



AQU@Sense MB

Online Water Bioburden Analyzer

2025

Challenges of Traditional Methods

Plate count method takes up to 5 days, causing delays in release.



“Only 0.1-1% of microbial populations are detected, leaving potential risks hidden.”
<USP 1223>



Manual processes increase the chance of errors and do not meet modern data integrity standards.



What an Online Water Bioburden Analyzer provides

Plate count method takes up to 5 days, causing delays in release.



Fast results



"Only 0.1-1% of microbial populations are detected, leaving potential risks hidden."
<USP 1223>



Accurate quantification of bioburden levels



Manual processes increase the chance of errors and do not meet modern data integrity standards.



Automation



Fast results



**Accurate quantification of
bioburden levels**



Automation



Early Warning

Detects water quality **trends** before they
become critical issues.



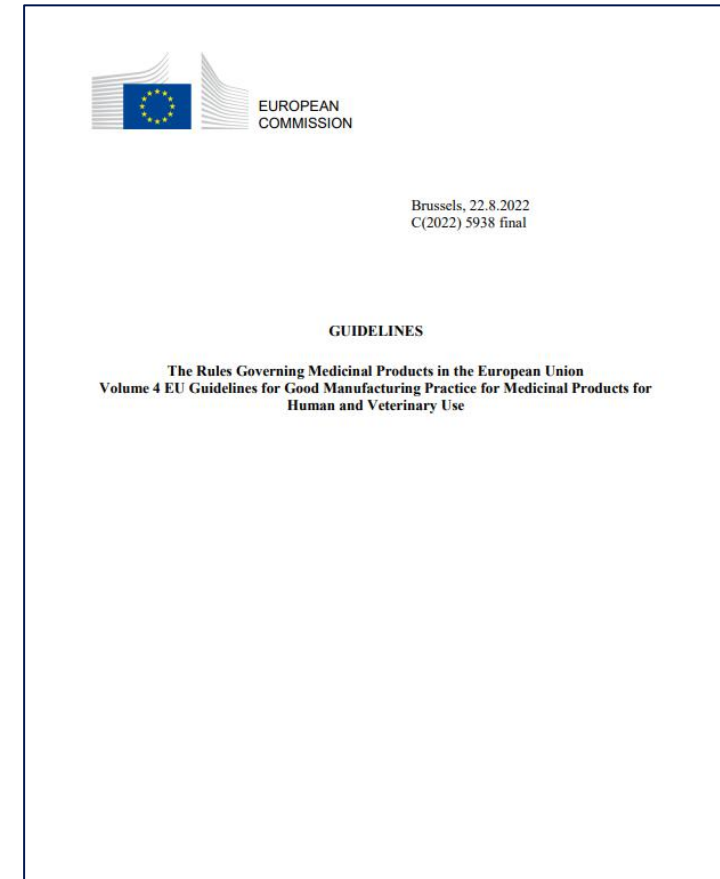
Faster Approvals

Release of water after sanitization
procedures within hours.



EU GMP Annex I from August 2022

- Alternative method as IPC requires no further validation (PQ) = use of engineering purposes only
- Use of suitable alternative methods is recommended to increase safety
- Trend analyses should be carried out for critical parameter

