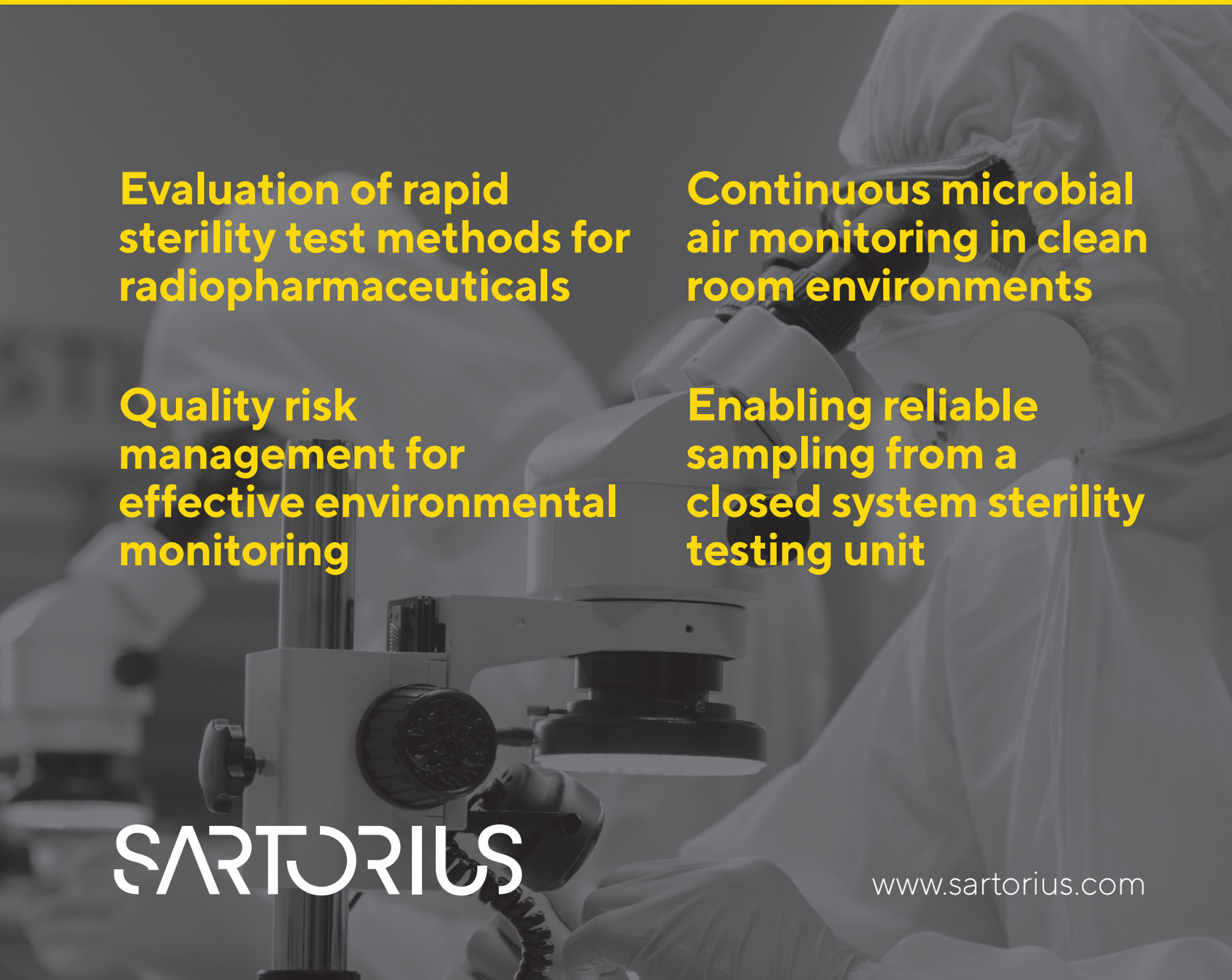
Important Considerations for Every Bio-pharmaceutical Microbiology QC Lab



Evaluation of rapid sterility test methods for radiopharmaceuticals

Continuous microbial air monitoring in clean room environments

Quality risk management for effective environmental monitoring

Enabling reliable sampling from a closed system sterility testing unit

SARTORIUS

www.sartorius.com

SARTORIUS

Contents

Introduction

The quality control (QC) microbiology laboratory plays a critical role in control of the pharmaceutical manufacturing environment and product release. As microbiology QC scientists you are a crucial part of this manufacturing process and carrying out all tests in a reliable, accurate and timely manner, following quality procedures and regulatory requirements protects patient health and enables products to be released on time.

Safety and compliance are key to all processes, and this ebook highlights important considerations for your microbiology QC lab, with particular focus on:

- The importance of continuous air monitoring for cleanrooms according to Annex 1 regulations
- Sterility testing for final release of cell therapy products
- Sampling from a closed system sterility testing unit

Developing well-designed environmental monitoring programmes requires an understanding of the risks, contamination sources, and strategies to reduce those risks. Dr Tim Sandle explores why risk assessments are requisites for building a compliant environmental monitoring regime, to meet the regulations in the revised EU GMP Annex 1, while delivering an effective programme.

