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REAL TIME MICROBIAL DETECTION BY LASER INDUCED SPECTROSCOPY AND ITS APPLICATION IN CONTAMINATION CONTROL

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Real time microbial detection by laser induced spectroscopy and its application in contamination control

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Abstract

A technology based on optical spectroscopy has been developed for real time detection of environmental microbes and continuous monitoring of the environment. This technology utilizes Mie scattering for airborne particle size measurement in the range of bacterial matters, and the detection of intrinsic UV-induced fluorescence from certain bio-chemicals inside bacterial cells as a biological marker. The optical instrumentation techniques used to achieve the requisite detection sensitivity and the procedure for its validation test will be presented.

One necessary aspect for applying this technology in contamination control is how to correlate its results to compendial microbiological methods and how to set the new limits based on this new method. A statistical process control (SPC) approach to the environmental monitoring of bioburden in parenteral aseptic facility is proposed to achieve this goal. This presentation will cover (1) the procedure of setting new limits using a new instrument, (2) the control chart methodology of utilizing environmental monitoring data to assess the state of control in a parenteral production environment and to troubleshoot the root cause when a variation from normal trend is detected.

Some application examples will be used to illustrate how to utilize the real time microbial detection features of this technology in the environmental monitoring and contamination control. Topics of discussion include sensor placement, data analysis, graphic data display, and trend analysis.

Key words: real time microbial detection, environmental monitoring, trend analysis.

1. Introduction

The compedial method for environmental monitoring in pharmaceutical manufacturing environments is growth media method. These methods require a number of days to elapse while samples, collected intermittently, are incubated. Advances in optical technology now promise both an instantaneous and continuous means for detecting airborne microbes.

In recent years, several of such rapid microbial methods have been developed for pharmaceutical contamination control applications. The examples of these real time optical microbial detectors are BioVigilant System's IMD™-A detector, Particle Measuring Systems' BioLaz and TSI's BioTrak. A typical optically based microbial detector instrument can instantaneously and continuously detect a particle's size and the laser induced auto-fluorescence from metabolite chemical compounds inside microbes (e.g. NADH, riboflavin and dipicolinic acid) as biological marker to differentiate microbes from inert dusts. As an example, the optical schematic of BioVigilant's IMD-A is shown in Fig. 1 below. The detection of microbes by IMD-A is done in real time as the target particles flow through the instrument.

