GMP–
PHARMA
CONGRESS

#sharing challenges
and solutions in practice

Review of Online water monitoring analyzers (OWBA) and their potential application

GMP/FDA Compliance Conference

Dr. Hans-Joachim Anders, Novartis Pharma

Part of PharmaCongress – Düsseldorf/Neuss, 31 May–1 June 2022

Dr. Hans-Joachim Anders

- **University Ulm**
  - Study of biology with focus on microbiology and molecular biology
  - PhD in microbiology on the anaerobic metabolism of aromatic compounds in denitrifying bacteria.
- **MSD (Merck, Sharp & Dohme) and Fresenius Home Care**
  - Field service sales and patient care
- **Novartis Pharma AG, Stein, Aargau, Switzerland**
  - Lab Head, Senior QA Facilitator,
    Teamlead Analytical Science & Technology Microbiology,
    microbiological quality assurance and control

- **Member of the expert group of the European Pharmacopoeia for pharmaceutical water**

Agenda

- Online Water Monitoring technologies
  - Biofluorescent particle counters
  - Flow Cytometry
  - Validation aspects
- Evaluation study Flow Cytometry
  - Result interpretation – higher counts and possible explenation
- Potential use of Online Water Monitoring Technologies
- Views of the industry working group collaboration (BPHOG/OWBA)
  - Who we are?
  - Expected benefits and vision of the future
  - Why isn't it reality already?

Online Water Monitoring Technologies

- **Online detection methods**
  - Continuous monitoring.
  - Direct connection of the analyzer to the pharmaceutical water treatment or distribution system at line.
  - No laboratory analysis necessary.
  - Biofluorescent Particle Counter
    - ⇨ Detection of the microorganism by excitation with a laser.
    - ⇨ Instruments measure the auto-fluorescence of the microorganisms.
  - Flow Cytometry
    - ⇨ Viability stain
    - ⇨ Detection of stained viable cells

Online Water Monitoring Technologies

- **Examples manufacturer and instruments**

Mettler-Toledo RMS7000
https://www.mt.com/

Biofluorescent particle counters

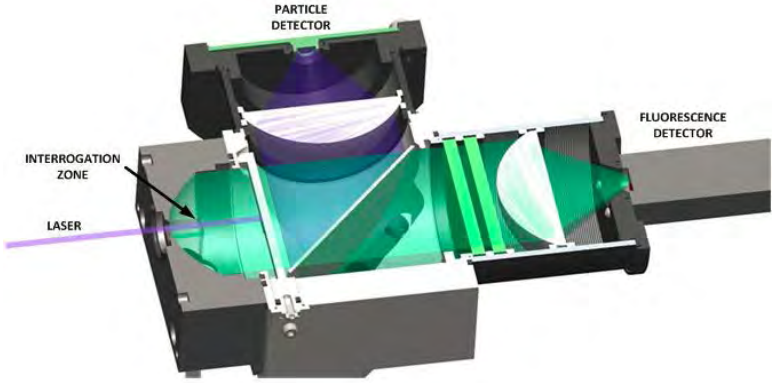
Sentinel MOBA
http://www.sentinelmonitors.com/

AQU@Sense MB
https://www.bwt-pharma.com/

Flow Cytometry counter

Online Water Monitoring Technologies

- **Biofluorescent particle counter**
  - Detection principle

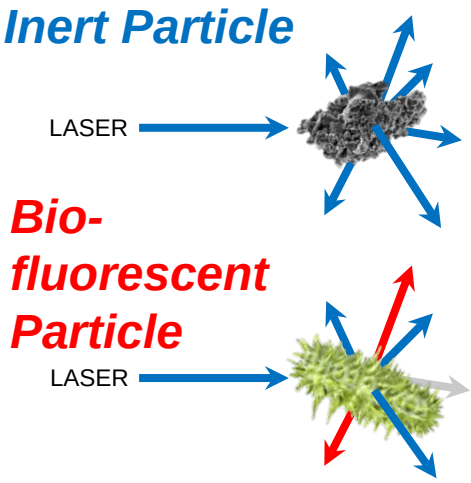


RMS 7000 Flow Cell

Online Water Monitoring Technologies

- **Biofluorescent particle counter**

  Similarly to a *Total Particle Counter*…



Scattered Light
Same Wavelength

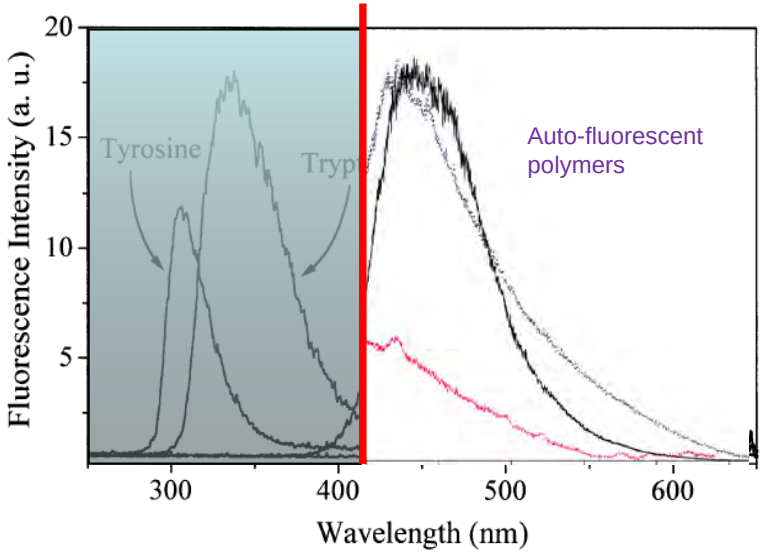
Scattered Light & **Fluorescence Light**
Longer Wavelengths

Each particle is assessed for:
- Scattered light intensity
- Fluorescent light intensity

Continuous, real-time outputs:
- Total particle counts
- Auto-fluorescence counts (AFU)

Online Water Monitoring Technologies

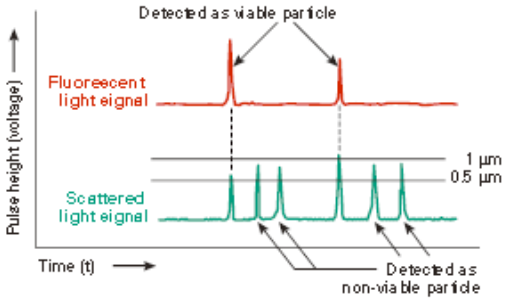
- **Biofluorescent particle counter**
  - Detection principle
    - ⇨ Excitation with 405 nm
    - ⇨ Autofluorescence of ATP, NAD(P)H and Riboflavine



