Microbial Monitoring of Air, Surfaces, and Liquids in Food Processing Environments

Microbial contamination primarily enters a food processing facility by being carried in on the air, on humans, on product, or equipment. Microorganisms often spread through touch and through the air when aerosolized droplets are created while washing equipment or food product. Aerosolization can also occur simply by the operation of equipment as pockets of contamination are loosened and dispersed. The contaminated droplets eventually settle onto surfaces where they can again be spread by contact. An effective monitoring program should thus include frequent air, surface, and liquids sampling. InnovaPrep has developed rapid sample collection and concentration tools that can dramatically help improve your level of detection in air, surface, and liquid samples when paired with Rapid Microbial Methods (RMM's) for a comprehensive, yet streamlined monitoring program.

It is the food processors responsibility to develop and implement an effective environmental microbial monitoring program for contamination control. A monitoring program should be capable of detecting an adverse drift in microbiological conditions quickly so that meaningful and effective corrective actions can be implemented to mitigate the problem.

Contamination enters a processing facility by any number of ways, most commonly from being carried in on humans, on product, on equipment, or on outdoor air entering the facility. Microorganisms often spread through the air when aerosolized droplets are created when washing equipment or food product, or even by the operation of equipment. These contaminated droplets then settle onto surfaces where they can again be spread by contact. An effective and comprehensive monitoring program should thus include frequent air, surface, and liquids sampling.

The swift advancement of Rapid Microbial Methods (RMM's) such as qPCR marks a dramatic transition for streamlined sampling in the industry. Cutting time-to-detection provides huge benefits through reduction in holding times, recalls, incidence of harm and losses in revenue.

While RMM's are becoming an essential part of a complete monitoring program, widespread use of these technologies is long overdue. The lag is likely due to the fact that the sample collection and sample preparation required for RMM's requires a growth step to reach the limit of detection for trace contamination. Current growth methods require 48-72 hours making "rapid detection" seem less awesome. Further, growth methods do not address the viable-but-not-culturable (VBNC) microbes and spores that also pose a threat.

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To fully realize the promise of RMM's, new collection and sample prep technologies will also need to be adopted - but these must not add complexity. For example, new active air sampling methods will replace agar-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.

About InnovaPrep

InnovaPrep technologies address this need for rapid collection and concentration of air, surface, and liquid samples as a front end to RMM's for a truly *rapid* sampling program. These tools increase sensitivity and enable a faster, easier, and more efficient means of delivering a highly concentrated sample for subsequent analysis using RMM's thus providing sample to answer within minutes to hours without a need for a growth step. The company's 21 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.

InnovaPrep originally got its start with biosurveillance applications, quickly becoming a leader for 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 environmental monitoring, drinking water, and industrial microbiology as well as myriad research applications.

InnovaPrep Technology Overview:

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. This is an improved method for microbial monitoring in food processing environments compared to current methods such as agar based systems. Its active sampling mode and ability to be paired with RMM's such as qPCR for same-shift results without incubation will improve any contamination monitoring program.

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

The ACD-200 Bobcat (left) 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).

The Bobcat collector is light, about the size of a large flashlight, has an internal battery, and built-in tripod. It collects up to 200 liters per minute onto a dry 52 mm electret filter.

Electret filters are produced from non-woven dielectric polymer fibers that hold inherent positive and negative electrical charges. 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.

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 (as pictured to the right). 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 RMM's.

Rapid Filter Elution Kit

To reduce the analytical workload, samples can be split between an aliquot and a pool. Pooled samples can be quickly concentrated using the *Concentrating Pipette* shown below.

Liquid Sample Concentration

Concentrating Pipette Select™

The *Concentrating Pipette* is an automated, rapid bio- concentration device for modern sample preparation. The system replaces the older methods including centrifugation and enrichment as a front-end to microbial detection. It is highly efficient and effective for bacteria, parasites, molds, fungal spores and fragments, whole cells and viruses.

The one-pass method works by filtration through its high- flow single-use pipette tips to remove microorganisms from the fluid sample matrix. Once the microorganisms are captured, the instant and automated wet foam elution process recovers and delivers the microorganisms into a microliter volume of clean buffer ready for analysis by modern or classical methods. The one-pass method provides rapid automated sample volume reduction and simultaneous clean buffer exchange.

Single-use 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 3 Liters 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 exponential concentration factors. The elution process is initiated by the press of a button and takes seconds. The concentrated sample is then ready for analysis with either

RMM's such as qPCR or classical culture methods if quantitation is required. The tip is then discarded and replaced for the next sample without no decontamination step required.

Applications for the Concentrating Pipette within food Processing Environments include:

- Process water
- Vat rinses
- Equipment rinses
- Produce wash water
- Surface samples collected in a liquid
- Air samples collected into a liquid

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 into 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.

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.

Surface Sampling

A new research article published in the ASM mSphere Journal by University of Wisconsin—Madison and the Biotechnology and Planetary Protection Group, at the NASA Jet Propulsion Laboratory describes Microbial Observatory Experiments on the International Space Station (ISS) using a method of recovering surface samples that included the InnovaPrep Concentrating Pipette.

The study outlines an efficient method for collecting samples from large area surfaces. In summary, a pre-wetted 9"x9" 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 InnovaPrep Concentrating Pipette. To view the publication, please follow this link: http://msphere.asm.org/content/msph/1/5/e00227-16.full.pdf

Current common surface sampling methods usually involve small swabs that are placed in a culture media. These methods are generally inadequate not only in recovery efficiency but because of the limitation of sampling such small surface areas. A wipe + concentration method is much more effective for large surface areas than general swabbing and the concentration step means detection limits are significantly improved. The method is effective for almost any surface in a food processing environment.

(InnovaPrep is currently developing systems and methods for rapid recovery of samples for large surface areas.)

Conclusion

An environmental control program should be capable of detecting an adverse drift in microbiological conditions in a timely manner and 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.

Is your lab up to speed?