Claudia Scherwing and Jasmin Bunke present the results of a study, Continuous Microbial Air Monitoring in Clean Room Environments, which aimed to establish whether a continuous sampling (and multisampling point assay) provides effective monitoring for the entire production process.

Time-to-result is an important attribute of testing for short shelf-life cellular therapeutics, and a study from Luebbers et al compares the microbial detection capability of a highly sensitive and broad range microbial detection system with the compendial sterility test.

Arjan Langen and Kim van Boxtel evaluate rapid sterility test methods to assess if a rapid, non-compendial sterility test can be performed prospectively, while the final study in this ebook from Puttana et al evaluates the Sterisart® closed system sterility testing device, with a septum, for the recurrent sterile extraction of samples.

The biggest challenge for quality control in the biopharma industry is in reducing the risk of contamination. This ebook is designed to give you the tools you need to address the challenges that you face every day in the lab, ensuring that patient health is not affected by the release of a contaminated product, and that product release is timely.

SARTORIUS

Application Note

February 2019

Keywords:
continuous air monitoring, clean room, gelatin filter, MD8 Airscan®, EU GMP Guideline Annex 1

Continuous Microbial Air Monitoring in Clean Room Environments

Claudia Scherwing[1], Jasmin Bunke[2]

1. Product Development Lab Consumables Microbiology, Sartorius Stedim Biotech, Göttingen, Germany
2. Product Management Lab Essentials Microbiology, Sartorius Lab Instruments, Göttingen, Germany

* Correspondence
E-Mail: kai.nesemann@sartorius.com

Abstract

Environmental monitoring is an important part of quality assurance for the production environments of sterile pharmaceutical products. Especially for aseptic filling lines where products are filled without a terminal sterilization step it is of utmost importance for product safety and thus an essential part of the quality control strategy. Such ISO 5 graded manufacturing environments are required to have < 1 colony-forming unit (CFU) per m^3 of air.

A typical method for monitoring contamination of air is to actively draw air and filter it through special gelatin filters.

According to Annex 1 to the EU GMP guide a minimum sample volume of 1 m^3 of air should be taken per sample location. Considering an 8 hours work shift 1 m^3 is a too low sample volume to reliably judge the air quality of the manufacturing environment. One approach to improve product safety would be the implementation of a continuous air monitoring covering the complete production process (at multiple sampling points).

Unlike agar plates, which would dry out during long-term sampling, the Gelatin membrane filters can be used for the whole 8 h period. Human intervention, such as change of agar plates, could then be avoided, thus lowering the risk of secondary contaminations to nearly zero.

Introduction

The following study aimed to establish whether a continuous sampling (and multisampling point assay) provides effective monitoring for the entire production process (8 h) by determining whether trapped organisms can withstand long-term drying stress with unaltered recovery.

This study examined the recovery and viability of micro organisms captured on gelatin filters during 8 h of filtration with HEPA-filtered air from a laminar flow hood, using the MD8 Airscan® system. Stressed and unstressed filters were compared with parallel-run reference filters as controls. The CFU were counted and the genus of the identified microorganism populations determined to examine any changes in microbiological flora occurring during continuous long-term sampling.

Compared to the unstressed reference filters, neither total recovery nor recovered bacterial diversity changed. No statistically significant differences in CFU/m³ were found between test filters and reference filters, and no differences in the microbiological flora between test filters and reference filters. CFU populations were comparable.

8 h continuous air sampling on gelatin filters with the MD8 Airscan® system did not affect total recovery or change the diversity of recovered microorganisms when comparing test filters to reference filters.

Monitoring microbiological contamination of air in production areas is of major importance because aseptic filling is the step in the production process of the pharmaceutical industry that harbors one of the highest risks for contamination[2]. Aseptic filling lines are increasingly used in the pharmaceutical industry because increasing numbers of biotechnology products cannot be sterilized after production without the sterilization process affecting their quality. Filling lines are defined as ISO 5[1], and air actively sampled in these environments must have less than one colony forming units per cubic meter (CFU/m³), with a minimum sample volume of one m³ of air taken per sample location, according to Annex 1 to the EU GMP guide. Considering an 8 hour work shift, one m³ may be too low a sample volume to reliably judge the air quality of the manufacturing environment.

Thus, the development of a continuous production-monitoring tool to minimize risks for contamination and increase the overall standard of quality control is required. A method is needed, which continually surveys all cycles of the production process and allows sampling at multiple points.

To determine if continuous air sampling using gelatin membranes can effectively monitor the entire production process over an eight hour shift, the viability of microorganisms on gelatin filters during sterile air long-term filtration, i.e. whether trapped organisms can withstand long-term drying stress and yield unaltered recovery, was examined.

