IMPROVED VIABLE PARTICLE DISCRIMINATION

TSI BIOTRAK REAL-TIME VIABLE PARTICLE COUNTER MODEL 9510

APPLICATION NOTE CC-129
(9/16/2021) Rev A (US)

Contents

Introduction—Improved Viable Particle Discrimination for Pharma

This application note describes the performance of a new algorithm developed for use by the BioTrak® Real-Time Viable Particle Counter to determine the viability of a particle based on its measured optical properties. The new algorithm will be compared relative to the previously validated high-sensitivity algorithm—specifically describing the impact of the new algorithm on viable particle discrimination with respect to test aerosols of microorganisms and interferents (non-viable particulates).

The new algorithm is a conservative improvement to the previously validated version. This means that any particle that was considered viable by the original high-sensitivity algorithm is also considered viable by the new algorithm.

The new algorithm was developed chiefly to improve the detection capability of molds. Molds were not readily aerosolized during the development of the original algorithm and, therefore, it was not specifically trained to detect those optical signatures. Improvements made in the detection of molds are clearly demonstrated in the results section.

Background—Evolving Particle Detection Algorithms

During initial product development of the BioTrak Real-Time Viable Particle Counter, two viable particle detection algorithms, labeled as high-sensitivity and normal, were created. The normal sensitivity algorithm was developed with the intent to balance false positive and false negative counts to produce an accurate estimate in samples with relatively high counts. The high-sensitivity algorithm was more conservative in that it prioritized the decreasing likelihood of false negative counts at the risk of slightly increasing the likelihood of false positive counts. Given the desire to optimize the detection of as many viable particles as possible in Grade A areas, the pharmaceutical industry most widely adopted the use of the high-sensitivity setting. For this reason, the high-sensitivity algorithm was utilized for validation testing conducted in 2013.

The algorithms were empirically derived from the data generated by the optical sensors in the viability chamber of the instrument while sampling aerosolized microorganisms and interferents. Aerosolization of microorganisms during testing was achieved using an Ink Jet Aerosol Generator (IJAG). Some microorganisms proved to be sensitive to this method and retained little to no viability upon dissemination. Limited data was available for algorithm development and some validation tests, most notably those related to detecting mold spores.

To obtain a better understanding of the BioTrak Real-Time Viable Particle Counter's ability to detect viable particles composed of these microorganisms, different aerosolization methods were explored. A method that closely emulates the natural dispersal of mold spores proved to be effective. It provided an opportunity to reliably obtain optical data. This data indicated that these particles did have consistent optical signatures, however, they were largely falling outside the algorithms. The high-sensitivity algorithm was expanded to optimize the detection of these microorganisms without significantly affecting the exclusion of interferent particles. The available historical data of other microorganisms was also assessed with additional modifications being made to the algorithm to further optimize detection.

The result of this work is an updated viability determination algorithm that demonstrates improved detection of a broader array of microorganisms. Due to the historical prevalence in the use of the high-sensitivity algorithm and the improved accuracy of the new algorithm, the new algorithm will replace both the high-sensitivity and normal algorithm options.

Scope—Changes Associated with Updating the Algorithm

This change to the BioTrak Real-Time Viable Particle Counter alters the viable discrimination algorithm, impacting the viable particle counts only. It does not impact the total particle counts in any way.

The new algorithm is a change to the firmware code only—requiring no changes to the instrument hardware. It utilizes the same exact raw optical data as the originally validated algorithm.

The new algorithm does not affect instrument communication with external software platforms.

Methods—Comparing Algorithms

The data presented herein is only intended to assess the relative detection capability of one algorithm versus another. The validation of the instrument'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 Real-Time Viable 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 a viable particle or a non-viable particle.

