BioTrak Real-Time Viable Particle Counter — Sample and Collection Efficiency

Application Note CC-104 Rev E (A4)

Introduction

Biofluorescent particle counting is an alternative microbiological method for viable air monitoring. Instead of waiting for growth for detection, biofluorescent particle counters (BFPC) rely on the intrinsic fluorescence of molecules present in microorganisms for detection. This allows for detection of viable particles, also known as autofluorescence units (AFU), in real-time. When used in Grade A environments, they provide greater assurance of product quality than traditional methods by allowing for proactive actions in the event of as excursion and through the elimination of risk from monitoring interventions.

Annex 1 Requirements for Air Monitoring
9.17 The grade A area should be monitored continuously (for particles ≥0.5 and ≥5 µm) and with a suitable sample flow rate (at least 28 litres (1ft3) per minute) so that all interventions, transient events and any system deterioration is captured.   *9.24 Continuous viable air monitoring in the Grade A zone (e.g. air sampling or settle plates) should be undertaken for the full duration of critical processing…The monitoring should be performed in such a way that…any risk caused by interventions of the monitoring operations is avoided.*   *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.*

The BioTrak™ Real-Time Viable Particle Counter, a BFPC, is an instrument that has been designed to meet the requirements of Annex 1. Not only does it perform continuous viable monitoring, it provides total particle counts and has a method for collecting particles to allow for identification. This makes it a complete solution for monitoring air in Grade A environments.

Flow Rate and BFPC Design

Particles are counted by an optical particle counter by detecting the light that is scattered by a particle as it passes through a laser beam. Measuring light scatter is also involved in the detection of an AFU in a BFPC, but, as mentioned earlier, so is detecting fluorescence. Unfortunately, the intensity of the fluorescence signal is much weaker than that of scattered light. BFPC therefore need a slower flow rate for reliable detection of AFUs than conventional particle counters generally use. In fact, the flow rate for AFU detection needs to be significantly less than the 28 lpm minimum flow rate required for monitoring total particles in Grade A per Annex 1. This would mean that a single instrument could not perform both total particle and viable monitoring. However, there are a number of benefits for using a single instrument for both. This includes having less equipment in the Grade A area and minimizing airflow disruptions. Having less equipment also makes for easier integration with filling equipment and reduced maintenance and calibration.

The BioTrak™ BFPC uses an innovative approach in order to meet the GMP requirements for both tests to achieve this goal of monitoring with a single instrument. It has a sample flow rate of 28.3 lpm that initially passes the sample through an optical counter to obtain the total particle count before continuing on to a particle concentrator. The particle concentrator concentrates the particles into a reduced volume of the sample air, with the remaining air being exhausted. This drops the flow rate to 1 lpm before proceeding to the BFPC, thus allowing for AFU detection, see Figure 1.

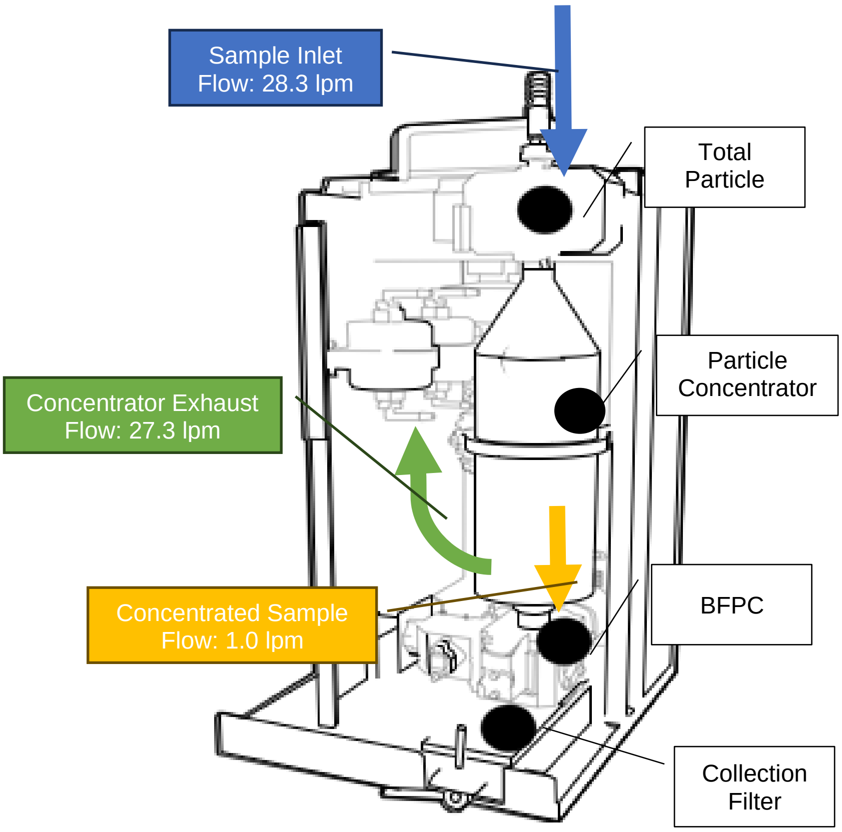

Figure 1
Sample flow path of the BioTrak BFPC.

