Modern Microbial Method Support of a Contamination Control Strategy

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Agenda

- M^3 Collaboration Industry Working Group Overview
- Modern Microbial Methods (MMM)
- MMM Support of a Contamination Control Strategy (CCS)
- Bio-Fluorescent Particle Counter (BFPC) Example

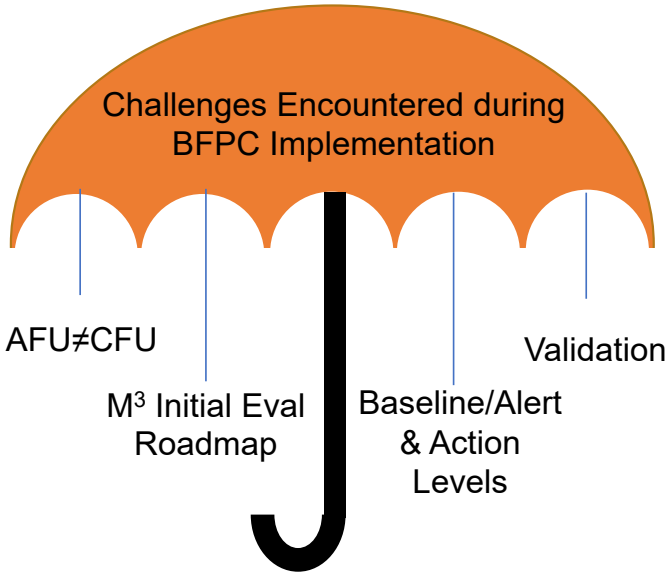
M^3 Modern Microbial Methods Collaboration

Mission – to support the implementation and use of modern microbial method technologies within the pharmaceutical and related industries

M^3 Collaboration – Est. 2021

Sub-team #1	• Bio-Fluorescent Particle Counter (BFPC) Validation, non-equivalence, challenges
Sub-team #2	• Establishing a BFPC baseline and setting alert/action levels
Sub-team #3	• Modern Microbial Method evaluation and implementation toolbox
Sub-team #4	• Air BFPC implementation

M³ Collaboration - Publications



Modern Microbial Methods Supporting a Contamination Control Strategy

by Allison Scott, Particle Measuring Systems, et al. PUBLISHED ON Jan 8, 2025

[Author's note: The authors are part of a collaboration of industry working groups that joined forces in 2021 to support the awareness and adoption of modern microbial methods. These groups include the BioPhorum Operations Group, the Kilmer Community Rapid Microbiology Methods group, the Online Water Bioburden Analyzer working group and the Process and Environmental Monitoring Methods (PEMM) working group.]

The *EU GMP Annex 1: Manufacture of Sterile Medicinal Products* addresses the manufacture of sterile medicinal products and includes the requirement for a documented contamination control strategy (CCS) (1).

At a high level, the CCS should outline the scientific evidence leveraged to support the prevention and detection control measures that enable successful aseptic manufacturing. While concepts related to contamination control are not new to the industry, the emphasis on using modern microbial methods is a new addition to the guidance. Annex 1 now underscores the importance of using validated and reliable methods for monitoring and controlling microbiological contamination in sterile product manufacturing. This article outlines the types of modern methods currently available and where they can be implemented to align with the principles outlined in Annex 1 to support the quality and safety of sterile medicinal products.

Background

The term modern microbial method (MMM) is used to describe a method that is an alternative to or an enhancement of the compendial agar-based method. Other similar terms used to describe such methods are rapid microbiological methods and alternative methods, as used in Annex 1 (1). These methods can offer advantages over the compendial method, including but not limited to a shorter time to detection, real-time reporting of results, continuous monitoring, higher sensitivity and a lower false negative rate (e.g., due to detection of viable but not culturable (VBNC)) (2). Such advantages can be used to better support the detection of contamination and its prevention through a better understanding of the environment than intermittent sampling with the compendial method might provide.

MMM includes technologies based on the use of intrinsic fluorescence, extrinsic fluorescence (e.g., viability staining), bioluminescence, enzyme indicators, Raman spectroscopy, flow cytometry, solid phase cytometry, polymerase chain reaction (PCR) and automated colony detection and counting. Although described as modern compared to a method that has been used for over a century, many of these alternative methods are based on technologies that have been used for decades.

