Replacing traditional Viable EM Methods for Air in Grade A by Implementation of Biofluorecent Particle Counters (BFPC): a Case Study

Kurt Jaecques

SENIOR ADVISOR, GSK VACCINES STERILITY ASSURANCE CENTRE OF EXCELLENCE AT GLAXOSMITHKLINE VACCINES

PDA Parenteral Drug Association

2024 Pharmaceutical Manufacturing and Quality Conference

CONNECTING PEOPLE SCIENCE AND REGULATION®

Scope and General Background

- The majority of EM performed currently relies on conventional methods based on the recovery & growth of microorganisms using solid or liquid microbiological culture media

- these methods (active & passive air – surfaces) are often limited by:
  - slow microbial growth rates
  - long incubation periods
  - unintended selectivity of culture media
  - the inherent variability of microorganism recovery rates due to their non-specific response to culture methods

- Total airborne Particles (non-viable) using Laser light Scattering Devices (DPC)

Rapid methods (RMM) that provide faster time-to-result may offer an advantage for EM tests over current, conventional approaches.

Reference: PDA TR 13 Fundamentals of an Environmental Monitoring Program (update 2022)

Scope and General Background

We would like to move towards a next generation of Environmental Monitoring that enables:

Real-time viable & Total Particle EM within Grade A using RMM Technology: Bio Fluorescent Particle (BFPC) counting.

Value drivers: real-time reaction, efficiency and cost reduction

From

- Manual operations from sampling to analysis
- Agar management creates a real ergonomic pain
- No real time result for immediate investigation
- No opportunity for segregation leading to WO

To

- Gain process insights (continuous/on-line monitoring)
- Faster root cause detection
- Automate data capture and use data analytics (IoT)

Benefit

- Reduce costs & WO
- Increase productivity (manual operation reduction)
- Eliminate risks associated to manual operation in grade A/B
- Fully digitalized processes

Scope and General Background: Regulatory Drivers

PREVIOUS

NEW

EU Annex 1 2022

Alternative methods (RMM) not mentioned within the guidance !!

9.28 The adoption of suitable **alternative monitoring systems such as rapid methods should be considered** by manufacturers in order to expedite the detection of microbiological contamination issues and to reduce the risk to product. These rapid and automated microbial monitoring methods may be adopted after validation has demonstrated their **equivalency or superiority to the established methods**.

The manufacturer should scientifically justify the limits applied and **where possible correlate** them to CFU.

Scope and General Background: Regulatory Drivers

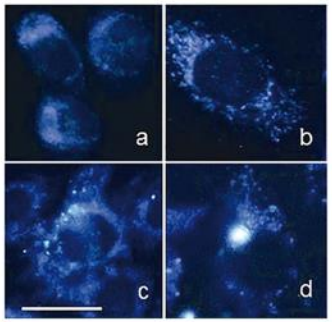
European Pharmacopoeia (EP)

- The **chapter § 5.1.6**. of the European Pharmacopoeia facilitates the implementation and use of alternative microbiological methods:
  - where this can lead to **efficient microbiological control and improved assurance** for the quality of pharmaceutical products.
  - Alternative methods for the control of microbiological quality have shown **potential for real-time or near real-time results with the possibility of earlier corrective action**. These new methods, if validated and adapted for routine use, can also offer significant improvements in the quality of testing.
  - Alternative methods may be used for in-process samples of pharmaceutical products, particularly for the application of Process Analytical Technology (PAT), **for environmental monitoring** and for industrial utilities (e.g. production and distribution of water, steam etc.), thereby contributing to the quality control of these products.

