Microbial Contamination and Control Conference

Modern Microbial Method Support of a Contamination Control Strategy

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Agenda

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

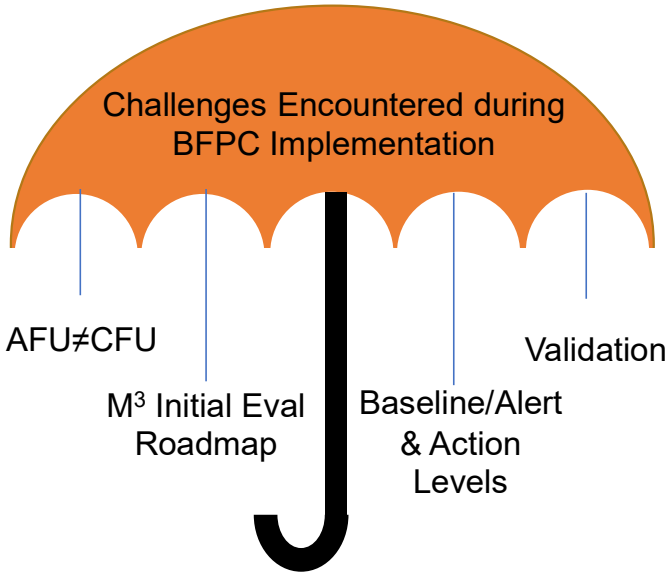
M³ Modern Microbial Methods Collaboration

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

M³ Collaboration - Publications



Modern Microbial Methods Supporting a Contamination Control Strategy

by Allison Scott, Particle Measuring Systems, et al. Published on Jan 9, 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

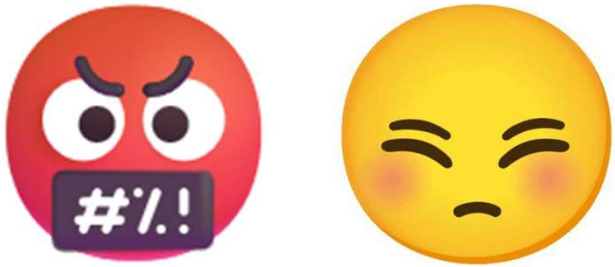
The term modern microbial method (MMM) is used to describe a method that is an alternative to or an enhancement of the compendial agar-based method. Other similar terms used to describe such methods are rapid microbiological methods and alternative methods, as used in Annex 1 (1). These methods can offer 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).



IMDB Image – Doris Day and Rock Hudson in Pillow Talk (1959)

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)

Rapid (RMM) or Modern (MMM) Microbial Methods

RMM - A method that provides a faster time-to-result than traditional methods

MMM - A method that is an alternative to or an improvement upon the traditional agar-based method

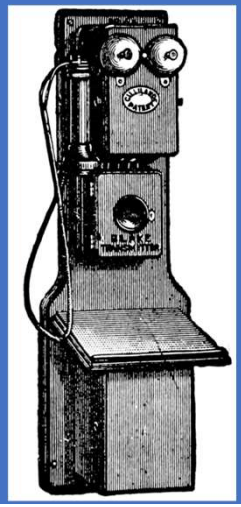


Photo File:Bicho-preguiça 3.jpg - Wikimedia Commons
Photo File:Florida Box Turtle Digon3.jpg – Wikipedia

MMM

Technology	High Level Description
Intrinsic Fluorescence	Use of light to induce fluorescence from molecules and metabolites (e.g., NADPH) already present in a cell
Extrinsic Fluorescence	Use of light to excite stains or fluorophores added to a cell
Bioluminescence	Qualitative and/or quantitative assessment of ATP, a marker of cell viability, based on luciferin-luciferase cascade emitted light
Enzyme Indicators	Compounds or methods used to detect or measure enzyme activity (e.g., colorimetric or fluorescent indicators)
Respiration Methods	Detection of an increase in CO_2 or decrease in O_2 as an indication of microbial growth (e.g., gaseous headspace analyzers, colorimetric or fluorometric sensors)

