INTERPRETING OUT-OF-TOLERANCE FLUORESCENCE DETECTOR FINDINGS

TSI BIOTRAK REAL-TIME VIABLE PARTICLE COUNTER

APPLICATION NOTE CC-130
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Introduction—Viable Particle Discrimination

This application note describes the performance of the BioTrak® Real-Time Viable Particle Counter to determine the viability of a particle based on its measured optical properties when the fluorescence detection system is determined to be out-of-tolerance (OOT). The document is intended to aid in assessing the impact of these findings on the functionality of the instrument.

The fluorescence detection system exposes particles to ultra-violet (UV) laser light and assesses the viability of each using three optical parameters: scattered light intensity and fluorescent light intensity at two distinct wavelength bands. The bands of fluorescent light are detected with two photomultiplier tubes (PMTs), one detecting low wavelengths and one detecting high wavelengths.

The system is calibrated using reference fluorescent particles. If during as-found testing the expected signal is higher or lower than the allowable tolerance, the impact on viability detection cannot easily be deduced (e.g., a calibration parameter found to be OOT above the upper tolerance level will not always correlate to more particles detected as viable). This document provides a method for assessing the impact of the condition on viable particle discrimination.

NOTE—The analysis presented here is intended for use with algorithm version 7004524 (the algorithm version is displayed on the information screen of the instrument interface). The primary validation of the BioTrak Particle Counter using this algorithm was completed in 2021 (*TSI BioTrak Real-Time Viable Particle Counter Biological Detection Performance Validation Report; Document No: 10000054369*).

Methods—Simulating Out-of-Tolerance Photomultiplier Tubes Accuracy Conditions

The data presented herein is only intended to assess the relative detection capability of the BioTrak Particle Counter when OOT PMT accuracies are observed. The validation of the particle counter's bio-detection capabilities compared to traditional methods—such as active air sampling—is outside the scope of this document. This validation work has been performed as per applicable guidance, USP <1223>, EP 5.1.6, and PDA TR33, and is detailed elsewhere in separate validation plan and report documents.

For each particle that enters the viable detection optics of the BioTrak Particle Counter, three parameters are measured: 1) scattered light intensity, 2) fluorescent light intensity at relatively low wavelengths, and 3) fluorescent light intensity at relatively high wavelengths. During normal operation, these parameters are assessed in real-time by an algorithm that counts each particle as either viable or non-viable.

During normal operation, only the result of the algorithm is reported, not the raw optical data. However, TSI has the ability to operate the BioTrak Particle Counter in a proprietary instrument mode to obtain the raw measurements from the optical sensors for each particle. The raw optical data can be adjusted to simulate a signal that is higher or lower than the original signal and fed into the algorithm to simulate the effect of an OOT condition on the detection of viable particles.

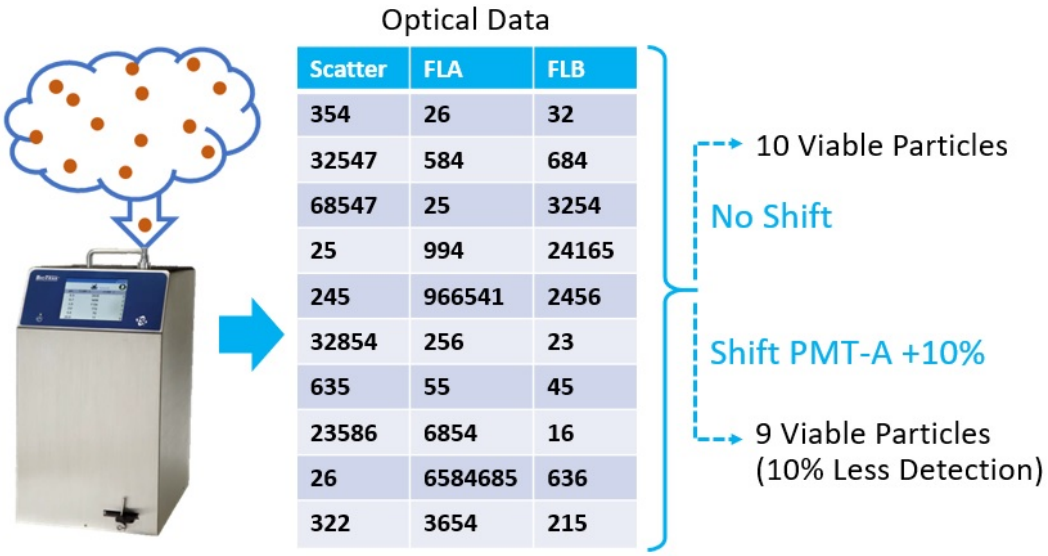

Figure 1. Test Process Flow Schematic. Left to right: 1) sample a test aerosol, 2) record optical parameters for each particle, 3) using the detection algorithm assess the optical data with and without the adjustment. In the hypothetical example shown here, the signals from PMT-A for each particle are adjusted to be 10% more intense than the original data.

Results

The data presented below was collected during the primary validation of the BioTrak Particle Counter in 2021 (*TSI BioTrak Real-Time Viable Particle Counter Biological Detection Performance Validation Report; Document No: 10000054369*). A typical data acquisition replicate includes the analysis of thousands of individual particles in the sample volume.

For each table below, all experimental replicates of the microorganism group that were included in the primary validation were used. The average number of particles detected as viable is considered the baseline measurement. For each cell in the table, the raw optical data from PMT-A and PMT-B was adjusted and fed into the detection algorithm. The resultant change in the number of the viable particles detected is expressed as a percentage. For an OOT PMT accuracy condition, the impact of viable particle detection can be estimated for each microorganism group according to the cell in the table that corresponds most closely with the actual as-found PMT accuracy test observations.

