BIOTRAK® REAL-TIME VIABLE PARTICLE COUNTER
SAMPLE AND COLLECTION EFFICIENCY

APPLICATION NOTE CC-104

Introduction

The BioTrak® Real-Time Viable Particle Counter is a full-featured Rapid Microbial Method (RMM) instrument that detects the total number of particles in the air as well as determines which of those particles are viable micro-organisms. Additionally, the BioTrak Particle Counter incorporates a particle collection filter so the optically analyzed particles are available for subsequent speciation analysis. The BioTrak Particle Counter incorporates proven technologies leveraging TSI's experience in particle measurement theory, instrument development, and calibration. Figure 1 shows the primary components of the BioTrak Particle Counter.

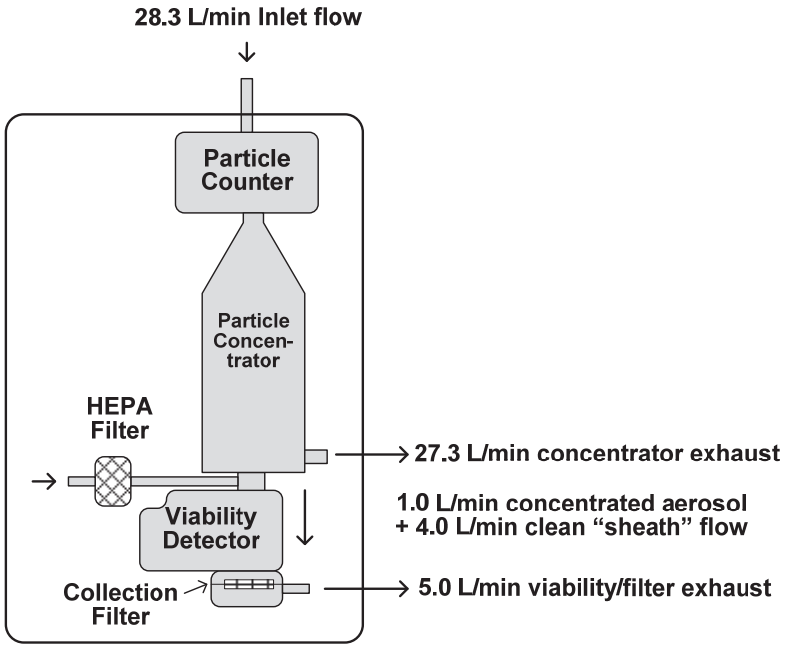

Figure 1. Key Components of the BioTrak Real-Time Viable Particle Counter

This Application Note presents information related to aerosol sampling and collection efficiency at various stages along the aerosol path in the BioTrak Particle Counter, and relates those efficiencies to other methods, like Active Air Samplers. When applying a viable particle counter, or any particle device in a cleanroom, proper characterization of the product is important so results can be understood, leading to root cause identification, and ultimately to continual improvement.

BioTrak Particle Counter

Particles sampled by the BioTrak Particle Counter first enter the Optical Particle Counter (OPC) section, which is an ISO-21501-4 compliant airborne particle counter, similar to TSI AeroTrak® Portable Particle Counters. A high flow rate (28.3 L/min) is needed to inspect the sample volumes mandated by regulatory requirements in a reasonable time, to provide sufficient particle counts for statistical accuracy, and to enable a high probability of capturing contamination events. For more information on this measurement, please refer to TSI Application Note CC-102, *Importance of Good Non-Viable Measurements*.

Particle Sampling Efficiency in the Particle Concentrator

The Viability Detector shown in Figure 1 cannot make viability measurements at the high flow rate of the total Particle Counter due to the low intensity of the fluorescence signals emitted by viable particles. In general, the optical sensitivity of an Airborne Particle Counter (APC) is proportional to the sample flow rate - the amount of detected light is proportional to the time a particle is present in a light beam of a given intensity. The intrinsic fluorescence from microbes is much smaller (by a factor of 10^{-2} to 10^{-3}) than the scattered light, so adequately detecting fluorescence is not practical at the 28.3 LPM (1 CFM) of a typical APC. Thus, to achieve good fluorescence sensitivity, the inertial Particle Concentrator shown in Figure 1 is used to deliver most of the particles from the 28.3 L/min OPC outlet flow into the 1 LPM inlet flow of the Viability Detector.