Scope and General Background: application

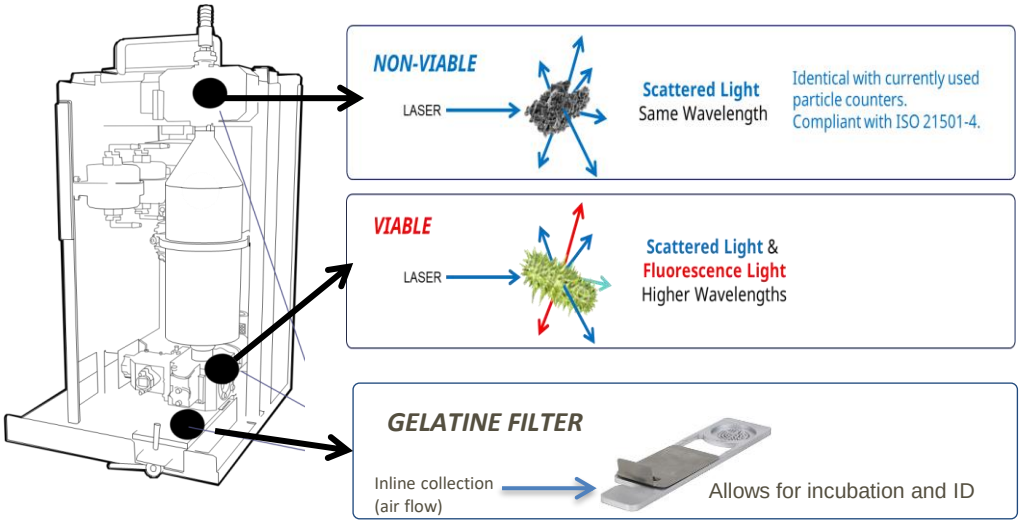
Replace Traditional EM Methods for Air By Implementation of Biofluorescent Particle Counters

Cells naturally fluoresce!



Contain many fluorescent molecules!

Molecule	Approximate Peak Fluorescence (nm)
NAD(P)H	450
Retinol	500
Riboflavin	550
Folic acid	450
Pyridoxine	400
Tyrosine	305
Tryptophan	325
Flavin	540



1. Show equivalence or superiority in detection

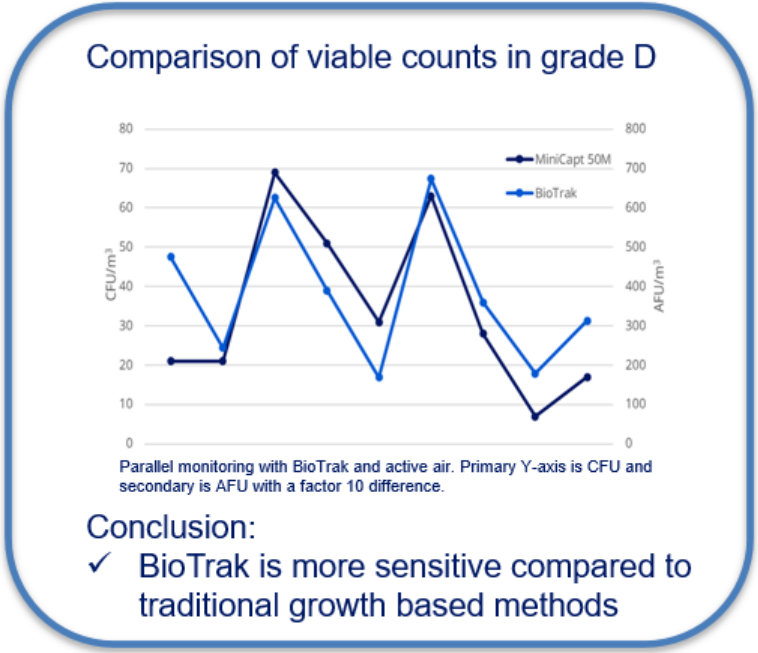
2. Particle loss in tubing: to be qualified for all configurations

3. Identification in terms of AFU detection

4. Response to an AFU hit

Equivalence or superiority in detection: How to confirm?