1. *"Real-time Measurement of Fluorescence Spectra from Single Airborne Biological Particles "*, Hill et al., Field Analytical Chemistry and Technology, 3, 221–239 (1999) (265 nm excitation)
2. "*High performance recycling of polymers by means of their fluorescence liftetimes"*. Heinz Langhals et al. Dept of Chemistry, LUM University of Munich, Munich, Germany. August 2014 (365 nm excitation)

Online Water Monitoring Technologies

- **Biofluorescent particle counter**
  - Result in Autofluorescence Units – AFU

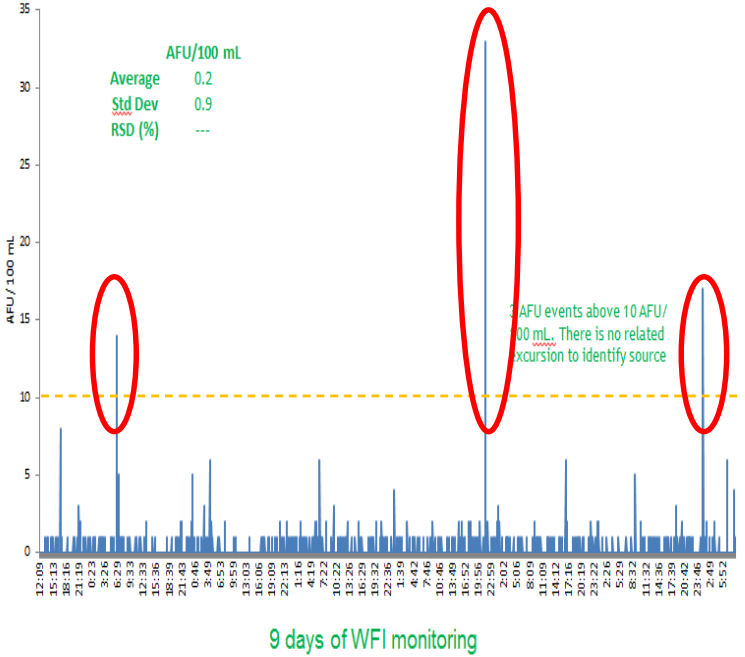


AFU ≠ CFU

  - **Colony-forming unit (CFU)** is a unit that gives an estimate of the number of microorganisms in a sample.
  - **Auto-Flourescence Unit (AFU)** is a unit that takes into account fluorescence as well as size of a particle.

Online Water Monitoring Technologies

- **Biofluorescent particle counter**
  - Points to consider:
  - Qualification aspects:
    - ⇨ False-positive rate
    - ⇨ Calibration standard
      - Calibration
      - Suitability
  - Validation aspects



9 days of WFI monitoring

Online Water Monitoring Technologies

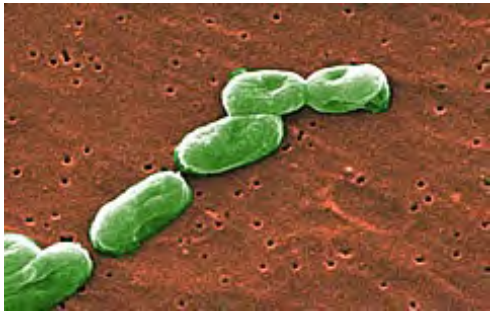
- **Biofluorescent particle counter**
  - Reason for False-positive signals
    - ⇨ Microorganisms in the VNBC stage, Viable but not culturable, described for pharmaceutical water.
    - ⇨ Microorganisms that do not grow on the media used but can be detected during online measurement (95-99% of all microorganism species or have not been cultivated with traditional methods so far, e.g. because the media/conditions used do not allow growth).
    - ⇨ Polymers (EPDM, Teflon), dead cells, pollen, some solvents (isopropanol), rouge, metal abrasion (reflective properties) can lead to false positive results.

Online Water Monitoring Technologies

- **Biofluorescent particle counter**
  - Validation aspects:
  - AFU ≠ CFU and
    rate of false-positive results and their origin
    - ⇨ Will make comparative studies using the traditional method difficult or impossible.
    - ⇨ Most test criteria to validate an alternative microbiological method according to EP 5.1.6, USP<1223> or PDA TR33 can't be tested
      - especially equivalence to traditional method.
      - Spiking experiments are of limited significance due to particle contamination and viability stage of the organisms compared to organisms in pharmaceutical grade water
    - ⇨ Possible solutions
      - Signal induced auto sampler – will allow traditional anlysis of the peak
      - Alternative assessment of equivalence according to USP1223 - Decision Equivalence.

Online Water Monitoring Technologies

- **Biofluorescent particle counter**
  - Calibration of the instrument
    - ⇨ No model organism available
    - ⇨ Vitality and size of the microorganisms and thus the fluorescence signal depends on the growth phase in which the microorganisms are located, i.e. an organism cultured on medium is not comparable with a microorganism adapted to pharmaceutical water.
    - ⇨ OWBA working group is therefore pursuing the establishment of fluorescent beads similar in size to *Ralstonia pickettii*.
    - ⇨ *Ralstonia pickettii* is a gram-negative rod-shaped bacterium that can often be isolated from pharmaceutical water.



http://www.higieneambiental.com/calidad-de-aire-interior/ralstonia-pickettii-patogeno-oportunista-emergente

Online Water Monitoring Technologies

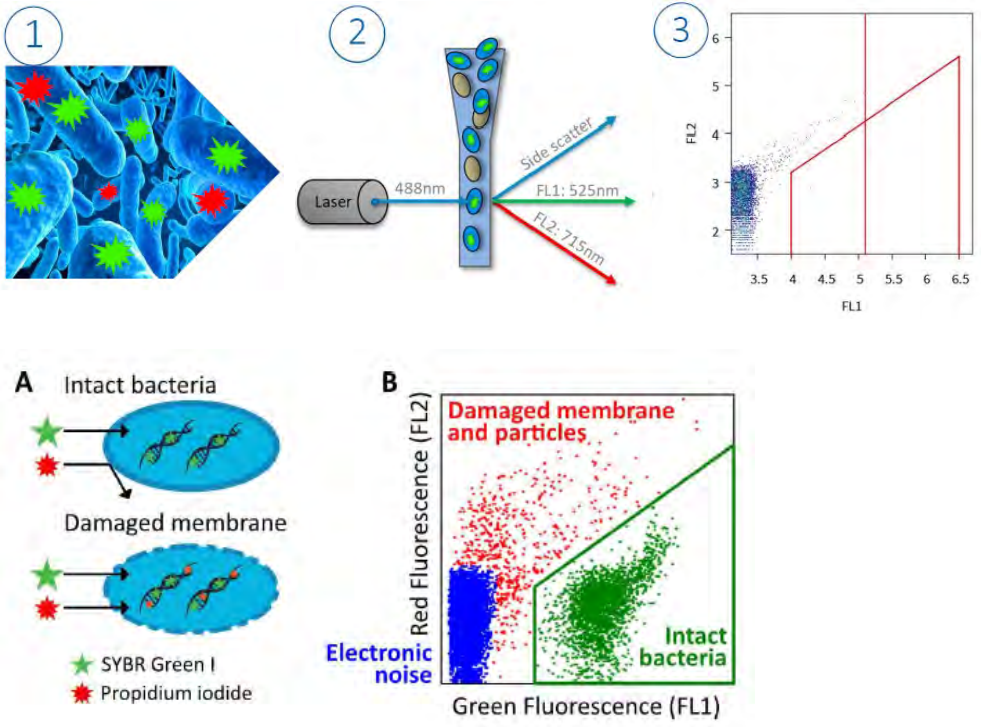
- **Biofluorescent particle counter**
  - Calibration of the instrument
    - ⇨ In collaboration with NIST (National Institute of Standards and Technology), a replacement microorganism in the form of fluorescent beads will be developed.
    - ⇨ Like online TOC with artificial standards sucrose/benzoquinone.
    - ⇨ The following properties must be met by the standard:
      - Number
      - Size
      - Fluorescence (critical parameter, because the fluorescence of commercial beads are too high)
      - Stability
    - ⇨ Comparative studies with *Ralstonia pickettii*.

