Instantaneous microbial detection demonstrated for air & water

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ABSTRACT

BioVigilant Systems, Inc. Instantaneous **M**icrobial **D**etection are enabling biosensor devices with application for Life Sciences & Healthcare; Military-Civil Defense & Security; and for Emerging Requirements Impacting Human Health, Safety, Industry, and Environmental Monitoring. Many studies show growth techniques, standard in environmental monitoring, are inadequate to evaluate quality and quantity of microbial content: (1) to show environmental microbial content data in time enough for curative action; and (2) to indicate with fidelity entire bioburdens of samples. IMD™ is shown capable of instantaneously detecting particle size and auto-fluorescence. IMD™ presents high fidelity data about airborne biological content, with on-going tests for liquids. Rigorous field tests of IMD™ have been conducted for current users in manufacturing cleanrooms, aseptic isolators, and independent and government testing facilities in uncontained environments.

Keywords: microbe, detection, biosensor, optical, monitoring

1 MICROBE DETECTION

BioVigilant Systems, Inc. IMD™ are enabling biosensor devices with application for Life Sciences & Healthcare; Military-Civil Defense & Security; and for Emerging Requirements Impacting Human Health, Safety, Industry, and Environmental Monitoring.

Traditionally, growth based techniques have been the standard in environmental monitoring for evaluating the quality and quantity of microbial content. This family of culturing techniques relies on the efficacy of the growth media (TSB, SCDA, etc.) to induce replication and growth in the microbe.

However, many studies have underscored the inadequacy of these techniques: (1) to provide environmental microbial content data in a manner sufficiently expeditious for timely curative action; and (2) to faithfully indicate the entire bioburden of a sample.

BioVigilant Systems, Inc. has designed and built the IMD™ System, which is capable of instantaneously detecting airborne particle size and auto-fluorescence. In this manner, the instrument provides information of high fidelity about airborne biological content, with additional applications projected for liquid environments, as well.

The IMD™ System has been field tested in numerous environments, including the cleanrooms and aseptic isolators of pharmaceutical companies. The IMD™ System has also been subjected to rigorous field tests by independent and government testing facilities, which show its efficacy in uncontained environments and as compared to instruments currently in Security-Military sector systems use.

Current amplifications of the IMD™ include prototype testing of a liquid environment model, and in other applications which bring together biological, optical, and engineering sciences in advanced ways to help secure a clean environment and public safety.

BioVigilant's new optical technique has been established to determine the microbial content of a sample. Microbial efficacy is necessarily linked to the ability of a cell to sustain metabolic activity. Thus, the presence of metabolites such as NADH, riboflavin, and others is a reliable indicator of true microbe viability. Use of the inherent auto-fluorescent properties (tendency to fluoresce when exposed to ultraviolet light) is key to IMD™ enabling technology.

What follows is designed to show the IMD™ Systems' efficacy for various environments, with primary focus on demonstrated uses for the Pharmaceutical industry; for field environments and other uses in the Security-Military sector; where excessive microbial contamination is present in environments otherwise measured to be within thresholds using traditional growth-based methods; and other applications and environments for which IMD™ Systems capability to detect particle size and auto-fluorescence can be validated, including liquid and water environments, and other applications which bring together biological, optical, and engineering sciences in advanced ways to help secure a clean environment and public safety.

2 COMPANY DESCRIPTION

BioVigilant Systems™ is a Tucson, AZ-based company, now employing 30+ professionals. Founded in November 2002, BioVigilant originates, researches, designs, manufactures, markets and distributes instruments with unique, proprietary capabilities to detect extremely small particles, such as bacteria or microbes, and to determine simultaneously if each individual particle is biologic or inert. This critical BioVigilant-branded process -- Instantaneous Microbial Detection -- provides real time & continuous quantification of microbes present in air or

liquid, and determines what general character the particles' optical signatures reveal.

BioVigilant develops, builds and maintains a family of environmental monitoring instruments to assure clean environments for use in pharmaceutical manufacturing, military and civil security, building safety, and other emerging applications. Through the novel use of IMD™ technology, BioVigilant's scientists have created instruments with entirely new classes of functionality and unparalleled sensitivity and accuracy than had been possible using existing methodology. BioVigilant, based in Tucson, AZ, maintains development operations in Austin, TX and Boston, MA.

IMD™ products replace & augment existing products & methods of testing for microbial contamination, including the century-old process of microbial culturing. The advantages of IMD™ to manufacturing are substantial & dramatic -- to reduce production down time, inventory & scrapped product costs. In validating safety or remedying contamination, IMD™ should enable improvements in the air quality of congested and microbially-challenged areas, and to expedite validations of water purification, improving capacities for safe, healthy and sustaining lives.

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3 TARGET MARKETS

Market research shows that market size applicable to the company's line of IMD™ instruments, in pharmaceutical manufacturing alone, projects in excess of $2 Billion. Pharmaceutical manufacturers, the Company's primary market focus, see the value of IMD™ from substantial reductions in production costs & product quality improvements.

Under current methods (i.e., microbial culturing), test results for contamination are not known for several days. The consequence is several days' work of production -- worth hundreds of thousands of dollars & more -- are wasted. With IMD™ ™, contamination is detected in an instant, allowing immediate corrective action, saving time, drug batches, & the bottom line.

IMD™ also supports Military-Security applications, and the Company is currently partnered with significant sector primaries, as well having successfully made demonstration for transportation, other high-value asset & densely populated sites. Other uses projected are for securing "safety zone" from microbial "contamination zones" in hospitals, laboratories, & for clean water validation.

