

# Rapid Microbiology Newsletter

A Newsletter for the Rapid Micro Users Group™



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## Letter from the Editor

Summer is upon us and the RMUG staff is hard at work putting together the Annual RMUG Conference, which will be held September 25-27 in Crystal City, VA. With so much to offer this year, the conference has been extended to two full days, including fun events planned to make it the rapid micro event not to miss. Significant savings on the conference can be yours by registering by July 22. Don't be left out, sign up today!

This newsletter explores the optical technology developed by BioVigilant, which can determine viable vs. non-viable contamination in real time. I hope you find the article to be informative.

Stay cool and don't forget the sunscreen!

Best Wishes,

*Michele*

Michele Conway  
Executive Editor

## Vendor Spotlight Instantaneous Microbial Detection

By Chuck Bolotin  
BioVigilant Systems Inc.

BioVigilant has developed an optical technology that can determine the quantity and size of 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 devices provide instantaneous 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, both regulatory and internal requirements call for testing in order to determine the level of microbial contamination. Existing conventional testing methods 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,
- the process is slow, with results generally not available for two to five days.

Among these undesirable attributes, the consequences of waiting for results are generally the most significant, and include costly planned and unplanned halts in production, as well as continued production under incorrectly assumed acceptable conditions; finding out later that the product manufacturing conditions were not within regulatory requirements, thereby triggering expensive and timely investigations.

## BioVigilant's Technology

BioVigilant's technology was originally developed to meet the demanding specifications of the U.S. Military and Homeland Security for real time detection of the presence of airborne weaponized bio-agents such as *Bacillus anthracis*, which is in the size range of 1 - 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 that would keep BioVigilant from accomplishing its task. A new approach with a fundamentally different design would be 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 not possible using existing technology. As an additional benefit, this new technology allowed BioVigilant to concurrently determine, for each individual particle, if that particle was inert or biologic. In addition to its application within homeland security, this combination of technologies is now being applied to the field of pharmaceutical manufacturing.

The following is a brief description of BioVigilant's fluorescence sensor technology with regard to airborne monitoring (liquid monitoring is very similar), using BioVigilant's model BAT detector.

The BAT consists of three components: (1) an optical assembly to measure individual particle size; (2) a concurrent optical detector 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 dust particles.

The optical assembly uses the well-known and often used Mie scattering detection scheme<sup>1</sup>, but applies it in a novel way, enabling BioVigilant's devices to make highly accurate measurements of airborne particles with size ranges from 0.5 microns to 20 microns. This capability of being able to make fine distinctions in size is important in order to determine the class of microbe, because different classes of microbes have different size ranges, as depicted in Figure 1.

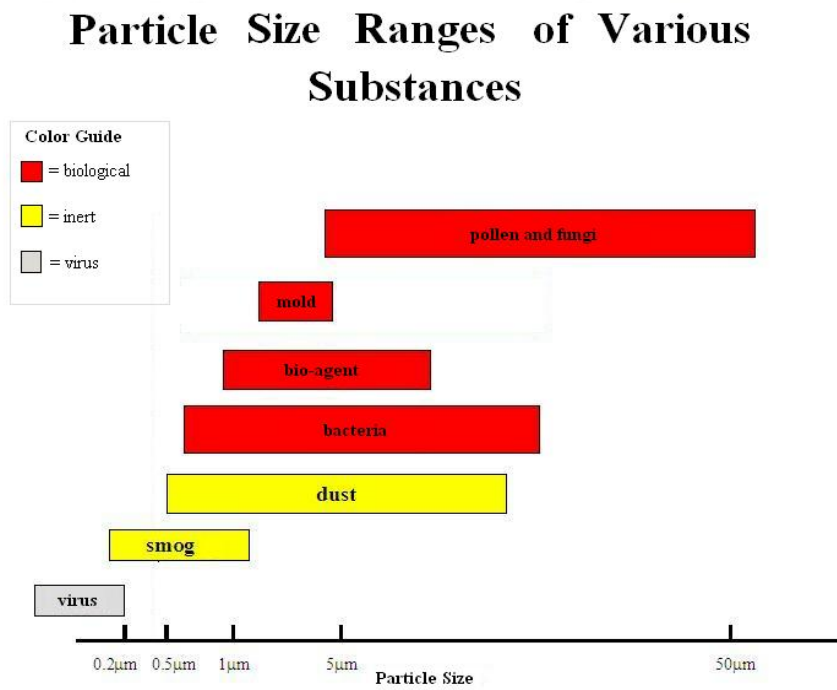


Fig. 1 Particle size ranges of several airborne particulates, including both inert and microbes.