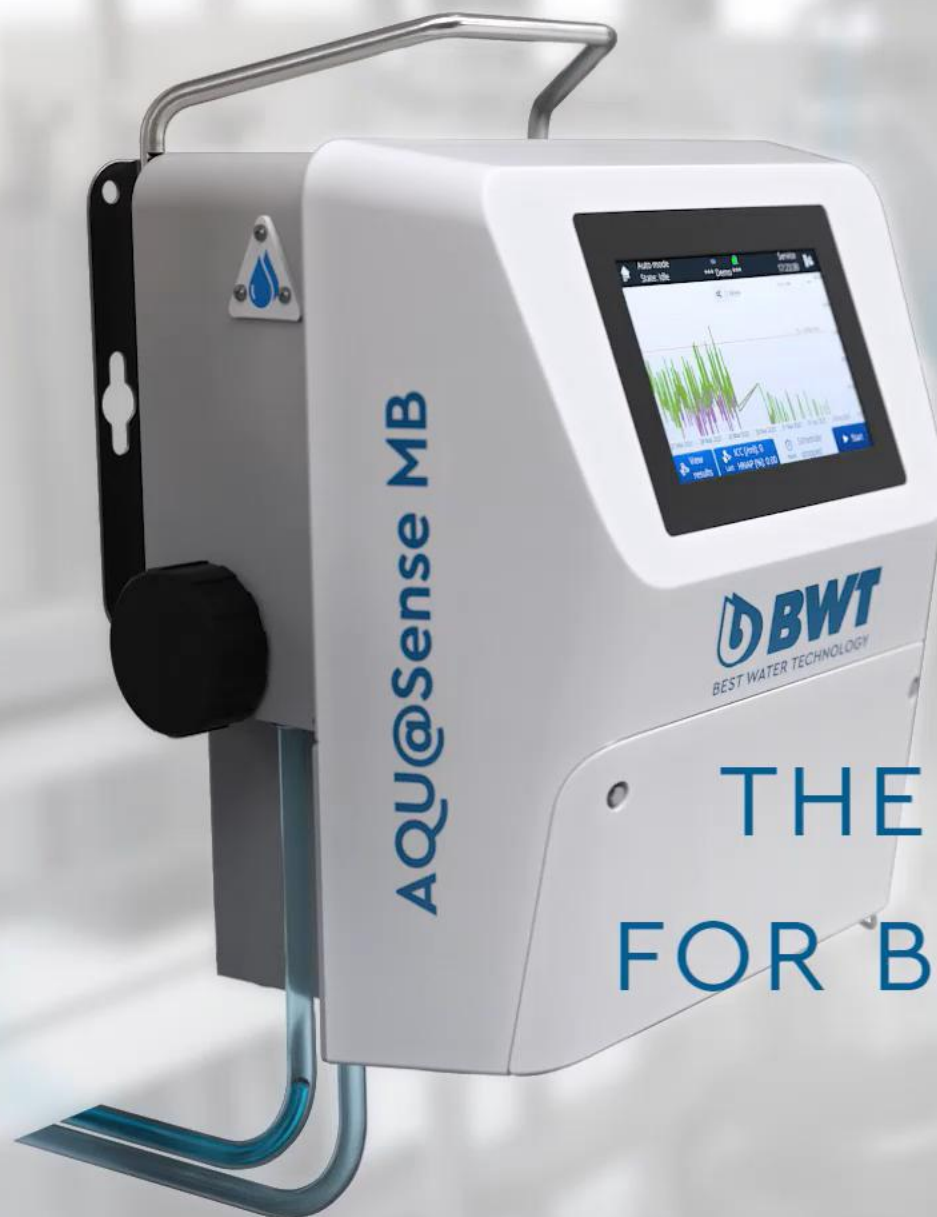


Technology behind AQU@Sense MB

Based on Flow Cytometry



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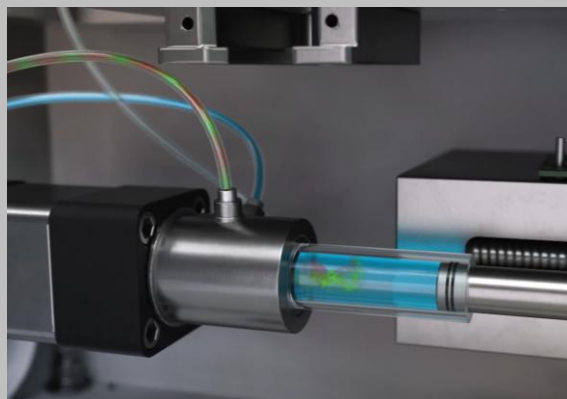