The CCS elements discussed in Annex 1 include the design of both the facility and manufacturing process, premises and equipment, personnel, utilities, and raw material controls - including in-process controls, product containers and closures, vendor approval, management of outsourced activities, process validation, validation of sterilization processes, preventive maintenance, cleaning and disinfection, monitoring systems (including alternative methods), prevention mechanisms and continuous improvement (1). As it is intended in this article to communicate the elements of a CCS that MMMs can support, the CCS elements from Annex 1 have been combined into the following four categories with investigational tools having application to all:

www.modernmicrobialmethods.com modernmicrobialmethods@gmail.com www.linkedin.com/company/modern-microbial-methods/

Scott, A et al. Modern Microbial Methods Supporting a Contamination Control Strategy. PDA Letter (2025).

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Modern Microbial Methods (MMM)

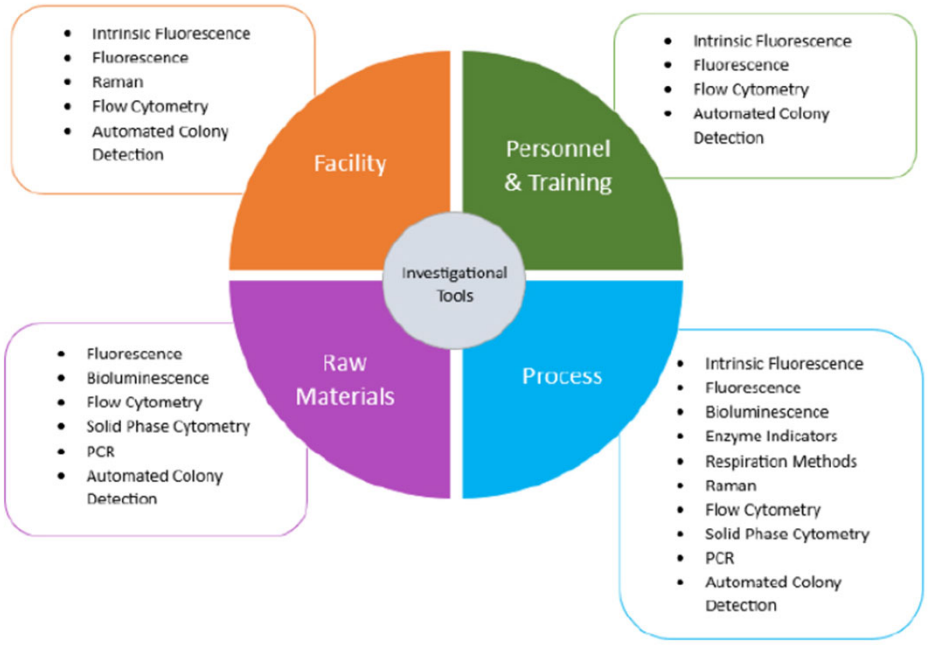
- Can include technologies based on the use of
  - Intrinsic fluorescence
  - Extrinsic fluorescence
  - Bioluminescence
  - Enzyme indicators
  - Respiration methods
  - Raman spectroscopy
  - Flow cytometry
  - Solid phase cytometry
  - PCR
  - Automated colony detection & counting

Article Goal – Highlight MMM currently available and where they could be used to support elements of a Contamination Control Strategy (CCS)

Contamination Control Strategy

- Annex 1 – *A planned set of controls for microorganisms, endotoxin/pyrogen and particles, derived from current product and process understanding that assures process performance and product quality.*
  - Includes 16 CCS elements for consideration
- Five elements were derived from the 16 mentioned in Annex 1
  - **Facility** (includes premises and equipment, utilities, and environmental monitoring)
  - **Personnel and training**
  - **Raw materials** (includes raw materials controls and product containers and closures)
  - **Process** (includes process controls, process validation, validation of in-process sterilization, preventative maintenance, cleaning and disinfection)
  - **Investigational tools** (prevention mechanisms)

MMM Support of a CCS



- MMM can offer advantages over the traditional method
  - Shorter time to detection
  - Real or near real-time reporting of results
  - Continuous monitoring
  - Automation
  - Higher sensitivity
- Potential limitations
  - Destructive technique
  - Specialized equipment
  - Challenging validation/implementation
  - Limit of detection

Scott, A et al. Modern Microbial Methods Supporting a Contamination Control Strategy. PDA Letter (2025).