Online Water Monitoring Technologies

- **Flow Cytometry – Aqu@Sense**
  - Overview
  - Automated Flow Cytometry
  - Continuous monitoring of microorganisms in pharmaceutical grade water
  - Measurement interval from 0,5 – 6 h
  - Stand alone (Offline) or integrated use (Online) possible
  - Instrument sanitizable with Ozone or hot water

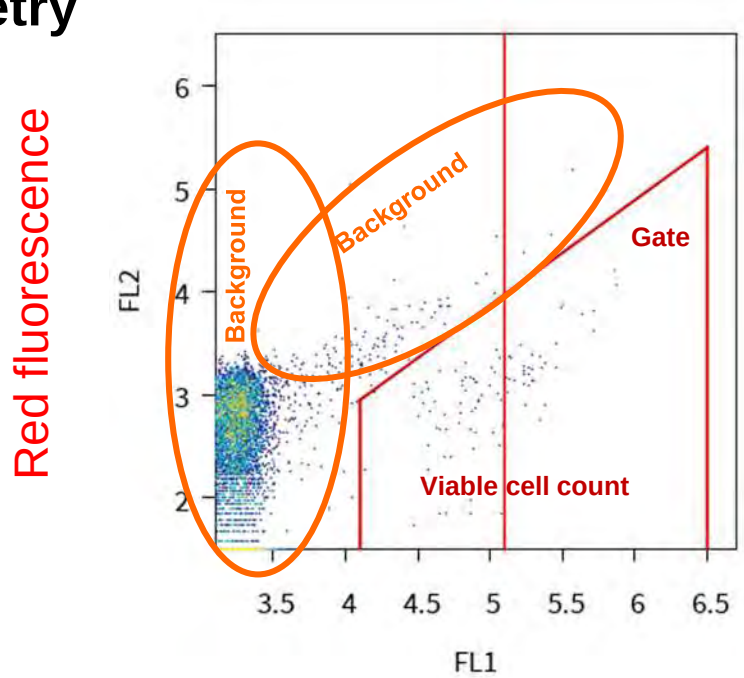
Online Water Monitoring Technologies

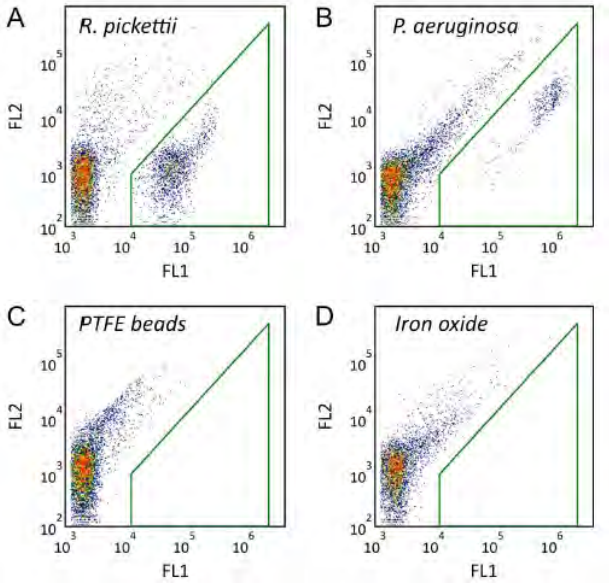
- **Flow Cytometry**
  - Viability stain



Online Water Monitoring Technologies

- **Flow Cytometry**
  - Counting





Online Water Monitoring Technologies

- **Flow Cytometry**
  - Validation aspects:
    - ⇨ Instrument/Method could be validated according EP 5.1.6, USP<1223> or PDA TR33.
    - ⇨ Cell count vs. CFU
    - ⇨ Based on viability staining, interfering particles probably play a lesser role.
    - ⇨ Offline mode will allow to use discrete samples for validation testing.
    - ⇨ Spiking and equivalence tests are possible.

Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study**
  - **Feasibility Study Tests:**
    - ⇨ Accuracy and Precision – with *P. aeruginosa*, *S. maltophilia*, *S. aureus*, *M. radiotolerans*
    - ⇨ Equivalence – «real» samples compared to plate count
  - **Additional tests:**
    - ⇨ Linearitiy – different dilutions of microorganisms
    - ⇨ Background
    - ⇨ Detection of Spore-forming microorganism

Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Accuracy/Precision**

Stenotrophomonas maltophilia

Accuracy

The mean of Aqu@Sense values for *S. maltophilia* is significantly greater.

Precision

Stdevs are NOT significantly different

		Run 1	Run 2	Run 3
per mL (90 µL analyzed)	AquaSense replicate 1	1978	1974	2134
	AquaSense replicate 2	1866	1855	2172
	AquaSense replicate 3	1918	2004	2326
	AquaSense replicate 4	1808	1888	2825
	AquaSense replicate 5	2035	2166	2264
per mL (90 µL plated)	Pour plate replicate 1	1622	1611	1611
	Pour plate replicate 2	1256	1722	1789
	Pour plate replicate 3	1322	1611	2178
	Pour plate replicate 4	1311	1467	1511
	Pour plate replicate 5	1344	1356	1633

Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Accuracy/Precision**

Methylobacterium radiotolerans

Accuracy

The mean of Aqu@Sense values for *M. radiotolerans* is NOT significantly greater.