Molds

		PMT-A Accuracy (%)							
		-20	**-15**	**-10**	**-5**	**5**	**10**	**15**	**20**
PMT-B Accuracy (%)	**-20**	1%	0%	-1%	-2%	-4%	-5%	-7%	-8%
	-15	2%	1%	0%	-1%	-3%	-4%	-5%	-7%
	-10	2%	2%	1%	0%	-2%	-3%	-4%	-6%
	-5	2%	2%	1%	1%	-1%	-2%	-3%	-4%
	5	2%	1%	1%	1%	-1%	-1%	-2%	-3%
	10	1%	1%	1%	0%	0%	-1%	-2%	-3%
	15	0%	1%	1%	0%	-1%	-1%	-2%	-2%
	20	-1%	0%	-1%	-1%	-1%	-1%	-2%	-3%

Table 1. Changes in the detection levels of molds (*A. brasiliensis, C. cladosporioides, and P. chrysogenum*) for various PMT accuracy conditions. Cells are colored by the magnitude of the change in detection: white <±5% and green >±5%.

Vegetative Bacteria

		PMT-A Accuracy (%)							
		-20	**-15**	**-10**	**-5**	**5**	**10**	**15**	**20**
PMT-B Accuracy (%)	**-20**	-2%	-2%	-2%	-2%	-6%	-10%	-14%	-19%
	-15	-2%	-1%	-1%	-1%	-4%	-7%	-11%	-15%
	-10	-2%	-1%	0%	0%	-3%	-5%	-8%	-12%
	-5	-2%	-1%	0%	0%	-2%	-3%	-6%	-9%
	5	-2%	0%	0%	0%	0%	-2%	-3%	-6%
	10	-2%	-1%	0%	0%	0%	-1%	-3%	-5%
	15	-3%	-1%	0%	0%	0%	-1%	-3%	-5%
	20	-3%	-2%	-1%	0%	0%	-1%	-2%	-4%

Table 2. Changes in the detection levels of vegetative bacteria (*S. epidermidis, B. subtilis, R. Pickettii, C. acnes, and C. striatum*) for various PMT accuracy conditions. Cells are colored by the magnitude of the change in detection: white <±5%, green >±5% and orange >±10%.

Bacterial Spores

PMT-B Accuracy (%) \ PMT-A Accuracy (%)	-20	-15	-10	-5	5	10	15	20
-20	-5%	-4%	-4%	-4%	-5%	-6%	-7%	-7%
-15	-3%	-3%	-3%	-3%	-3%	-3%	-4%	-5%
-10	-2%	-2%	-2%	-2%	-2%	-2%	-2%	-3%
-5	-1%	-1%	-1%	-1%	-1%	-1%	-1%	-2%
5	1%	1%	1%	1%	1%	0%	0%	0%
10	1%	2%	2%	2%	1%	1%	1%	1%
15	1%	2%	2%	2%	2%	2%	2%	1%
20	2%	2%	2%	2%	2%	2%	2%	2%

Table 3. Changes in the detection levels of bacterial spores (*B. subtilis*) for various PMT accuracy conditions. Cells are colored by the magnitude of the change in detection: white <±5% and green >±5%.

Yeast

PMT-B Accuracy (%) \ PMT-A Accuracy (%)	-20	-15	-10	-5	5	10	15	20
-20	-21%	-17%	-16%	-14%	-13%	-13%	-14%	-14%
-15	-20%	-16%	-12%	-8%	-6%	-6%	-7%	-8%
-10	-18%	-14%	-10%	-7%	-2%	-1%	0%	-1%
-5	-17%	-13%	-9%	-6%	1%	2%	3%	3%
5	-16%	-11%	-7%	-2%	4%	6%	8%	9%
10	-17%	-10%	-5%	-1%	6%	8%	9%	11%
15	-17%	-10%	-5%	-1%	5%	9%	10%	12%
20	-18%	-11%	-6%	0%	7%	10%	11%	12%

Table 4. Changes in the detection levels of yeast (*C. albicans*) for various PMT accuracy conditions. Cells are colored by the magnitude of the change in detection: white <±5%, green >±5% and orange >±10%.

Summary and Conclusion

The impact of OOT PMT accuracy readings can be estimated by purposefully adjusting raw optical data collected under in-tolerance conditions, and rerunning the detection algorithm. The results of many such calculations is presented here to allow users to assess the impact of their unique OOT condition.

The risk the condition introduces must be holistically considered. Therefore, the tables are presented according to microorganism groups. These groups are present in cleanrooms at differing levels and should be considered during the impact assessment. For reference, typical levels of microorganism groups, according to Sandle et. al.[1], are presented in Table 5.

Distribution of Pharmaceutical Cleanroom Air Sample Isolates in Grade A & B	
Veg. Bacteria	87%
Bac. Spores	12%
Fungi	1%

Distribution of Pharmaceutical Cleanroom Air Sample Isolates in Grade C & D	
Veg. Bacteria	88%
Bac. Spores	9%
Fungi	4%

Table 5. Distributions of microorganism groups by cleanroom grade.

[1] Sandle T. A review of cleanroom microflora: types, trends, and patterns. PDA J Pharm Sci Technol. 2011 Jul-Aug;65(4):392-403. doi: 10.5731/pdajpst.2011.00765

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