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Instantaneous Microbial Detection

BioVigilant has invented an optical technology that can determine the quantity and size of very small particles in liquid or air, and simultaneously determine whether each particle is inert or biologic, all in real time. The result is that BioVigilant's instruments provide instantaneous and continuous microbial detection, thereby creating wide-ranging and profound positive consequences for pharmaceutical manufacturers.

This paper briefly discusses the problems BioVigilant's technology solves, how its technology works, and how it is applied.

How It's Done Now and the Consequences

Within the pharmaceutical manufacturing environment, for both internal and regulatory reasons, testing is required in order to determine the level of microbial contamination. Existing conventional testing methods, however, all have several undesirable attributes in common:

- the cost per test is high;
- the process is labor intensive (many times requiring significant set-up, monitoring and counting, and also the chance of additional contamination from the humans who do the testing); and
- the process is episodic and slow, with results generally not available for two to five days or more.

Among these undesirable attributes, the consequences of the time spent waiting for results are generally the most significant, and include costly planned and unplanned halts in production, as well as continued production under conditions that could be discovered later to be outside regulatory or internal requirements. This could then result in product being thrown out, as well as necessitating expensive and time-consuming investigations.

BioVigilant's Technology

BioVigilant's technology was originally developed to meet the demanding specifications of the U.S. military and Department of Homeland Security for real-time detection of airborne weaponized bio-agents such as *Bacillus anthracis*, which is in the size range of 1 to 7 microns. After considering the technical requirements and analyzing the suitability of existing technology for particle detection and sizing (much of which was invented 20

or more years ago), BioVigilant decided that existing methods contained fundamental design limitations. A new approach with a fundamentally different design was required.

Using an innovative optical design, BioVigilant invented a unique method and technology to count and size very small particles to a sensitivity level and at lower costs than possible using existing technology. As an additional benefit, this new technology allowed BioVigilant to determine concurrently, for each individual particle, if that particle was inert or biologic. In addition to its application within homeland security, this combination of technologies (now called Instantaneous Microbial Detection) is being applied to pharmaceutical manufacturing.

The following is a brief description of BioVigilant's fluorescence sensor technology with regard to airborne monitoring using BioVigilant's model IMD-A instrument.

The IMD-A consists of three components: (1) an optical assembly to measure individual particle size; (2) an integrated and parallel optical assembly to detect a UV laser-induced fluorescence signal from certain metabolites inside microbial cells and spores; and (3) an algorithm for differentiating airborne microbes from inert particles.

The optical assemblies employ the well-known and often-used Mie scattering detection scheme¹, but apply it in a novel way, enabling BioVigilant's instruments to make highly