During normal operation, only the result of the algorithm is reported, and not the raw optical data. However, TSI has the ability to use a proprietary instrument mode to obtain the raw measurements from the optical sensors for each particle. Since it is this raw data that is entered into an algorithm, once collected, it can be entered into other proposed algorithms to compare effectiveness (see Figure 1). This provides a powerful tool for developing an improved algorithm and to demonstrate its relative detection capabilities in comparison to the originally validated high-sensitivity algorithm.

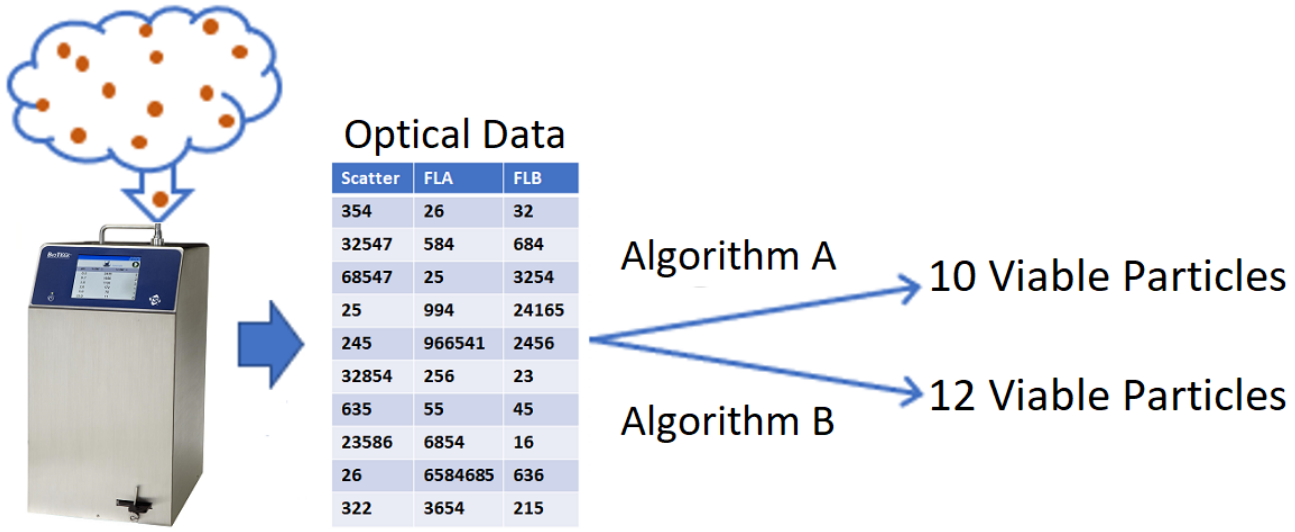

Figure 1. Test Process-flow Schematic. Left to right: 1) sample a test aerosol, 2) record optical parameters for each particle, 3) assess the optical data with different algorithms.

Results—Proof is in the Data

Microorganisms

Several bioaerosols were used to assess the new, improved algorithm. During these tests, four replicate samples were taken and the optical data recorded. The optical data was evaluated using the original high-sensitivity algorithm and the newly improved algorithm. A comparison of the results obtained can be seen in Figures 2–4. Since the new algorithm is an expansion of the original high-sensitivity algorithm, a higher count was obtained for all replicates of each microorganism when using the new algorithm.

