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An Overview of Biological Sample Collection and Concentration Solutions for Pharma Applications from InnovaPrep

Microbiological Monitoring for Environmental and Product Integrity

Regarding the recent revision to The United States Pharmacopoeia (USP) chapter <1116>, moving from measurement of microbial contamination based on conventional enumeration of colony forming units (CFUs) to Contamination Recovery Rates (values expressed in maximum allowed percentage of contaminated samples), the author suggests the changes will shift the industry to wide-spread use of Rapid Molecular Methods (RMMs). To fully realize the promise of RMMs, new collection and sample prep technologies will also need to be adopted. This white paper provides an overview of novel front-end technologies for microbiological monitoring for use with RMMs. The technologies apply to biological and particulate contamination monitoring in the environment as well as monitoring the integrity of ingredients, like water, intermediate products, such as cell culture media, and finished products, including parenteral fluids, and compounded drugs.

Biological Collection and Concentration Technologies for Microbial Monitoring

The United States Pharmacopoeia (USP) chapter <1116> Microbiological Control and Monitoring of Aseptic Processing Environments was recently revised. The revision proposes measurement of microbial contamination based on Contamination Recovery Rates (values expressed in maximum allowed percentage of contaminated samples), rather than the conventional enumeration of colony forming units (CFUs) currently endorsed by aseptic guidance. This opens the door for adoption of Rapid Molecular Methods (RMMs) to finally replace traditional outdated growth methods.

The revised guidance suggests this imminent shift in the following excerpt: "*An environmental control program should be capable of detecting an adverse drift in microbiological conditions in a* ***timely manner*** *that would allow for meaningful and effective corrective actions. It is the responsibility of the manufacturer to develop, initiate, implement, and document such a microbial monitoring program."* Current growth methods require 48-72 hours to detection. New available methods allow detection within the same shift/day.

The swift advancement of RMMs marks a dramatic transition for the industry. While rapid analytical methods are becoming an essential part of a complete monitoring program, widespread use of these technologies is long overdue. Cutting "time-to-detection" will provide widespread benefits through reduction in holding times, recalls, and incidence of harm. These are significant changes that will make pharmaceutical products safer and protect the industry from losses in revenue.

To fully realize the promise of RMMs, new collection and sample prep technologies will also need to be adopted. These must not add complexity. For example, new active air sampling methods will replace media-based methods, but it will be necessary that sample recovery be fast and simple to perform. Additionally, sample preparation for analysis with these new methods must also be quick, easy, and ideally, automated.

InnovaPrep® has developed innovative technologies for front-end collection and concentration of microorganisms from a wide range of sample types, including air, surfaces, and liquids. The company's 30 pending and awarded patents apply to a novel biological particle elution process termed Wet Foam Elution™ that greatly improves and widens the utility of new state-of-the-art analytical methods. These products apply to monitoring contamination in the environment as well as monitoring the integrity of ingredients, like water and liquid diluents, intermediate products, such as cell culture media, and finished products, including parenteral fluids, and compounded drugs.

The company began with biodefense applications and has become a leader in sample collection and concentration systems for the Department of Defense and large-scale defense systems

integrators. Since the commercialization of the company's flagship product, *The Concentrating Pipette,* its markets have expanded to include general-use microbiology, consumer product QA/QC, Life Sciences R&D, food and beverage process and safety monitoring, environmental monitoring, drinking water monitoring, clinical and veterinary research and most recently pharma QA/QC.

Sample Concentration Technology Overview:

Figure 1. Wet Foam Elution

InnovaPrep concentration products are based on the principle of capturing particles/organisms from relatively large liquid volumes onto a membrane filter and then recovering the particles/organisms into a comparatively small liquid volume using a process termed Wet Foam Elution, (WFE). WFE uses standard buffer solutions, such as phosphate buffered saline or Tris buffer along with a very low concentration of a surfactant, such as Tween 20, added as a weak foaming agent. A dissolved gas, such as carbon dioxide or nitrous oxide, is used to create the foam as the buffer solution is released onto the filter from a small canister. When dispensed over the filter, the fluid expands to six times its initial liquid volume and is rapidly swept over the membrane filter surface, recovering the target particles. The foam then quickly breaks down into a liquid, leaving a highly concentrated sample ready for subsequent sample preparation and analysis. An image of WFE during early R&D is provided above in Figure 1.

The unique properties of wet foam make it a superior method for recovery of particles from membrane filters when compared to elution with aqueous solutions. Expansion of the elution fluid to six times its original volume, coupled with a significant increase in the liquid viscosity, and other unique wet foam properties, enables concentration factors that often exceed those achieved with other approaches by two or even three orders of magnitude. The WFE method can be used not only to provide a substantial sample concentration factor, but also to allow the use of larger filter membrane surface areas – enabling the concentration of target organisms from difficult matrices.

Liquid Sample Concentration

Figure 2. Concentrating Pipette Select

The Concentrating Pipette Select is an automated, rapid micro-particle concentrator developed for general microbiology use. The system performs "mechanical enrichment" as a front-end to rapid microbial detection eliminating the need for centrifugation or culture enrichment steps.

As liquid samples are drawn into the single-use Concentrating Pipette Tips, particles are captured on an internal membrane filter. When the entire sample has been processed, the Wet Foam Elution process is triggered to efficiently recover the captured particles (bacteria, spores and vegetative bacteria, fungal spores, molds and yeasts, whole cells, viruses, or other particles) in a highly concentrated form. The one-pass method provides rapid automated sample volume reduction and simultaneous clean buffer exchange.

