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EU GMP ANNEX 1:

Continuable Viable Monitoring and the Role of Lower Flow Rates in Air Samplers

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Overview

Continuous viable monitoring is essential in aseptic manufacturing, especially under the EU GMP Annex 1 guidelines established in 2022. These guidelines emphasize real-time environmental monitoring in Grade A areas to quickly identify and address contamination risks. By utilizing lower flow rates, such as the 10L/min ActiveCount Remote Air Sampler, you can significantly enhance your monitoring processes while also achieving cost efficiencies.

Lower flow rates lead to fewer sampling plates, which reduces media consumption and minimizes the labor required for incubating and processing samples. This approach not only aligns with Annex 1's directive to minimize human interventions but also decreases the risk of contamination associated with operator presence in critical areas. Furthermore, implementing lower flow rates streamlines data management, allowing your quality assurance team to focus on real-time results rather than extensive reports.

By adopting this method, you not only ensure compliance with regulatory standards but also promote environmental sustainability by reducing overall resource consumption. Lower flow rate sampling thus supports effective contamination control while enhancing operational efficiencies, making it a practical solution for maintaining the integrity of aseptic environments in pharmaceutical manufacturing.

EU GMP Annex1 Continuous Microbial Monitoring

EU GMP Annex 1: 2022 places significant emphasis on continuous environmental monitoring (EM) in aseptic manufacturing, particularly in Grade A environments. The guidelines aim to reduce contamination risks by ensuring that environmental conditions, including microbial and particulate counts, are constantly monitored in real-time, ensuring any deviation is immediately detected. This approach enhances contamination control by allowing rapid responses to any adverse conditions that may arise during production. Lower flow rates for microbial air sampling, as validated in this study, support the continuous monitoring model of Annex 1, offering practical benefits in reducing both operational complexity and associated costs.

Cost and Operational Efficiencies with Lower Flow Rates

Continuous monitoring at lower flow rate (10L/min), as demonstrated with the ActiveCount Remote Air Sampler, offers several efficiencies aligned with Annex 1's principles. The guidelines recommend using systems that minimize environmental and operational impact, reducing not only contamination risks but also overall resource consumption. Lower flow rates allow for fewer sampling plates, reducing the frequency and volume of samples needed to maintain compliant monitoring, which brings several advantages:

1. **Reduced Media Requirements & Cost Savings**

With fewer TSA plates needed for continuous monitoring, operational costs decrease significantly. Annex 1 emphasizes optimizing resources in aseptic environments, and by using lower flow rates, the facility can reduce its media consumption by avoiding excessive sampling frequency. This directly translates to savings in:

- **Labor Costs:** Lower media usage means less time spent incubating plates, processing results, and preparing reports.
- **Incubation and Storage Resources:** Fewer samples mean reduced space and energy needed for incubation, an essential aspect in high-throughput facilities where space and resources are at a premium.
- **Report Generation and Data Management:** With fewer plates, the process of compiling, reviewing, and storing microbial monitoring data is streamlined, reducing workload and allowing resources to be allocated more efficiently.

2. **Minimizing Operator Interventions and Reducing Contamination Risk**

Continuous monitoring at lower flow rates also aligns with Annex 1's emphasis on minimizing human interventions in aseptic processes. Each operator entry into a Grade A environment introduces potential contamination risks. By requiring fewer media replacements and sample transfers, lower flow rates reduce the need for operator presence in critical areas, thus maintaining the aseptic integrity of the environment.

- **Annex 1, Section 9.28** specifies that "human interventions should be minimized," noting that any activity in the aseptic zone increases contamination risks. Lower flow rate sampling supports this requirement by reducing operator handling and frequency of interventions.

3. **Enhanced Efficiency and Environmental Sustainability**

The reduced need for high-volume air sampling aligns with Annex 1's encouragement of efficient resource use, supporting environmental sustainability by decreasing both media and energy consumption. Lower flow rate sampling reduces HVAC system burden, aligning with the Annex's guidance on sustainable cleanroom practices by lessening energy consumption in the air handling units (AHUs) that support air quality in Grade A and B environments.