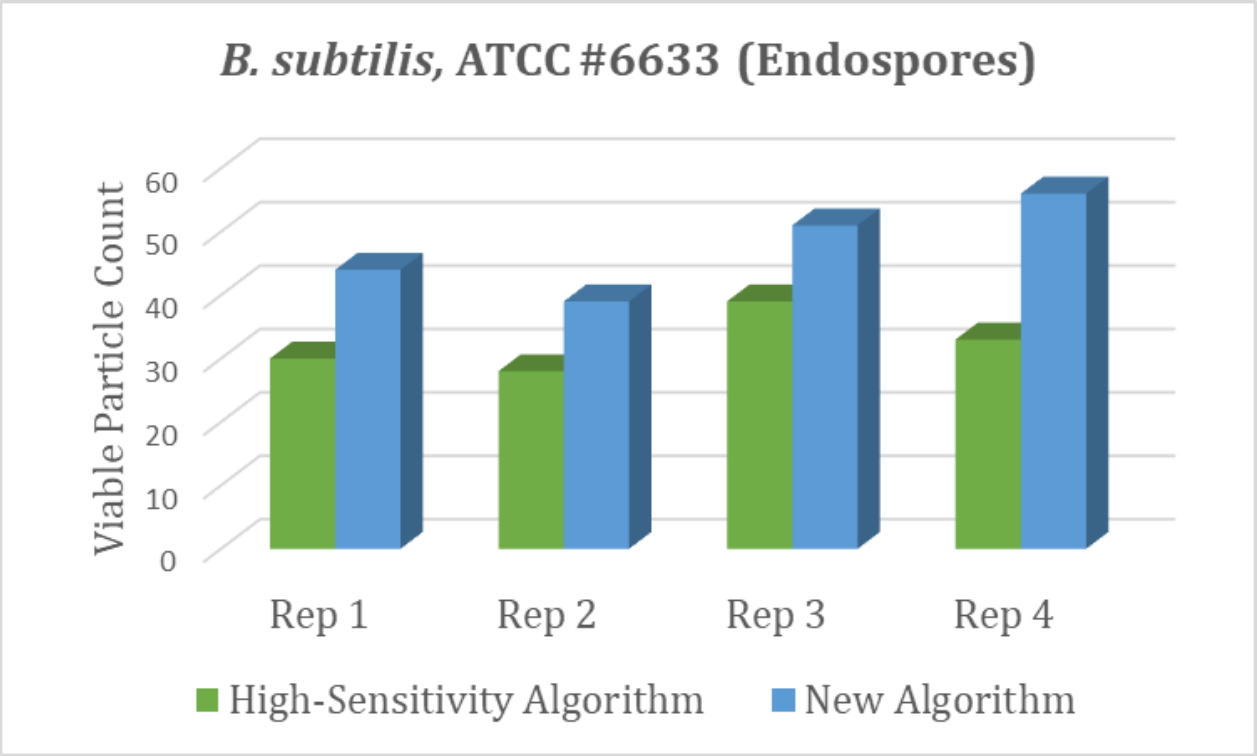

Figure 2. Comparison of the high-sensitivity algorithm to the new, improved algorithm for the detection of bacterial endospores.