THE IDEAL SOLUTION FOR BIOBURDEN MONITORING

Flow Cytometry in a nutshell

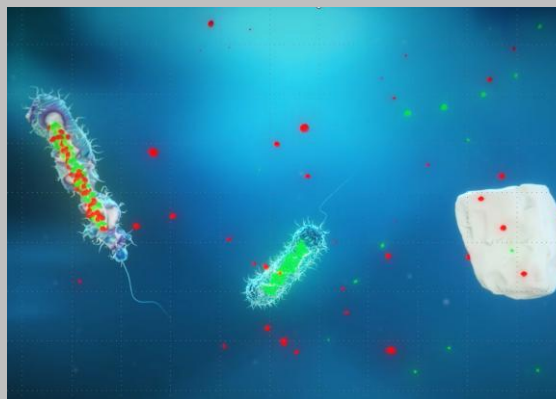
Staining

With two DNA specific dyes



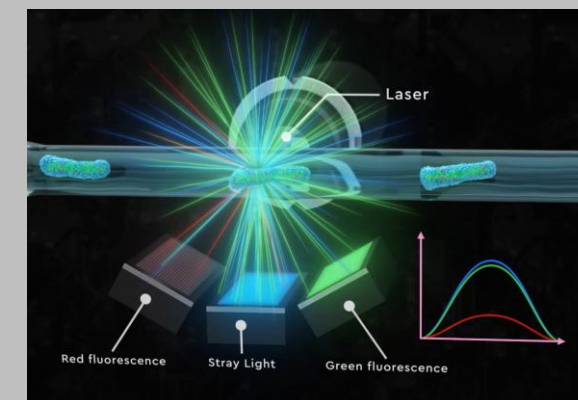
Incubation

Only one dye can pass an intact cell membrane

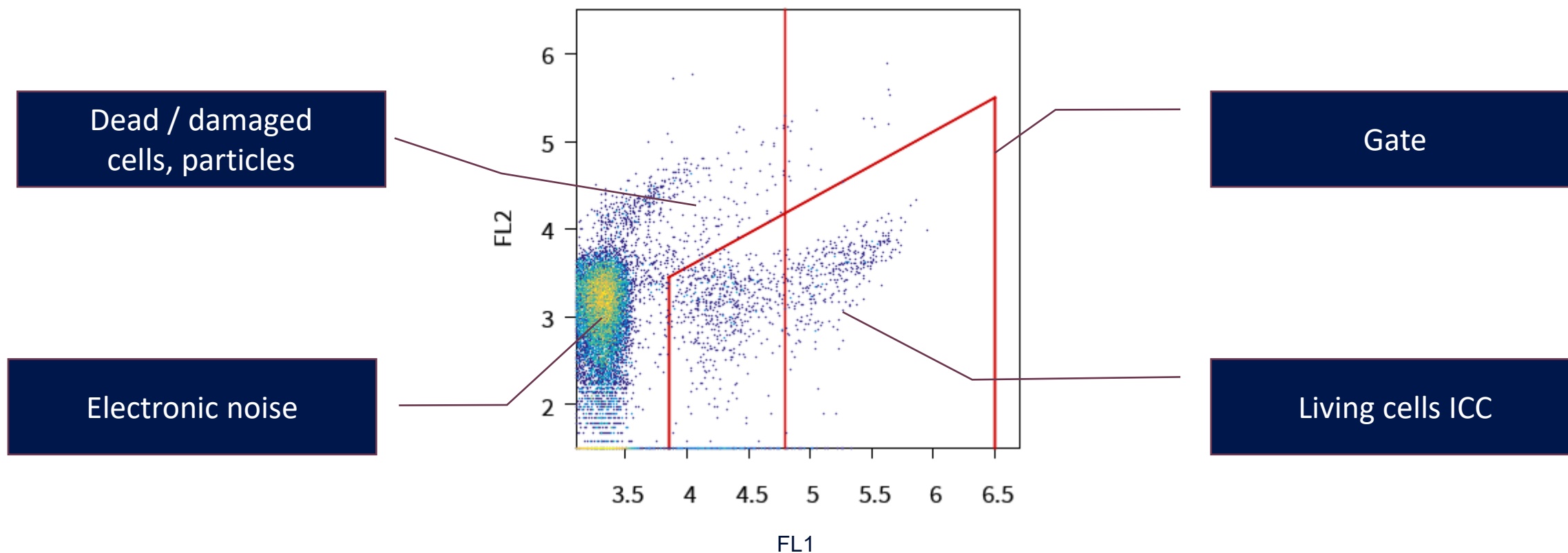


Counting

Analysis of specific straylight and fluorescence signals



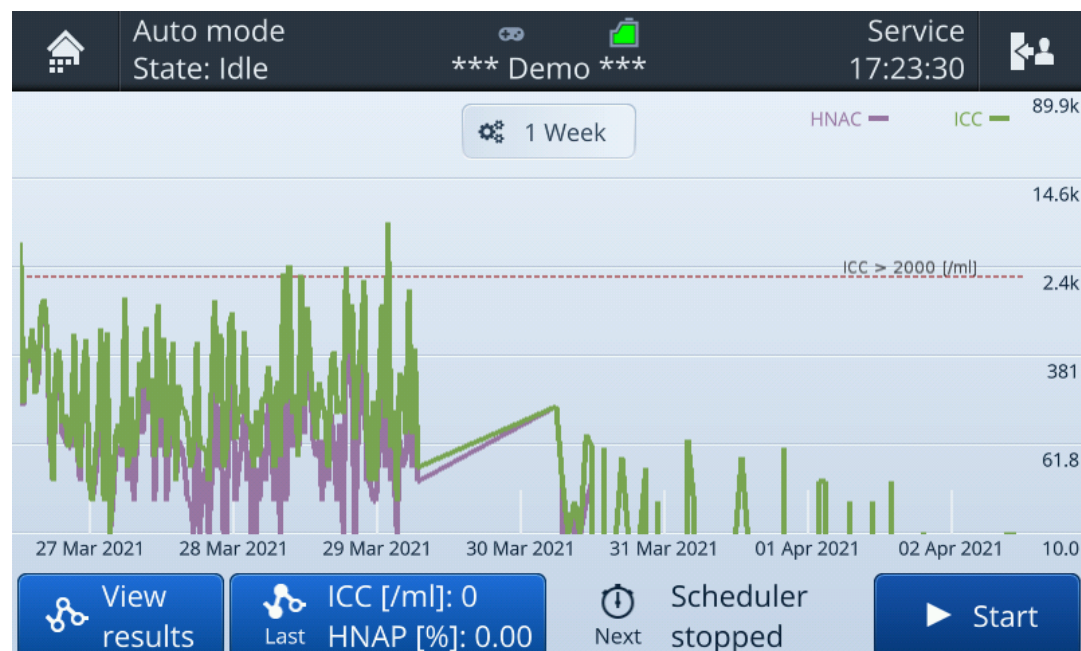
DOT-Plot - Example



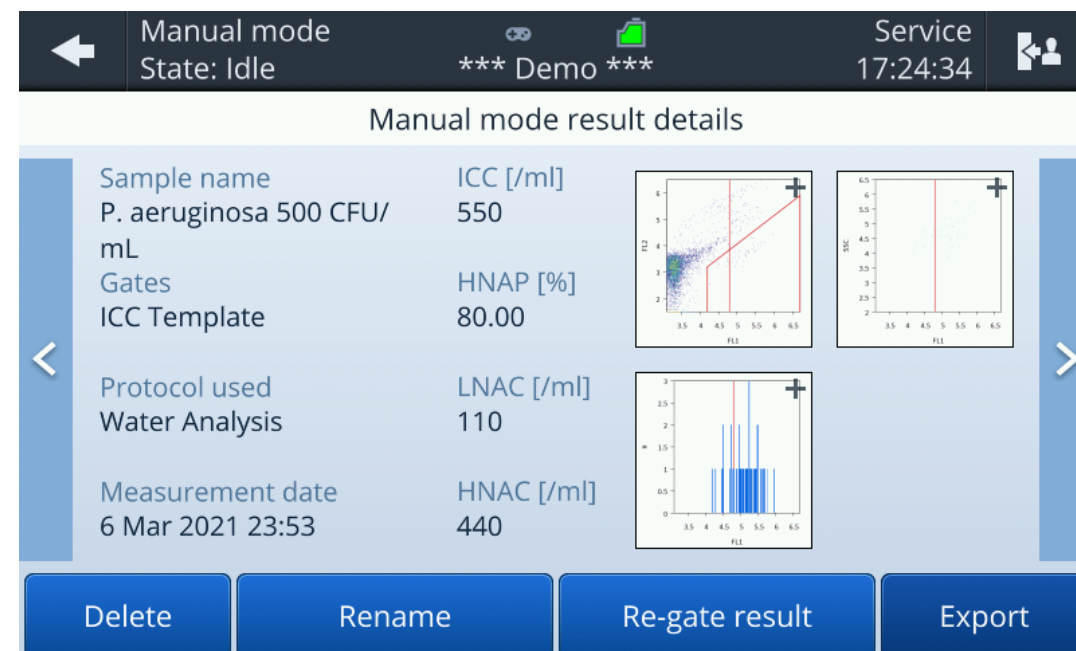
ICC = Intact Cell Count

Results displayed on the device

Online measurement



Manual mode



A new Unit: Intact Cell Count (ICC)

ICC $\neq$ CFU

ICC provides a precise count of every intact cell in a sample, while CFU only indicates colony growth without revealing its origin or composition.



Plate Count



vs.

AQU@Sense MB

Population	
A	Individual culturable organisms
B	Conglomerates (microorganisms attached to particle)
C	Biofilm fragments
D	VBNC-Organisms
E	Biofilm attached to surface

← detectable

← +/- detectable

← Not detectable

It takes **5 days** until the result can be evaluated.

Population	
A	Individual culturable organisms
B	Conglomerates (microorganisms attached to particle)
C	Biofilm fragments
D	VBNC-Organisms
E	Biofilm attached to surface

← detectable

← Not detectable

Results within **30 minutes**

Use of an AQU@Sense MB

Fast results

A sample of 90µl is measured within 30 minutes



Accurate quantification

Detects intact cell counts (ICC) and VBNC (viable but non-culturable cells)



Automation

Integration onto water systems allows automated data collection.