- **Annex 1, Section 8.2** emphasizes that facilities should ***"take measures to optimize the environmental impact of operations,"*** which includes reducing unnecessary resource consumption. By optimizing sampling rates to the minimum required for accurate monitoring, lower flow rates contribute directly to this objective.

Operational Example: Continuous Monitoring Workflow with Lower Flow Rates

By implementing lower flow rate monitoring, facilities can operate a streamlined workflow that is both cost-effective and compliant with Annex 1:

- **Sampling Frequency:** The 10 L/min samplers allow for extended monitoring without frequent plate replacements. This approach fulfills Annex 1's continuous monitoring requirements while reducing **operator exposure and media consumption.**
- **Data Review and Reporting:** With fewer samples to manage, QA teams can focus on real-time data from critical samples, reducing overall reporting requirements and allowing rapid identification of any microbial deviations.
- **System Setup and Maintenance:** Lower flow rate samplers require less frequent servicing and calibration, which simplifies maintenance scheduling and ensures compliance with **Annex 1, Section 5.12**, which requires regular qualification and maintenance of monitoring equipment.

Aligning Lower Flow Rate Sampling with EU GMP Annex 1

Lower flow rates for continuous microbial monitoring, as validated in this study, provide clear advantages in aseptic manufacturing. By reducing media and resource demands, minimizing operator interventions, and aligning with the environmental goals of EU GMP Annex 1, these samplers provide a compliant and cost-effective solution for real-time microbial monitoring in cleanroom environments. Lower flow rate sampling thus enhances contamination control while supporting operational efficiencies and sustainability in line with EU GMP standards, establishing a foundation for streamlined, compliant, and environmentally responsible aseptic production practices.

Validation of 10L/min Sampling and Media Qualification

Lighthouse Worldwide Solutions contracted a 3rd party company Bactrimm Pharmaceutical Microbiology to qualify the ActiveCount Remote Air Sampling system with 10L/min flowrate to demonstrate media exposure times achievable with recovery and dehydration meeting Industry regulatory requirements.

Importance of Media Validation

For microbial monitoring, the sampling media must not only support growth but also maintain stability during prolonged exposure to airflow, avoiding dehydration (keeping it below 40%) and ensuring biological recovery rates above 70%. The TSA plates containing 1% glycol were specifically selected to maintain moisture and facilitate microbial recovery, making them ideal for continuous monitoring applications in cleanrooms.

Objectives

The study's objective was to qualify the ActiveCount Remote Air Sampler at 10L/min for extended air sampling under Grade A (ISO 5) conditions. Air sampling was conducted across varying volumes (1,000, 1,500, 2,000, and 2,500L) to assess both the effectiveness of the sampler and the integrity of the TSA plates. Growth promotion tests were performed to ensure the TSA plates could support microbial growth after air sampling.

Materials and Methods

Test Products

- **Air Sampler:** ActiveCount and Remote Impactor Kit model at 10L/min.
- **Media:** 90mm TSA plates with 1% glycol, sourced from BioTrading, stored at 2-8°C to ensure stability before use.

Test Strains

The study used a range of microbial strains that represent potential contaminants in cleanroom environments:

Reference Strains:

Pseudomonas aeruginosa
ATCC 9027 | PR230813-140

Bacillus subtilis spores
ATCC 6633 | 1066633-713

Staphylococcus aureus
ATCC 6538 | PR230813-139

Candida albicans
ATCC 10231 | PR230813-142

Aspergillus brasiliensis
ATCC 16404 | PR230813-143

House Flora Isolates:

Micrococcus luteus
BHF19-99, BHF19-310314-99

Janibacter hoylei
BHF19-83, BHF19-201213-83

Staphylococcus hominis
BHF19-101, BHF19-250610-101
representing typical environmental flora.