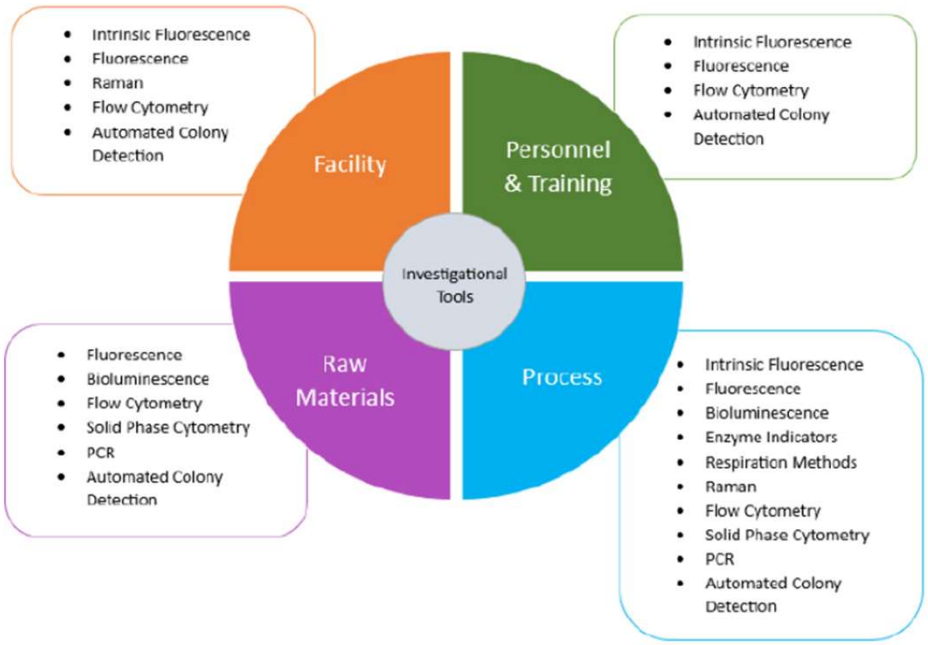
MMM

Technology	High Level Description
Raman Spectroscopy	Use of light to interact with chemical bonds in a sample to produce a spectrum characteristic of each sample/particle
Flow Cytometry	Use of light to induce scatter and fluorescence in individual fluorescently-labelled microorganisms in a water stream
Solid Phase Cytometry	Collection of a sample on a filter, sample staining, and viable cell enumeration using a laser or imaging system
PCR	Nucleic acid amplification-based technology for microbial detection and identification
Automated Colony Detection and Counting	CFU enumeration through detection of intrinsic fluorescence and/or growth using optics/camera

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 o fin-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

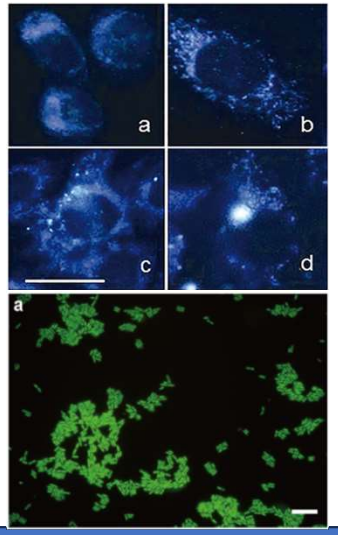
Intrinsic molecules[1]
(e.g., NAD(P)H, riboflavin)

→ Light Excitation

  - Extrinsic – reaction with applied stains/dyes

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

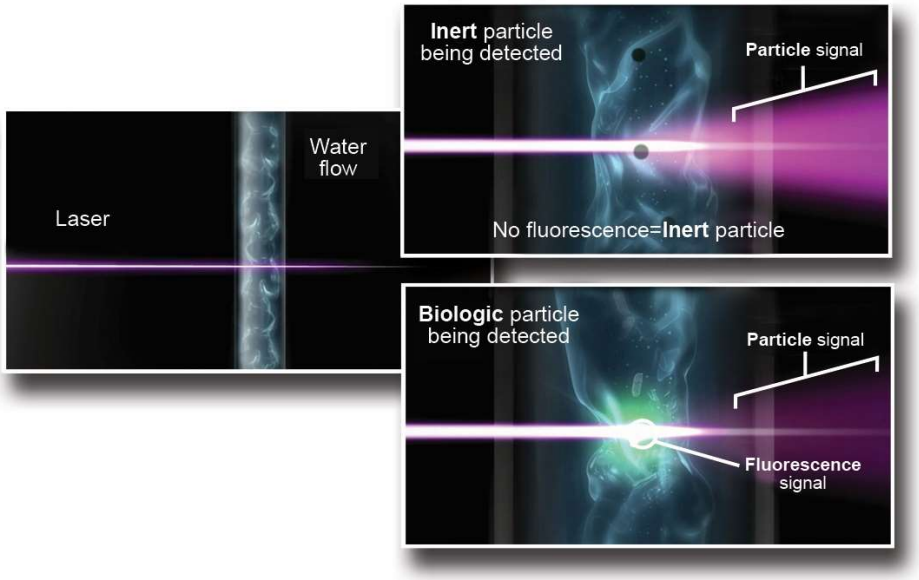
→ Light Excitation



[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).