Former tests showed that gelatin filters with an inlet velocity of 0.25 m/s had average retention rates of 99.9995 % for Bacillus subtilis varniger spores and 99.94 % for T3 coliphages[5].

Materials and Methods

The study examined whether the viability of microorganisms on gelatin filters was maintained during the long-term filtration of filtered air. The expression "filtered air" describes the ISO 5 HEPA-filtered air of the used Class 2 biological safety cabinet.

Test and reference gelatin filters were first exposed to non-sterile air for 30 minutes. The MD8 Airscan® air samplers (set at an air flow rate of 2.0 m³/h (0,144 m/s)) had been located in a non-controlled laboratory environment (autoclave room) approx. 30–40 cm apart from each other. This sampling location had been chosen in order to build up special environmental conditions. There, a higher relative humidity (~ 57 ± 6 % and temperature: ~ 21 ± 1 °C) was expected (thus increased amount of drying stress sensitive, waterborne microorganisms (e.g. gram-, generating a "worst case" scenario). Further, a general higher content of airborne microorganisms per cubic meter was expected than in the "normal" laboratory. Because of that, it was postulated that the following 8 hours of drying stress would show a clearly visible and statistical detectable effect.

Following, the test filters were used to sample filtered air for a further 8 hour period.

For the filtration of ISO 5 graded air, the MD8 Airscan® sampling heads were placed under a laminar flow hood (relative humidity: ~ 43 ± 3 % and temperature: ~ 23 ± 1 °C), thus, there was no additional high relative humidity while the 8 hour stressing.

The reference filters were subjected to only 30 minutes filtration of non-sterile air without further aeration. They were placed on soybean-casein-digest agar medium directly after sampling.

At the end of the 8 h filtration period under the laminar flow hood, the test filters also were placed on soybean-casein-digest agar medium plates and incubated at 32 °C for 4 days.

The colonies that developed were counted and recorded as CFU/m³ a total of 26 times. Then, the CFU/m³ were compared for the test and reference filters. Additionally, the genus of each colony was identified to determine if the microbiological flora had changed during continuous long-term sampling.

Results

Figure 1 shows the mean CFU/m³ on test (gold bar, mean = 69 colonies, sd = 51 colonies) and reference filters (grey bar, mean = 64 colonies, sd = 32 colonies). A mean difference of 5 CFU/m³ (not statistically significant according to the paired T-test) was found, but observed no general trend upon comparison of test and reference filters (see Fig. 2). In 12 cases, there were more CFU/m³ on test filters than on reference filters, but the opposite was examined in 13 cases (see Fig. 2). The standard deviation in test and reference filters can be attributed to the broad fluctuation of microorganisms naturally occurring in the ambient air of non-controlled environments.

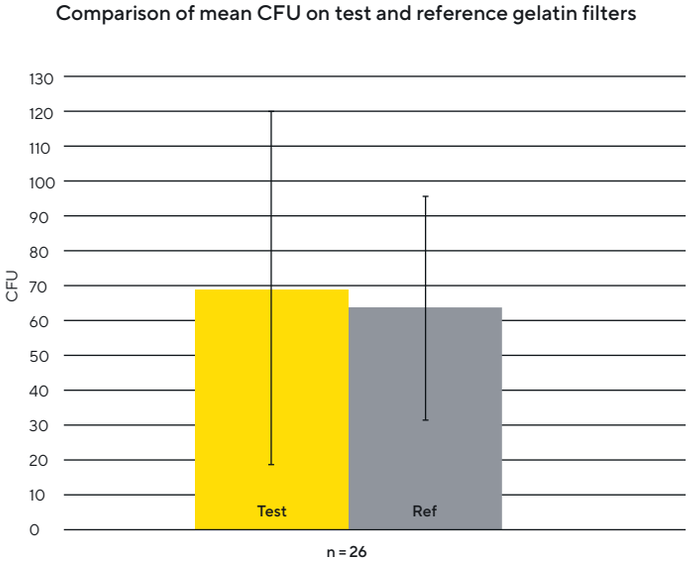


Figure 1: Comparison of mean CFU on test and reference gelatin filters.