Test strains, stored as frozen stocks (-80 °C, Viabank System (Medical Wire & Equipment)), were derived from the Bactimm Master Cell Bank and comply with PhEur. *Bacillus subtilis* spore suspension was obtained commercially from Biotrading.

All strains met PhEur standards, with Bacillus subtilis spores sourced from Biotrading, ensuring compliance with validated microbial quality.

Media and Other Reagents

NaCl 0.9% {FZ): sodium chloride, 4.3 g; potassium phosphate, 1.5 g; sodium hydrogen phosphate, 3.5 g; purified water, 1000 ml. The solution was sterilized using a validated process. Supplier: Bactimm B.V., batch: 14E2201.

Maximum recovery diluent with 0.1 % Tween 80 {PFZ+ Tween): peptone, 1.0 g; sodium chloride, 4.3 g; polysorbate 80, 1.0 ml; potassium dihydrogen phosphate, 1.5 g; disodium hydrogen phosphate, 3.5 g; purified water, 1000 ml. The pH was adjusted so that after sterilization it was 7.0 ± 0.2. The solution was sterilized using a validated process.

Supplier: Bactimm B.V., batch: 14D1501.

Equipment and disposables

Incubator 30-35°C, BAC006
Cross flow cabinet, BAC118
Vortex, BAC051
Pipette, BAC162, BAC163
Pipette tips, Supplier: Acila, batch G1296/14043
Scales + printer, BAC057 + BAC058

Sampling Procedures

Exposure to 1000L air

The ActiveCount Remote Air Sampler 10 L/min was placed in a Grade A cabinet and air was sampled for 100min (1 h 40min). Hereafter, growth promotion tests were performed on the plates.

Exposure to 1500L air

The ActiveCount Remote Air Sampler 10L/min was placed in a Grade A cabinet and air was sampled for 150min (2h 30min). Hereafter, growth promotion tests were performed on the plates.

Exposure to 2000L air

The ActiveCount Remote Air Sampler 10L/min was placed in a Grade A cabinet and air was sampled for 200min (3h 20min). Hereafter, growth promotion tests were performed on the plates.

Exposure to 2500L air

The ActiveCount Remote Air Sampler 10L/min was placed in a Grade A cabinet and air was sampled for 250min (4h 10min). Hereafter, growth promotion tests were performed on the plates.

Sampling Protocol

Sampling volumes and durations were set to simulate continuous operation conditions, critical for Grade A (ISO 5) environments in aseptic manufacturing. Sampling durations were structured as follows:

1,000 L Sample: 100 minutes for 10L/min
1,500 L Sample: 150 minutes for 10L/min
2,000 L Sample: 200 minutes for 10L/min
2,500 L Sample: 250 minutes for 10L/min only

Growth Promotion Test

Growth promotion tests were performed by inoculation of the TSA plates after exposure to air with not more than 100 CFU of the microorganisms listed. After inoculation the TSA plates were incubated for not more than 3 days (reference bacteria) or 5 days (C. albicans, A. Brasiliense's, and house flora isolates) at 30-35 °C. A TSA plate (Bio Trading) freshly removed from the packages served as reference.

Weight Loss Measurements

The weight loss per plate after exposure to 1000, 1500, 2000 and 2500L air was determined. Hereto, the plates were weighted prior to and after exposure to 1000, 1500, 2000 and 2500L air and the average weight loss per ActiveCount Remote Sampler and per volume air was calculated.

Exposure to:	Avg Weight Loss (gram)
1000L air 4 L/min	3.5
1000L air 10 L/min	2.7
1500L air 4 L/min	5.4
1500L air 10 L/min	3.9
2000L air 4 L/min	6.8
2000L air 10 L/min	5
2500L air 10 L/min	6.4

Test Specifications

Inoculum Count (reference plates)

The inoculum count is valid if the mean number of CFU of the inoculum is not more than 100.