- BFPC do not only detect viable culturable organisms – also VBNC; damaged cells – some interfering polymers & solvents (typically detect 10 more)
- Equivalence/superiority through parallel testing (standard/BFPC)
  - In High(er) Bioburden Cleanroom
    - *Proposal: Grade D active air vs BFPC*
  - Equivalence/superiority by doing parallel testing in Grade A (i.e. 1000h)
    - *Proposal: to assess per barrier technology (1 RABS/1 Isolator)*
    - *Proposal: statistical equivalence by using e.g. Rare event Control Charts (as majority are zero counts)*



Source: Novo Nordisk PDA Micro Conference 2021

Particle loss in tubing

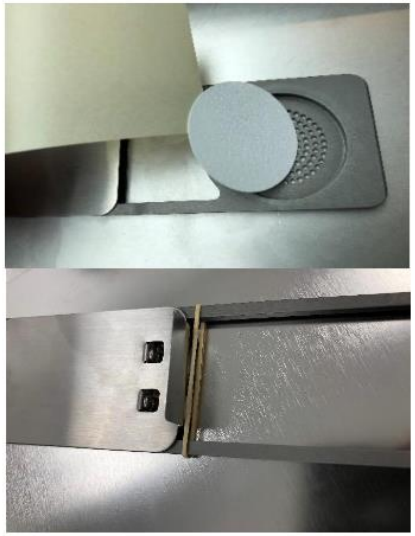
Proposal to minimize particle loss & how to qualify it

- Sampling through a system of pipes and tubes does not affect the biological efficiency. The sampling system affects only the physical efficiency.
- Ref. institute to do this qualification (independent)
- As we know we loose particles (especially 5 micron) – minimize tube length – use appropriate tubing ID – minimize bends
- We can not accept 50% particle retention – requires optimal design – See challenge related to the location of the counter later

Identification upon AFU detection

Proposal

- BFPC detects not only culturable viables
- Equipment can not be installed in Grade A (Grade C or by preference in base of machine to reduce tube length – not hindering operators !)
- Gelatin requires transfer on agar (where?) – Risk for cross-contamination
- Some challenges related to the qualification of the gelatin membranes (e.g. effect holding time on recovery)
- **Do we really need an ID related to each AFU hit knowing that following an AFU hit it will be considered as a CFU (with same associated actions) ?**



Source;: BIOTRAK EU User Meeting 2022 (Novo)

Position of Regulators related to the RMM application

Biggest challenge: not having a microbial ID !

- EMA Innovation Task Force (ITF):
  - Not performing a **microbial ID** upon detection of an AFU goes against the EU Annex 1 requirement § 9.31 ("*Microorganisms detected in Grade A & B should be identified to species level*")
  - Using settle plates post AFU hit: is not **maximizing** the attempt to **recover** a potential contaminant
  - ITF recognizes the specific challenge related to the use of a non-culture based method – need for regulatory harmonized position on microbial ID !
- FDA Emerging Technology Team (ETT):
  - One should **maximize the recovery** of the potential contaminant
  - An investigation without an ID is not deemed to be a thorough one

Position of Regulators related to the RMM application

Biggest challenge: not having a microbial ID !

- Arguments for not performing an ID:
  - Remediation action following an AFU the same as for a confirmed CFU
  - RMM is **much more sensitive and is measuring continuously** – much higher level of detectability (culture-based methods are semi-quantitative!)
  - Trying to recover the gelatin implies cross-contamination risks
  - Trying to recover the gelatin require access to the gelatin support (so device not to be installed in machine base – longer tubing/bends required)
  - An AFU might originate from a VBNC organism; damaged cell, interference…so success to recover it limited

Guidance on good manufacturing practice and good distribution practice: Questions and answers

Human | Veterinary | Compliance and inspections | Regulatory and procedural guidance | Research and development

Guidance on good manufacturing practice and good distribution practice: Questions and answers | European Medicines Agency (europa.eu)

4. Is rapid method valid for the detection of microorganism within grade A and B? H+V Jan 2024

Rapid method is one of the alternative monitoring systems that may expedite the detection of microorganisms pending that the requirements of annex 1 points 9.28, 9.30 and 9.31) are fulfilled.