Particle Sampling Efficiency in the Particle Concentrator

Impaction air samplers have traditionally been the most commonly used instruments for viable air monitoring. These collect particles onto the surface of an agar plate that has been positioned inside the sampler. When sampling, air is actively pulled through holes or slits above the plate. Once inside the sampler, this air needs to abruptly turn to go around the plate. Larger particles that are in the sampled air will have too much inertia and be unable to make this turn. These particles will fall out of the airflow and impact onto the surface of the plate. Conversely, smaller particles will remain entrained in the airflow and be exhausted out. This is visualized in Figure 2.

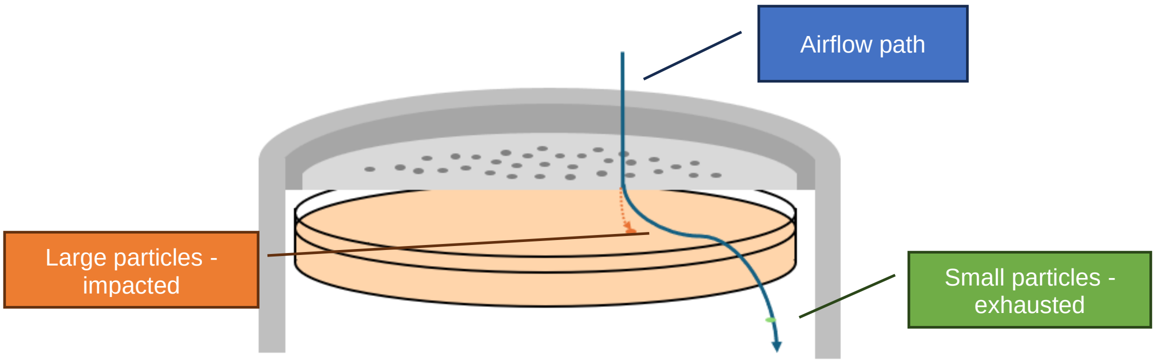

Figure 2
Particle collection in an impaction air sampler.

No impaction samplers can collect all particles. Based on the geometries of their design (e.g. hole diameter and distance to the agar surface), samplers vary in collection efficiency. This efficiency, known as physical efficiency, is characterized by the D_{50}. The D_{50} is the particle size at which half will impact on the plate and half will be exhausted.

However, physical efficiency does not paint a complete picture for the efficiency of an impaction sampler because not only do these particles need to be collected, they need to grow. This means that the impaction velocity needs to be slow enough that otherwise culturable microorganisms will not be damaged during impaction to the point that they will no longer grow sufficiently for detection. This overall efficiency is known as the biological efficiency. The design of these instruments must therefore balance particle collection with maintaining culturability to maximize the biological efficiency.

A particle concentrator works in a similar way to an impaction air sampler. Upon entering the concentrator, the majority of the sample air makes a sudden turn. However, instead of causing the larger particles to fall out and impact on a plate, these particles continue on to the BFPC. And again, smaller particles are exhausted out, see Figure 3. The balance that needs to be struck here is to concentrate as many particles as possible while slowing the flow sufficiently for reliable fluorescence detection. In the case of the BioTrak BFPC, the flow is reduced to 1 lpm.

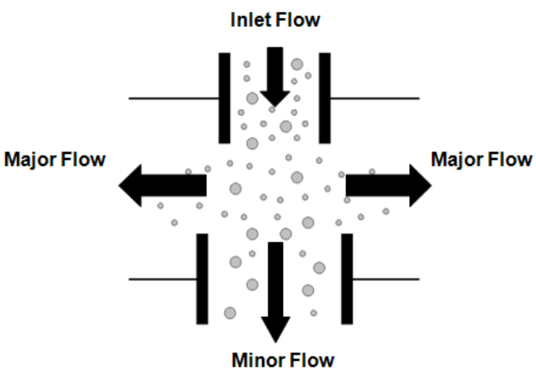

Figure 3
Mechanism for particle concentration.

In either case, using an impaction air sampler or using a BFPC, some amount of smaller particles are sacrificed to maximize the detection of the larger particles. Fortunately, the impact of this is minimal because airborne viable particles tend to be larger particles[1].

In addition to allowing the BioTrak BFPC to do both total particle and viable monitoring with the same instrument, utilizing a particle concentrator has another significant benefit. By having a higher sampling flow rate, a much larger volume of air is being testing during continuous sampling. With the high degree of cleanliness in Grade A environments, the larger the volume of air sampled, the better the chance there is to detect a contaminant. And, after all, what is the purpose of viable air monitoring? Is it to try to detect everything in a sample, even if you have to reduce the sample size significantly to do that? Or, is it to detect risk to product?

At a flow rate of 28.3 lpm, the sampled volume is 28.3 times greater than what would be sampled by a 1 lpm instrument. Even compared to a 5 lpm instrument, the volume sampled is 5.7 times greater. Trading the detection of small particles to obtain a significantly larger sample volume makes the use of a particle concentrator the preferred choice for Grade A monitoring.

[1] Whyte W, Green G, Albisu A. Collection efficiency and design of microbial air samplers. *Journal of Aerosol Science* 2007; **38:** 101.

Conclusions

The BioTrak BFPC includes total particle counting at a flow rate of 28.3 lpm, continuous viable sampling, and a method to collect viable particles for identification, making it a complete solution for monitoring Grade A environments. Meeting the Annex 1 flow rate requirement for particle counting is made possible by the use of a particle concentrator to slow the flow down sufficiently for fluorescence detection. The particle concentrator also improves the likelihood of detecting a viable particle by sampling larger volumes of air.

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