BioAerosol Monitoring System (BAMS)

How it supports 2022 EU GMP Annex 1

Table of Contents

1. Introduction to Cherwell

Cherwell is a leading supplier of prepared microbiological media and environmental monitoring instrumentation for the pharmaceutical and related industries.

Cherwell started life in 1971 and is still a family-owned business, our current MD is the second generation. We are a dedicated group who truly believe in doing things right. Our values of Reliable, Adaptable and Partnership play to the importance we place on not only working together, but more importantly on focusing on our customer's needs. And delivering on those wishes. We believe in making high quality products, not cheap products, that truly help our customers to be more effective.

Our business relies on individuals who are dedicated to doing things to a high standard, every time and against defined procedures. Therefore, attention to detail is vital as every element is critical in producing a top-quality product.

The leading provider of standard and tailored cleanroom microbiology solutions for effective management of controlled environments and processes.

- Environmental Monitoring Solutions
- Prepared Media Manufacturer
- Viable Air Sampling and Monitoring

2. BioAerosol Monitoring System (BAMS)

2.1 OVERVIEW

- 5 LPM flow rate, 405nm laser, one particle detector and one fluorescence detector
- Measures particle size and biologic status simultaneously
- Continuous, real-time results without need for staining or reagents (up to 6 hours while on battery power)
- ISO 21501-4 compliant particle counter
- 8” touchscreen usable while wearing 2 layers of latex gloves
- Can auto-trigger a sample with the Bio-Aerosol Sampler (BAS) to capture a traditional microbial aerosol sample
- Can be used to sample compressed air when connected to the Electric High Pressure Diffuser (EHPD)

2.2 BAMS SPECIFICATION

Specification	BioAerosol Monitoring System	Specification	BioAerosol Monitoring System
Alarm	Audible built-in alarm	Battery	10.8V, 9000mAh, rechargeable lithium battery
Calibration frequency	Once a year	Capture the biological contamination sample	Connect the BioAerosol Sampler (BAS) via WIFI/USB to collect the biological contamination sample in real-time
Communication	RJ45, USB, SENSER-HUB, WIFI	Concentration limit	4,000,000 particles/ft^3 at 10% coincidence loss
Count efficiency	50% ± 20% for 0.5µm, 100% ± 10% for > 0.75µm (meets ISO 21501-4 and JIS B9921)	Cycles	1,000 samples on one location
Data reliability	Compliant with 21CFR Part 11	Data security	Authority management, authority level divide into admin, operator and supervisor
Data storage	119G	Delay	0-99 hours 59 minutes 59 seconds
Dimensions (HxWxD)	1O (H) x 7.87 (W) x 10.39 (D) in 255 (H) x 200 (W) x 264 (D) mm (with handle and foot mat)	Display	8.0" touch screen
Enclosure	316L Stainless Steel and anodized aluminium	Exhaust	Internal HEPA filter (>99.997% @ 0.3µm)
Export file	PDF file or EXCEL file	Flow rate	5L/min with ± 3%
Flow rate control	Electronic, automatic closed-loop	Interval	0-99 hours 59 minutes 59 seconds
Language	Chinese, English	Laser source	Long life laser
Operating conditions	Temperature: 5°C - 35°C / 41°F - 95°F Relative humidity: 5 - 90% non-condensing	Power	AC 100 - 240V, 50 Hz / 60 Hz
Print	Auto, off-line	Reports	ISO / EUGM P / CHINESEGM P

Specification	BioAerosol Monitoring System	Specification	BioAerosol Monitoring System
Safety	FCC Part 15, Subpart B, EN 61010 - 1:2010, EN 61326-1:2013, EN 6132 6-2-2:2013, EN 61000-6-1:2007, EN 61000-6-3:2007+A1, EN 300 32 8 V2.1.1: 2016, ETSI EN 301 489-1 V2.2.0: 2017, ETSI EN 301 489-17 V3.2.0: 2017, EN 62311:2008, EN 62479: 2010, EN 60825-1 :2014, ASTM D 4169 DC13, FCC IDENTIFIER: 2AV6V-M 110	Sampling mode	Manual, auto, cumulative count ∑/ differential count Δ or concentration
Sampling time	0.1 seconds - 999 hours 59 minutes 59 seconds	Size channels	0.5µm, 1.0µm, 2.0µm, 3.0µm, 5.0µm, 10.0µm
Size range	0.5µm to 25µm	Size resolution	<15% @ 0.5µm (meets ISO 21501-4)
Storage conditions	Temperature: 5°C - 35°C / 41°F - 95°F Relative humidity: 5 - 90% non-condensing	Warranty	1 year after activation
Weight	12.81bs / 5 .8kg (without battery)	Zero count	<1 count / 5 min