4 TECHNOLOGY DISCUSSION: CURRENT LIFE SCEINCE APPLICATIONS

While BioVigilant Systems™ began its current approach to environmental detection spurred by the impact in military-civil defense and security markets following September 11, 2001, the Company's activities in the life sciences sector have been well received, particularly by pharmaceutical manufacturing interests. Notably, evaluation of BioVigilant's Instantaneous Microbial Detection system to monitor microbial populations in environmental air was conducted by Vishvesh K. Bhupathiraju and Brandon Varnau of Bayer HealthCare in Berkeley, California; along with Jerry R. Nelson of Nelson Laboratories, Salt Lake City, Utah; and J.P. Jiang & Chuck Bolotin, BioVigilant Systems Inc., Tucson, Arizona. Their collaborative work was reported in: "Evaluation of an Instantaneous Microbial Detection System in Controlled and Clean Room Environments," pp. 35-6+, BioPharm International, September 2007 (vol. 20, no. 9), and referred throughout this discussion as "Bayer." Nelson Laboratories and laboratories at US Army Dugway Proving Ground provided use of microbial barrier test chambers during the Bayer study.

The Bayer study conducted tests of the IMD™ in controlled microbial barrier test chambers and in clean room environments, comparing results from the IMD™ with those obtained using conventional environmental air monitoring methods. Also, microbial populations in environmental air in an unclassified environment were evaluated using the IMD™-A and the all gas impingement (AGI) method coupled with ScanRDI.

The Bayer study report showed:

> "The mean microbial recoveries obtained from the AGI were higher but in the same order of magnitude recovered by the IMD™ units. In the classified environments examined, the microbial recoveries from the SAS air sampler were substantially lower than the microbial counts detected by the IMD™." (Bayer, p.35)

Further, the study states that:

> "Results suggest that the IMD™ has the potential to provide a reliable evaluation of the microbial populations in environmental air instantaneously creating a positive impact on the pharmaceutical manufacturing industry." (Bayer, p.35)

Microbial air monitoring is fundamental for maintaining secure, safe, and uncontaminated pharmaceutical, as well as other life science, clean room environments. Both particulate and microbial content of the air are monitored in pharmaceutical research and manufacturing environments, with particulate content at the 5.0 and the 0.5 micron level observed using total particulate monitoring systems. Environmental air is evaluated for microbial content, especially, with both active and passive air monitoring devices.

Typically, active microbial content in environmental air has been evaluated using a variety of systems, including Anderson Air, liquid impinger, the SMA air sampling systems, for example, or other similar systems.

Active air monitoring often involves use of devices in which microorganisms from known volumes of air are captured on media plates. Alternatively, a liquid is infused with subject air, and microorganisms in the liquid, passed

through a membrane filter, are captured, transferred to media plates, and grown for observation, evaluated by the plate count method using settling plates, which then yields an inferential air contamination rate. Results from these "trap and grow" methods of microbial monitoring of environmental air are obtained 30 hours to 5 days after the tests are performed.

The key limitation of these conventional environmental monitoring methods is the several days' wait for a microbial growth yield of monitoring data. Over the past decade, several culture and non-culture based rapid methods have been developed with the capability of much faster turnaround for microbial data, including one which uses the ScanRDI system , a semi-automated method for measuring total viable organisms without the need for culturing, using complex sampling methods elsewhere described (Bayer, pp.36+) .

Many in the pharmaceutical and life sciences industries generally suggest that an ideal system for microbial air monitoring in clean room environments would require little or no sample preparation or manipulation. Moreover, such an ideal system would provide environmental microbial air monitoring data in real time (Bayer, 36+).

BioVigilant's IMD™ system is a non-culture based system, using optical particle-sizing and fluorescence sensor technologies. The IMD™ system has the unique, proven ability to determine the quantity and size of biological particles in environmental air, and to simultaneously determine whether each particle is inert or biologic, all in real time (see: [2] Jiang, J. P. 2005.).

The IMD™ 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 metabolites within microbial cells and spores; and (3) an algorithm for differentiating airborne microbes from inert dust particles (see: Figure Available at NTSI/CSI Conference & Trade Show, Boston, MA 2008).

The optical assembly uses the Mie scattering in its detection scheme to make accurate measurements of airborne particles in a size range from 0.5 microns to 20 microns. Further, use of Mie scattering facilitates UV light illumination and examination of **each** particle as it passes through the "interrogation zone" for the presence of the metabolites NADH and riboflavin, which are necessary metabolic intermediates of living organisms, including bacteria and fungi.

Viability of a microbial cell is necessarily linked to its ability to metabolize and replicate. Metabolites are optically excited by ultraviolet light, emitting broadband fluorescence. (see: [3] Morris, Scott. 2008.) If these metabolites are present in the observed environment, excited by the UV photon energy to emit auto-fluorescent light, they are detected by the IMD™ sensor.

IMD™ technology is not capable of identifying genus or species of microbes, however, the IMD™ uniquely view each particle to instantaneously determine the size of the particle and then determine whether it is biologic or inert.

The BioVigilant IMD™ System has a unique utility in monitoring environments for pharmaceutical manufacturing areas because of its ability to evaluate microbial quality of environments in real time.

The Bayer study identifies several areas in which the IMD™ has potential for immediate benefit: 1) Investigations; 2) Reduction of Shutdown Length; 3)Training; and 4) Modification of Aseptic Process. Bayer is exploring the use of the IMD™ for these purposes while pursuing additional studies and the question: "What will it take to replace environmental monitoring of air with the IMD™?" (Bayer, p.44+).

Similar questions are being asked by primary contractors in the Military-Civil Defense & Security sector, in water and wastewater processing. Other applications in air and liquid environments (e.g., food processing and packaging) are emerging. As climate change reveals new microbial contamination zones, other novel IMD™ applications will emerge which bring together biological, optical, and engineering sciences in advanced ways to help secure a clean environment and public safety. Information is available to expand on these issues.

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