<sup>1</sup> Mie scattering is an optical phenomenon where 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 of the particle size. Using this principle, one can determine the sizes of particles by measuring the light intensity scattered by those particles.

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 BioVigilant's sensor. While BioVigilant's technology is not capable of identifying the genus or species of microbes, and viruses are too small and lack the metabolism for detection, BioVigilant's ability to simultaneously, and for each particle, determine the size of the particle and if it is biologic or inert, indicates to the user if there has been contamination.

### How BioVigilant's Instant Microbial Detection Devices Can Be Applied to Pharmaceutical Manufacturing

BioVigilant's devices can be used as warning devices where levels of contamination are monitored in pharmaceutical clean rooms. Relative to airborne monitoring, BioVigilant's devices can sample the air continuously or be used as a spot check, and give an indication or alarm when microbes are detected. Figures 2 and 3 are typical BioVigilant device data displays. Figure 2 shows a clean air sample with no microbes, whereas Figure 3 shows the display when a burst of baker's yeast powder was sprayed in the air.

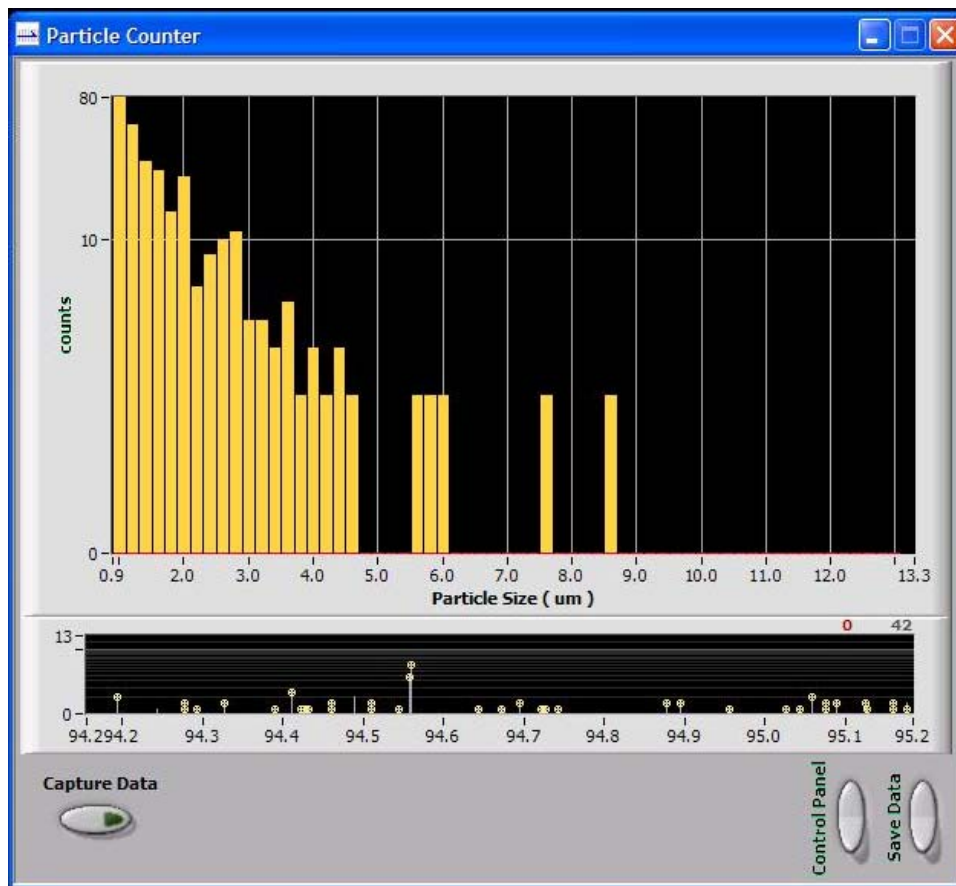


Fig.2 Typical data display of clean air

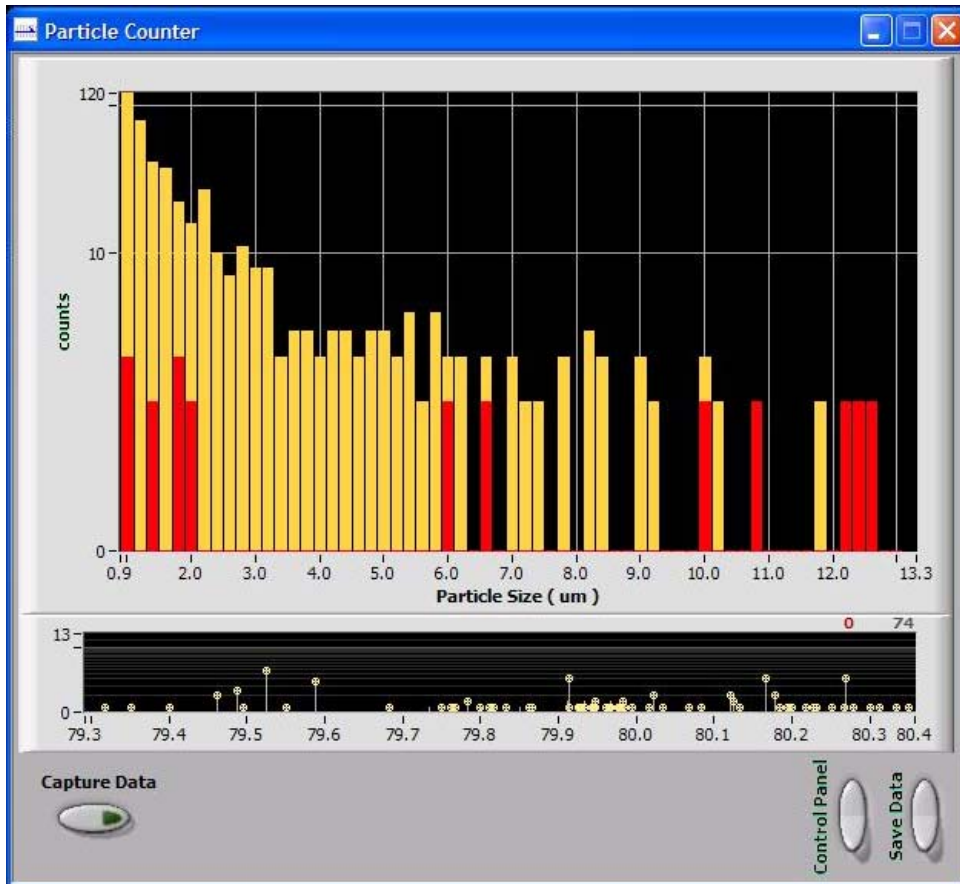


Fig. 3 Example display when baker's yeast powder was present in an air sample.

In these sample displays, airborne particle distribution histograms are shown, where the x-axis represents particle size range and the y-axis represents the particle counts per liter of air. The yellow bars in both displays denote inert particles, while the red bars in Figure 3 denote the presence, size and count of microbes. According to specific clean room requirements, an alarm protocol can alert facility managers in the event of microbial detection.

In addition to helping to comply with existing regulatory and internal requirements, BioVigilant's unique use of technology makes its devices 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 quality, as well as testing for microbial contamination of liquids.