3. 2022 EU GMP Annex 1

The 2022 Annex 1 revision was released on 25 August 2022

- This is a revision of the previous 2008 version of Annex 1
- The release comes two years after the last draft version of the document
- The length has increased from 16 to 59 pages

Industry has one year to implement

- Deadline for coming into operation is 25 August 2023
- Only point 8.123 is given two years and a date of 25 August 2024

One reason listed for the updates made is:

- To clarify how manufacturers can take advantage of new possibilities deriving from the application of an enhanced process understanding by using innovative tools as described in the ICH Q9 and Q10 guidelines

A significant increase in detail and guidance has been included in the **2022 Annex 1 revision.**

Alternative approaches, rapid/alternative methods, continuous monitoring systems and trend analysis are all mentioned, often with regards to supporting **QRM** and a **Contamination Control Strategy**.

- Note that none of these topics were discussed or mentioned in the 2008 Annex 1 and support the forward thinking displayed in this revision

"Continuous total particle and viable monitoring is recommended, primarily for grade A, and a significant departure from the previous revision"

Trending is also stressed throughout the document to support **Quality Risk Management**, a timely identification of adverse trends or a change in the environment and potential for **faster resolution** if identified.

3.1 ENVIRONMENTAL & PROCESS MONITORING

Section 9 includes total particle environmental monitoring (EM) and viable particle EM and personnel monitoring (classification is covered in Section 4)

- 9.10 – 9.12 all relate to trending of data, which is supported by continuous rapid microbiological methods much more significantly than the traditional method
    - 9.10 Alert levels for grade A (total particle only) grade B, grade C and grade D should be set such that adverse trends (e.g. a numbers of events or individual events that indicate a deterioration of environmental control) are detected and addressed
    - 9.11 Monitoring procedures should define the approach to trending. Trends should include, but are not limited to:
        - Increasing numbers of excursions from action limits or alert levels
        - Consecutive excursions from alert levels
        - Regular but isolated excursion from action limits that may have a common cause, (e.g. single excursions that always follow planned preventative maintenance)
    - 9.12 The monitoring of grade C and D cleanrooms in operation should be performed based on data collected during qualification and routine data to allow effective trend analysis

3.2 ENVIRONMENTAL & PROCESS MONITORING – TOTAL PARTICLE

Table 5: Maximum permitted total particle concentration for monitoring

Grade	Maximum limits for total particle ≥ 0.5 µm/m3		Maximum limits for total particle ≥ 5 µm/m3	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined[(a)]	29 300	Not predetermined[(a)]

[a]For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data, where applicable.

Note 1: The particle limits given in the table for the 'at rest' state should be achieved after a short 'clean up' period defined during qualification (guidance value of less than 20 minutes) in an unmanned state, after the completion of operations (see paragraph 4.29).

Note 2: The occasional indication of macro particle counts, especially ≥ 0.5 µm, within grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation.

- Significant changes are present within section 9 on continuous monitoring, and monitoring for the duration of critical processing so that excursions can be identified, and action taken.