Growth Promotion Test

The TSA plates promote growth if the number of CFU for each microorganism differs not more than a factor two from the number of CFU counted for the inoculum, e.g. if the inoculum contains 100 CFU the maximum number of CFU for the addition control is 200 and the minimum number of CFU is 50.

Results

Exposure to 1000L air 10L/min

Micro-organism	Exposure to 1000L air 10 L/min	Positive Control	Lower Limit	Upper Limit	Within Specification
P. aeruginosa	30	28	14	56	C
B. subtilis spores	66	69	35	138	C
S. aureus	74	62	31	124	C
C. albicans	43	47	24	94	C
A. brasilensis	50	45	23	90	C
M. luteus	>100	>100	N/A	N/A	NC
*M. luteus**	65	62	31	124	C
J. hoylei	2	0	0	0	NC
*J. hoylei**	7	7	4	14	C
S. hominis	40	46	23	92	C

C= Conform | NC= Non-Conform | * = Retest

Results

Exposure to 1500L air 10L/min

Micro-organism	Exposure to 1000L air 10 L/min	Positive Control	Lower Limit	Upper Limit	Within Specification
P. aeruginosa	40	28	14	56	C
B. subtilis spores	60	69	35	138	C
S. aureus	73	62	31	124	C
C. albicans	51	47	24	94	C
A. brasilensis	53	45	23	90	C
M. luteus	>100	>100	N/A	N/A	NC
*M. luteus**	78	62	31	124	C
J. hoylei	5	0	0	0	NC
*J. hoylei**	6	7	4	14	C
S. hominis	42	46	23	92	C

C= Conform | NC= Non-Conform | * = Retest

Test Specifications (continued)

Results

Exposure to 2000L air 10L/min

Micro-organism	Exposure to 1000L air 10 L/min	Positive Control	Lower Limit	Upper Limit	Within Specification
P. aeruginosa	22	28	14	56	C
B. subtilis spores	68	69	35	138	C
S. aureus	61	62	31	124	C
C. albicans	42	47	24	94	C
A. brasilensis	50	45	23	90	C
M. luteus	>100	>100	N/A	N/A	NC
*M. luteus**	87	62	31	124	C
J. hoylei	1	0	0	0	NC
*J. hoylei**	23	7	4	14	C
*J. hoylei***	5	3	2	6	
S. hominis	40	46	23	92	C

C= Conform | NC= Non-Conform | * = Retest | ** = 2nd Retest

Results

Exposure to 2500L air 10L/min

Micro-organism	Exposure to 1000L air 10 L/min	Positive Control	Lower Limit	Upper Limit	Within Specification
P. aeruginosa	3	27	14	56	C
*P. aeruginosa**	19	21	11	42	
B. subtilis spores	29	25	35	138	C
S. aureus	51	49	31	124	C
C. albicans	27	24	24	94	C
A. brasilensis	45	40	23	90	C
M. luteus	3	23	12	46	NC
*M. luteus**	87	98	31	124	C
J. hoylei	0	1	1	2	NC
*J. hoylei**	6	5	4	14	C
S. hominis	68	55	23	92	C

C= Conform | NC= Non-Conform | * = Retest

A couple out of specification results were obtained during this study. The growth promotion tests out of specification were repeated and all within specification.

In combination with Trypcase Soya Agar plates containing 1 % glycol (K438P090MC, batch: 1409043815), the ActiveCount Remote Air Sampler with a flowrate of 10L/min are suitable to use for several hours under a Grade A (ISO 5) cabinets.

The specifications of the growth supportive activity of P. aeruginosa, B. subtilis spores, S. aureus, C. albicans, A. brasiliensis, M. luteus, J. hoylei and S. hominis were met for 1000, 1500 and 2000L air for 10L/min Air Samplers. The growth promotion test was also conforming specifications for 2500L air using the 10L/min Air Sampler. The 90mm TSA+ 1 %glycol plates used in this study are suitable to use for air sampling for at least 4 hours and 10 minutes.