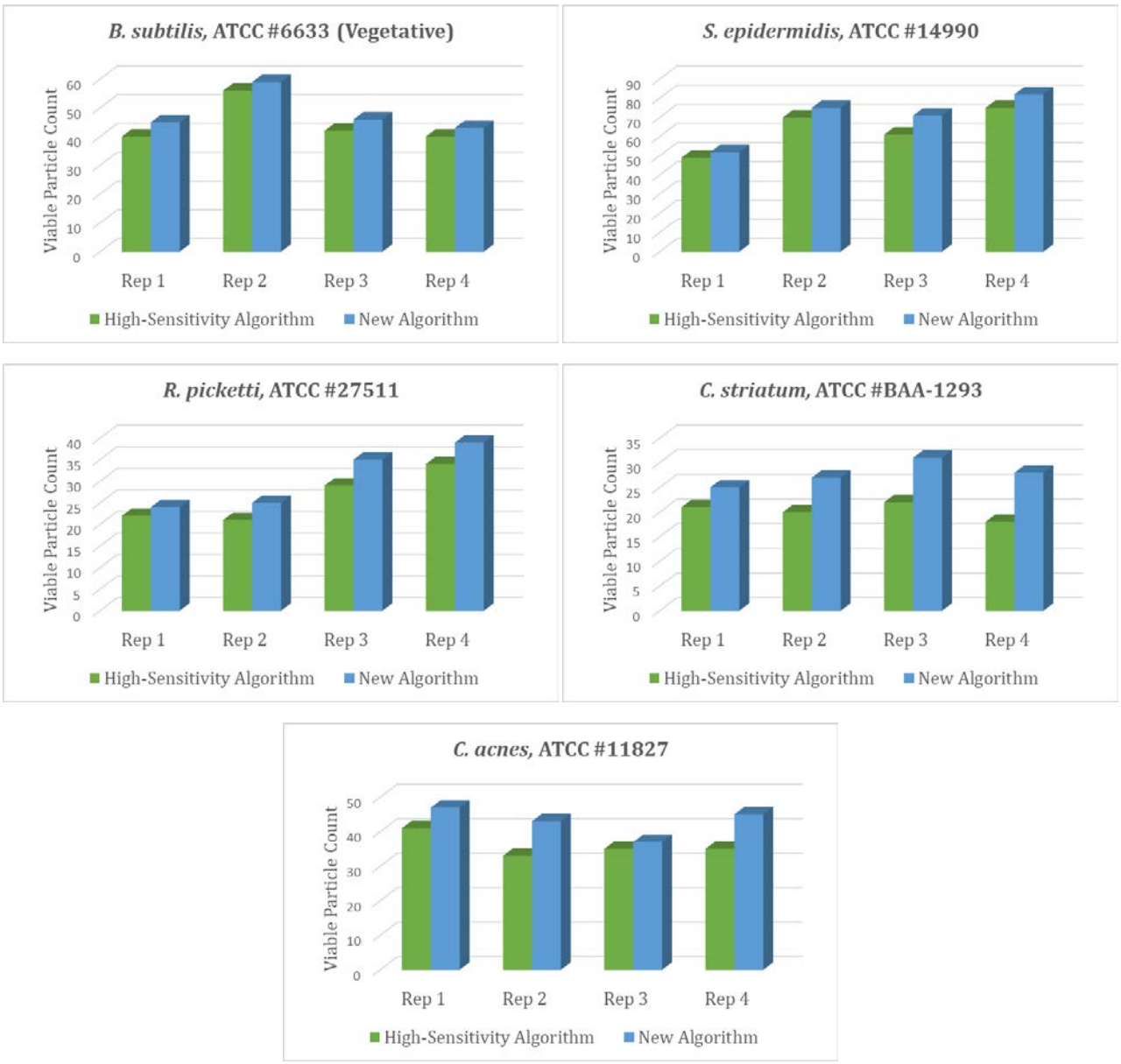

Figure 3. Comparison of the high-sensitivity algorithm to the new, improved algorithm for the detection of vegetative bacteria.