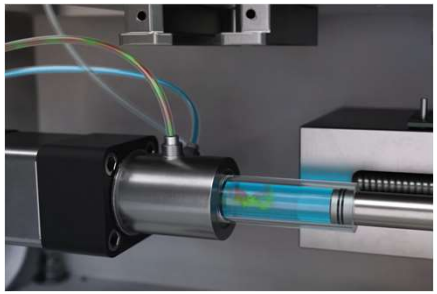
BFPC

Laser Induced Fluorescence (LIF)

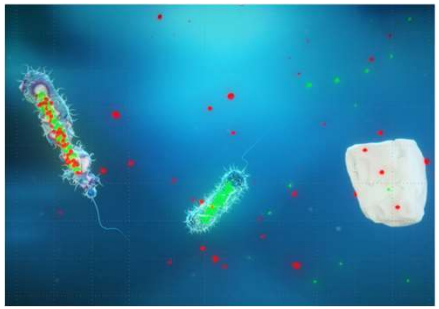


Flow Cytometry

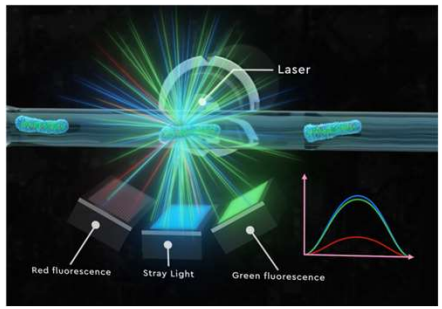
Staining



Incubation



Counting



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 nut non-culturable cells in a sample

Air Monitoring

- Facility
  - Environmental monitoring
  - Compressed gases

Continuous EM and robotic sampling

MicronView BAMS Robot Brochure v1.1.

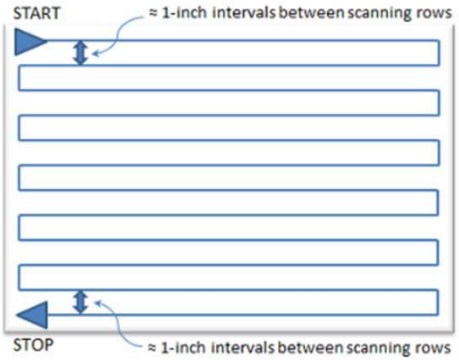
- Personnel and Training
  - Aseptic technique
  - Gowning

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

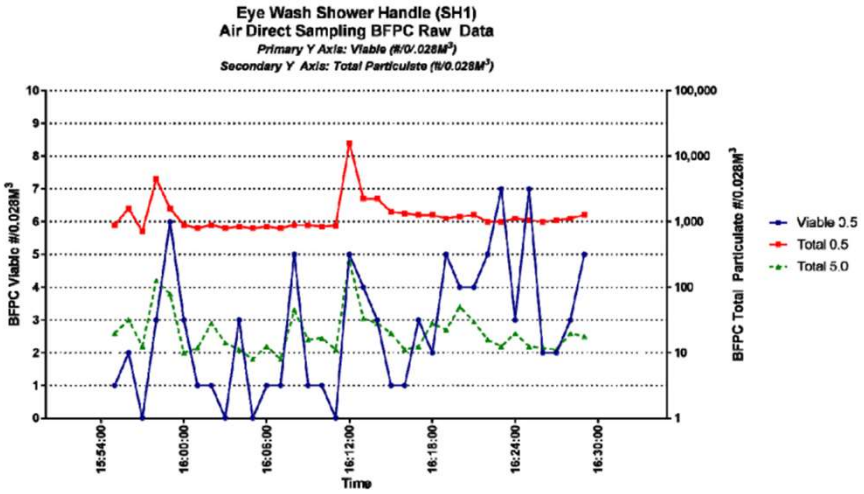
- Process
  - Preventative maintenance
  - Cleaning and disinfection