### **A Comparison of Current Method vs. Proposed Method**

The table below compares BioVigilant's Instant 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.	Using the conventional method creates more planned and unplanned halts in production and greater potential for contamination, significantly increasing costs and lowering production.
Level of detection.	Detection and sizing of viable microbes. No identification of microbial types or viruses.	Microbial detection and identification.	When using BioVigilant's device, for those who need speciation, culturing would have to be done after BioVigilant's device detected contamination.
Mode of detection.	Continuous monitoring and real time feedback of results.	Intermittent monitoring and time-delayed feedback of results.	Continuous monitoring increases accuracy and lowers chances of contamination; 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 read results.	Human intervention creates more possibilities for inaccuracies.
Cost per test.	Limited to maintenance of device and low cost disposables.	Can be significant.	BioVigilant's method lowers per test cost.

## Summary

BioVigilant's devices provide instantaneous microbial detection, which make them extremely useful tools to:

- implement PAT;
- comply with both regulatory and internal requirements for microbial monitoring; and,
- significantly reduce costly time delays in the pharmaceutical manufacturing processes.

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**The 4<sup>th</sup> Annual RMUG™ Conference**  
**September 25 – 27, 2005**  
**Sheraton Crystal City, Crystal City, Virginia**

**Early Bird Registration ends July 22!**  
**Don't miss your opportunity to sign-up now and save!**

We have expanded the conference to two full days and are working hard to provide new content on the latest technology, regulatory updates, and what users are requesting. With a new session added to the agenda and the opportunity to network with your peers at the **International Spy Museum** in Washington, D.C., this is the one rapid micro conference you don't want to miss!