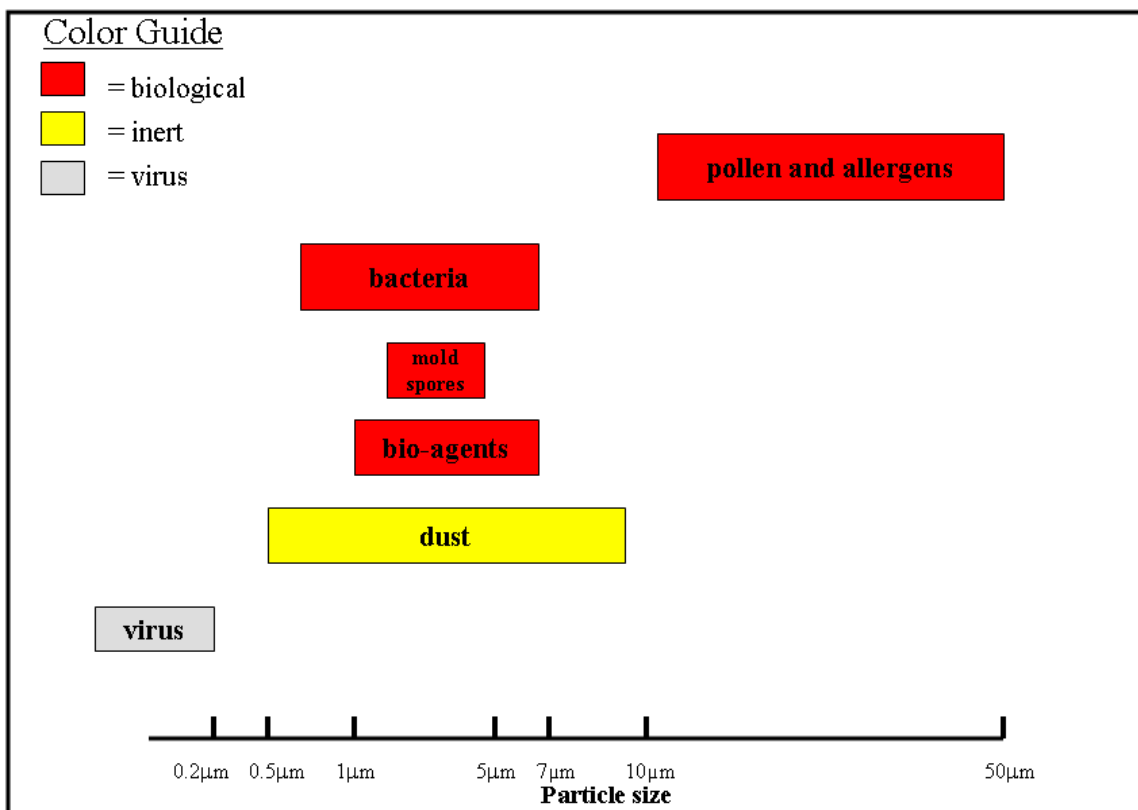


Fig. 1 Particle size ranges of several airborne inert and microbial particulates.

accurate measurements of airborne particles with size ranges from 0.5 microns to 15 microns. This ability to make fine distinctions in size is important in order to determine the class of microbe, because different classes of microbes generally have different size ranges, as depicted in Figure 1.

BioVigilant's unique use of Mie scattering also facilitates the use of UV light illumination to concurrently examine each particle for the presence of the metabolites NADH and riboflavin, which are necessary intermediates for metabolism of living organisms, and therefore exist in microbes such as bacteria and fungi. If these chemical compounds exist in a bio-aerosol, they are excited by the UV photon energy and subsequently emit auto-fluorescence light which is detected by the IMD instrument. While BioVigilant's technology is not capable of identifying the species of microbes (and viruses are too small and lack the metabolism necessary for detection), the IMD instrument's ability to simultaneously, and for each particle, determine the size of the particle and whether it is biologic or inert indicates to the user the presence or absence of microbial contamination.

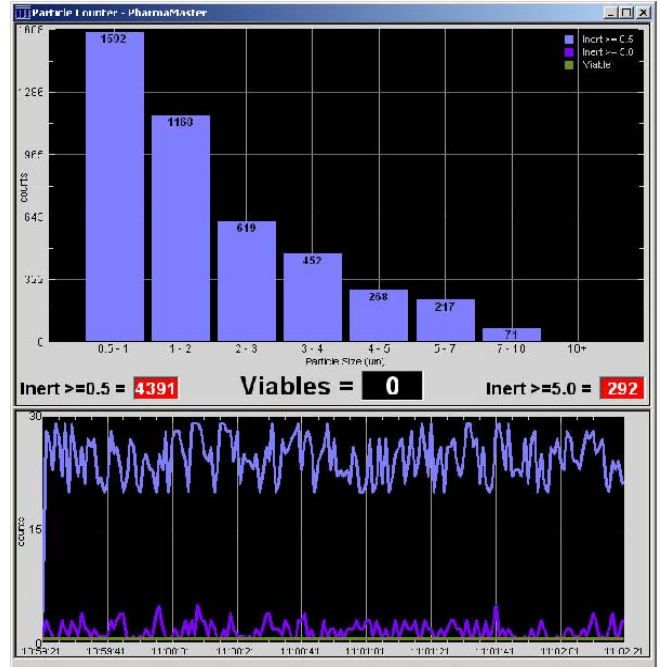


Fig. 2 Typical data display of clean air

How BioVigilant's Instantaneous Microbial Detection Instruments Can Be Applied to Pharmaceutical Manufacturing

BioVigilant's IMD-A instruments can be used in pharmaceutical manufacturing clean rooms (I) as warning instruments; (II) as continuous monitoring and trending instruments; and, (III) to verify if remediation was successful.

(I) As Warning Instruments

BioVigilant's IMD-A can sample the air continuously or do spot checks and give an indication or alarm when microbes are detected. Figures 2 and 3 are examples of IMD-A PharmaMaster displays. In each sample display, the blue bar denotes inert particles, the green bar denotes viable particles, the blue and purple lines denote inert particles and the green line denotes viable particles. An alarm protocol can alert facility managers in the event of microbial detection, according to specific clean room requirements.

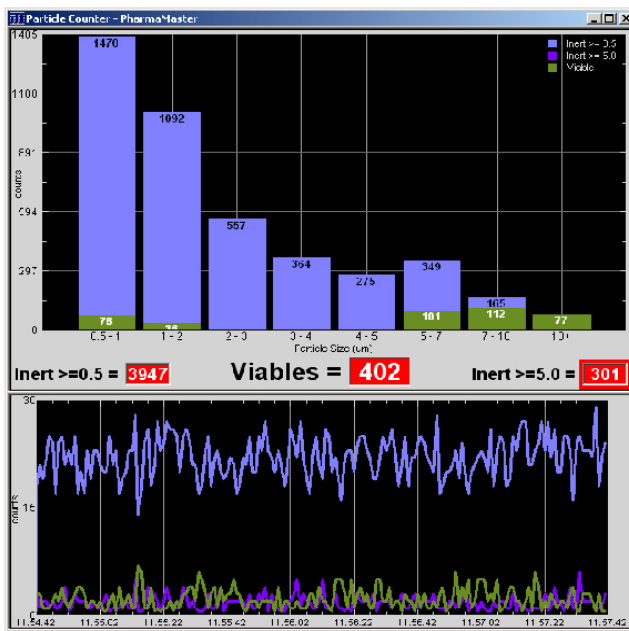


Fig. 3 Example display when baker's yeast powder was present in an air sample.