HEPA filter integrity testing

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

- Investigational Tool
  - Root cause assessment

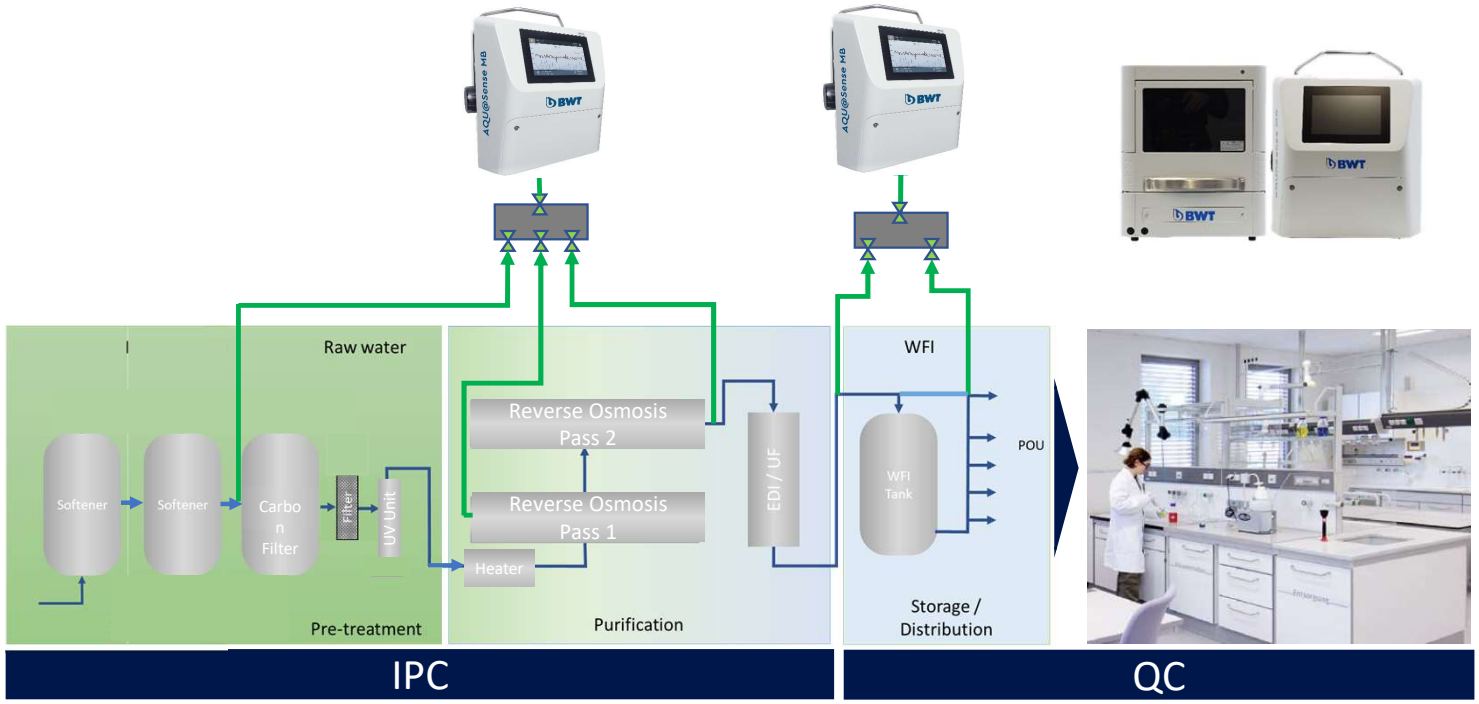


Identification of a contamination source

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
  - Continuous water system monitoring
- Raw Materials
  - Manual sampling
- Process
  - Preventative maintenance
  - Sanitization
- Investigational Tool



WFI Loop – Continuous Monitoring and Sanitization

- Objectives
  - Understand the AFU trend with water system hydraulic events
  - Evaluate microbial response to heat sanitization
  - Assess elevation of bioburden due to water system microbial contamination
- Concluded that intrinsic fluorescence based BFPC provided greater visibility and real-time insight than CFU alone.

