BIOTRAK® REAL-TIME VIABLE PARTICLE COUNTER EVALUATION GUIDANCE

APPLICATION NOTE CC-124 (US)

Introduction

The BioTrak® Real-Time Viable Particle Counter provides real-time detection of airborne viable and total particles in controlled environments. It is comprised of a 1 CFM flow rate particle counter, a particle viability detector, and a collection filter that allows for the further characterization of the viable particles that were detected. Unlike growth based methods, the BioTrak Real-Time Viable Particle Counter assesses the viability of particles in real-time by exposing the particles to a laser and detecting for fluorescence in a process known as laser-induced fluorescence (LIF). Viable results are reported in counts making them appear more similar to results obtained from a particle counter than from traditional microbiological methods that are reported in colony forming units (CFU). This guidance provides methodology for familiarizing the user with the operation of the BioTrak Real-Time Viable Particle Counter and for evaluating the data obtained against those produced by the methods currently utilized.

Evaluation Study Goals

The primary goals of this evaluation are:

- Compare the ability of the BioTrak Real-Time Viable Particle Counter to detect airborne viable contaminants to that of the current active air sampling method.
    - Compare BioTrak Real-Time Viable Particle Counter viable particle counts to CFU counts recovered by an active air sampler in areas of varying levels of cleanliness.
    - Determine the benefits of real-time, time-related, viable particulate results to time delayed, discrete, active air sampling results.
    - Compare the ability of the BioTrak Real-Time Viable Particle Counter's particle collection gelatin filter to recover viable contaminants for characterization to that of the active air sample plates.
    - Compare the time to perform testing and to obtain results between the BioTrak Real-Time Viable Particle Counter and the active air sampler.
- Compare the ability of the BioTrak Real-Time Viable Particle Counter to detect airborne particles to that of the current particle counter.
- Evaluate the proof of concept of using the BioTrak Real-Time Viable Particle Counter to replace, reduce, or augment current particle counting and active air sampling methods.

Evaluation Study Guidance

Box 1: What to Expect Before you Begin

The concentration of viable particles detected by the BioTrak Real-Time Viable Particle Counter will usually, if not always, be higher than the concentration of CFU detected by active air sampling except in aseptic areas where both techniques typically yield a count of zero. This is primarily due to the higher sensitivity of the detection method employed by the BioTrak Real-Time Viable Particle Counter. The BioTrak Real-Time Viable Particle Counter uses laser induced fluorescence (LIF) to detect all particles with fluorescent characteristics consistent with a viable microorganism, irrespective of its metabolic state or culturability.

Guidance for performing an evaluation is outlined below. Examples of data sheets that can be used to record the testing performed can be found attached to the end of this document. Modifications can be made as necessary to meet the requirements of the user.

Determine Sampling Locations

A variety of locations should be selected to be able to compare results under different conditions. Recommended locations are described below:

- Select a number of known "dirty" locations in ISO 7 (Grade C) or higher rooms where there is a high degree of certainty that a countable number of viables will be recovered by the active air sampler. This will assure that both non-viable and viable results obtained via the BioTrak Real-Time Viable Particle Counter can be compared to those obtained via the current methods.
- Select one or two "clean" locations in ISO 5/6 (Grade A/B) areas that are expected to yield predominantly counts of zero using the current methods. This will allow for the assessment of BioTrak Real-Time Viable Particle Counter results for monitoring aseptic operations.

Box 2: Sampling Location Considerations

- Sampling in dirty locations will demonstrate the ability of the BioTrak Real-Time Viable Particle Counter to detect a higher number of viable particles through the use of LIF in comparison to CFU counts obtained using traditional microbiological methods.
- In ISO 5/Grade A areas (such as isolators or RABs) used for aseptic operations, any false positives could lead to unnecessary and costly actions. Therefore, any method that would produce background counts consisting of false positives would render the data produced in these environments meaningless to the user. Sampling in clean locations will demonstrate that the discrimination capabilities of the BioTrak Real-Time Viable Particle Counter will predominantly produce counts of zero, thus demonstrating its suitability for use in monitoring aseptic operations.
- If sampling in an isolator, it will not be reasonable to place the BioTrak Real-Time Viable Particle Counter inside the isolator. If a port does not exist that allows for the installation of an additional isokinetic probe, then the BioTrak Real-Time Viable Particle Counter may be able to utilize the probe that is already installed for particle counting. The probe must be designed for use with a 1 CFM flow rate particle counter and use tubing with an inside diameter of 3/8" or 1/2". Unless two probes are installed, it will not be possible to directly compare BioTrak Real-Time Viable Particle Counter to the existing particle counting equipment.