User Friendly

Intuitive interface and gathered data is available in .xlsx, .csv and .pdf



Manual and Automated modes

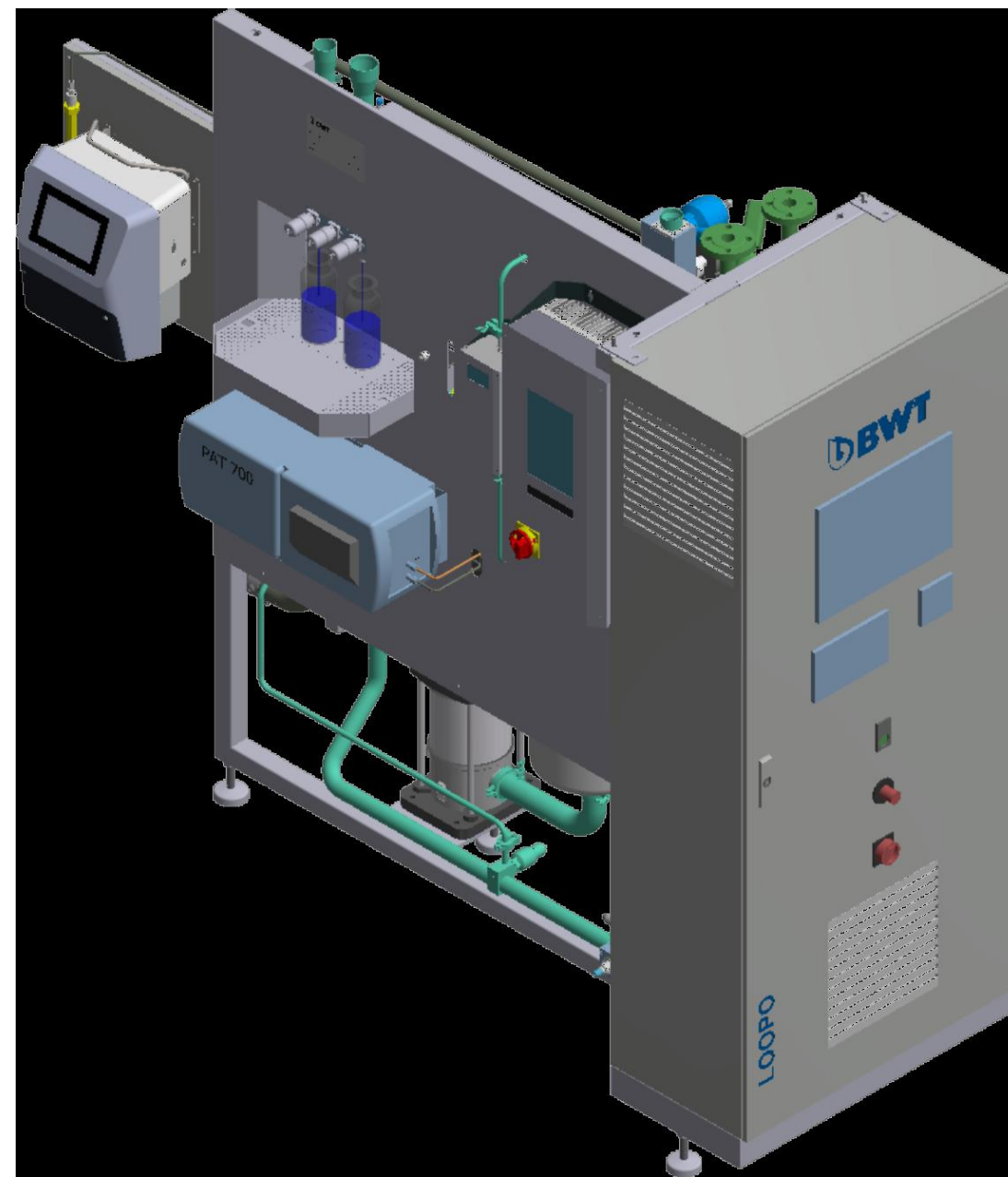
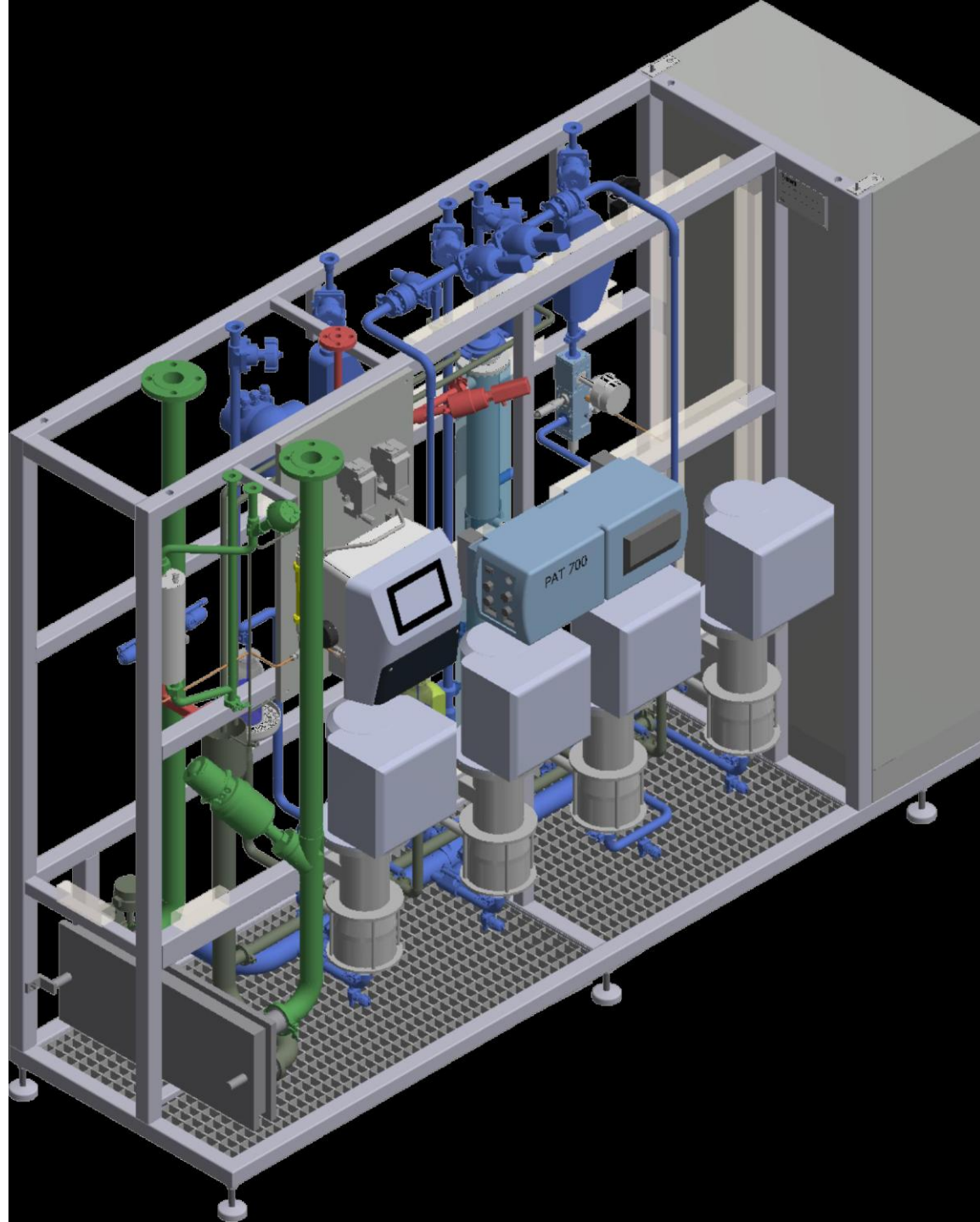
Operates in both, manual (offline benchtop) and automated (installed online) modes.