Precision

Stdevs are NOT significantly different

		Run 1	Run 2	Run 3
per mL (90 µL analyzed)	AquaSense replicate 1	1807	769	474
	AquaSense replicate 2	2079	922	571
	AquaSense replicate 3	2017	971	675
	AquaSense replicate 4	2028	922	748
	AquaSense replicate 5	2138	761	761
per mL (90 µL plated)	Pour plate replicate 1	2489	1111	767
	Pour plate replicate 2	2667	1033	767
	Pour plate replicate 3	2589	1211	700
	Pour plate replicate 4	2756	1144	656
	Pour plate replicate 5	2456	1067	711

Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Accuracy/Precision**

Pseudomonas aeruginosa

Accuracy

The mean of Aqu@Sense values for *P. aeruginosa* is significantly less.

Precision

Stdevs are NOT significantly different

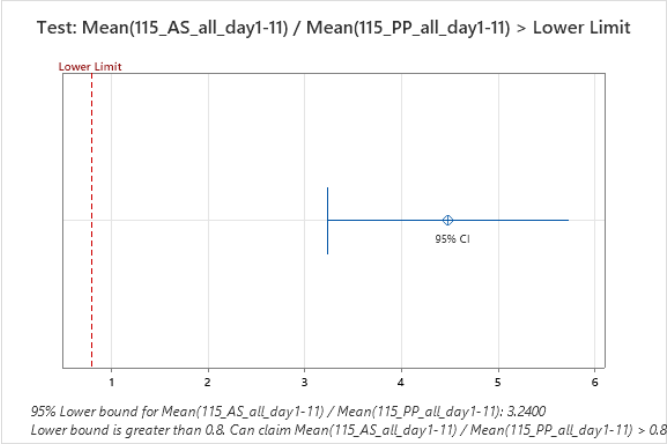
		Run 1	Run 2	Run 3
per mL (90 µL analyzed)	AquaSense replicate 1	441	458	571
	AquaSense replicate 2	886	740	495
	AquaSense replicate 3	852	842	709
	AquaSense replicate 4	969	731	776
	AquaSense replicate 5	943	1006	970
per mL (90 µL plated)	Pour plate replicate 1	789	911	1144
	Pour plate replicate 2	1067	933	1278
	Pour plate replicate 3	856	722	1156
	Pour plate replicate 4	1067	856	1144
	Pour plate replicate 5	1078	889	1033

Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Equivalence**

After Reverse Osmosis stage 1

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
AquaSense replicate 1	**per ml**	66	76	131	76	43	121	77	76	109	101	109
AquaSense replicate 2		54	99	110	67	54	123	65	99	98	143	88
Pour plate replicate 1	**per ml**	11	0	56	22	0	44	44	11	0	16	20
Pour plate replicate 2		11	0	22	11	22	78	11	22	0	23	17

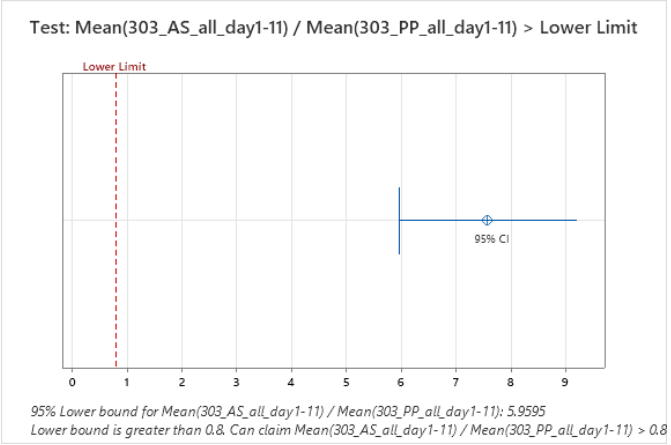


Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Equivalence**

After Softener

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
AquaSense replicate 1	per ml	5038	3677	6164	4985	3327	4194	2955	3041	3275	3068	4677
AquaSense replicate 2		5490	3624	6622	5083	3514	3666	2596	3377	3674	3123	4477
Pour plate replicate 1	per ml	167	1256	933	388	289	244	178	922	667	389	511
Pour plate replicate 2		278	1500	833	411	356	289	189	700	544	389	389



Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies?**

    Two water samples from two sampling points after Softener, were tested in duplicates with the ScanRDI, Aqu@Sense and Pour plate method.

    ScanRDI: 1 ml of each sample was filtrated with 9 ml of sterilized Purified Water and then processed according the direct detection protocol.

    Aqu@Sense: 2 x 90 µL of each water sample are analyzed automatically by the Aqu@Sense BW. Result is given per mL!

    Pour plate: 2 x 90 µL and 2 x 1 mL of each water sample are pipetted in an empty petri dish and covered with approximately 20 mL R2A Agar, 30-35°C, 5 – 7 days

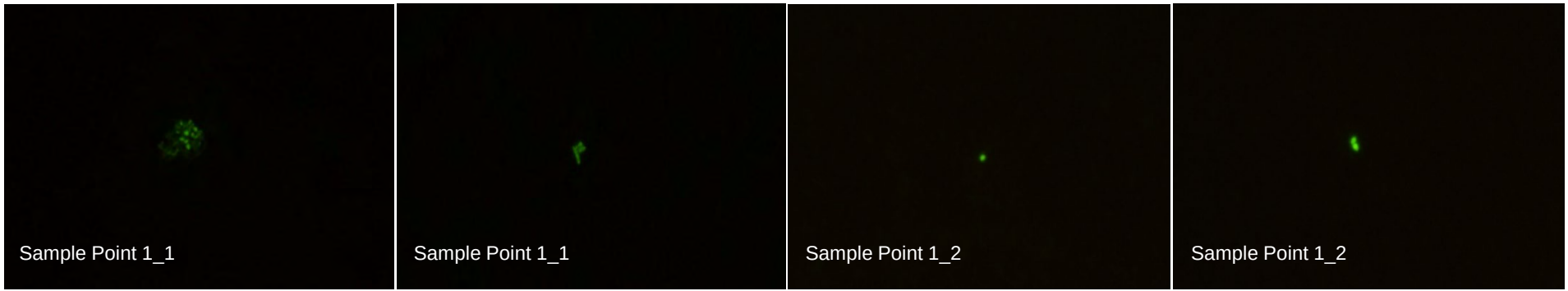
Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies?**

Method	Sampling Point 1 Sample1	Sampling Point 1 Sample 2	Sampling Point 2 Sample1	Sampling Point 2 Sample 2
ScanRDI (events/MO per ml)	46	43	115	107
Aqu@Sense (ICC/ml)	1403	1453	2084	1572
Pour plate (cfu/ml)	0	4	38	23