Conclusion

In combination with Trypcase Soya Agar plates with 1% glycol (K438P090MC, batch: 1409043815), the ActiveCount Remote Air Sampler 10L/min is suitable to use for at least 4 hours and 10 minutes. Results of growth promotion tests after air sampling of 1000, 1500, 2000 and 2500L air were all within specification.

Microbial Recovery

All microbial strains tested demonstrated compliance with growth promotion standards, with CFU counts remaining within acceptable thresholds. Each plate type supported microbial growth after air sampling volumes, indicating the effectiveness of TSA plates with 1% glycol in maintaining viable conditions for microbial recovery.

Plate Weight Stability

Weight loss across different sampling volumes showed that the TSA plates with 1% glycol could sustain prolonged sampling without significant dehydration. For example:

2,000 L at 4 L/min: 6.8g average weight loss
This confirms that the plates maintain adequate hydration for at least 4 hours and 10 minutes with the 10L/min sampler, meeting GMP standards.

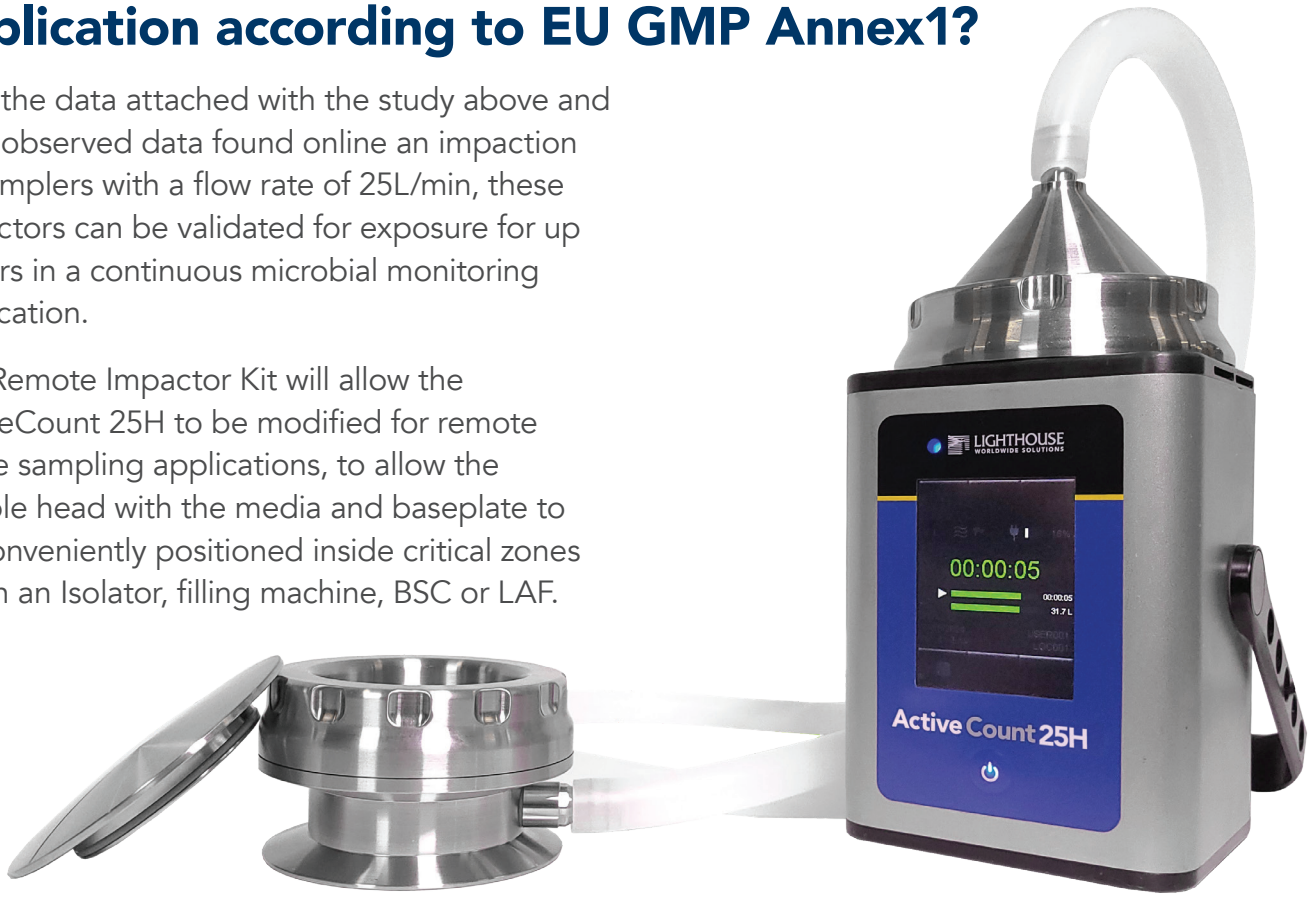
Compliance with GMP Standards

The ActiveCount Remote Air Sampler with flowrate of 10L/min, showed no interference with microbial viability. The growth promotion results aligned with the EU GMP Annex 1: 2022 guidelines, which emphasize that continuous air monitoring must not compromise microbial recovery.