Single-use Concentrating Pipette Tips are available in a range of pore sizes and surface area configurations for a variety of applications. The system will concentrate volumes up to 3L to a final sample volume as low as 200 µL at speeds up to 200 mL per minute (depending on pore size and matrix), providing a concentration factor of up to 12,000X (at 80% efficiency) . The elution process is initiated by the press of a button and takes seconds. The concentrated sample is then ready for analysis with either RMMs such as qPCR, or classical culture methods if quantitation is required. The tip is then discarded and replaced for the next sample without a decontamination step.

Applications for the Concentrating Pipette within Aseptic Processing Environments include:

- Process water
- Parenteral fluids
- Bulk liquid drug substances
- Liquid intermediates
- Liquid excipients
- Surface samples from production areas and medical devices (see below)
- Medical device and or equipment rinses
- Air samples collected in a liquid
- Media

Mycoplasma

The use of cell culture in biopharma is growing extensively. In order to achieve reproducible results from cells, good cell culture conditions are vital. However, it is estimated that about 5% to 30% of the world's cell lines are contaminated with mycoplasmas. The lack of a cell wall in mycoplasmas makes them invisible to the naked eye. They are resistant to common antibiotics, and they cannot be detected visually by turbidity of the media or under the inverted microscope. Testing is extremely expensive and requires up to 2-3 weeks for a determination.

Concentrating media using the Concentrating Pipette has shown quick and excellent detection (less than 1 CFU per mL) of mycoplasma in culture media using the 0.05 um pipette tip, a DNA extraction kit, and qPCR.

Surface Sampling

InnovaPrep is currently developing systems and methods for rapid recovery of samples from surface swabs and wipes for large surface areas. The company has performed proof-of-concept testing for rapid recovery and concentration from surface wipes such as the Swiffer® brand wipes, followed by a rinse in a buffered solution to release the particles, and subsequent concentration on the Concentrating Pipette with superior results compared to common methods.

A new research article published in the ASM mSphere Journal by University of Wisconsin-Madison outlines an efficient method for collecting samples from large area surfaces. In summary, a pre-wetted 9in x 9in polyester wipe using sterile water was used to sample a 1-meter squared area. The wipe was then transferred to a to a 500-mL sterile bottle containing 200 mL of sterile PBS. The bottle with the wipe was shaken for 2 min followed by concentration with the Concentrating Pipette. To view the publication, please follow this link: http://msphere.asm.org/content/msph/1/5/e00227-16.full.pdf

Air Monitoring

The ACD-200 Bobcat Dry Filter Air Sampler with Rapid Filter Elution Kit has become the sampler of choice for Department of Defense applications, for its dry collection method, ease of sample recovery, portability, compatibility with rapid analytical methods, and excellent collection efficiency. InnovaPrep is now introducing the technology for Pharma microbiological monitoring. It is an improved method over current methods such as media-based systems, for its active sampling mode and ability to be paired with RMMs such as qPCR for same-shift results without incubation.

Figure 3 Collector deployed (left) and with inlet open to recover filter for elution (right)

The ACD-200 Bobcat (Figure 3) has been developed to address a broad range of air sampling requirements. It is ideally suited for the collection of bioaerosols and particulate matter; including submicron-sized particles, airborne molecular contamination, and particulates. It can be custom configured to be triggered by other systems, or it can be operated as a stand-alone unit. Operational modes include pre-defined single sample collection, externally triggered collection, continuous sampling, and programmed intermittent sampling for long-term monitoring. The unit can be operated using an internal rechargeable battery or plugin (110/220 V 50-60Hz).

The Bobcat collector is light, about the size of a large flashlight, has an internal battery, and built-in tripod. It collects at up to 200 liters per minute onto a dry 52 mm electret filter. Electret filters are produced from non-woven dielectric polymer fibers that are made with a combination of positively charged and negatively charged fibers. This substantially increases the collection efficiency of the filter for any potential contaminant including bacteria spores and vegetative bacteria, fungal spores, vegetative molds and yeasts, whole cells, viruses, or other particles.

Figure 4 Rapid Filter Elution Kit

Following aerosol collection, the Filter Cassette is removed from the Collector, capped on one side, then snapped onto the Sample Cup. This provides a primary container for transport. To extract the captured particles from the filter, a Canister containing the elution foam is pressed to a fitting on the Elutor Cap (Figure 4). The elution foam is released from the Elution Canister evenly through the filter. The wet foam passes through the interstitial spaces of the filter to extract captured particles. Sample elution takes seconds and produces 6 to 7 mL of liquid sample. Within seconds, the foam collapses back to a liquid in the Sample Cup, available for sample processing and analysis using RMMs.

To reduce the analytical workload, samples can be combined. Pooled samples can be quickly concentrated using the Concentrating Pipette, then analyzed. If a pool is determined to be contaminated, the aliquots can then be analyzed to determine the specific sample that is contaminated.

Conclusion

The revised USP <1116> guidance states, "*An environmental control program should be capable of detecting an adverse drift in microbiological conditions in a* ***timely manner*** *that would allow for meaningful and effective corrective actions. It is the responsibility of the manufacturer to develop, initiate, implement, and document such a microbial monitoring program."* Current growth methods require 48-72 hours to detection. The above methods allow contamination detection within the same day for immediate corrective action. These may include on-the-spot corrective personnel training, detecting an external source of contamination, or pulling a defective product from the line before it's packaged.

Is your lab up to speed?