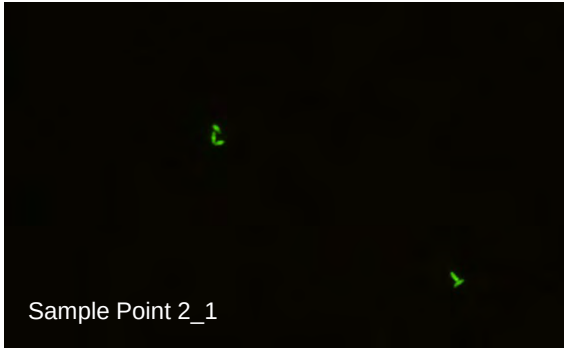
Online Water Monitoring Technologies

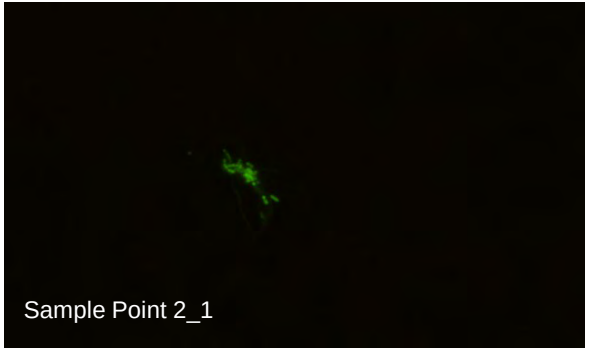
- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies?**

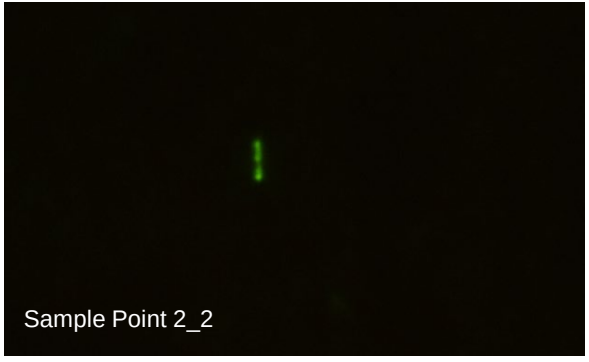


Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies?**

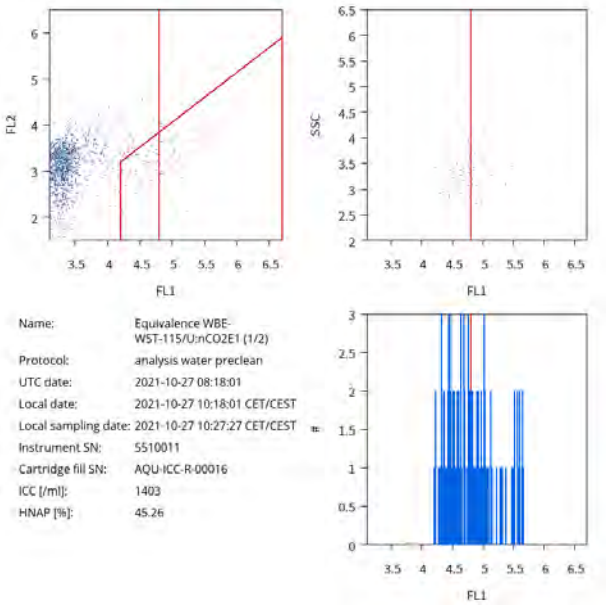


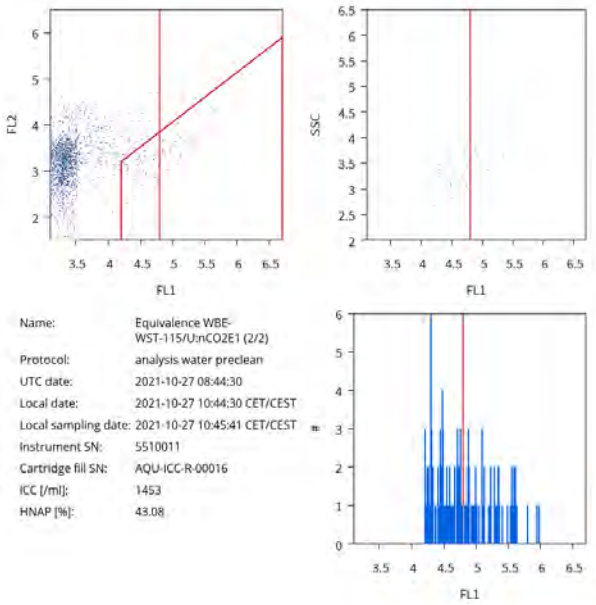




Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies? – Diagrams**





Online Water Monitoring Technologies

- **Flow Cytometry – Feasibility Study results**
  - **Why higher counts in Equivalence studies? – Conclusion**
    - ⇨ The cell count measurement with the Aqu@Sense method for samples after Softener is approx. 32/16 times higher than with the ScanRDI method.
    - ⇨ Viable but not culturable cells – with Scan RDI the could be detected, could explain the lower plate count results.
    - ⇨ Plate Counts and ScanRDI counts lower due to clustering of organisms, as can be seen in the ScanRDI pictures.

Online Water Monitoring Technologies

- **Both Technologies**
  - Potential use of the Online instruments in the view of OWBA workgroup:
    - ⇨ Optimization of processes like sanitisation
    - ⇨ Revalidation after maintenance work, Pre- und Post-Maintenance measurement
    - ⇨ Biofilm-Monitoring
    - ⇨ Reducing of grab sampling in water treatment plants
    - ⇨ Publication: Anders HJ, Ayers F, Fitch B, Forng RY, Hooper S, Luebke M, Mateffy J, Noverini P, Termine B, Yan L, and Weber J of the Online Water Bioburden Analyzer Workgroup(2017): Practical Application Of Online Water Bioburden Analyzers In Pharmaceutical Manufacturing

https://www.pharmaceuticalonline.com/doc/practical-application-of-online-water-bioburden-analyzers-in-pharmaceutical-manufacturing-0001?vm_tId=2016150&user=f7b92eb0-a95c-4087-a7dd-dfd35afdbc01&utm_source=et_6214180&utm_medium=email&utm_campaign=PHARM_08-15-2017&utm_term=f7b92eb0-a95c-4087-a7dd-dfd35afdbc01&utm_content=Practical+Application+Of+Online+Water+Bioburden+Analyzers+In+Pharmaceutical+Manufacturing.