Particle concentrators separate particles within a flow using particle inertia and aerodynamic drag to separate particles into a low volume sample flow (Minor Flow) and high volume exhaust flow (Major flow) . As shown in Figure 2, the inlet flow, which in the BioTrak Particle Counter's detector has been measured by the Airborne Particle Counter, is directed into the concentrator's flow separation region. Larger particles have sufficient inertia to be carried into the 1 L/min minor flow path leading to the viability detector. Most of the inlet flow air, along with the majority of the relatively smaller, low-inertia particles, follows the 27.3 L/min major flow path that is filtered and exhausted. Concentrator performance is dictated by the geometry and dimensions of the flow separation region.

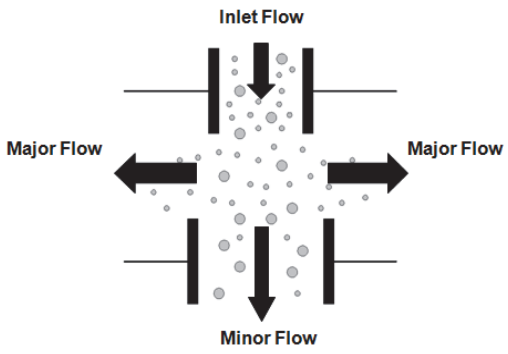

Figure 2. In a Particle Concentrator, the inertia of larger particles (>~2 µm) carries them into the lower "minor flow" volume path, while most of the air is carried off in the higher "major flow" volume path

The concentrator's performance determines how effectively particles are delivered to the BioTrak Particle Counter's Viability Detector. The concentrator's efficiency varies with particle size and is the ratio of the number of particles of a given size in the minor flow to the number of particles of that size in the inlet flow.

$$\text{Particle Concentrator Efficiency}_{(\text{Particle Size X})} = \frac{\#\text{ of Particles Minor Flow}_{(\text{Particle Size X})}}{\#\text{ of Particles Inlet Flow}_{(\text{Particle Size X})}}$$

For any Particle Concentrator, the Particle Concentration Efficiency will vary by particle size. Figure 3 shows a curve of the concentration efficiency versus particle size for three BioTrak Particle Counters measuring *Bacillus globigii* spore clusters. There are two key operating characteristics of an aerosol particle concentrator that describe its performance:

- The D_{50} cut point, the smallest particle diameter for which the concentration efficiency is 50%
- Midrange efficiency—this is the region that has the highest efficiency, between the reduced efficiency due to small particles, and the region where larger particles start efficiency is reduced due to settling and impaction forces