9.31 Microorganisms detected in the grade A and grade B areas should be identified to species level and the potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Consideration should also be given to the identification of microorganisms detected in grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may indicate a loss of control, deterioration in cleanliness or that may be difficult to control such as spore-forming microorganisms and moulds and at a sufficient frequency to maintain a current understanding of the typical flora of these areas.

In the end…ALL BFPC to be installed outside of the machine base!

What is the correct response to an AFU hit

Proposal

- Although an AFU does not per definition mean it is 100% sure it poses a microbial risk for the aseptic process (interference, cell debris/non culturable etc.) – data show that typically within a correct operating Grade A, AFU hits are very rare (even UDAF in Grade B). So, for this reason:
- GSK proposes:

AFU excursion

In Grade A
AFU = CFU

Any AFU Hit (>0) is an action limit

Handling of Incident

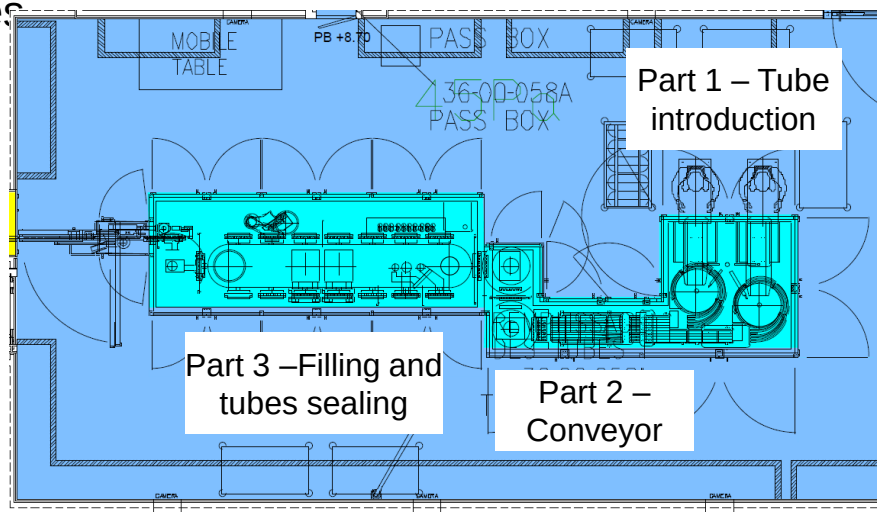
1. Immediately stop the line
2. Operator alerted, and an investigation is performed in order to assess for rootcause
3. Potentially affected units: rejected automatically!
4. 1 AFU = trigger to confirm contamination risk & ID

Continue fill

Following appropriate containment & consideration of resanitization

Project Strategy for GSK Vaccines:

Start off with a pilot on Rotarix oral Vaccine Filling line – final goal: All Grade A within GSK Vaccines



- **Existing** commercial tube filling line used for **sterile** vaccine
- **Grade A** (Open RABS) surrounded by a Grade B area
- EM performed by:
  - particles counters
  - viable air sampling
  - Settle plates

  => Change of settle plates every 4h meaning **stops of the filling activities** and **intrusion** in the grade A

Project Strategy for GSK Vaccines:

Replace settling plates (passive air sampling) and active air sampling by Biofluorescence Particle Counters in grade A

- No change on the current sampling locations
- No change on surface sampling (swab/finger touch)

REGULATORY STRATEGY

VALIDATION STRATEGY

OPERATIONAL STRATEGY/CHALLENGES

Sterility assurance improvement

- **Risk decrease**: Currently 98 settle plates per batch ➔ 98 ingressions for settle plate change & ingress of new settle plates
- **Robustness increase**: A real time result is giving the opportunity to immediately investigate and take action
- **Reduction of unplanned events**: Last year, 30 deviations linked to settle plate issues occurred: cracks in the gel, opening time exceeded, settle plate falling down