    - 9.16 For grade A, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly

    - 9.17 **The grade A area should be monitored continuously** (for particles ≥0.5 and ≥5 µm) and with a suitable sample flow rate (at least 28 litres (1ft3) per minute) so that all interventions, transient events and any system deterioration is captured. The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms including the consideration of additional microbial monitoring

    - 9.18 **It is recommended that a similar system be used for the grade B area** although the sample frequency may be decreased. The grade B area should be monitored at such a frequency and with suitable sample size that the programme captures any increase in levels of contamination and system deterioration. If alert levels are exceeded, alarms should be triggered

    - 9.21 The size of monitoring samples taken using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of cleanrooms and clean air equipment. Monitoring sample volumes should be justified

3.3 ENVIRONMENTAL & PROCESS MONITORING – VIABLE PARTICLE

Table 6: Maximum action limits for vialbe particle contamination

Grade	Air sample CFU / m^3	Settle plates (diam. 90mm) CFU / 4 hours[a]	Contact plates (diam. 55mm) CFU / plate[b]	Glove print, including 5 fingers on both hands CFU / glove
A	No growth[c]			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

[a] Settle plates should be exposed in grade A and B areas for the duration of operations (including equipment set-up) and changed as required after a maximum of 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used).

- For grade C and D areas, exposure time (with a maximum of 4 hours) and frequency should be based on QRM.
- Individual settle plates may be exposed for less than 4 hours.

[b]Contact plate limits apply to equipment, room and gown surfaces within the grade A and grade B areas. Routine gown monitoring is not normally required for grade C and D areas, depending on their function.

[c]It should be noted that for grade A, any growth should result in an investigation.

Note 1: It should be noted that the types on monitoring methods listed in the table above are examples and other methods can be used provided they meet the intent of providing information across the whole of the critical process where product may be contaminated (e.g. aseptic line set-up, aseptic processing, filling and lyophilizer loading).

Note 2: Limits are applied using CFU throughout the document. If different or new technologies are used that present results in a manner different from CFU, the manufacturer should scientifically justify the limits applied and where possible correlate them to CFU.

"Grade A maximum action limits for viable particle contamination have changed from <1 to no growth as any growth in grade A should result in an investigation."

- Note 1 mentions use of monitoring methods other than those listed in the table
- Note 2 refers to use of new technologies that do not report in CFU
- It will remain to be seen how companies interpret the "No growth requirement" for grade A when using more sensitive technologies not dependent upon growth
- As included for total particle monitoring, there is now mention of **continuous viable air monitoring in grade A with a similar approach recommended for grade B**
    - 9.22 Where aseptic operations are performed, microbial monitoring should be frequent using a combination of methods such as settle plates, volumetric air sampling, glove, gown and surface sampling (e.g. swabs and contact plates). The method of sampling used should be justified within the CCS and should be demonstrated not to have a detrimental impact on grade A and B airflow patterns. Cleanroom and equipment surfaces should be monitored at the end of an operation
    - 9.24 **Continuous viable air monitoring in grade A (e.g. air sampling or settle plates) should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and critical processing**. A similar approach should be considered for grade B cleanrooms based on the risk of impact on the aseptic processing. The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and any risk caused by interventions of the monitoring operations is avoided
- Efficiency and validation data are both mentioned as needed in this section for sampling methods selected
    - 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
    - 9.29 Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. Supporting data for the recovery efficiency of the sampling methods chosen should be available