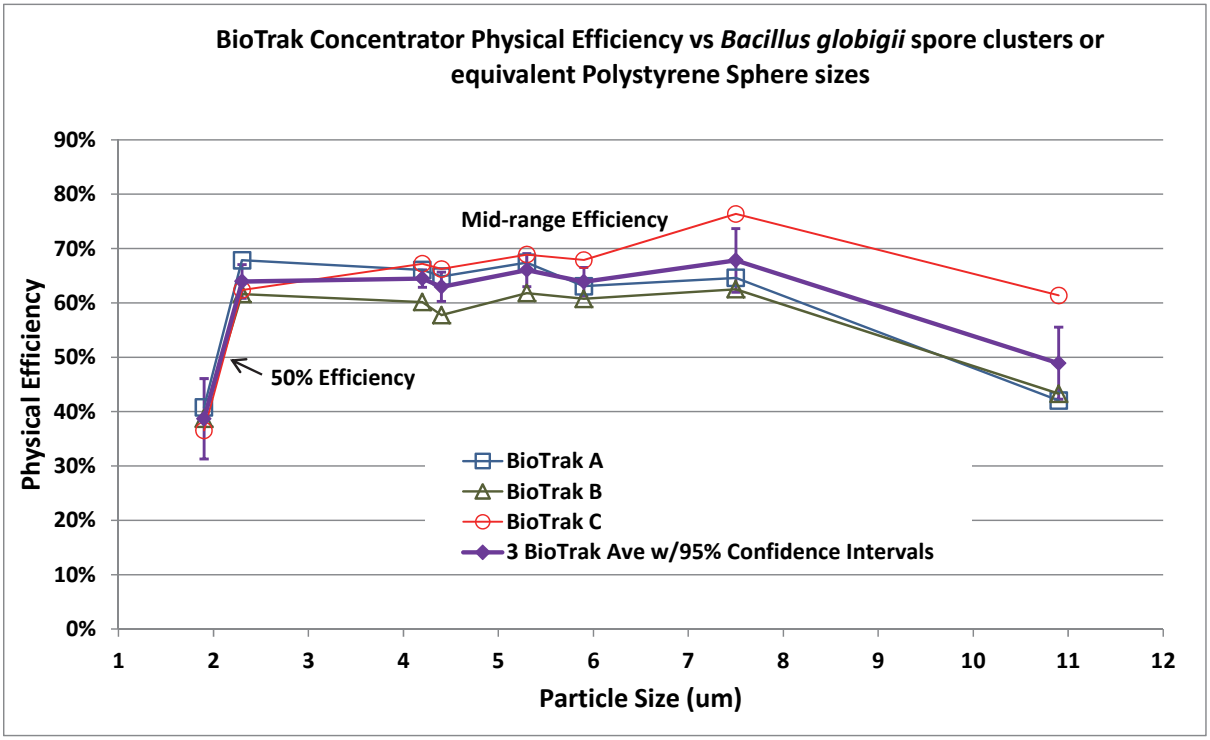

Figure 3. BioTrak Particle Counter concentrator efficiency versus particle size. The key characteristics are the 50% efficiency size and the efficiency over the midrange of particle sizes

Most viable microbial particles in the environment are in the 2 to 10 µm size range, so it is important that the concentrator have good efficiency over this size range. The BioTrak Particle Counter's Particle Concentrator is based on a novel design licensed from and co-developed with Texas A&M University (Haglund 2007)[1], which has high efficiency over a wide range of particle sizes.

As can be seen in Figure 3, the BioTrak Particle Counter Concentrator has a midrange aerosol efficiency of approximately 65% in the 2 to 8 µm size range. In controlled laboratory tests with *Bacillus globigii*, at a higher relative humidity, there was a degradation of concentrator efficiency. For the specific controlled test only, at 55% relative humidity, the concentrator efficiency dropped to 45%. The type of micro-organisms and environmental conditions found in each environment varies, which is why it is important to perform an evaluation to compare BioTrak Particle Counter data with Active Air Sampling data for each application.

[1]The BioTrak 9510-BD Viable Particle Counter incorporates the following patented technologies: Patent Numbers 6,167,107; 5,701,012; 5,895,922; 6,831,279; 7,261,007.

Sampling Efficiency in Air Samplers

Figure 4 below shows a typical Active Air Sampler. Each Air Sampler has a different sampling efficiency.

