in grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may indicate a loss of control, deterioration in cleanliness or that may be difficult to control such as spore-forming microorganisms and molds and at a sufficient frequency to maintain a current understanding of the typical flora of these areas.*

In order to meet the regulatory expectation to identify viable contamination as outlined in Annex 1, the BioTrak™ Real-Time Viable Particle Counter incorporates a method to collect the particles detected. The collected particles can then be cultured to allow for identification.

Collection of particles is performed through the use of gelatin membrane filters. Gelatin membrane filtration is a well-established, highly efficient method for collecting viable particles. When a filter is installed, the particles that pass through the biofluorescent particle counter will be captured by the filter. After removal from the instrument, the filter is placed directly on solid media where it dissolves on the moist surface. Once dissolved, the plate can be incubated for growth. It is critical that proper technique is used to assure that the organisms cultured are from the air being sampled and not due to contamination during processing.

Box 1: Alternative Methods for Processing of Gelatin Membrane Filters

Placing the gelatin membrane filters on solid media is the most common method for processing to achieve growth. However, depending on the user's needs, other methods may be employed. This could include dissolving the filter in liquid media or in a sterile liquid that is subsequently processed via membrane filtration.

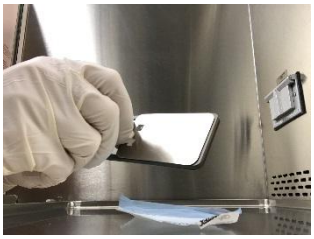
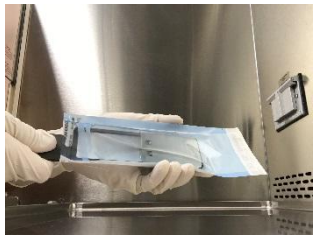
Viable Particle Collection

When monitoring in aseptic areas, the best practice is to position only the sample probe in the aseptic, Grade A, area with the instrument installed locally in the Grade C/D area. It is in this area where the collection filter will be inserted and removed from the instrument. The TSI Aseptic Gelatin Filter Holder (P/N 700216) was designed to protect the gelatin filter from this environment and assure the organisms cultured are only those that were collected during sampling.

Box 2: Handling Gelatin Membrane Filters
Gelatin membrane filters are pre-sterilized and individually packaged in media transfer paper. Aseptic technique should be used at all times when handling the filters. Filters should only be touched with the media transfer paper, sterile forceps, or other sterile implements as needed. The filters are brittle; care must be taken during handling to prevent breakage.

The recommended procedure for using the filter holders is provided in Table 1.

Table 1: Viable Particle Collection Using the Aseptic Gelatin Filter Holder

Prepare for Use	1. Clean holder if visibly soiled. 2. Place holder in a sterilization pouch, assure the cover on the holder is open.  3. Steam sterilize using an appropriate wrapped goods cycle. 4. For a continuous manufacturing process, please prepare a second aseptic gelatin filter holder using the process above.
Load Filter	1. In a biosafety cabinet, or other suitable clean environment, remove the holder from the pouch. 2. Load a filter onto the holder. 3. Close the cover and return to the pouch.    4. At the location of the instrument, remove the holder from the pouch (assure the cover is closed before removing). 5. Insert the holder into the instrument. If necessary, hold the cover closed until the tab comes in contact with the instrument case to prevent accidental exposure of the filter. 6. Turn the locking handle into the vertical position. 7. Begin sampling. 

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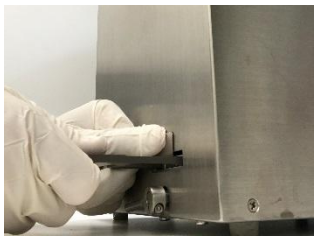
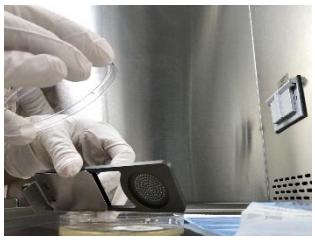
Unload Filter	1. Turn the locking handle to release the holder. 2. Remove the holder while pressing the tab of the cover against the instrument case until the cover is closed and return to a sterilized pouch. Note: If continuously monitoring, and sampling was not stopped prior to unloading, a new filter holder must be loaded within 30 seconds to avoid a flow error.	
Culture and Identify	1. In a biosafety cabinet, or other suitable clean environment, remove the holder from the pouch and aseptically transfer the filter onto the media surface. 2. Allow the filter to dissolve. If filter curls, lay flat with sterile forceps or inoculating loop. 3. Invert the plate and incubate. 4. Use desired methods to identify the recovered organisms.	

Box 2: Culturing of Viable Particles

As with all culture-based methods, many factors affect the ability of viable particles to grow. Some of these factors include the selection of growth media, incubation time and temperature, and the health of the organisms collected. Due to these factors, some, or all, of the viable particles collected may not result in growth.

Maintenance

To prevent contamination of the filter from the instrument itself, purging the flow path and cleaning the collection filter interface should be performed as applicable. Instructions for performing both can be found in the Operation Manual (P/N 6005481). Purging removes residual particles from the flow path that may have fallen out of the airflow during prior sampling. A zero check will confirm that residual particles have been removed up to the viability detector. Cleaning the interface will remove contaminants that may have entered into the interface from the surrounding environment. It is important to note that when using the aseptic gelatin filter holder, the cover prevents the filter from being exposed until it is in the flow path.

Knowledge Beyond Measure.

TSI Incorporated – Visit our website **www.tsi.com** for more information.

USA	**Tel:** +1 800 680 1220	**India**	**Tel:** +91 80 67877200
UK	**Tel:** +44 149 4 459200	**China**	**Tel:** +86 10 8251 6588
France	**Tel:** +33 4 91 11 87 64	**Singapore**	**Tel:** +65 6595 6388
Germany	**Tel:** +49 241 523030		

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