4. BioAerosol Monitoring System (BAMS) in Support of RU GMP Annex 1

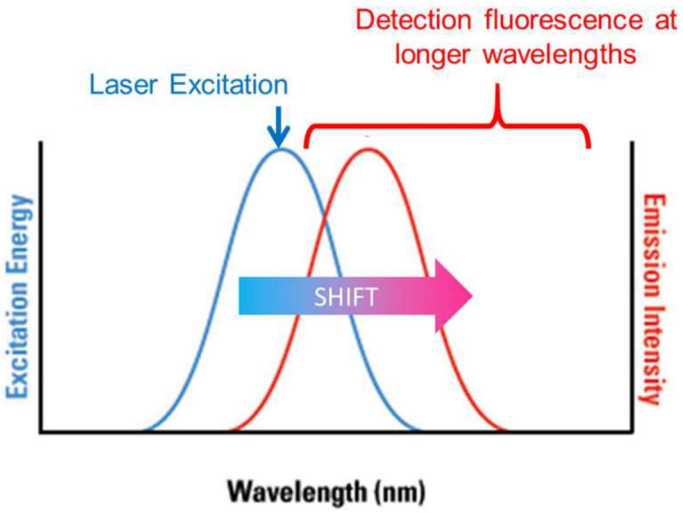
4.1 BENEFITS

- BAMS 5 LPM continuous microbial and total particle monitor
  - ISO-21501-4 compliant particle counter
  - Real-time microbial monitor based on intrinsic fluorescence detection (no sample preparation needed)
- BAMS Bio-Fluorescent Particle counter readily supports:
  - Continuous total particle and biologic particle monitoring
    - Reports total particle and total biologic counts in a range of sizing bins including ≥ 0.5 µm and ≥ 5 µm per m^3
    - With a 5 LPM flow rate, the system can be used for continuous total particle monitoring in Grade B environments but does not support the minimum 28 LPM flow rate for Grade A total particle monitoring
  - Personnel training due to real-time total particle and biologic count feedback
  - Minimisation of operator presence in critical environments
  - Continuous data stream to better support evaluation of trends
  - Micro-organism collection through an active air sampler trigger option

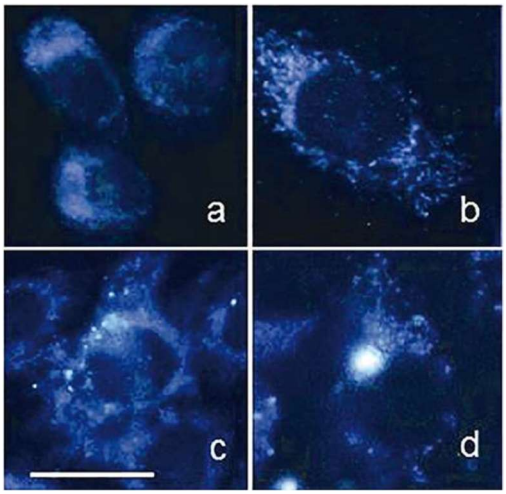
4.2 BIO-FLUORESCENT PARTICLE COUNTERS (BFPC)

- Bio-fluorescent particle counters (BFPC) are a form of enhanced particle counters, capable of the **continuous and real-time detection of inert particles and micro-organisms** in air and water
- These systems utilise the detection of scattered laser light for particle enumeration, and fluorescence detection for the classification of detected particles as BFP (i.e. biologic) or inert (i.e. non-biologic). As particles are sampled by the BFP-counting system, they are exposed to laser light, commonly at a 405nm wavelength

4.3 BIO-FLUORESCENT PARTICLE (BFP)



Cells naturally fluoresce



All cells 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

**Primary molecules detected by 405nm laser excitation*

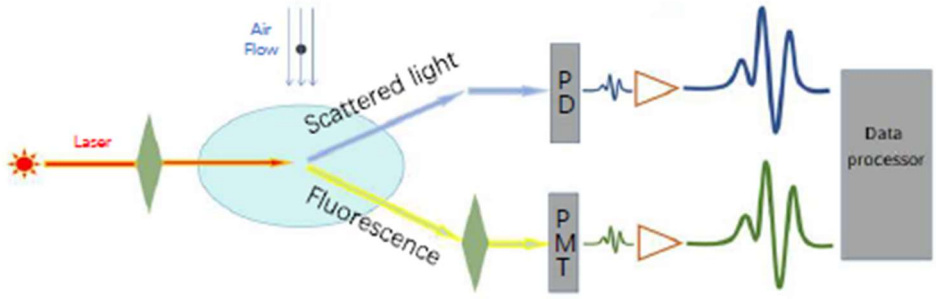
4.4 PROCESS CONTROL – BFPC INSIGHT AND ADVANTAGE

- BFPC systems sample continuously, and report total particle and biologic counts in real time
    - Continuous monitoring allows for improved understanding of the environment and risk assessment
    - Real time reporting allows for proactive process control decisions
    - Real time results facilitate faster root cause assessment and corrective action
- BFPC will generally detect more micro-organisms than the traditional method
    - BFPC detection is not dependent upon the ability of a micro-organism to grow, and therefore can detect a broader range of micro-organisms (e.g. VBNC, stressed and dead cells, if the cell wall is still intact)
    - Methods based on micro-organism culturability can have significant limitations to the number of micro-organisms detected, especially under traditional incubation times, temperatures and media selection
- BFPC is a complementary tool
    - BFPC systems do not identify
    - Traditional culture-based method can be used if an over action event is identified on the BFPC system, leading to more informed monitoring with the traditional method