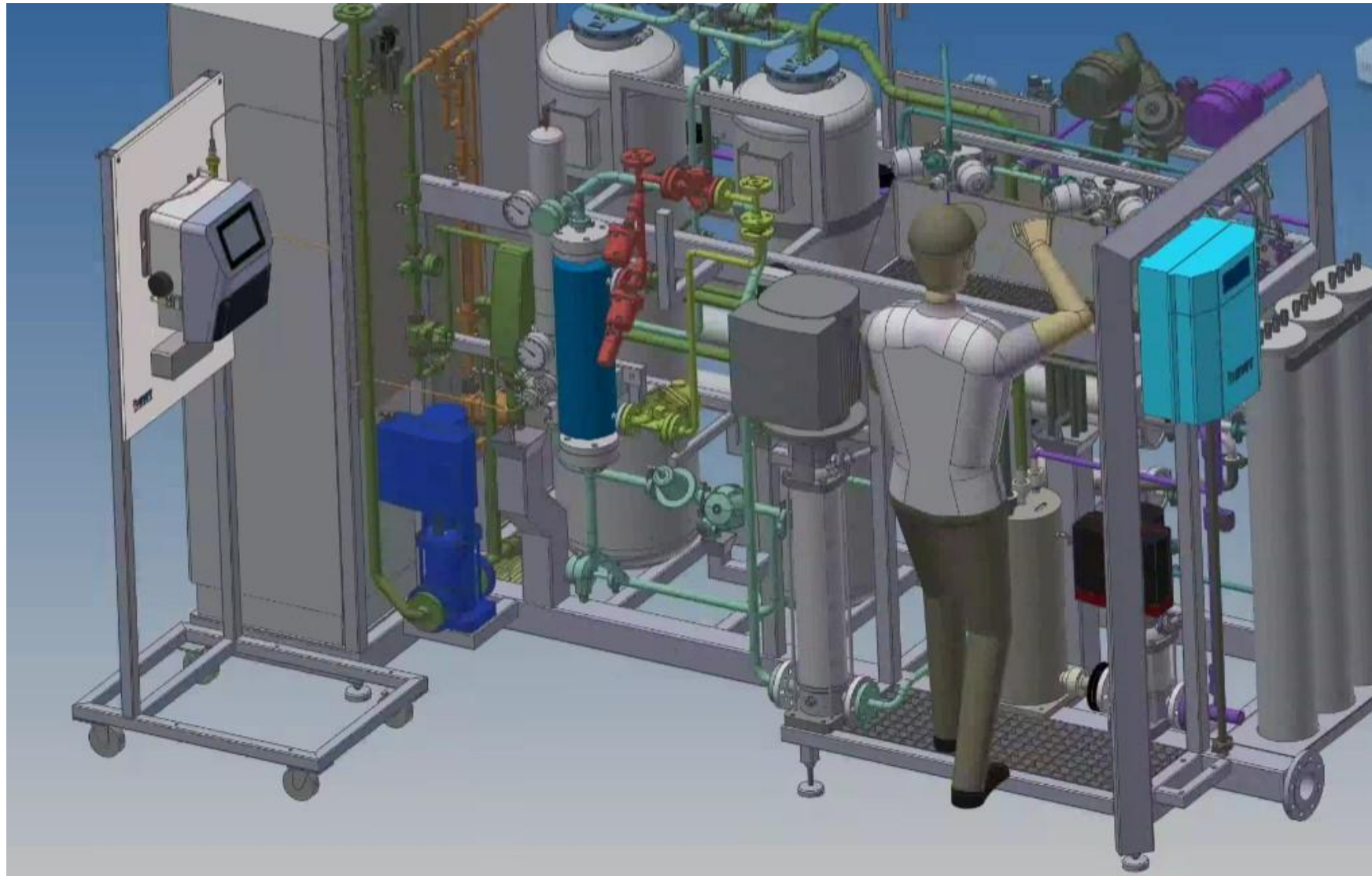
Installation Examples

AQU@Sense MB

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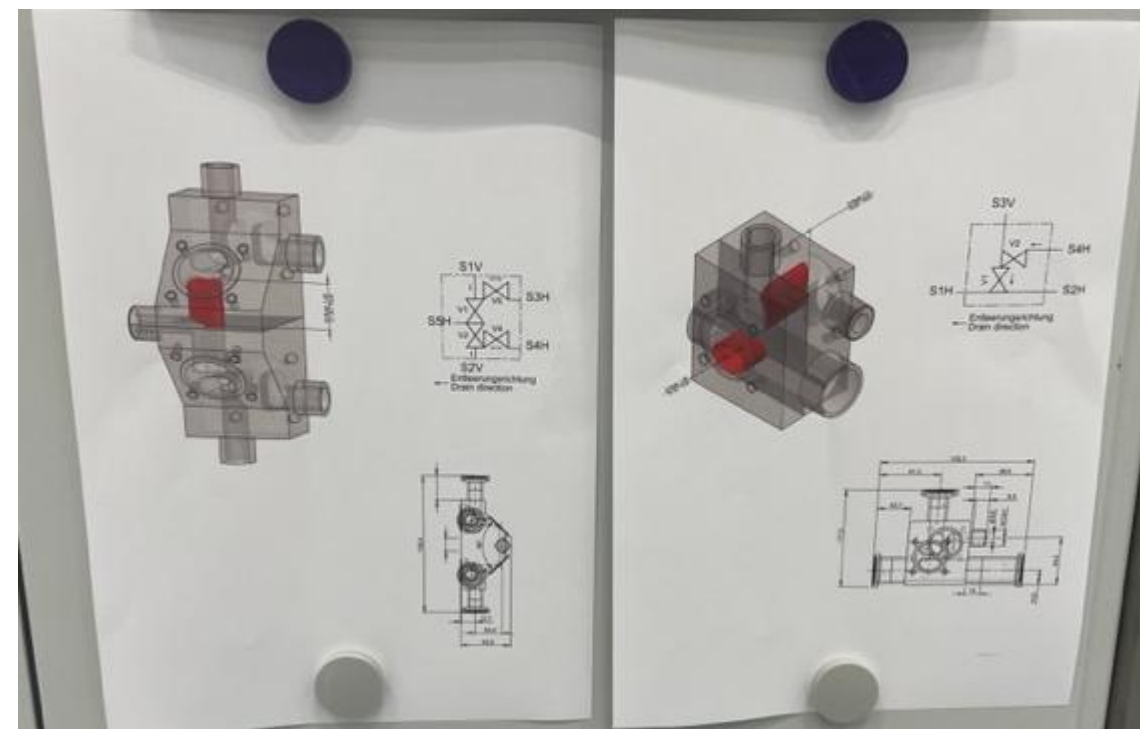




Loopoline



With 2 measurement points



Benefits with Multisampling Options



COST
EFFICIENCY



COMPACT
INSTALLATION



STREAMLINED
MONITORING



FLEXIBILITY AND
SCALABILITY

- ✓ Better control over the water quality at different process steps
- ✓ Better Compliance and Risk Management
 - ✓ Stronger validation and traceability