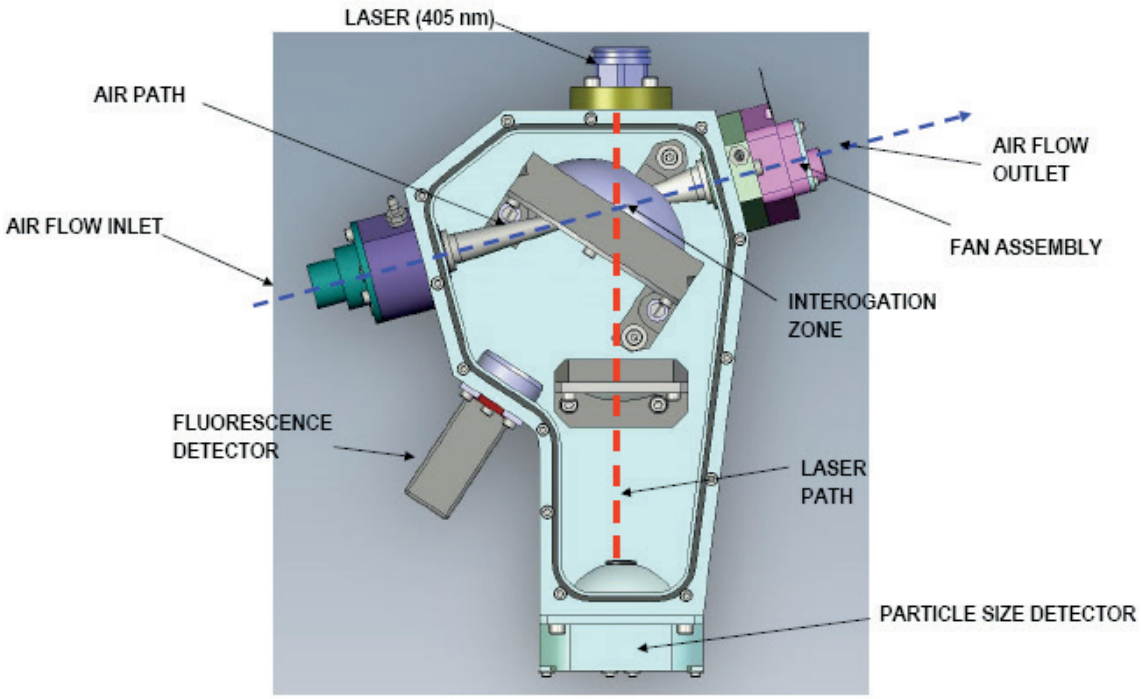


Fig. 1 Optical schematic of IMD-A microbial detector

As shown in Fig. 1, the environmental air is sampled through the instrument and the particles in the air flow will be interrogated by a laser: the size of the particle is measured by the intensity of the scattered laser light, and if the particle is a microbe the laser induced fluorescence will be measured and that particle is marked as biologic. All these measurements are done in real time and enable instantaneous detection of airborne microbes.

The real time detection feature of the optical microbial detectors could be beneficial for pharmaceutical contamination control and manufacturing process improvement. The author wishes to discuss several topics related to the implementation of real time microbial detector technology for such applications: (1) suggestion on how to set the new limits for the new method; (2) trend analysis using Statistical Process Control methodology; (3) utilizing the real time feature of optical detector to validate operating procedures in clean room; (4) as a convenient tool to verify and visualize the air flow in a clean room.

2. Setting new limits for optical detector

Since the optical spectroscopy based microbial detector technology differs significantly from growth media based compendial method, in order to implement the new microbial method it will be necessary to establish a procedure to set new limits for it. A suggestion is made here on the procedure of setting new limits for optical detector method. This suggested procedure is based on the methodology of Statistical Process Control (SPC), which was recommended by recent FDA guideline (FDA 2011). The basic premise of this procedure is that when the environment in a clean room is in a state of statistical control, the airborne particles and microbes are distributed randomly. Under such condition, two different instruments probing the same population of microbes can be correlated by statistical treatment. This correlation can then be used to set the new limits for the new method.

The procedure to set new limits for a new microbial detector is described as follows:

(1) Perform side-by-side comparison test of new method and compendial growth media method in the facility for intended implementation. This test shall be done while the test site is in normal working condition and in a state of control;
(2) Process the comparison test data to obtain statistical parameters such as means and standard deviations of the counts of new method and compendial method: X (mean count of the new method), □ (standard deviation of new method) and Y (mean count of the compendial method);
(3) Setting new limits:
    a. Alert level: it is an indicative parameter of whether the environment at that site is in a state of control. The mean count X and standard deviation □ of new method shall be used in the context of Statistical Process Control (SPC)'s Upper Control Limit: X + 3 □ and use it as Alert level (Hussong and Madsen 2004);
    b. Action level: it is mandated by regulatory requirement for the class of the clean room. Since the sensitivities of new method and compendial method may be different, a link between these two methods needs to be established. One way of establishing such a link is to calculate the ratio of the mean counts of the two methods X/Y. The Action level is then modified for the new method by (value of Action level) ×X/Y.

3. Trend analysis

The real time reporting feature of the optical microbial detector could be useful in doing trend analysis for process validation and improvement. The following example illustrates a practical application using a real time detector IMD-A during an aseptic filling operating.