Can an ActiveCount with a flowrate of 25 L/min also be used for up to 4hrs in a continuous microbial monitoring application according to EU GMP Annex1?

With the data attached with the study above and from observed data found online an impaction air samplers with a flow rate of 25L/min, these impactors can be validated for exposure for up to 4hrs in a continuous microbial monitoring application.

The Remote Impactor Kit will allow the ActiveCount 25H to be modified for remote viable sampling applications, to allow the sample head with the media and baseplate to be conveniently positioned inside critical zones within an Isolator, filling machine, BSC or LAF.



FDA Guidelines:

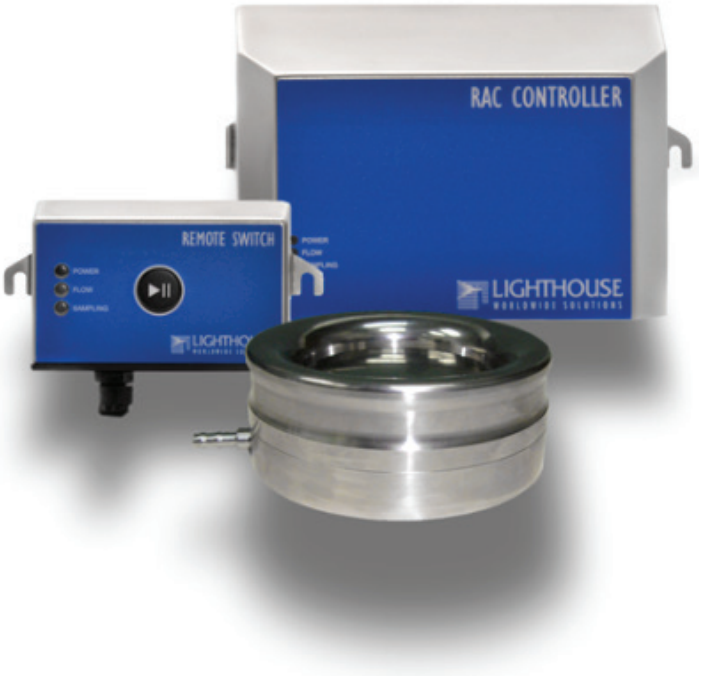
The FDA's "Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice" (2004) emphasizes the importance of growth promotion testing for culture media. This testing confirms that the media can support the growth of microorganisms, ensuring the reliability of environmental monitoring results. The guidance states:

> ***"Culture media used in environmental monitoring should be tested for growth promotion to ensure they are capable of supporting the growth of microorganisms."***

"Growth promotion testing of culture media is an important part of microbiological testing in support of pharmaceutical quality. The growth promotion test is a quality control requirement that confirms the ability of a new batch of media to support growth of a predetermined selection of representative microorganisms."