Don't have a registration form? Interested in exhibiting or sponsoring? Please contact Mark Pfitzenmaier at [mpfitzenmaier@vectech.com](mailto:mpfitzenmaier@vectech.com) or (248) 478-5820.

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**PHARMEUROPA – Nucleic Acid Amplification Techniques**

By Jeanne Moldenhauer  
Vectech Pharmaceutical Consultants, Inc.

Nucleic acid-based amplification techniques are based upon the use of polymerization techniques to multiply (Xerox) copies of DNA fragments. One example of this type of technique is Polymerase Chain Reaction, PCR, which has been described in detail in previous RMUG™ Newsletters. The multiplication achieved for the fragments is exponential.<sup>1</sup> It is also possible to use these types of procedures with RNA, providing that the RNA was transcribed into cDNA. This is achieved by use of reverse transcriptase, and is called Reverse Transcriptase PCR (RT-PCR).

There are other methods used for amplification of RNA, including Nucleic Acid Sequence-Based Amplification (NASBA) or Transcription Mediated Amplification (TMA).<sup>2</sup>

There are a variety of ways that the amplified fragments of nucleic acid may be analyzed including:<sup>3</sup>

- Size of the fragment
- Determination of the sequence in the fragment
- Combination with a fluorescent probe via re-hybridization

The choice of method used for analysis impacts whether the results obtained are qualitative, semi-quantitative or quantitative.

Nucleic acid amplification techniques are considered genotypic methods.

Some of the typical uses of these methods include: identification of microorganisms, detection of a specific organism or pathogen, and in some cases, can be used to quantify organisms. When

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<sup>1</sup> PHARMEUROPA, (October 2004), "Alternative Methods for Control of Microbiological Quality", Vol. **16(4)**: 555-566.

<sup>2</sup> PHARMEUROPA, (October 2004), "Alternative Methods for Control of Microbiological Quality", Vol. **16(4)**: 555-566.

<sup>3</sup> PHARMEUROPA, (October 2004), "Alternative Methods for Control of Microbiological Quality", Vol. **16(4)**: 555-566.

DNA methods are used, both living and dead microorganisms are detected because both have DNA. Use of RNA analysis, specifically mRNA, may be advantageous since mRNA degrades rapidly in dead microorganisms.<sup>4</sup>

Primers are selected to identify specific microorganisms, or groups of microorganisms. In some cases, use of multiple primers (in sequence of operations) can improve the methodology and reduce other sources of variability in the method.

These technologies are widely used in many non-pharmaceutical based operations.

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### In and Around the Rapid Micro Community

- **September 25 – 27, 2005. The 4<sup>th</sup> Annual RMUG™ Conference. Sheraton Crystal City, Crystal City, VA. Book now and save. Early bird registration ends July 22, 2005!**
- October 15 – 19, 2005. UW-River Falls 25th Food Microbiology Symposium "Current Concepts in Foodborne Pathogens and Rapid and Automated Methods in Food Microbiology." University of Wisconsin-River Falls. For more information please contact Doreen Cegielski at (715) 425-3704, visit <http://www.uwrf.edu/food-science> or e-mail [foodmicro@uwrf.edu](mailto:foodmicro@uwrf.edu).

If you know about, or would like to include information on conferences or events of interest, please provide the appropriate information to [rmug@vectech.com](mailto:rmug@vectech.com).



### **\*\*New Releases\*\***

#### **Pharmaceutical Quality by Richard Prince**

*Pharmaceutical Quality* is a collection of essays that offer an examination of quality from the perspectives of senior experts working in industry, government and academia from around the world.

#### **Quality Assurance Workbook for Pharmaceutical Manufacturers by Michael Jahnke**

*Quality Assurance Workbook for Pharmaceutical Manufacturers* shows you how your facility, through carefully planned processes, implemented at an early stage, can achieve and maintain a high production standard.

### **\*\*Coming September 2005\*\***

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<sup>4</sup> PHARMEUROPA, (October 2004), "Alternative Methods for Control of Microbiological Quality", Vol. 16(4): 555-566.

**Environmental Monitoring**  
**Edited by Jeanne Moldenhauer**

*Environmental Monitoring* compiles the extensive experience of a variety of experts in the field of aseptic processing and provides practical guidance on how to establish and maintain a monitoring system that will be meaningful, manageable and defensible.

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