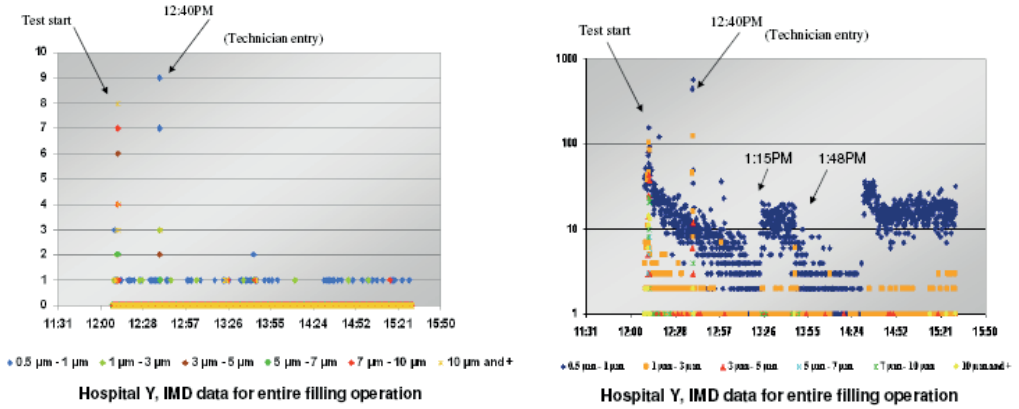


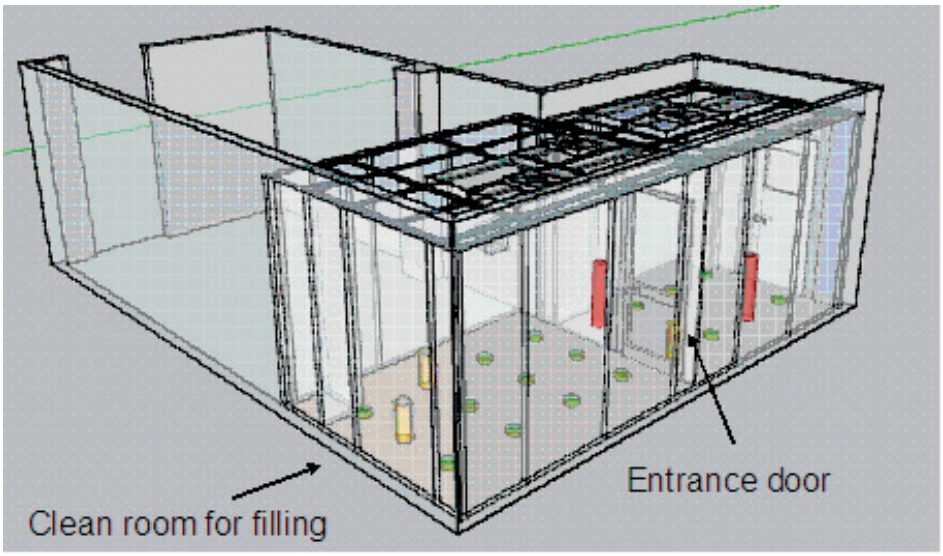
Fig. 2 IMD-A data during an aseptic filling operation in a hospital pharmacy.(Left) IMD microbial counts; (Right) IMD particle counts. Both are plotted against time of the filling operation.

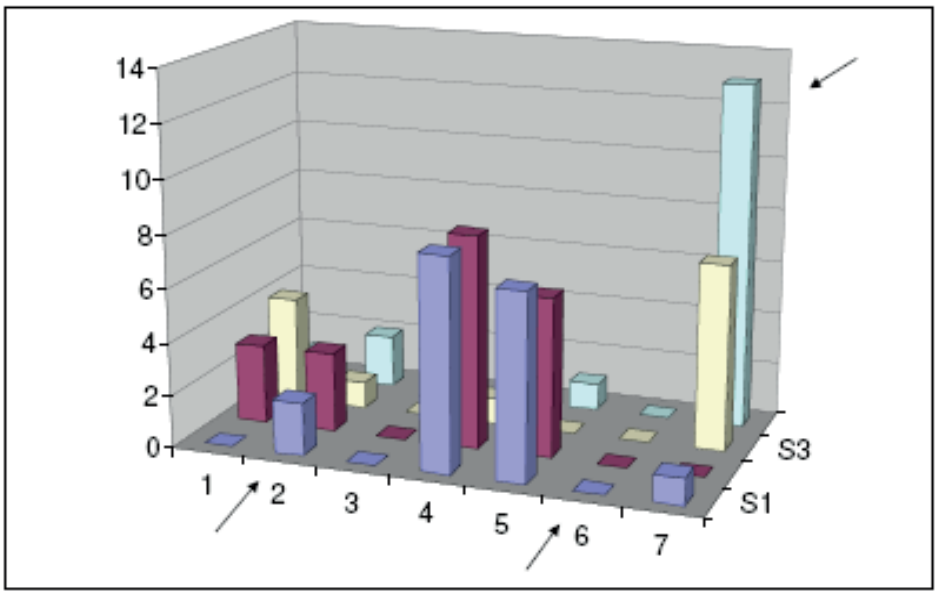
An IMD unit was used to monitor an aseptic filling operation in a hospital pharmacy. The filling was done in a clean room under a laminar flow hood. The probe of the IMD unit was placed inside the laminar flow hood during the entire filling operation (from 11:31 to 15:21). Figure 2 show time series data graphs from IMD monitoring of this filling operation. Several salient points are shown in these graphs: (a) in IMD microbial count graph (Fig. 2 (Left)), 2 sharp spikes are observed at 12:10 and 12:40. The first spike coincided with the start of test and setting up of IMD unit under the laminar flow hood, and the second spike appeared when the technician started the filling operation. It is well known that human operators accounts for the majority of microbial excursion inside clean rooms. The observations of this IMD monitoring are consistent with the human operator factor; (b) The IMD microbial counts