4.5 TECHNICAL PRINCIPLE OF BIOAEROSOL MONITORING SYSTEMS (BAMS)

- Sampled air is continuously interrogated by a 405nm laser
- All particles present in the sampled air are assessed for scattered light, used to size and enumerate particles, and fluorescence, for particle characterisation
    - Fluorophores, like NADH and riboflavin, present in all cells will emit fluorescence when irradiated with 405nm laser light
    - Other non-biologic, inert materials, can also emit fluorescence
- BAMS detects scattered light, using a photodiode, and fluorescence, using a photomultiplier tube, simultaneously and in real time

- Detected signals are passed through system algorithms to categorize fluorescent particles as biologic or inert



4.6 2018-2022 INDUSTRY PUBLICATIONS & WEBINARS

- Montenegro-Alvarado JM, et al. Pfizer case study: rapid microbial methods for manufacturing recovery after Hurricane María. Pharm Online. 2018 July
- Montenegro-Alvarado JM. Pfizer Leveraging rapid microbiological methodology in forensic evaluation to identify elusive root cause. Amer Pharm Rev. [Internet]. 2018 Sep
- Online Water Bioburden Analyzer Workgroup. A better approach to pharmaceutical water testing – user requirements for an online water bioburden analyzer. Pharm Online. 2018 Nov
- Weber J, et al. BPOG Continuous microbiological environmental monitoring for process understanding and reduced interventions in aseptic manufacturing. PDA J Pharm Sci Technol. 2019 Mar/Apr;73(2):121-134
- Russ M. Genentech Webinar – Changing a Paradigm: Implementing a Real Time Microbial Detection Analyzer in Pharmaceutical Water. Amer Pharm Rev. 2019 Mar 14
- Ayers F, et al. PEMM Bio-Fluorescent Particle Counter-Based Real-Time Feedback and Control of Processing Conditions, Eur Pharm Rev, Aug 2019 ed
- Benkstein K, et al. Evaluating changes to Ralstonia pickettii in high-purity water to guide selection of potential calibration materials for bioburden analyzers. J Ind Microbiol Biotechnol. 2019 Jul; 46: 1469-1478
- Bar R. Charting and Evaluation of Real-Time Continuous Monitoring Water Bioburden. PDA J Pharm Sci Technol. 2019 Sep; 73 (5) 496-509
- Prasad A, et al. BPOG Practical applications of bio-fluorescent particle counting in Environmental Monitoring Investigations. PDA J Pharm Sci Technol. 2020 Jan/Feb;74

- Hjorth J, et al. GMP Implementation of Online Water Bioburden Analyzers. Pharmaceutical Engineering. 2021 Jan/Feb
- Scott A, et al. PEMM A Discussion on Bio-Fluorescent Particle Counters: Summary of the Process and Environmental Monitoring Methods Working Group Meeting with the FDA Emerging Technology Team. Pda J Pharm Sci Technol. 2021
- Briglia C, et al. M3 Initial Evaluation Roadmap for Modern Microbial Methods. PDA Letter. 2022 Apr
- Scott A, et al. M3 Challenges Encountered in the Implementation of Bio-Fluorescent Particle Counting Systems as a Routine Microbial Monitoring Tool. Pda J Pharm Sci Technol. 2022

4.7 BFPC APPLICATIONS

- Timely return to production
    - Routine maintenance
    - New construction or equipment
    - Return from shutdown acceleration
- Improved Risk Assessment
    - Sample site selection
    - Dynamic modelling
    - FMEA
- Informed Investigations
    - Excursions
    - Root cause investigates and troubleshooting
    - Verification of CAPA effectiveness
- Green & Training Initiatives
    - HVAC flow reduction studies
    - Gowning training
    - Aseptic technique

cherwell

an analytichem company

7 & 8 Launton Business Centre,
Murdock Road, Bicester, OX26 4XB,
United Kingdom.

T: +44 (0)1869 355500
W: www.cherwell-labs.co.uk
E: sales@cherwell-labs.co.uk