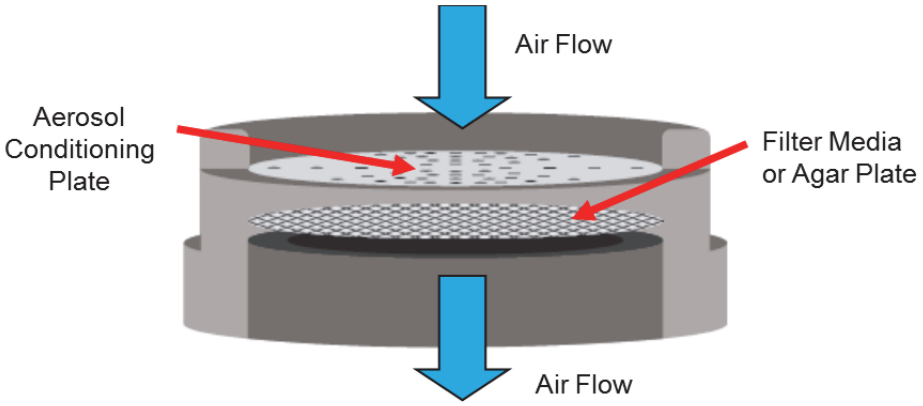

Figure 4. Drawing of a Typical Active Air Sampler

The same physical factors that affect the particle size dependent sampling performance of aerosol particle concentrators also apply to all active air samplers used in cleanroom applications. ISO 14698-1 describes a complex and experimentally challenging method for characterizing the physical and biological efficiency of active air samplers. Vellutato (2005)[2] presents a useful discussion on the validity of ISO14698-1 methods and the difficulty in implementing them, and proposes an alternative air sampler characterization approach.

Regardless of the characterization method, the particle size dependent efficiency is an important, and sometime overlooked, parameter when considering environmental air sampling technology and aerosol based viability detection. Yao & Mainelis (2006)[3] conducted tests on active air samplers to evaluate their efficiency. Experimental efficiency results from two different instruments are shown below in Figure 5. The data indicated by the solid square is the effective collection efficiency defined as the particles which are impacted onto the collection media compared to the particles present at the inlet of the sampler. The results show that different commercial active air samplers have significantly different efficiency performance.

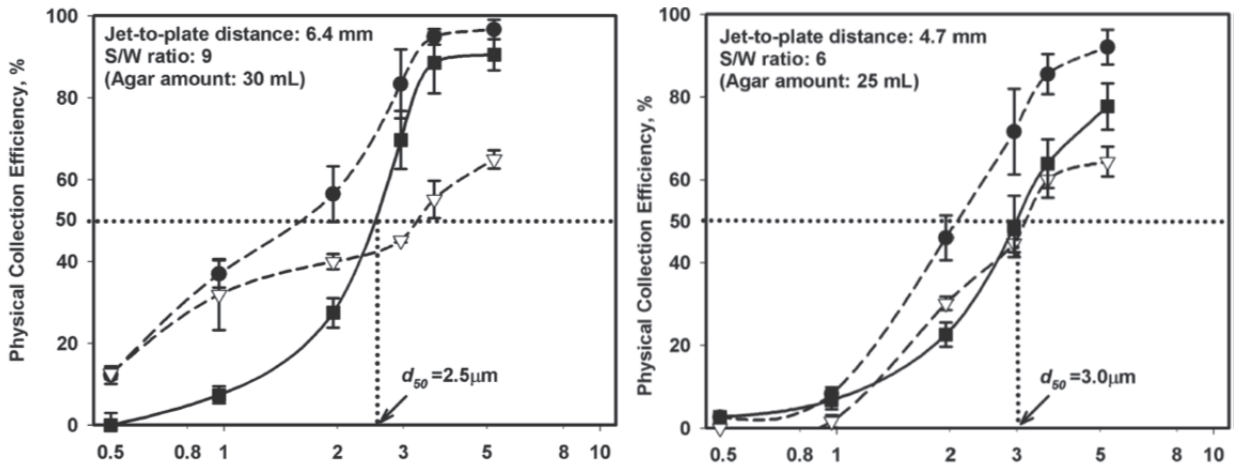

Figure 5. Sample active air sampler data from Yao & Mainelis (2006)[2] test

[2]Vellutato, A. (2005) "Sampling Equipment" in Mouldenhauer, J. *Environment Monitoring: A Comprehensive Handbook* Vol 1, pp 219–268, PDA, Bethesda, MD.