MMM Assessment and Selection

Step	Assessment
Initial Technology Assessment	Company goal and need alignment, applications, ease of implementation
Technical Considerations	Technology capabilities, limitations, validation review, data review
Data and Compliance Risk	Connectivity, data retrieval, *21 CFR Part 11*
Cost Considerations	Initial and long term
Instrument Evaluation	Overall assessment of instrument for application(s)

Fluorescence Based Detection

Fluorescence – luminescence that is caused by the absorption of radiation at one wavelength followed by nearly immediate reradiation usually at a different wavelength (Merriam-Webster)

- Intrinsic Fluorescence – naturally occurring fluorophores within an object

Photo: https://commons.wikimedia.org/wiki/File:Sorpion_Under_Blacklight.jpg

- Extrinsic Fluorescence – external fluorophores or dyes added to an object

Photo: Meow Wolf/Facebook

Bio-Fluorescent Particle Counters (BFPCs)

- Detect and count microorganisms in real or near-real time
- Use light scatter and fluorescence to enumerate microorganisms in an air or water environment (non-growth based)
- Fluorescence can be either
  - Intrinsic – excitation of molecules already present in the cell

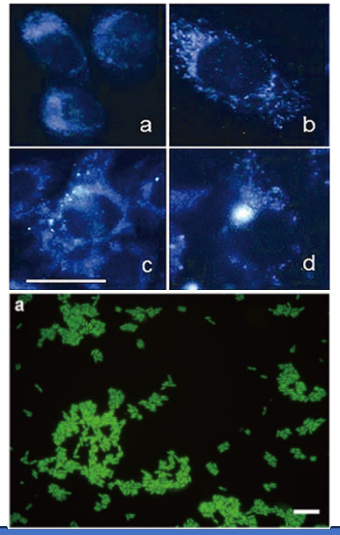
Intrinsic molecules[1]
(e.g., NAD(P)H, riboflavin)

→ Light Excitation

  - Extrinsic – reaction with applied stains/dyes

Added stains/dyes[2]
(e.g., SYBR Green, Propidium Iodide)

→ Light Excitation



[1]Croce, A. Light and Autofluorescence, Multitasking Features in Living Organisms. Photochem. 1(2), 67-124 (2021).
[2]Morono, Y. Accessing the energy-limited and sparsely populated deep biosphere: achievements and ongoing challenges of available technologies. Progress in Earth and Planetary Science. 10 (2023).

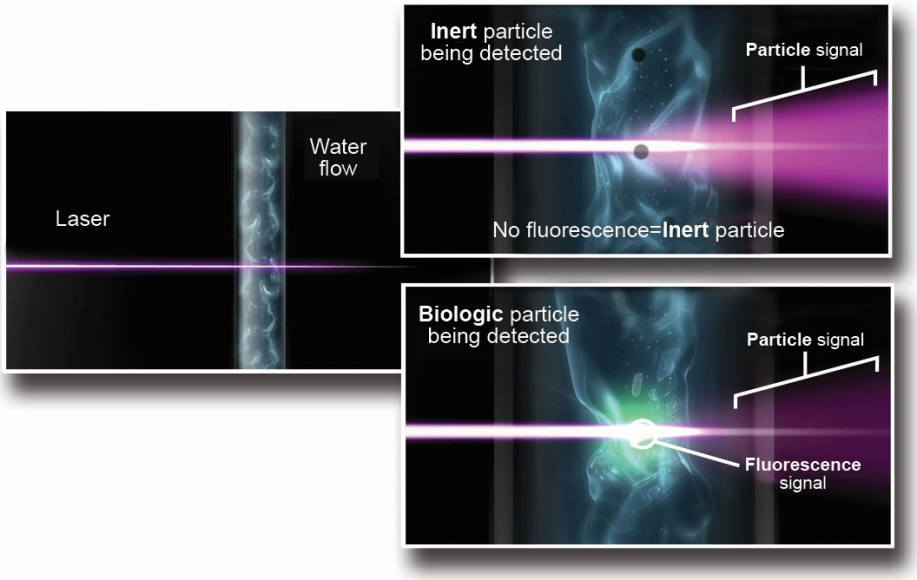
Same Analyte, Different Signals

CFU ≠ AFU ≠ ICC

- Colony-forming unit (CFU) is a unit used to estimate the number of viable and culturable bacteria or fungal cells in a sample
- Auto-Fluorescent Unit (AFU) is a unit that reflects both size and fluorescence of the particle that can detect viable but non-culturable cells in a sample
- Intact Cell Count (ICC) is a unit that reflects fluorescence emitted by intact cells that can detect viable but non-culturable cells in a sample

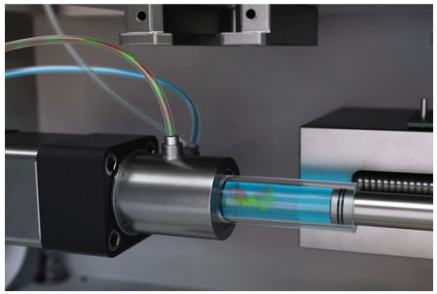
BFPC

Laser Induced Fluorescence (LIF)