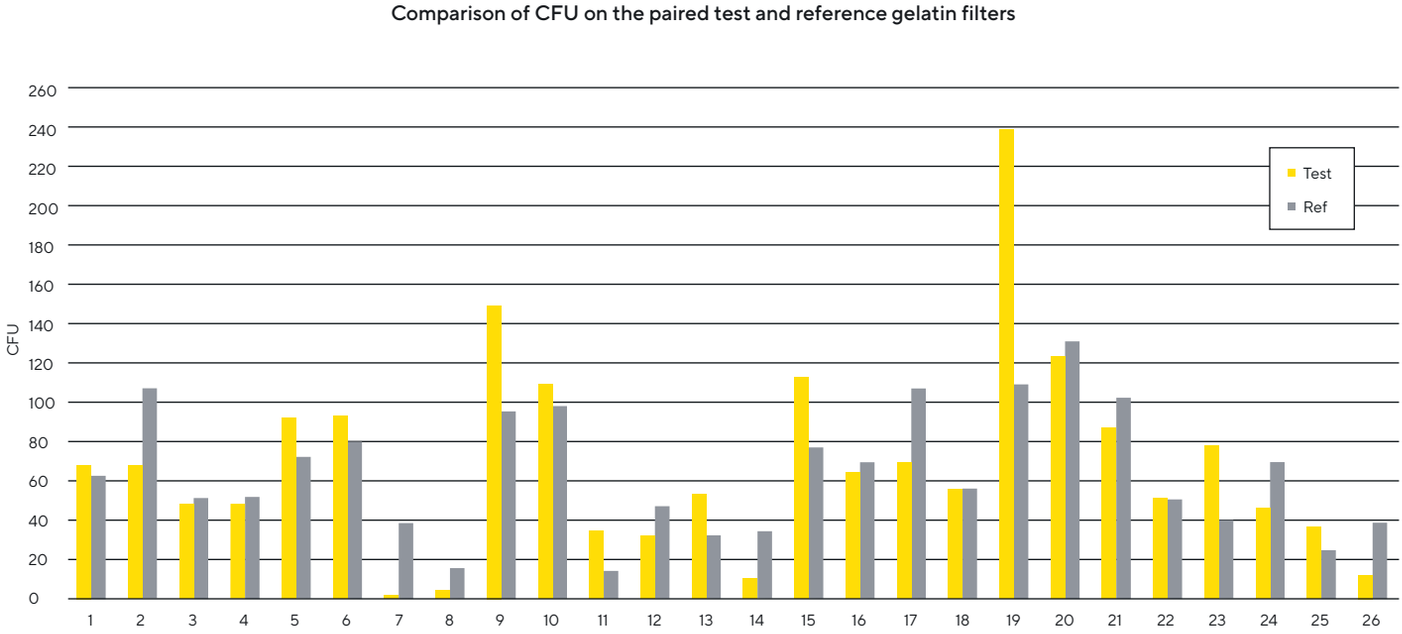


Figure 2: Comparison of CFU on the paired test and reference gelatin filters.

No statistically significant difference in the growth of microorganisms on test versus reference filters could be observed. Figures 3 and 4 show a representative soybean-casein-digest agar medium plate with microbiological flora grown on the paired test (left) and reference filters (right). This visual impression shows that the microbiological population found on the test and reference filters is comparable. The genus identification data from a macroscopic comparison of the microbiological flora shown in Figure 5A and 5B confirms the visual impression that the microbiological population on the test and reference filters is comparable.

No statistically significant difference in mean CFU/m³ between test and reference gelatin filters. The gold bar shows a mean CFU/m³ of 69 colonies, with a standard deviation (sd) of 51 colonies for the test filters (counted 26 separate times). The grey bar shows a mean CFU/m³ of 64 colonies, with an sd of 51 colonies for the reference filters (counted 26 separate times). The mean difference of 5 CFU/m³ between test and reference gelatin filters was not statistically significant.

No general trend of CFU/m³ upon comparison of test and reference filters. The gold bar shows CFU/m³ for 26 replicates of the test filters, and the grey bar shows CFU/m³ for the reference filters.

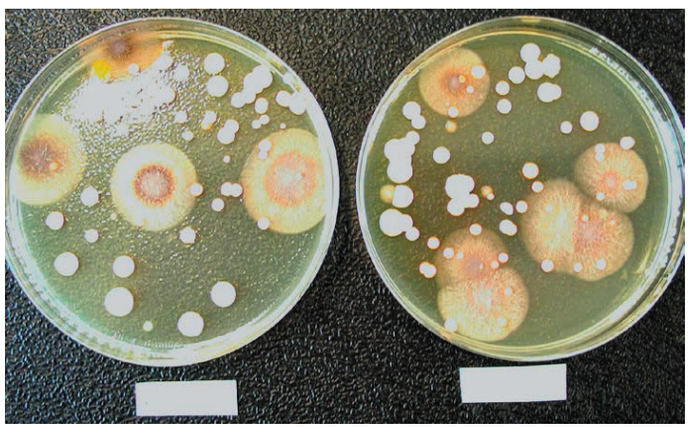


Figure 3: Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right). The composition of the microbiological population found on the test and reference filters is comparable. Representative soybean-casein-digest agar medium plates showing the microbiological flora grown on a test filter (left) and its corresponding reference filter (right).