As examples, Figure 2 shows a clean air sample with no microbes, whereas Figure 3 shows the display when a burst of baker's yeast (*Saccharomyces* stems, Inc 2005

cerevisiae) powder was sprayed in the air.

(II) As Continuous Monitoring and Trending Instruments

The ability of BioVigilant's IMD instruments to provide instantaneous microbial detection also enables them to provide continuous monitoring and trending, which is useful functionality not possible using existing conventional methods. In addition to aiding compliance with existing regulatory and internal requirements, this unique feature of BioVigilant's technology makes its instruments especially suitable for implementation of the FDA's Process Analytical Technologies (PAT) initiative by providing a process analyzer tool for microbiological monitoring of clean room air.

In addition to demonstrating control of the environment, within an aseptic manufacturing facility it is advantageous to be able to observe trending patterns of microbial and particulate distribution so that a microbial excursion can be detected and action taken as soon as possible. BioVigilant's IMD instruments are able to obtain the trending from analyses of a continuous stream of data, and thereby spot anomalous events. The bottom sections of both figures 2 and 3 above show how the IMD can be used for data trending.

This ability for data trending suggests several additional useful applications for IMD technology for pharmaceutical manufacturing, among them:

- the deviation from the background distribution in a controlled environment can signal either the failure or the imminent failure of the containment or filtration system;
- strategically placed IMD instruments can provide a two-dimensional map of microbial and particulate distribution which can be used to pinpoint the location of a microbial excursion;
- IMD instruments can be used as entryway or sensitive area sentries to alert of the presence of improperly sanitized personnel; and,
- IMD instruments can be used to discover deviations from standard operating procedures by human operators.

Additionally, because of the technology used by BioVigilant's IMD instruments, samples are not destroyed. As a consequence:

- ongoing samples may be taken, thereby making the sampling more representative of the entire batch and any contamination that exists more likely to be discovered earlier in the production process; and,
- at certain points in the production process (such as when vials are being filled), continuous sampling of entire batches is possible, thereby making it more likely that, if contamination is encountered, a smaller batch of production will have to be discarded than would be otherwise.

(III) To Verify If Remediation Was Successful

BioVigilant's IMD instruments can be used to obtain immediate results, for example, after remediation of microbial contamination has taken place, to determine, in real time and immediately, if the remediation was successful.

A Comparison of Current Method vs. Proposed Method

The table below compares BioVigilant's Instantaneous Microbial Detection method against the current plate culture method.

Feature	BioVigilant	Conventional Plate Culture Method	Consequence
Time from measurement to results.	Instantaneous.	Typically from one to five days or more.	Using the conventional method creates more planned and unplanned halts in production and greater potential for contamination, significantly increasing costs and lowering production. Conventional methods also are very slow to certify if remediation was successful, causing slowdowns and making it more difficult to determine the cause of contamination.
Mode of detection.	Continuous monitoring and real-time feedback of results.	Episodic monitoring and time-delayed feedback of results.	Continuous monitoring increases accuracy, allows for trending to spot problems early, lowers chances of contamination, reduces the need to dispose of production, reduces downtime and the time needed to remediate, and is conducive to PAT.
Time to set up sample.	None. Just turn it on.	Can be significant.	Conventional method requires higher labor costs and time delays.
Human intervention.	Minimal.	Required to set up samples, transport, and to read results.	Human intervention creates more possibilities for inaccuracies and contamination of the sample.
Cost per test.	Limited to maintenance of instrument and low cost disposables.	Can be significant.	BioVigilant's method lowers per- test cost.

Summary

BioVigilant's IMD-A instruments provide instantaneous and continuous microbial detection, which make BioVigilant's instruments extremely useful tools for pharmaceutical manufacturers to:

- understand and demonstrate control of the manufacturing environment;
- implement PAT;
- reduce the incidence and severity of microbial excursions;
- comply with both regulatory and internal requirements for microbial monitoring and remediation; and,
- significantly reduce costly time delays and product contamination.

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¹ Mie scattering is an optical phenomenon in which a beam of light is scattered by particles whose sizes are comparable to the wavelength of the light. In this case, the scattered light intensity is dependent on the particle size. Using this principle, one can determine the sizes of particles by measuring the light intensity scattered by those particles.