Online Water Monitoring Technologies

- **Both Technologies**

Pro's	Con's
Online Analysis	Cell count, AFU ≠ CFU
Continuous Monitoring	Authority acceptance?
No lab analysis, no dependency on media properties	Alternative method, Validiation acc. EP 5.1.6, USP<1223>, PDA TR33 ? Equivalence?
Sensitive Detection with Laser, Autofluorescence, Viability stain (no growth necessary)	Calibration ⇨ Calibration standard
Non destructive method, Auto sampler?	False-positive signals of particles (Kunststoffe, etc.), VNBC
Data Integrity	Investment costs

Views of the industry working group collaboration

- Industry working group established to discuss and push the implementation of Biofluorecent Particle counter
- Who we are:

Philip Villari, Merck & Co., Inc. (Author of the following slides)
Joanny Salvas, Pfizer (Author of the following slides)
Hans-Joachim Anders, Novartis Pharma Stein AG
James Cannon, Mettler-Toledo Thornton, Inc.
Anthony Cundell, Microbiological Consulting, LLC.
Michael Dingle, TSI Inc.
David Govezensky, Bio-Technology General (Israel) Ltd.
Patrick Hutchins, TSI Inc.
Cedric Joossen, Janssen
Chris Knutsen, Bristol-Myers Squibb
Petra Merker, Bayer AG
Stephanie Ramsey, Amgen Inc.
Margit Franz-Riethdorf, BioPhorum
Allison Scott, ANAD BioVigilant
Ans Vanbroekhoven, Sanofi

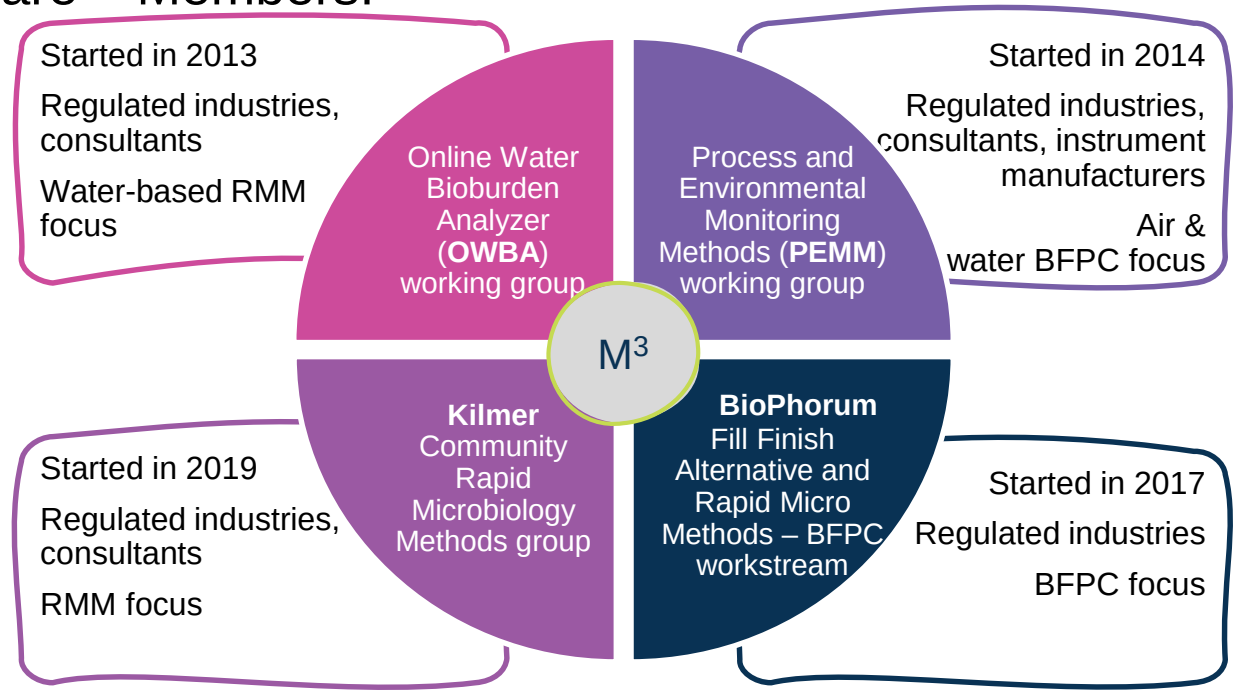
Views of the industry working group collaboration

Disclaimer

The information and opinions presented are those of the collaboration, and not necessarily the opinions of our individual employers.

Views of the industry working group collaboration

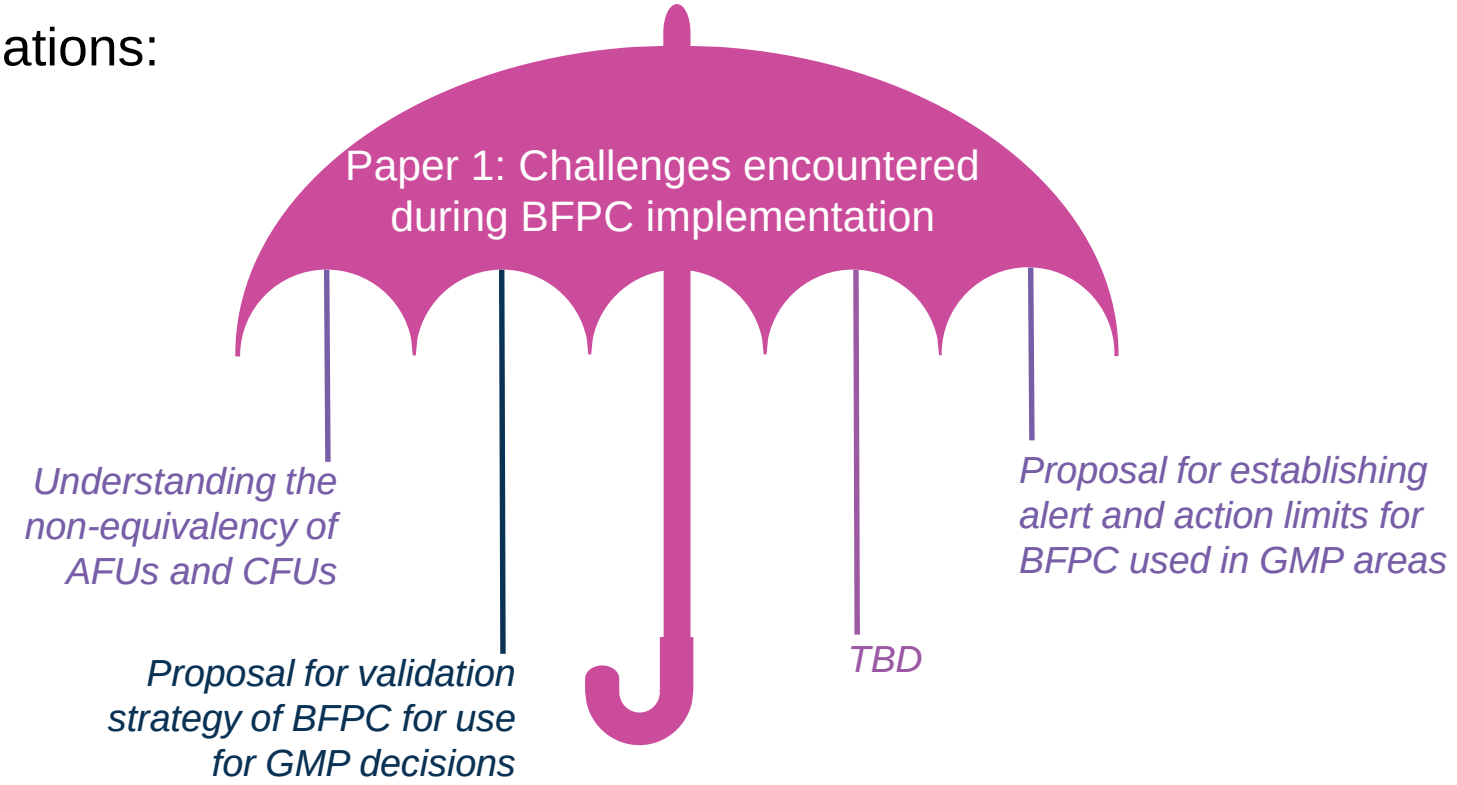
- Who we are – Members:



M³ - Modern Microbial Methods Collaboration

Views of the industry working group collaboration

- Publications:



Views of the industry working group collaboration

- Expected benefits and vision of the future

Improved Product Quality Control

- Real Time EM Data
- Reduced Manufacturing Risks
- Improved Root Cause Analysis
- Process Understanding

Enhances the Marketing and Supply of Safer and Effective Medicines to Patient Population

Views of the industry working group collaboration

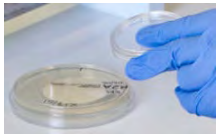
- Expected benefits and vision of the future

Water Sampling

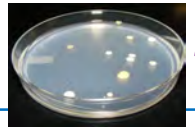
1. Collect water sample

2. Process sample (e.g. membrane filtration)

3. Plate sample filter



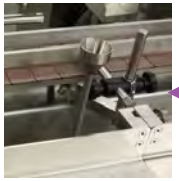
4. Incubate for 2-7 days



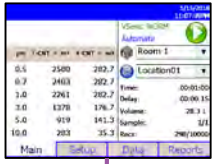
Time to result 2-7 days

BFPC

1. Sample for a specified time/volume



2. Automated counting in real-time



3. Automatically start next sample or operate continuously

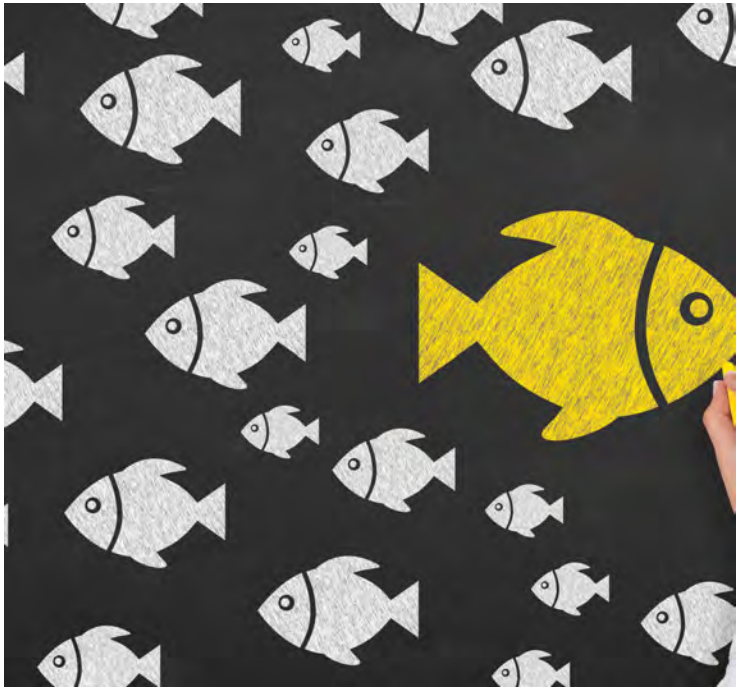
Real-Time Results

Views of the industry working group collaboration

- Why aren't Online Monitoring instruments reality already?

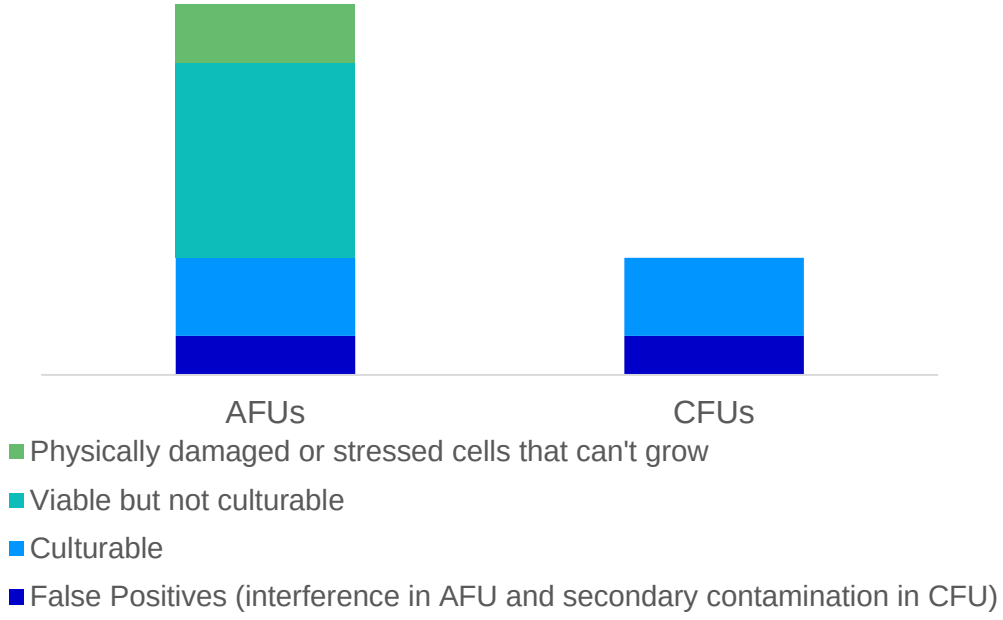
Colony-forming unit (CFU) is a unit used to estimate the number of viable and culturable bacteria or fungal cells in a sample