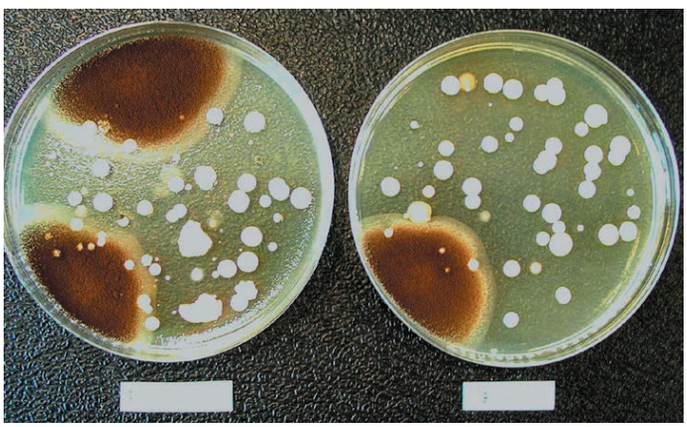


Figure 4: Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right). The composition of the microbiological population found on the test and reference filters is comparable. Representative soybean-casein-digest agar medium plates showing the microbiological flora grown on a test filter (left) and its corresponding reference filter (right).

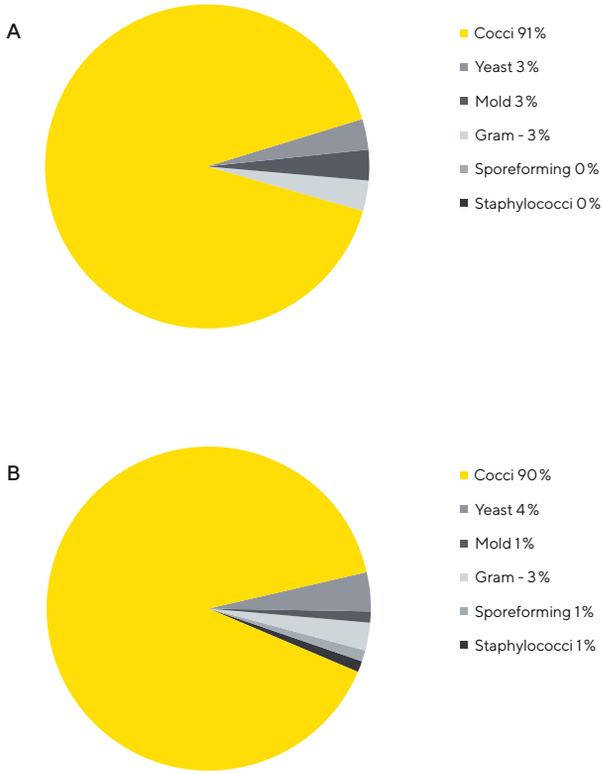


Figure 5: A. Composition of the microbiological population grown on the test gelatin filters. Almost all microbes grown on test filters is Cocci. This figure shows a breakdown of microbes grown on soybean-casein-digest agar medium plates from test filters. B. Composition of the microbiological population grown on the reference gelatin filters. Almost all microbes grown on reference filters are Cocci. This figure shows a breakdown of microbes grown on soybean-casein-digest agar medium plates from reference filters.

Conclusion

This study aimed to examine if gelatin filters manufactured by Sartorius Stedim Biotech GmbH are qualified for long-term (eight hours [8 h]) air sampling in production environments in the pharmaceutical industry. Specifically, if microorganisms collected on gelatin membranes can survive long-term filtration with filtered air. The 8-hour filtration period is representative of a typical work shift on an aseptic filling line.

The focus of the study aimed to establish whether long-term air filtration decreased the number of CFU/m³ on filters. Therefore, a non-sterile air sampling on test filters for 30 minutes, followed by a filtration of ISO 5 graded air for 8 h.

The experiment provided no statistically significant differences between test (stressed) and reference (unstressed) filters. The test filters had the same number of CFU/m³ as the reference filters (i.e., no microorganisms died during long-term filtration). The standard deviations in test and reference filters were attributable to the broad fluctuation of microorganisms naturally occurring in the ambient air of non-controlled environments. Moreover, no difference between the bacterial flora grown on the test and reference filters in either visual comparison or macroscopic comparison could be detected. Even gram-negative bacteria were found on stressed test filters. No statistical difference between stressed and unstressed gelatin filters.

In conclusion, this study showed that there was no statistical difference between stressed and unstressed gelatin filters, thus proving that gelatin membranes manufactured by Sartorius Stedim Biotech GmbH are qualified for continuous air monitoring in industrial pharmaceutical production environments covering a whole 8 h work shift without the need for human intervention.

References

- (Draft of the Revision of) Annex 1 of the EU Guidelines to Good Manufacturing Practice, November 2008 (December 2017)
- USP, Chapter 1116 – Microbiological Evaluation of Cleanrooms
- C. Scherwing, F. Golin, O. Guenec, K. Pflanz, G. Dalmaso, M. Bini, F. Andone, Continuous microbiological air monitoring for aseptic filling lines, PDA J Pharm Sci Technol, March | April 2007 61: 102–109
- CAMR Report 1993 "An assessment of the Sartorius MD-8 Microbial Air Sampler"

Author: **Dr Tim Sandle**

Quality risk management leads to more effective environmental monitoring

To be effective, microbiological environmental monitoring of cleanrooms needs to be risk based. This not only delivers an effective programme, Dr Tim Sandle finds, but also meets the regulatory expectation presented in the revised EU GMP Annex 1; hence, risk assessments are requisites for building a compliant monitoring regime.