The results of data collected from real-time viable particle counters are often compared to data obtained with highly variable active air samplers. For a real, unbiased evaluation, the efficiency of the active air sampler as well as that of the viable particle counter should be considered in the analysis. All high flow rate (above ~5 LPM) real-time viable particle detectors rely on some type of aerosol concentrator, so the particle size-dependent performance of the concentration technique needs to be characterized and any impact on results should be understood.

The BioTrak Particle Concentrator has a midrange aerosol efficiency of approximately 65%, meaning that the viable detector will generally see 65%of particles in the range of interest. Although this does introduce some bias, as long as the efficiency is characterized and its impact on results understood, it is not detrimental to use. For instance, a 65% efficient 28.3 LPM detector still analyzes more than 3 times as many particles per sample time than a 100% efficient 5 LPM sampler. The concept of effective sampling rate can be used where the effective sampling rate is defined as Flow Rate multiplied by efficiency (Effective Sampling Rate = Flow Rate x Efficiency).

Active air samplers have similar typical efficiencies as the BioTrak Particle Counter, so there is general comparability. However, each air sampler and high flow rate viable particle detector has different D_{50} and efficiency characteristics, so their impact must be considered for solid scientific evaluation and comparison purposes. Many active air samplers have had ISO14698-1 validation studies performed that characterize the aerosol efficiency of the sampler. This information should be available from the manufacturer.

BioTrak Particle Counter Collection Filter

As seen in Figure 1, the BioTrak Particle Counter incorporates an integrated viability preserving Collection Filter. The same particles that are optically analyzed in the Viable Detector are captured by BioTrak Particle Counter's Collection Filter, which captures virtually all the particles that are viewed in the Viable Detector. This allows for subsequent off-line speciation analysis of the optically interrogated particles that can support investigation of the contamination sources. Since collected particles may overlap on the Collection filter, it may not provide an accurate quantitative measure of cultured particles and does not replicate the quantitative collection of an active air sampler.

Putting it all Together

Figure 6 below provides a pictorial representation of the efficiencies at various stages found in the BioTrak Viable Particle Counter. In this diagram, the efficiencies shown in the left column are the efficiencies of each stage of the instrument. The right hand column shows the impact on a typical ambient particle distribution through each stage of the instrument. The largest impact on particle distribution is the effect of the concentrator which is included in the viability detector efficiency. All aerosol instruments have different aerosol transport efficiency characteristics. When evaluating real-time viability detectors, the aerosol efficiency of both the instrument under test and the active air sampler being used to collect reference samples must be considered to truly understand performance.

[3]Yao & Mainelis (2006) 'Efficiencies of Portable Microbial Samplers' *Aerosol Science & Technology*, **40:8**, 595-606.