Auto-Fluorescence 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



Views of the industry working group collaboration

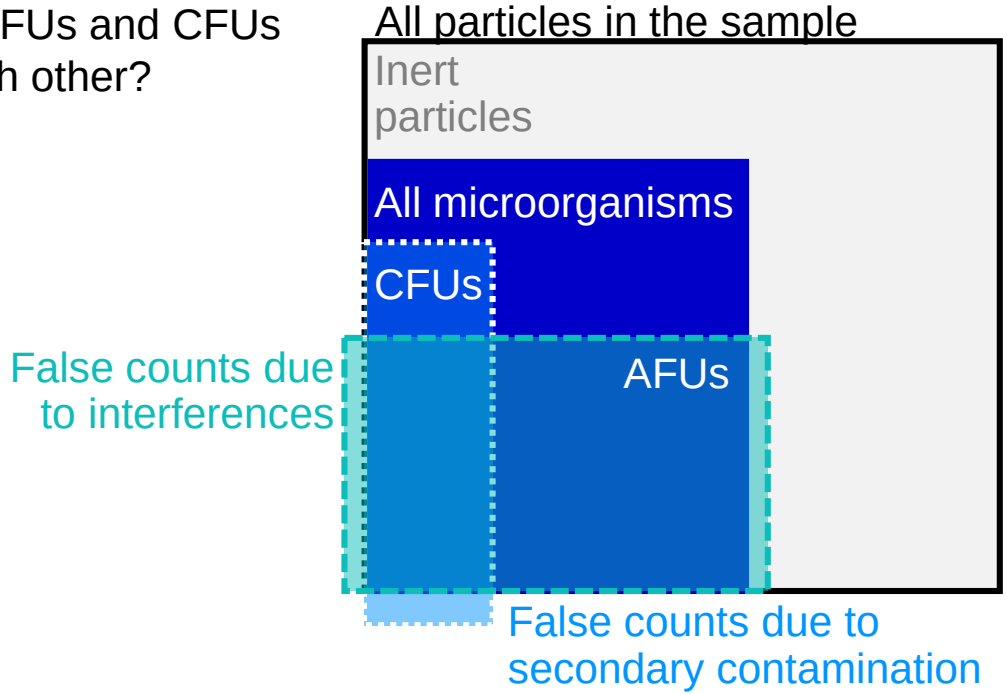
- Why aren't Online Monitoring instruments reality already?
  - What can be measured as AFUs and/or CFUs?



Relative sizes are for illustration purposes only and may differ from sample to sample

Views of the industry working group collaboration

- Why aren't Online Monitoring instruments reality already?
  - How could AFUs and CFUs relate to each other?



Relative sizes are for illustration purposes only and may differ from sample to sample

Views of the industry working group collaboration

- Why aren't Online Monitoring instruments reality already?
- Validation challenges:
  - **Why is validation a challenge?**
    - ⇨Require different methodology than traditional method (AFU ≠ CFU)
    - ⇨Interpretation of validation guidance can be difficult
    - ⇨Extensive validation is often expected
  - **Overcoming this challenge**
    - ⇨Utilize Industry Working Group and Regulatory Support
    - ⇨Determine extent of validation appropriate to application
    - ⇨FDA safe harbor principle and research exemption use as technology is evaluated and implemented
  - **Additional Information**
    - ⇨M^3 collaboration has a paper on validation in progress which will provide a detailed approach

Views of the industry working group collaboration

- Why aren't Online Monitoring instruments reality already?
- Validation strategy:
  - Primary vendor validation address Limit of Detection, Limit of Quantification, etc.
  - User establish non-inferiority to traditional method
  - USP <1223> offers four validation options regarding equivalence testing:
    - ⇨ Decision equivalence: most applicable
  - Compare ability of BFPC vs. Traditional to identify out-of-limit event
  - M^3 Working Group for validation strategy paper
- Qualification Strategy
  - BFPC testing in desired environment to establish baseline counts and potential interferents; "Tailor BFPCs to your needs!"
  - In situ testing in Grades C and D areas or water treatment plants (non-zero AFU and CFU counts)
    - ⇨ Obtain natural microflora information

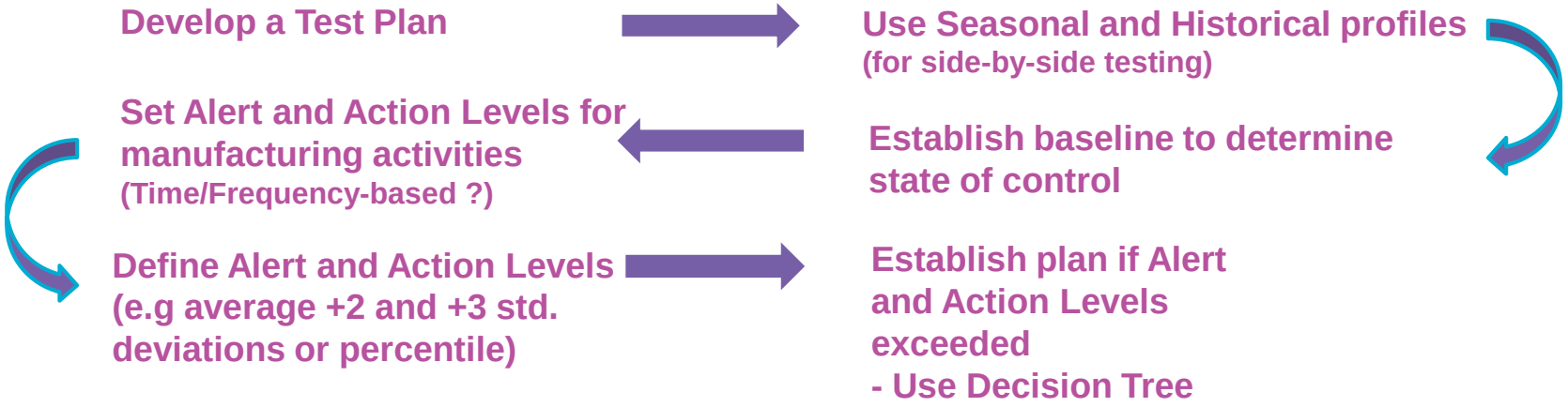
Views of the industry working group collaboration

- Why aren’t Online Monitoring instruments reality already?
- Setting Alert and Action Levels
  - AFU determination often more sensitive than CFU
  - Continuous monitoring can overestimate meaningful excursions
  - Difficult to determine sufficient side-by-side testing needed with traditional method

Views of the industry working group collaboration

- Why aren't Online Monitoring instruments reality already?
- Setting Alert and Action Levels

Overcoming the Challenge



M^3 collaboration has baseline paper to overcome challenges in progress

Views of the industry working group collaboration

- Dilemma?

Regulatory guidelines, such as the EU Annex I draft, have been encouraging the use of alternative technologies.

However, these same guidance documents continue to include limits defined in terms of CFU counts, and agencies continue to expect conventional capture and identification of any “hits” or AFUs.

BFPC and their mode of detection of microorganisms require a re-thinking across the pharmaceutical industry for manufacturers, inspectors, and regulators to enable a paradigm shift from the traditional to modern monitoring methods.

Thank you for your attention

QUESTIONS ?