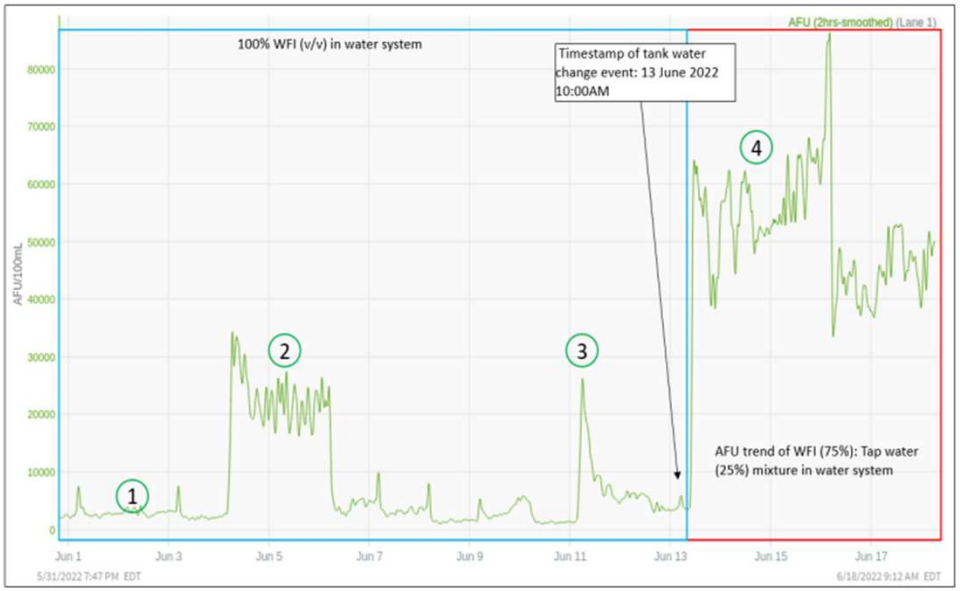


Figure 6: AFU/100mL: 1 June - 18 June 2022.

1. The baseline of the water system: 2600 AFU/100mL
2. Microbial content removed from heat sanitization: 22000 AFU/100mL
3. Microbial content removed from heat sanitization: 12500 AFU/100mL
4. Average AFU due to the microbial excursion: 51222 AFU/100mL.

Enhanced Microbial Monitoring with an On-line Bioburden Analyzer. Stilmas Case Study. Mettler Toledo , 2022. https://optimalbiotech.com/wp-content/uploads/2023/04/Case-Study-on-7000-RMS.pdf

Dialysis Water – Monitoring and Investigation

- Four locations monitored
  - SP1- End of pre-treatment, after carbon filters
  - SP2 – Primary treatment (after first RO)
  - SP3 – Secondary treatment (after second RO, dialysis water supply)
  - SP4 – Distribution system return
- Concluded flow cytometry based BFPC was a much faster and more sensitive method than HPC, allowing faster corrective action

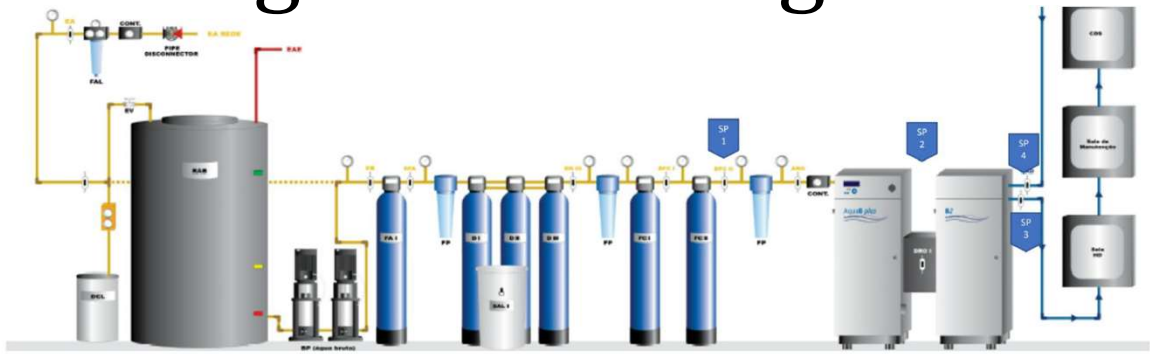


Fig. 1. Dialysis water treatment system and reference sampling points (drawing generated in Adobe Illustrator 2025, version 29.0.1; https://www.adobe.com/uk/products/illustrator.html).

Item	HPC (pour plate method / filtration method)	FCM
Sample preparation	Requires a specialized technician and samples must be transported under refrigerated conditions to the laboratory	Sample preparation is usually not needed and no refrigeration step if measurements are done onsite
Incubation time	Typically, 168 h	Typically, 30 min
Accuracy & reproducibility	Not all viable cells will grow on cultivation media (VBNC)	High level of information, accuracy & reproducibility
Normative frame	HPC remains the key variable in assessing microbiological quality of dialysis fluids (e.g., ISO standards)	Still to be considered in some standards (i.e., requires a validation)
Cost perspective	Lower investment, higher running costs	Higher investment, lower running costs

Table 2. Comparison of typical advantages and disadvantages of HPC and FCM methods.

Lucena, R. et al. Comparison of flow cytometry and heterotrophic plate count methods for dialysis water microbial monitoring. Nature Sicentific Reports (2025) 15:12809

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 new technologies

Thanks to the M³ Collaboration Team

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