 - ✓ Compliance with regulatory standards

Application of AQU@Sense MB



What is the AQU@Sense MB used for?



Early Detection of Trends

- Early intervention in system operation



Early Water Release

- Faster technical release for production



Quality Control

- Has already been validated as offline QC method for saline solutions



Reduces Downtimes of Water Systems

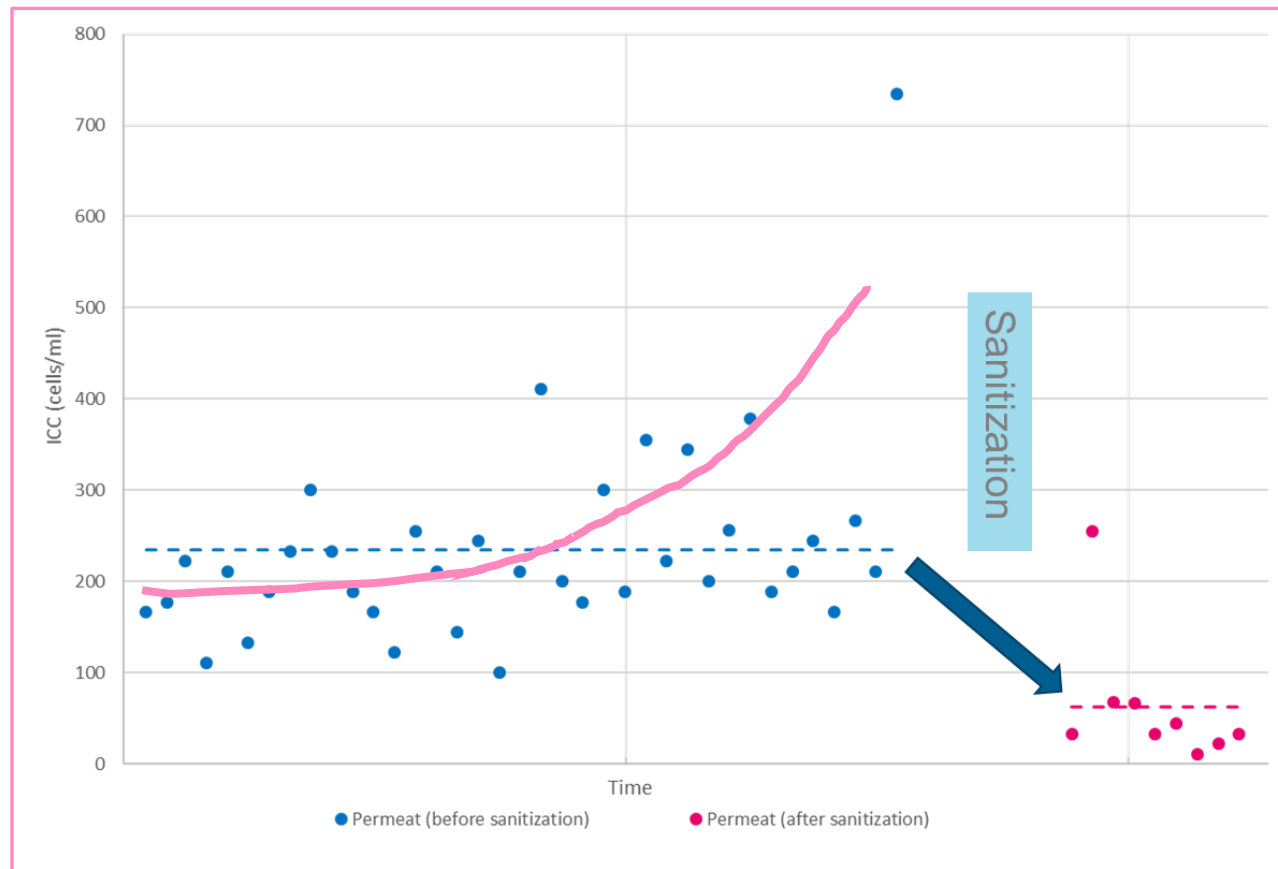
Enables PW or WFI systems to be released within **a few hours** post-sanitization!