Determine Test Parameters

Select the test parameters to make the results from the different test methods comparable and meaningful.

- The length of the sampling period should be determined by the nature of the sample location. Dirty locations are recommended to be tested over a period of two hours to assure that some viables are recovered on multiple active air samples. Due to the expected infrequency of counts at clean locations, it is recommended to sample for four hours to allow for the opportunity to detect one of these events.
- The BioTrak Real-Time Viable Particle Counter and site particle counter should be configured to sample continuously, with relatively short sample times and no hold time between samples. The instruments must be configured to have the same sample time and not sample volume so results can be compared over time. The initial delay should be the same; the pump on the BioTrak Real-Time Viable Particle Counter takes approximately 10 seconds to reach the flow set-point, therefore, an initial delay of at least 15 seconds is recommended.
- Configure the BioTrak Real-Time Viable Particle Counter and site particle counter to report cumulative results at the desired particle sizes.
- The BioTrak Real-Time Viable Particle Counter should be configured to report viable counts as cumulative counts for the 0.5 particle size.
- The sample volume/time used for the active air sampler should be what is mandated by the existing sampling standard operating procedure (SOP).
- The BioTrak Real-Time Viable Particle Counter has two sensitivity levels: Normal and High. Normal should be used for “dirty” locations and High for “clean” locations.

Box 3: BioTrak Real-Time Viable Particle Counter Sensitivity Settings; "Normal" and "High"

The primary goal of continuous, real-time viable particle counting is to ensure the cleanroom is under control. To best achieve this, the BioTrak Real-Time Viable Particle Counter can detect viable particles with High sensitivity, for use in very clean areas such as Grade A, or Normal sensitivity, for use in areas where microorganisms are relatively more abundant. The goal is to maintain dynamic range in the measurement in order to reliably detect changes in the concentration of viable particles. Where viables are essentially absent (i.e. Grade A) effective monitoring requires High sensitivity detection of any microbial presence; in less clean areas, Normal sensitivity is used to maintain the dynamic range in the measurement.

Prepare for Testing

- Prepare the particle counter and active air sampler as per existing SOPs. Alter the instrument configurations as needed to utilize the parameters determined above.
- Assure the time and date setting on all instruments is in agreement.
- Prepare the BioTrak Real-Time Viable Particle Counter for sampling, refer to the BioTrak Real-Time Viable Particle Counter Operation Manual for detailed instructions.
    1. Clean the collection filter interface.
    2. Install a zero count filter and perform a purge.
    3. With the zero count filter still installed, run a zero count to confirm that no residual particles are present in the system.
    4. Configure with the parameters determined above.
- Prepare the BioTrak Real-Time Viable Particle Counter collection filter.
    1. Disinfect or autoclave the filter holder.
    2. In a clean environment (biosafety cabinet or clean bench), load a gelatin filter into the filter holder. Take appropriate measures to assure filter does not become contaminated prior to sampling.
- Install the equipment at the sampling location.
    1. Co-locate sampling inlets of all equipment at the same height and in close proximity (within two feet is recommended), using approximately the same length and curvature of tubing.
    2. Install the filter holder into the BioTrak Real-Time Viable Particle Counter.

Perform Sampling

1. Coordinate as necessary to ensure that sampling begins on all instruments simultaneously. The site particle counter and BioTrak Real-Time Viable Particle Counter can be started at the same time if both instruments were configured to have the same initial delay. The active air sampler should have a plate installed and sampling initiated when the other instruments begin sampling.
2. Record activities being performed in the area as desired to allow for the correlation of counts observed to the activities being performed.
3. Once an active air sample is complete, remove the plate and install a new one. Coordinate the start of the active air sampler with the start of the next sample cycle of the site particle counter and BioTrak Real-Time Viable Particle Counter. Label the active air sample plates with the zone, location, sample start time, and date.
4. Stop sampling with the BioTrak Real-Time Viable Particle Counter and particle counter as necessary at the end of the sampling period.
5. Remove the filter holder from the BioTrak Real-Time Viable Particle Counter. Take measures to assure the filter does not become contaminated prior to transferring to a media plate.
6. In an appropriate environment, place the gelatin filter onto a media plate using good aseptic technique. Gelatin filters can become fragile, thus, care should be taken when handling.
7. Once the gelatin filter has dissolved, invert the filter and active air sample plates and incubate as per existing SOPs.
8. Remove plates at the end of incubation and enumerate the CFUs present on the active air sample plates.