AN ENVIRONMENTAL MONITORING programme needs to consider the detail of operations and the process flow within pharmaceutical product manufacturing. This is best achieved using quality risk management principles and supporting risk assessments for contamination control and monitoring (detectability of contamination event). Such an approach is embedded within the forthcoming changes being made to EU GMP Annex 1.[1] In addition, a risk-centric approach is also deemed essential in order to meet the expectations of the biocontamination control standard EN17141: 2020.[2] These guidances place environmental control above environmental monitoring, requiring contamination control measures to be designed into each part of the production process with the aim of reducing microbial, pyrogenic and particulate contamination. This requires the application of contamination controls such as cleaning, decontamination, sterilisation and transfer methods for primary packaging materials, consumables and intermediate product, in order to reduce the contamination risks as far as possible. Where contamination control is less than optimal, this will help to direct the location and frequency of environmental monitoring.[3]

As well as directing a series of proactive contamination control measures, quality risk management can also assist with shaping the primary aspects of the environmental monitoring programme. This article considers how and where risk assessment principles can be applied.

Fundamentals of risk assessment

Risk assessment can be expressed as a formal process – an activity based on a series of key steps. In short, these involve the following:[4]

- Establishing the context and environment that could present a risk
- Identifying the hazards and considering the risks these hazards present
- Analysing the risks, including an assessment of the various contributing factors
- Evaluating and prioritising the risks in terms of further actions required
- Identifying the range of options available to tackle the risks and deciding how to implement risk mitigation strategies.

Risk assessments begin through the identification of hazards. Hazards are agents and they include physical, chemical and biological factors. In the case of this article, we are interested in the microbiological factors. Hazards need to be evaluated on their potential severity and the likelihood of the hazard occurring. When the likelihood is at a frequency that could lead to a control breakdown, attempts should be made to lower this. Monitoring can be orientated to risks that cannot be mitigated to an acceptably low level, although care should be taken with environmental monitoring that detection, using methods with known metrological limitations, is not used as a substitute for poor control.

Application of quality risk management

There are several different risk assessment tools available, including those presented in ICH Q9.[5] Perhaps the most useful method for environmental monitoring is the Hazard Analysis Critical Control Point (HACCP).[6] This approach uses process flow mapping to help pinpoint contamination sources and includes steps such as:

1. Identification of all potential microbiological contamination sources and routes of contamination in the environment. This may also include selected microorganisms of interest (such as those with the potential for causing microbiological contamination of the product and/or harm to the intended recipient, such as spoilage of product; as well as organisms being indicative of a specific control breakdown; eg, the presence of fungi).

2. Assessing the risk from these sources and routes and, where appropriate, introducing or improving microbiological contamination control methods to reduce the identified risks. This necessitates understanding the activities associated with each individual manufacturing stage, such as the transfer of contact parts and components into critical areas, equipment and machine setup, and routine production.

3. Establishing a monitoring schedule with approximately selected sampling methods, in order to monitor the microbiological contamination source or their control methods or both. This will involve selecting between air, surface and personnel samples using either conventional sampling techniques or rapid microbiological methods.

Appropriate monitoring methods can be selected by posing and answering the following risk-based questions:

- What locations are in close proximity to processing activity?
- What sites or equipment are contacted by personnel or gloves?
- What sites represent the most difficult areas to clean and disinfect?
- Where is the greatest amount of activity?
- What are the material and personnel flows?
- Where are the entry points where materials transfer from lower to higher classification?

4. Establishing alert and action levels with measures to be taken when required, if these levels are exceeded.

5. Verification on a continuing basis, that the microbiological contamination control system is effective and meeting agreed performance parameters by reviewing product contamination rates, environmental monitoring results, risk assessment methods, control methods and monitoring limits and, where appropriate, modifying them accordingly.

6. Establishing and maintaining appropriate documentation including the education and training of all staff involved with the clean controlled environment.

This stepwise analysis assists with the selection of the locations for monitoring. Identified areas for directing monitoring will include areas where contamination is easy to transfer, such as personnel gowning change areas, pass-through hatches, and material transfer airlocks. It is also important to include open processing and areas of higher personnel activity within the cleanroom.

Monitoring frequencies can also be assessed through an examination of risk factors, where the higher the risk factors, the greater the potential for contamination; and hence consideration must be given to a higher frequency of monitoring. Examples of risk factors for this type of review include:

- Room activity – differences between process, storage, office/administration, washing, sterilising; such as, autoclave operation, sterile filtration and sterile filling.
- Exposure risk: for how long is the product exposed?
- Is open processing involved? If so, for how long?
- Room temperature – cold, warm or ambient?
- Process stage – raw material processing, intermediate manufacturing or final formulation?
- Duration of process activities – short, medium or long term relative to all operations
- Water exposure or wet area increases risks.