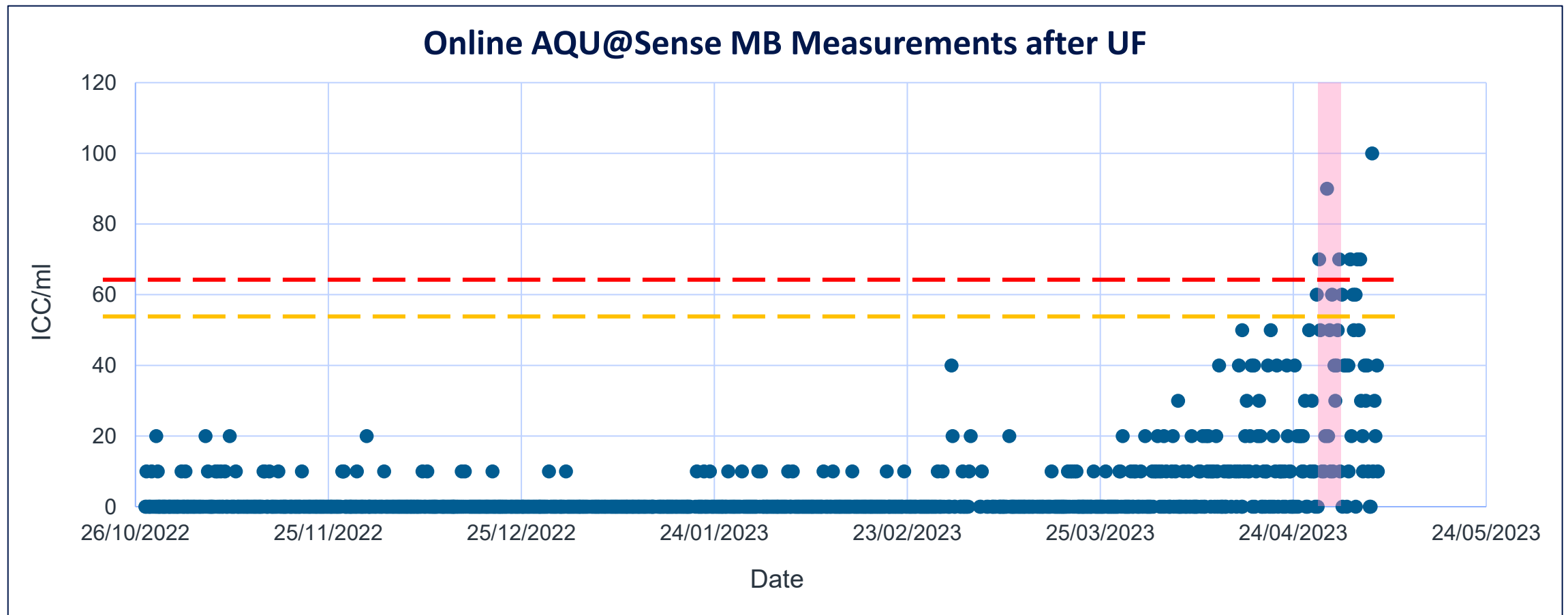
Heterotrophic Plate Count is traditionally used which takes **at least 5 days**.



Allows Early Detection of Changes



Trend Monitoring allows early detection of deviations or adverse trends in water purification systems which **allows proactive intervention**

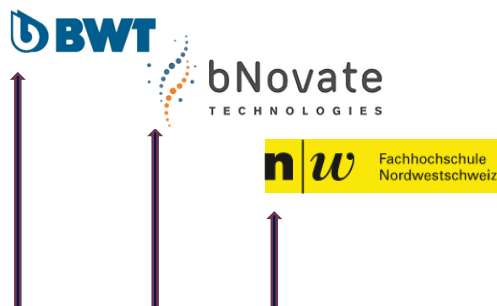


Validation of the AQU@Sense MB as new QC release method

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Primary Validation



Validation Summary Report AQU@Sense MB			
Approved by			
Position	Name/Signature	Company	Date
Project manager		BWT	15 July 2012
Head of production		BWT	15 July 2012



Validation Master Plan AQU@Sense MB			
Approved by			
Position	Name/Signature	Company	Date
Project manager		BWT	15 July 2012
Head of production		BWT	15 July 2012
Head of development		BWT	15 July 2012



TU/e



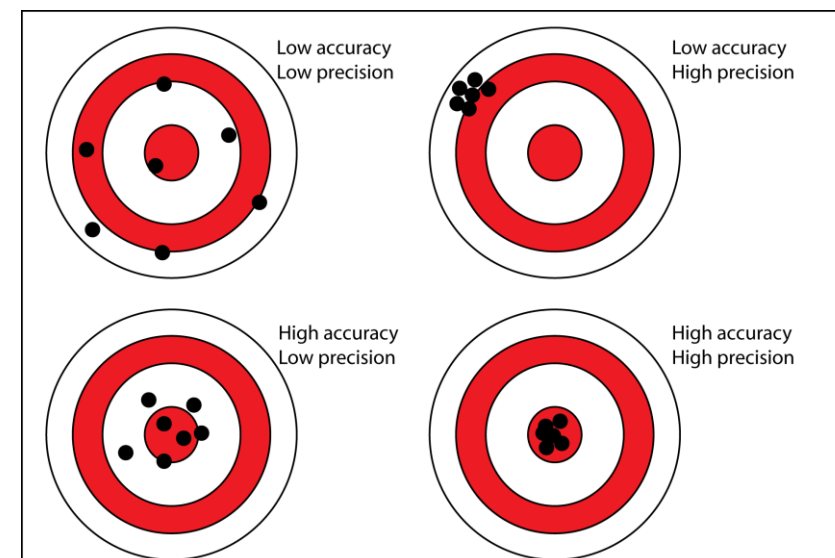
Microorganisms used

	Test?	Argument
Pseudomonas paraeruginosa	X	Biofilm-forming bacteria, Gram -, can be introduced through feed water, mentioned in "all" documents
Bacillus subtilis	X	Review of the test for Gram+, validation of R2A, formation of spores under stress
Staphylococcus aureus	X	Infectious in clinics; Gram+
Stenotrophomonas maltophilia	X	USP <1231>, "microbiota in UPW", "bacteria cont. UPW", M. Rieth
Ralstonia pickettii	X	USP <1231>, "microbiota in UPW", "bacteria cont. UPW", M. Rieth
Burkholderia cepacia	X	USP <1231>, "microbiota in UPW"
Penicillium sp.	X	Review of the test for fungi
Sphingomonas paucimobilis	X	Occurrence in WFI
Escherichia coli	X	USP <1231> Hygiene Indicator, Potential Internal Contamination in Water System Gram
Candida albicans	X	Ph. Eur. test for yeasts

Tested parameters

Parameter	Meaning
Accuracy	How accurate is the method?
Precision	Are the readings repeatable?
Specificity	A1: examination of the typical microorganisms A2: examination of stressed microorganisms B: examination of impurities
Limit of Detection (LOD)	Which is the lowest value that can be measured?
Limit of Quantification (LOQ)	How accurately can a small value be measured?
Linearity	How linear are the measurements compared to the HPC method?
Range	What is the measuring range?
Robustness & Ruggedness	How susceptible is the method to failure?