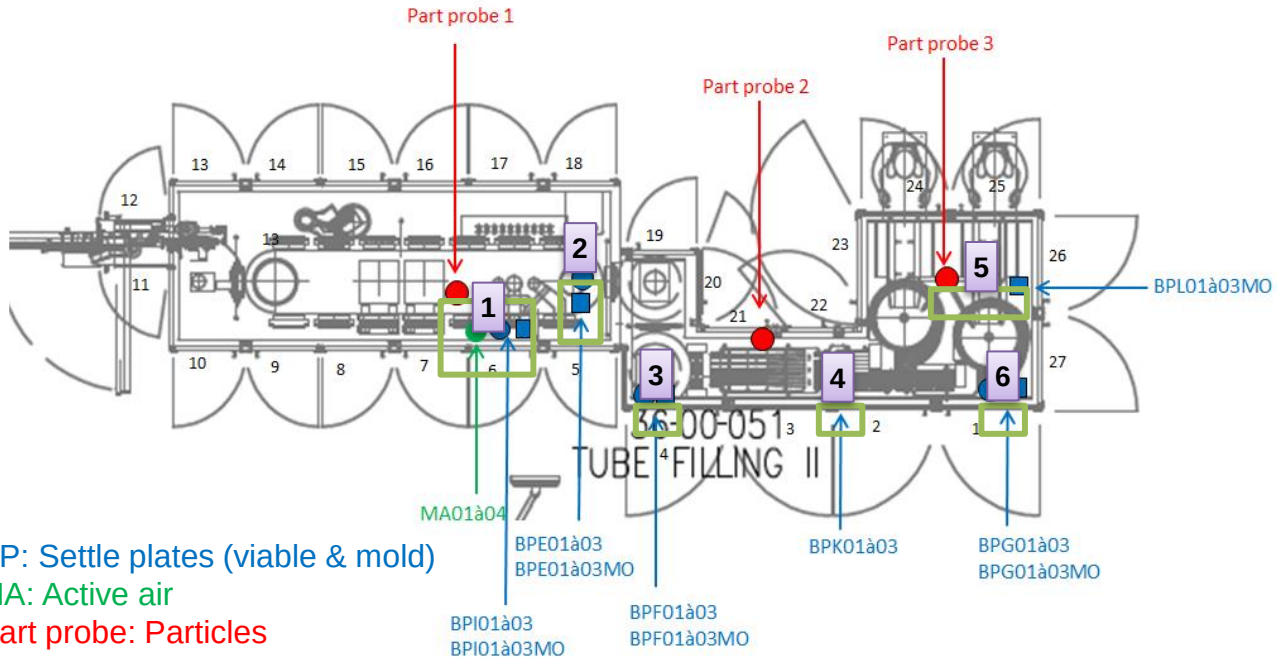
Productivity

- **Improve line productivity** by filling additional doses (7 times 20 Min saving per batch)
- **Operator time saving**: one grade A operator dedicated for this task