9. Determine the morphology of the colonies present on both the active air sample plates and the BioTrak Real-Time Viable Particle Counter collection filter plate.

Time Study

At one of more select locations, record the time it takes to perform the tasks related to using the BioTrak Real-Time Viable Particle Counter and the active air sampler. Also record the time it takes to obtain results from the end of sampling to the time that viable particle counts are available on the BioTrak Real-Time Viable Particle Counter and when active air plates have been counted.

Data Analysis

The following should be considered when analyzing the results obtained:

- Viable particle counts from the BioTrak Real-Time Viable Particle Counter are expected to be higher than the CFU counts obtained from active air sampling due the ability of the BioTrak Real-Time Viable Particle Counter to detect viable but not culturable organisms.
- The collection filter plates are only for the recovery of viable particles for use in characterizing the organism detected; they are not to be used for quantification.

Perform the following analyses to achieve the goals stated above:

- Compare the BioTrak Real-Time Viable Particle Counter results to the active air sampler results.
  - Compare raw results obtained for the time frames when sampling overlapped.
  - Normalize data for direct comparison as needed.
  - Compare the BioTrak Real-Time Viable Particle Counter data to the notes on room activity.
- Compare BioTrak Real-Time Viable Particle Counter particle counts to those obtained using the current particle counter.
- Compare the organisms recovered via the BioTrak Real-Time Viable Particle Counter's gelatin filter with those recovered on active air plates.
- Total the time spent testing with the BioTrak Real-Time Viable Particle Counter in comparison to active air sampling.
- Compare the time it takes to obtain results with the BioTrak Real-Time Viable Particle Counter to the time it takes to obtain results from performing active air sampling.

BioTrak Real-Time Viable Particle Counter Evaluation—Equipment and Material Record

Zone:		**Location:**		**Date:**	
Class/Grade:		**Room Status:**			

Description:

Sampling Equipment Information

	Site Particle Counter		BioTrak Particle Counter		Site Air Sampler	
Model/Equip No.						
Serial Number						
Cal Due Date						
Flow Rate (L/min)						
Parameter Settings	**Parameter**	**Setting**	**Parameter**	**Setting**	**Parameter**	**Setting**
			Count Mode	Automatic		
			Start Delay	00:00:15		
			Sample Time	00:01:00		
			Hold Time	00:00:00		
			Cycles	∞		
			Volume	28.3 L		
			Channels T	0.5 and 5.0 enabled		
			Count Units	∑ #		
			Channels V	0.5 enabled		
			Count Units	∑ #		
			Viable Sensitivity	High		

Material Information

Material	Lot No.	Exp. Date
TSA Plates		
Gelatin Filters		

BioTrak Real-Time Viable Particle Counter Evaluation—Sampling and Results Record

Zone:		Location:		Date:	

Sampling & Results

Site Particle Counter			BioTrak Real-Time Viable Particle Counter				Site Air Sampler		Activity Notes
Sample Start Time	Count		Sample Start Time	T-CNT		V-CNT (≥ 0.5µm)	Sample Start Time	CFU	
	≥ 0.5µm	≥ 5.0µm		≥ 0.5µm	≥ 5.0µm				

Incubation

Incubator:		Temp Range (°C):		Cal. Due Date	

Time In:		Time Out:	

BioTrak Real-Time Viable Particle Counter Evaluation—Characterization of Recovered Organisms

Zone:		Location:		Date:		Sample Time:	

Characterization

Colony Morphology						Gram Stain	Identification	Present On (Y/N):	
Color	Surface Appearance	Opacity	Shape	Margin	Elevation			Collection Filter Plate	Active Air Sampling Plate

BioTrak Real-Time Viable Particle Counter Evaluation—Time Studies

Zone:		Location:		Date:	

Work Time Study

Task	Time to Perform (min)		Comment
	BioTrak	Active Air Sampling	
Sample Plate Prep			
Gelatin Filter Prep			
Loading Plate			
Loading Gelatin Filter			
Unloading Plate			
Transfer Plates to Incubation			
Print/Download BioTrak Results			
Enumeration of Plates			
Total	**0**	**0**	

Time to Result

	Sampling Complete		Results Available		
	Date	Time	Date	Time	Total Time to Result
BioTrak					
Active Air Sampling					

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