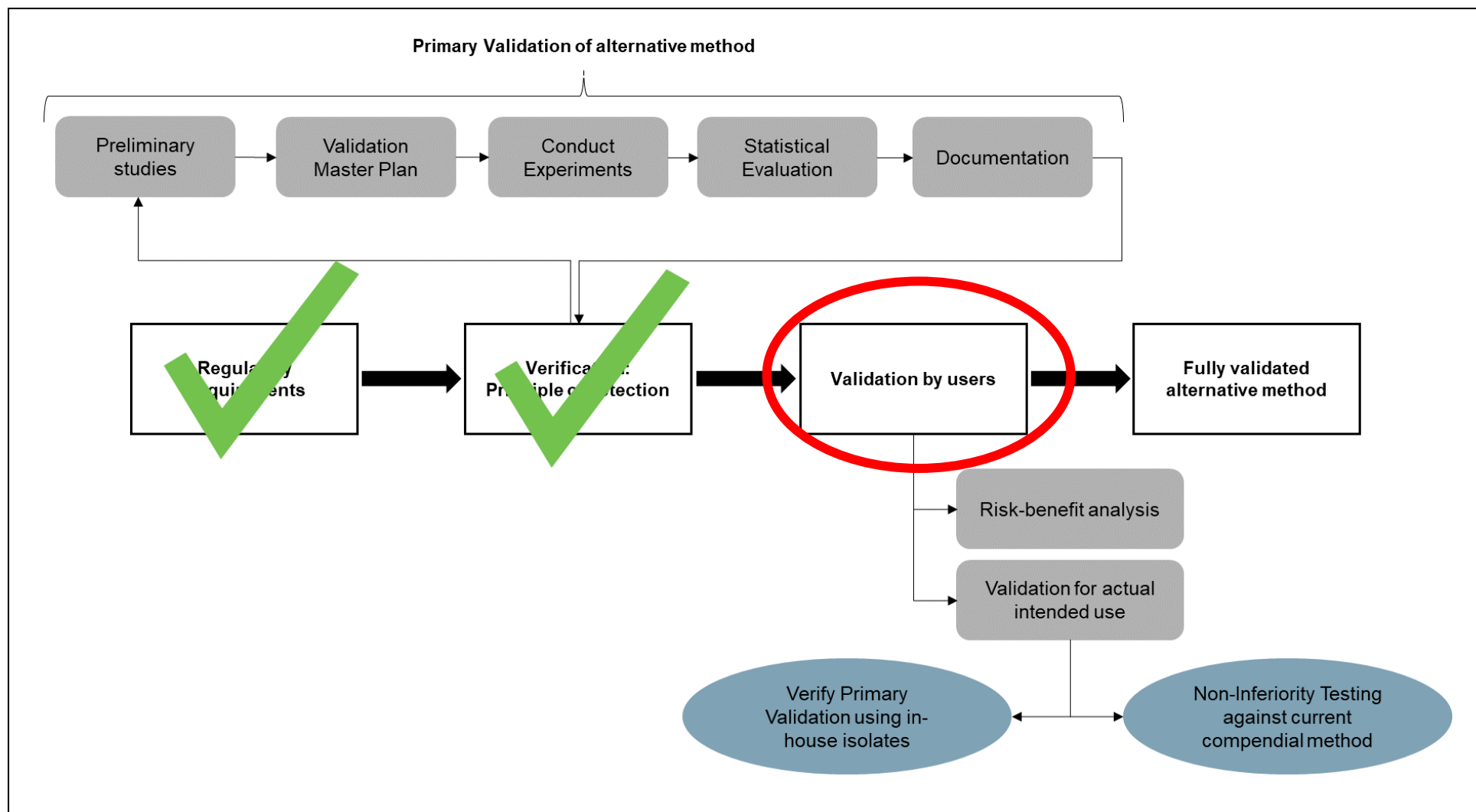
Accuracy vs. Precision



Results of the primary validation

Parameter	Result	Details
Accuracy	✓	not inferior to HPC (high above 70% ICC recovery)
Precision	✓	not inferior to HPC (RSD < 30%)
Specificity	✓	all species detected
LOQ	✓	not inferior to HPC
Linearity	✓	With spiked samples we can measure a correlation.
Range	✓	not inferior to HPC
Robustness	✓	Operational and environmental variables do not affect the test results
Ruggedness	✓	Impervious to vibrations
The primary validation successful		

Validation of alternative methods according to Ph. Eur.



There is a difference between IPC and QC

As **In-Process Control**, an online bioburden monitoring device can **immediately be adopted**, and its application is reinforced by the EU GMP Annex I from August 2022

As **QC Method**, alternative methods may be **adopted after validation**.

Summary

- 1. Flow Cytometry gives exact information about bioburden to**
 - Increase process safety
 - Detect potential risks within few hours
 - Optimizes sanitization cycles
 - Accelerate release of the water systems after maintenance / sanitization
- 2. Individual measurements become less important → the trend is important to understand the process fully.**

Thank you

Terms

RMM = Rapid Microbiological Method

Modern/Alternative Method = RMM, OWBA

OWBA = Online Water Bioburden Monitoring

HPC = Heterotrophic Plate Count (compendial Method)

Compendial /Traditional Method = Plate Count Method

Validation

- **EQ = Equipment Validation (IQ and OQ) = Installation and Operation Qualification**
- **PQ = Performance Qualification**
 - Demonstrates the installed equipment and the microbiological method provide results in accordance with expected outcomes and criteria
- **PRIMARY VALIDATION** = A set of experiments to prove the capabilities and limitations of a **method** in a scientifically sound way