Sustainability

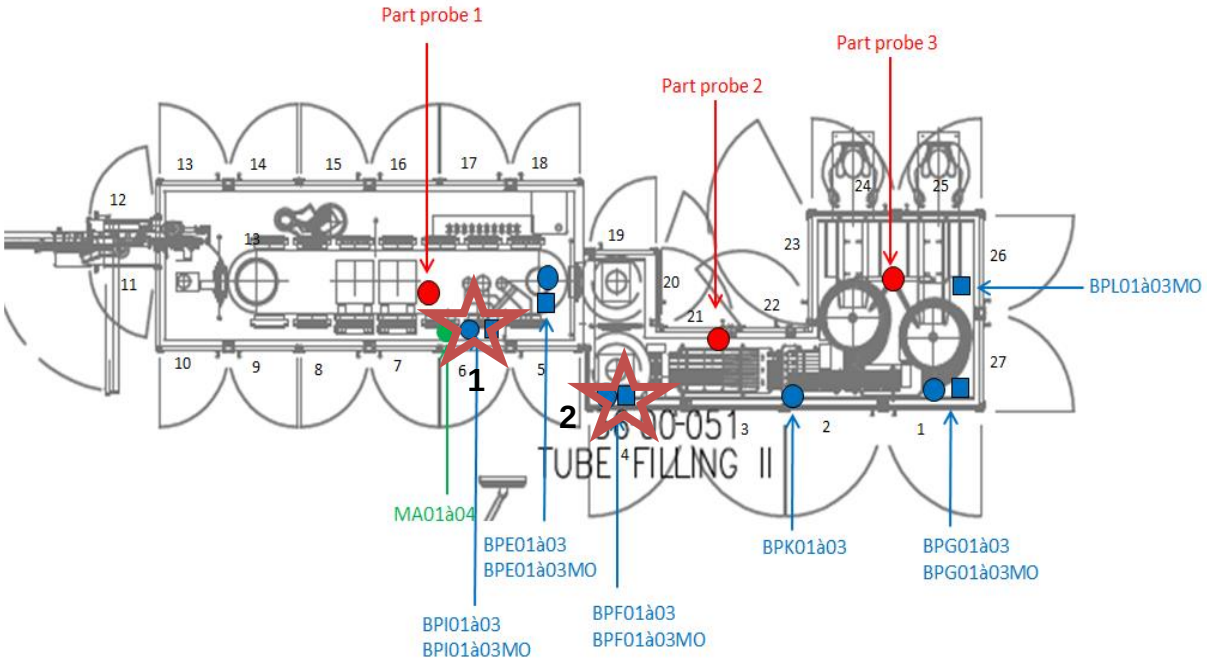
Real-Time EM Pilot Project

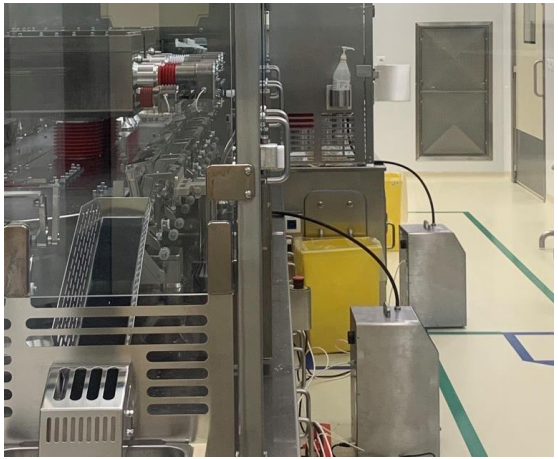
Particle Counters locations



BP: Settle plates (viable & mold)
MA: Active air
Part probe: Particles

Feasibility Study RABS Filling Line





Conclusions upon Pilot feasibility study

- AFU hits during filling of oral Vaccine (oral suspension in squeezable polyethylene tube):

Activity	AFU Hits ?
Start-up of BFPC	Yes (need to pre-flush)
At-rest of the filling line	No
In-operation (filling – RABS doors closed)	No
Open door interventions	No
Use of IPA wipes (upon change EM plates)	Yes (wipes – IPA – transfer ?)

Can't rule out the condition of sampling tubing used for this study

Can Rule out IPA as spraying directly above the device did not cause AFU hits

Overall conclusions & next steps

- BFPC seems to be superior to conventional culture-based methods (typically detection signal 10 x higher)
- Within Grade A – standard processes (even open door) seem not to increase level of detected above action limits
- Some interference observed from materials used (i.e. wipes) – need mitigation
- Regulators expect a maximum effort to obtain a microbial ID upon detection of an AFU (even for nonculture-based RMM – global regulatory position = ID)

Thank you

- GSK Internal Team
  - Aurélie Stragier
  - Vincent Jouvenne
  - Florence Mirarchi
  - Barbara Laurensis