EU GMP Annex 1:

The EU GMP Annex 1, which provides guidelines for the manufacture of sterile medicinal products, also underscores the necessity of validating culture media used in environmental monitoring.



It specifies that media should be tested for growth promotion to ensure they can support the growth of microorganisms that may be present in the manufacturing environment. The annex states:

Key Recommendations:

- **Growth Promotion Testing:** Before use, each batch of culture media should undergo growth promotion testing using a panel of standard test organisms and relevant environmental isolates to confirm its ability to support microbial growth.
- **Regular Validation:** Media validation should be conducted regularly and whenever there is a change in media formulation, supplier, or storage conditions.
- **Documentation:** All validation activities, including growth promotion test results, should be thoroughly documented and reviewed as part of the quality assurance process.

How Can You Get Aligned with Annex1:2022 Continuous Viable Monitoring?

Lighthouse Worldwide Solutions has viable monitoring solutions to enable sterile product manufactures to adhere to the latest EU GMP Annex1:2022 requirements for continuous viable monitoring. The ActiveCount can support both normal testing where a 1m3 sample volume is sampled with the ActiveCount 100H or 25H and with the 25H converted into a remote sampling system as shown above the simplicity of the ActiveCount, its small footprint and user touchscreen can be quickly implemented into your Environmental Monitoring program to satisfy both ISO 14644-2:2015 and EU GMP Annex1:2022 viable monitoring requirements.

For a completely automated solution with connection to real-time monitoring software the Remote Active Count is the best option and has a flow rate of 10L/min sample head. Allowing for media exposures above 4hrs.erators can locally load the media and start the sampling process. All sample parameters such as location, media ID, Operator and Location can be automatically captured.

Related Products

ActiveCount 25H and 100H

High-performance portable active air samplers suitable for use in cleanrooms and aseptic environments.

Learn More >

ActiveCount Remote Impactor Kit

Created to address the challenges of sampling in confined or hard-to-reach cleanroom settings.

Learn More >

ActiveCountR

Designed for ISO EN17141 and EU GMP Annex 1:2022 Compliance

Learn More >

References

1. *EU GMP Annex 1: 2022 – Guidelines on the Manufacture of Sterile Medicinal Products.*
2. *FDA (2004). Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice.*
3. *ISO 14698 – Cleanrooms and associated controlled environments - Biocontamination control.*
4. *EN 17141 – Cleanrooms and associated controlled environments - Biocontamination control.*
5. *EC GMP-GCLP.*
6. *Standard Operating Procedure BP05093001: Referentiestammen en bereiden inoculum suspensies (2011, in Dutch).*
7. *Standard Operating Procedure BP02020401: Hoofd SOP QC (niet) selectieve vaste media (2012, in Dutch).*
8. *Standard Operating Procedure BS09012802: Productspecifieke SOP Casein Soyabean Agar (TSA) (2014, in Dutch).*
9. *Qualification of the Remote ActiveCount Air Sampler 4 and 10 L/min -Bactimm project number P626140416.*

Founded in 1982, Lighthouse Worldwide Solutions is the world's leading supplier of real time contamination monitoring systems air samplers and airborne particle counters. The company has leveraged its superior software design, data integration ability and worldwide support offices to provide its customers with leading edge contamination monitoring solutions. These solutions have allowed Lighthouse's customers to maintain high product yields through continuously monitoring conditions that may have an adverse effect on their products. The Lighthouse Monitoring System and Lighthouse line of airborne particle counters have become the standard for many companies, such as Amgen, Genentech, Baxter, Pfizer, Bayer, Novo Nordisk, SpaceX, Tesla, Seagate, TSMC, Samsung, Lockheed Martin, Microchip, Medtronic, 3M, Boston Scientific and many more. www.golighthouse.com