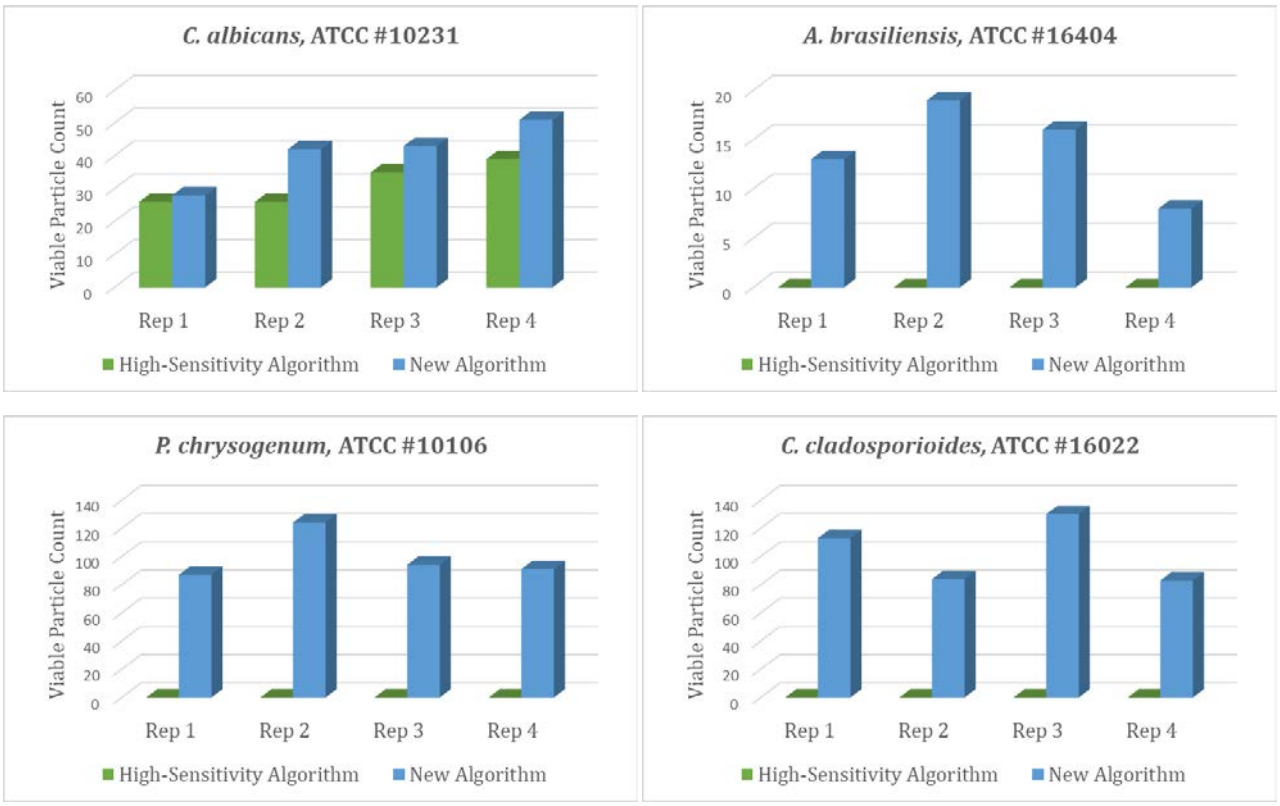

Figure 4. Comparison of the high-sensitivity algorithm to the new, improved algorithm for the setection of fungi (yeast and mold spores).

Non-Viable Particles (i.e., interferents)

Several aerosols of possible interfering non-viable particles were generated to assess the new, improved algorithm. Since most of the materials tested are manufactured to produce low numbers of particles, vigorous manual manipulations were performed in an effort to generate as many particles as possible during testing. This was performed inside a HEPA filtered cabinet with the instrument sampling from within the cabinet directly below the manipulations. The optical data was evaluated using the original high-sensitivity algorithm and the new, improved algorithm.