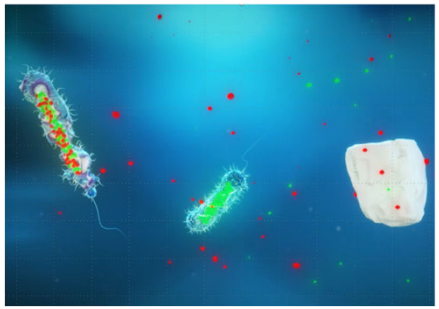


Flow Cytometry

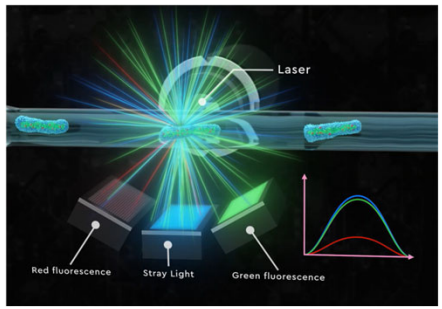
Staining



Incubation



Counting



Air Monitoring

- Facility
  - Environmental monitoring
  - Compressed gases

Continuous EM and robotic sampling

MicronView BAMS Robot Brochure v1.1.

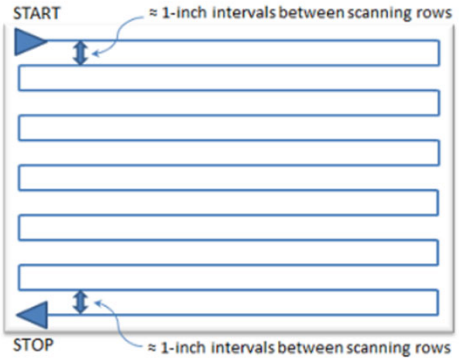
- Personnel and Training
  - Aseptic technique
  - Gowning

Real-time training feedback

Eaton, T. et al. Use of a Real-Time Microbial Air Sampler for Operational Cleanroom Monitoring. PDA J Pharm Sci and Tech 2014: 68, 172-184.