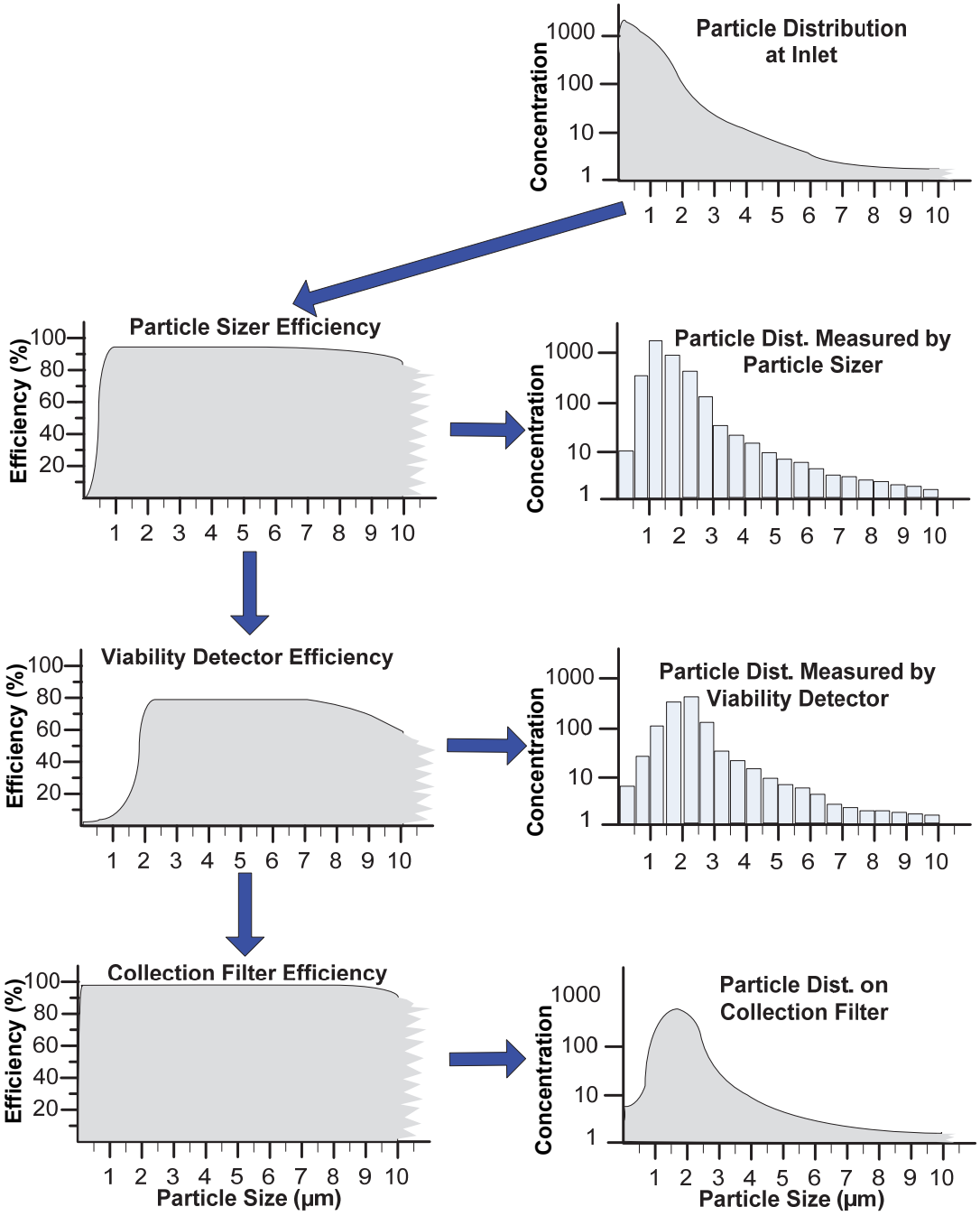

Figure 6. Sampling and Collection Efficiency, and related Particle Distribution throughout the different stages of the BioTrak Real-Time Viable Particle Counter.

Conclusion

When evaluating a new technology, it is critical to understand how the instrument works. TSI has fully characterized the aerosol efficiency of the BioTrak Real-Time Viable Particle Counter. The concept of effective sampling rate helps convey an understanding of the impact that particle sampling efficiency has on viable particle analysis. When evaluating real-time viable particle detectors, it is common practice to run comparability studies where the results obtained from the new method are compared to current methodologies. For the BioTrak Particle Counter, the results are typically compared to culture colony count results from an active air sampler. Thus, the aerosol sampling efficiencies of both the unit under test and the reference method must be incorporated into the analysis. With a good understanding of the measurement methods, including the advantages and limitations, you will be able to properly evaluate the BioTrak Particle Counter's performance compared to your existing method and identify applications where it provides maximum benefit.

Please contact TSI for more information regarding the BioTrak Particle Counter.

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

USA	**Tel:** +1 800 874 2811	**India**	**Tel:** +91 80 41132470
UK	**Tel:** +44 149 4 459200	**China**	**Tel:** +86 10 8219 7688
France	**Tel:** +33 4 91 11 87 64	**Singapore**	**Tel:** +65 6595 6388
Germany	**Tel:** +49 241 523030		

Distributed by:
Kenelec Scientific Pty Ltd
1300 73 22 33
sales@kenelec.com.au
www.kenelec.com.au

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