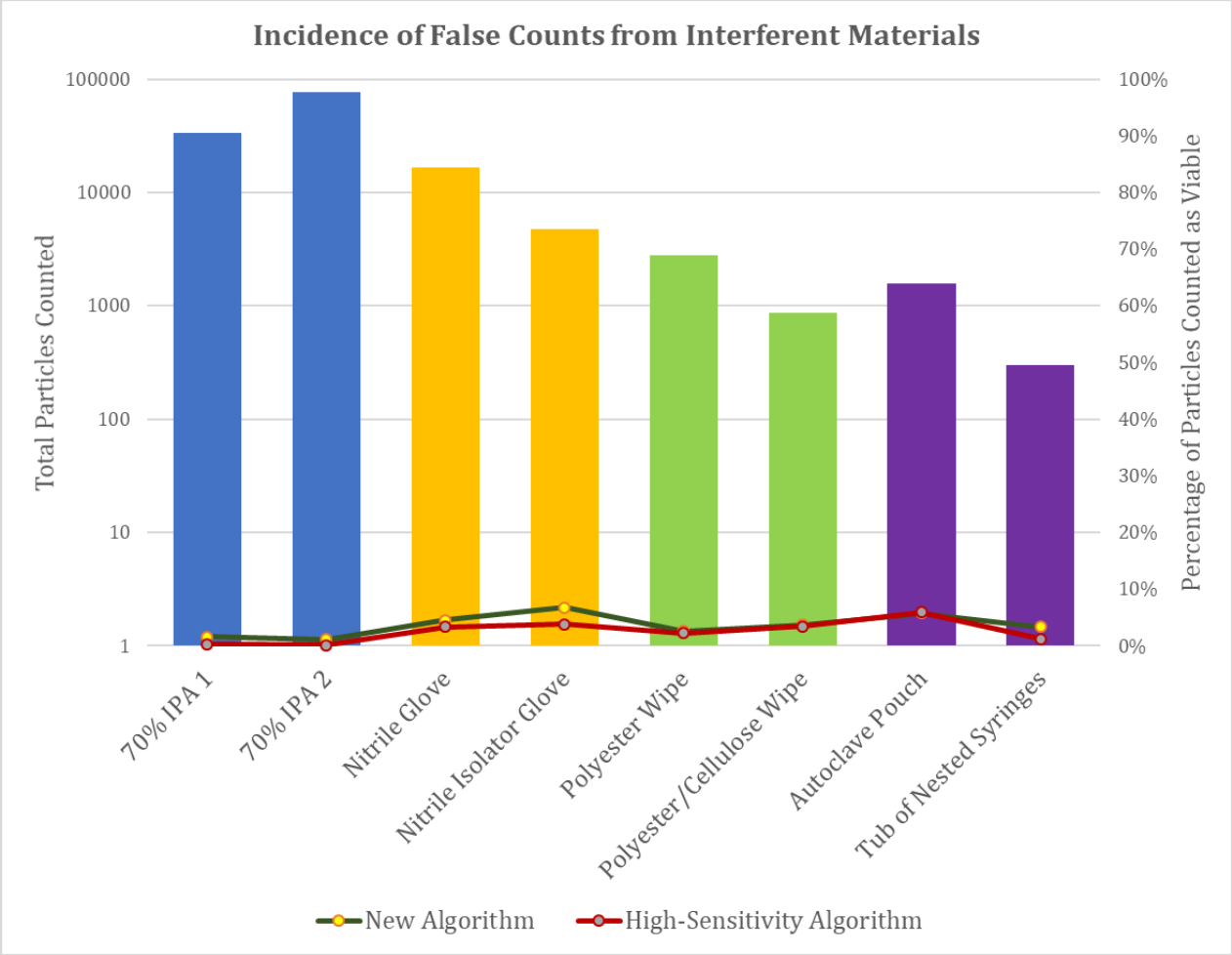

Figure 5. Comparison of the high-sensitivity algorithm to the new, improved algorithm for the detection of interferents.

Zero-Counting

No changes to the zero-count specification are associated with the new algorithm. Since no changes are made to the optical sensors, an algorithm change should not result in any spurious counts not related to a particle (i.e. instrument noise). When tested with a filter attached to the inlet, no spurious viable counts were detected.

Summary & Conclusions—TSI Delivers Trusted Detection

The new algorithm significantly improves the detection of microorganisms, most markedly molds. Since the changes were conservative in nature, some non-viable particles were detected as viable at slightly higher rates in comparison to the high-sensitivity algorithm. However, the overall interferent detection rate with respect to the total number of particles generated was still less than 7% in all cases. This continues to represent a very small risk for false positive counts given that these materials generate particles at a much smaller rate during routine use than produced during this testing.

It is clear that the new algorithm does indeed impact the discrimination and counting of viable particles. Therefore, validation tests of the original high-sensitivity algorithm would not implicitly transfer to the new algorithm. To address this, TSI performed a primary validation of the BioTrak Real-Time Viable Particle Counter running the new algorithm per industry guidance. Users that have performed testing should also consider the impact of the new algorithm on any conclusions drawn from that testing and determine what additional testing or retesting may be applicable for their intended use.

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