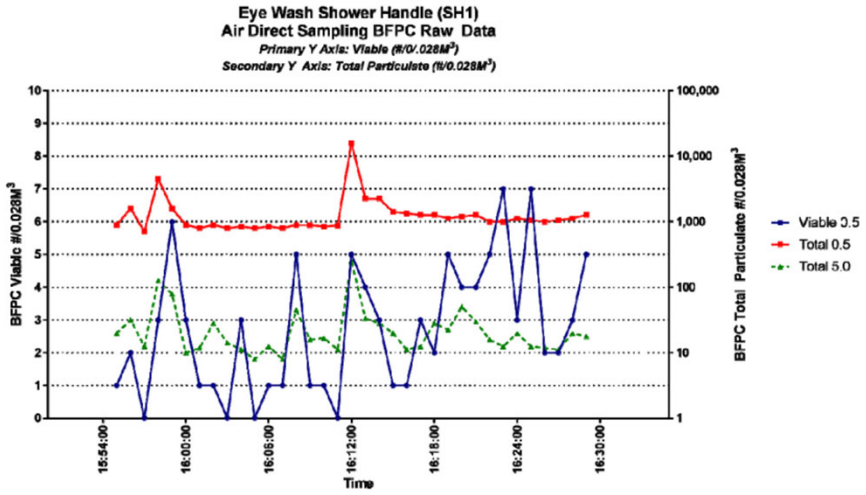
Air Monitoring

- Process
  - Preventative maintenance
  - Cleaning and disinfection



HEPA filter integrity testing

- Investigational Tool
  - Root cause assessment



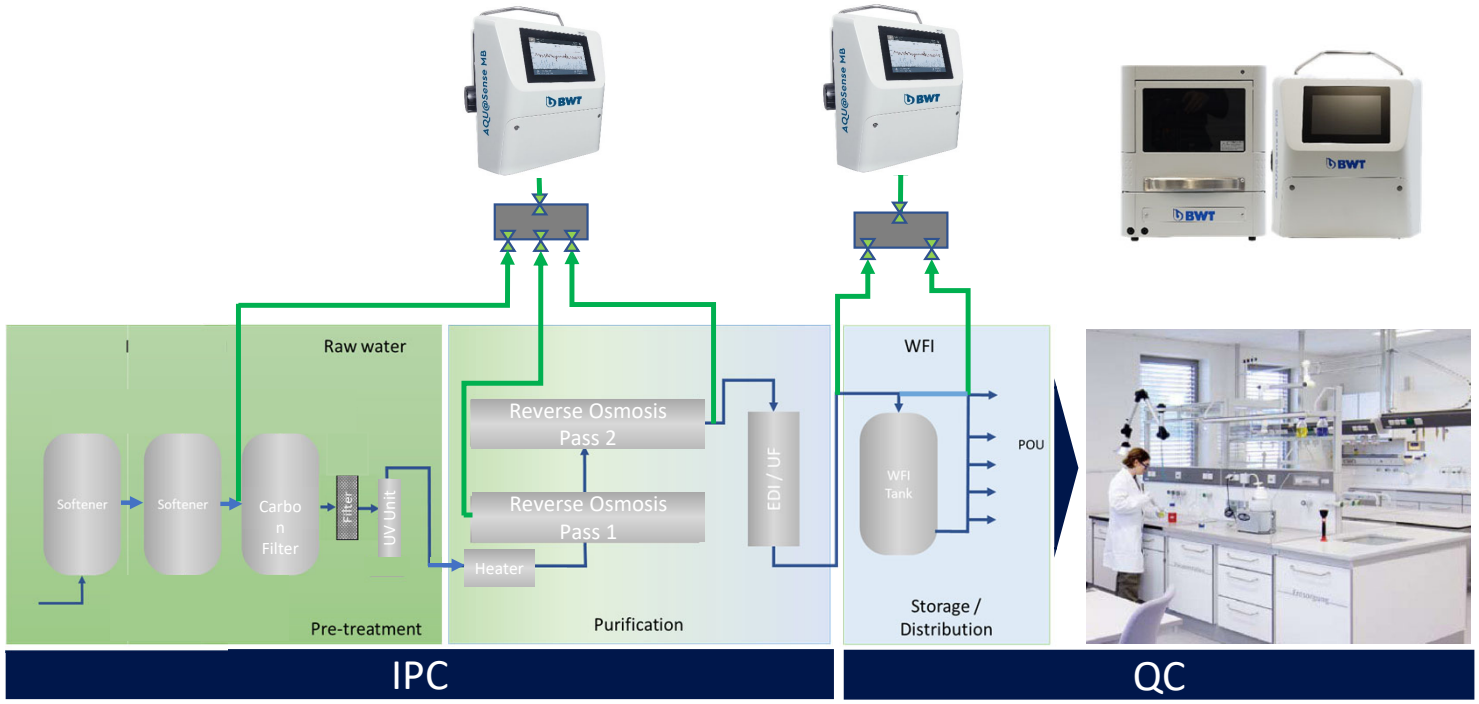
Identification of a contamination source

Montenegro-Alvarado, J. M. et al. Pfizer Case Study: Rapid Microbial Methods for Manufacturing Recovery After Hurricane Maria. Pharmaceutical Online (2018)

Prasad, A. et al. Practical Applications of Biofluorescent Particle Counting in Environmental Monitoring Investigations. PDA J Pharm Sci and Tech 2020: 74, 318-323.

Water/Liquid Monitoring

- Facility
  - Water system monitoring
- Raw Materials
  - Manual sampling
- Process
  - Preventative maintenance
  - Sanitization
- Investigational Tool



Summary

- There are a variety of modern microbial method technologies available today
  - Technologies may be able to support multiple elements of a CCS
- Important to evaluate company needs and goals along with technology capabilities and limitations
- Industry support is available as you evaluate and use new technologies

Thanks to the M³ Collaboration Team

Amanda McFarland
Ambili Menon
Arundhati Samanta
Caroline Dreyer
Cedric Joossen
Chris Knutsen
Cynthia Martindale
Hans-Joachim Anders
James Hauschild
Jess MacGregor
Jim Cannon
Joanny Salvas
Jon Kallay
Julia Geith
Kim Perkins
Lynn Johnson
Margit Franz-Riethdorf
Meghan Provenzano
Miriam Guest
Mike Dingle
Mike Russ
Nina Moreno
Olivia Venhuizen
Patrick Hutchins
Petra Merker
Conor Murray
Phil Villari
Pieta IJzerman-Boon
Sebastian Strandberg Rutell
Steffi Matthyssen
Stephanie Ramsey
Timothy Cser
Tim Emmerzaal
Tony Cundell
Ulrik Lytken Schack
Victoria Navarro Lahoz

Thank you!

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