remained low during the rest of the filling operation. This indicated that the environment in that aseptic filling room was in a state of control and the technicians were following correct clean room procedures; (c) In a pharmaceutical filling operation, it is common that the operation itself will generate airborne particles, as shown in Fig. 2 (Right). It is comforting to notice that the increases of IMD particle counts was not accompanied by corresponding increase of microbial counts, indicating that those particles were inert ones generated from the liquid pharmaceutical drugs during filling operation.

4. Tools for validating clean room operation procedures

The ease of operation and real time data reporting of optical microbial detectors can also facilitate the validation of a clean room condition and an aseptic operation procedure. The immediacy and ease of operation of this type of detectors make it convenient to visualize the condition of a clean room and root cause analysis.





Company X Fill Room IMD Biologic Counts 09/09/2008: two hoods at (2, S1) and (6, S1); entrance door at (7, S3)

Fig. 3 Grid pattern graph of IMD survey of an aseptic filling room. (Upper) architecture drawing of the fill room; (Lower) IMD microbial count grid pattern data graph.

An example of this application is shown in Fig. 3. An IMD-A detector was used to survey a pharmaceutical clean room for bio-burden. The architecture layout of the clean room is shown in Fig. 3 (Upper), in which a single entrance door and ceiling mounted HEPA filter can be seen. The room was surveyed by the detector in a grid pattern and IMD microbial counts in each grid cell are depicted in Fig. 3 (Lower). In scrutinizing Fig. 3 (Lower), one notices that the microbial counts peaks at grid cell (7, S3) where the entrance door is located. This observation led to the conclusion that this room was not positively pressurized as required for a clean room. After this realization, the Ventilation in the room was adjusted to maintain positive pressure inside the room. After this adjustment, a follow-up survey indicated a much reduced bio-burden in the same room.

A real time microbial detector can also serve as a tool for visualization of air flow and microbial distribution in a pharmaceutical clean room (Ljungqvist and Reinmuller 2006). The visualization capability is a useful aid in troubleshooting and root cause analysis. As shown in example of Fig. 3, an examination of the IMD grid pattern data graph, the origin of the problem (leakage through entrance door) and its root cause (lack of positive pressure inside) quickly became apparent. This led to a timely remediation and elimination of the problem.

It is well known that the process and personnel inside clean room are the most likely sources of contamination. It is therefore essential to validate clean room procedures and personnel operating protocol. Real time microbial detector can be useful in validating clean room operating procedure (as shown in Fig. 2), as well as in clean room personnel training as a training aid (an instant display of a person's action by a detector is usually more convincing to the trainee than mere classroom instruction).

5. Conclusions

New real time microbial methods based on optical detection technology can be useful tools for contamination control in pharmaceutical manufacturing. A procedure for setting new limits is suggested here to facilitate implementation of a new method for monitoring pharmaceutical manufacturing operation. The real time detection capability of these new methods shall be beneficial in both demonstration of the control of environment and root cause analysis.

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