This data-led approach enables relative risks to be compared. Importantly, once the monitoring frequency for each cleanroom has been determined, this should be reviewed at regular intervals. This may invoke changes to room status (and hence the monitoring frequency) or to changes for different sample types within the room, ideally captured through change control.

With data review, this should be undertaken by trend analysis in order to fully understand the nature of risk from a datum exceeding the action level. Invariably, a single excursion is of significance and more can be gained from trend assessments using graphical approaches that look at the counts obtained (in many cases, counts will begin to rise before an alert or action level is breached – effective action can be taken when upward trends are spotted as early as possible). The trending concept should be extended to microbial type and the frequency of excursions, as well as to actual data values. Trending also requires an understanding of what is 'normal', so that any variation can be compared.[7]

Risk assessments can also assist with the process of determining the significance of an excursion. A result at the action level may or may not indicate that the risk to the product or process is similarly high. It is important to weigh up the contamination transfer coefficient (that is, how could contamination get from A to B? What is the vector that could enable this to happen, eg, by air, via equipment transfer, by personnel touching and so on).

Summary

Developing well-designed environmental monitoring programmes requires an understanding of the risks, contamination sources and strategies to reduce risks. This is necessary in order to determine frequencies of monitoring; assess suitable monitoring locations; and for

understanding the significance of contamination events, should they occur. With any established environmental monitoring programme, it is important to review this programme regularly and to update or adapt the regime according to process changes, room design, shift changes, cleaning and disinfection levels, and to set and evaluate corrective and preventative actions put in place to address contamination events.

References

1. EMA (2020) Second targeted stakeholders' consultation on the revision of Annex 1, on manufacturing of sterile medicinal products, of Eudralex volume 4. Available at: https://ec.europa.eu/health/medicinal_products/consultations/2020_sterile_medicinal_products_en
2. EN 17141: 2020 "Cleanrooms and associated controlled environments – Biocontamination control", European Commission, Brussels
3. Sandle T. (2019) Biocontamination Control for Pharmaceuticals and Healthcare, Academic Press, London, UK
4. Sandle T. (2015) Contamination Control Risk Assessment. In McCullough, K.Z. and Moldenhauer, J. (Eds.) Microbial Risks and Investigations, DHI/PDA, River Grove, USA, pp783-836
5. ICH Q9, Guideline on Quality Risk Management (ICH, September 2015). Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-3.pdf
6. Jahnke M, Kuhn K-D. (2003) Use of the Hazard Analysis and Critical Control Points (HACCP) Risk Assessment on a Medical Device for Parenteral Application. PDA Journal of Pharmaceutical Science and Technology, 57 (1): 32-42
7. Hussong D, Masden RE. (2004). Analysis of Environmental Microbiology Data from Cleanroom Samples. Pharmaceutical Technology Aseptic Processing, Supplement 2004, pp10-15

Dr Tim Sandle

Tim has over 25 years' experience of microbiological research and biopharmaceutical processing. Tim is a member of several editorial boards and has authored 30 books on microbiology, healthcare and pharmaceutical sciences. Tim works for BPL in the UK and is a visiting tutor at both the University of Manchester and UCL.

SARTORIUS

Application Note

February 20, 2020

Keywords or phrases:
Rapid sterility testing of short shelf-life therapeutics; Equivalency of Real-Time PCR- and growth based detection

Equivalency of PCR-Based Rapid Sterility Testing and the Compendial Culture Method According to Ph. Eur. 2.6.1., JP 4.06 and USP <71>

Jan-Niclas Luebbers[1], Dr. Alexandra Mueller-Scholz[1]*, Dr. Diana Patzelt[1], Kai Nesemann[2]

1. Lab Essentials Applications Development, Sartorius, Goettingen, Germany
2. Product Management Lab Consumables, Sartorius, Goettingen, Germany

* Correspondence
E-Mail: PCR@Sartorius.com

Abstract

In this study, we compared the microbial detection capability of the Microsart® ATMP Bacteria and Microsart® ATMP Fungi Real-time PCR kits with the compendial sterility test. We spiked samples, using 6 different bacterial (*Bacillus subtilis, Staphylococcus aureus, Clostridium sporogenes, Pseudomonas aeruginosa, Streptococcus pyogenes,* and *Pseudomonas protegens)* and 2 fungal (*Candida albicans* and *Aspergillus brasiliensis)* species at concentration levels between 2.5 CFU/mL and 198 CFU/mL, and compared our results to the growth-based method performed in parallel at an external contract lab, according to Ph. Eur. 2.6.1, JP 4.06 and USP <71>[2,3,4]. Our results show full equivalency of Microsart® ATMP Bacteria and Microsart® ATMP Fungi with the compendial method. Moreover, the Microsart® ATMP Fungi detected *Candida albicans* with higher sensitivity.

Introduction

Sterility testing is a critical component of the release testing for any cell therapy product since microbial contamination of cell therapy products can potentially kill recipients. The current compendial sterility test for most bacteria takes 14 days and 28 days for mycoplasma testing before contamination can be ruled out with certainty[1,2,3]. However, time-to result is an important attribute of testing for short shelf-life cellular therapeutics, especially for autologous cell therapies intended to for terminally ill patients.

As a result, growth-independent rapid assays are in increasing demand. To fulfill this demand, we developed and comprehensively validated a highly sensitive and broad range microbial detection system, consisting of an efficient Microsart® ATMP Extraction DNA isolation protocol, and followed by a real-time PCR assay using the Microsart® ATMP Bacteria/Fungi/Mycoplasma kit.

We designed this validation study to evaluate the bacterial and fungal detection capability of this system, according to the requirements of the European Pharmacopeia chapter 5.1.6[1]. *In silico* sequence alignment analysis demonstrated that the Microsart® ATMP Bacteria kit can detect > 94% of Gram-positive and Gram-negative bacteria. Also, *in silico* analysis for Microsart® ATMP Fungi demonstrated an additional coverage of > 37% of all fungi species. Due to the higher variability in fungal genera, the fungal coverage is seemingly low; however, all USP/EP relevant species are covered, as well as further typical contaminants. In addition, PCR assays even allow the detection of bacterial and fungal contaminations that are not covered by growth tests.

In this study, we show that both PCR assays are comparable in detection of microbial contaminants to the compendial culture method. For comparison, spiked samples were tested in parallel at an external contract lab, according to Ph. Eur. 2.6.1, JP 4.06 and USP <71>[2,3,4].

Methods

EZ-CFU™ standards (quantified reference cultures; Microbiologics) for eight microbial species (Table 1) were rehydrated in 2 mL of rehydration buffer, according to the instructions for use[5]. The bacteria suspensions were diluted in DMEM + 5 % FBS and the fungal suspensions in DMEM to generate concentrations of 2x LOD_{95}, LOD_{95} and ½ LOD_{95}. Samples were split into aliquots. LOD_{95} values of the different bacterial and fungal species were determined during their respective PCR kit validation and are listed in Table 1.

An aliquot of each concentration was used for sterility testing (direct inoculation) at Labor LS. Each sample was cultivated in thioglycolate medium and soya-bean casein medium for 14 days, according to the recommendation of the guidelines. In parallel, aliquots were extracted in duplicates and analyzed using a CFX96 Real-time PCR instrument according to the instructions for use of the Microsart® ATMP Extraction kit, the Microsart® ATMP Bacteria, or the Microsart® ATMP Fungi detection kit,[6,7,8]. The test setup is described in Table 2.

Results

Our results are summarized in Table 3. For the Microsart® ATMP Bacteria and Microsart® ATMP Fungi results are given in Ct values (cycle threshold values). Ct values < 40 are positive.

Species	Strain	Atmosphere	LOD_{95}
Staphylococcus aureus	ATCC 6538	aerobic	25 CFU/ml
Clostridium sporogenes	ATCC 19404	anaerobic	50 CFU/ml
Pseudomonas aeruginosa	ATCC 9027	aerobic	5 CFU/ml
Streptococcus pyogenes	ATCC 19615	aerobic	99 CFU/ml
Pseudomonas protegens	ATCC 17386	aerobic	10 CFU/ml
Candida albicans	ATCC 10231	aerobic	50 CFU/ml
Aspergillus brasiliensis	ATCC 16404	aerobic	50 CFU/ml

Table 1: Bacterial and fungal strains used in this study, respective incubation conditions, and LOD_{95}

Comparison with culture method with defined starting material quantity at external contract lab		
1	Spiked DMEM + 5 % FBS with *B. subtilis, S. aureus, C. sporogenes, P. aeruginosa, S. pyogenes* or *P. protegens* at 2x LOD_{95}, LOD_{95} and ½ LOD_{95}. One aliquot without spike was processed as NC.	
	A 1 mL aliquot of each sample was used per each cultivation media (thioglycolate medium and soya-bean casein medium) at the external contract lab L+S AG.	DNA was extracted from 1 mL starting material with Microsart® ATMP Extraction. Real-time PCR was performed according to Microsart® ATMP Bacteria.
2	Spiked DMEM with *C. albicans* or *A. brasiliensis* at 2x LOD_{95}, LOD_{95} and ½ LOD_{95}. One aliquot without spike was processed as NC.	
	A 1 mL aliquot of each sample was used per each cultivation media (thioglycolate medium and soya-bean casein medium) at the external contract lab L+S AG	DNA was extracted from 1 mL starting material with Microsart® ATMP Extraction. Real-time PCR was performed according to Microsart® ATMP Fungi.

Table 2: Test setup for comparison between Microsart® ATMP Bacteria and Microsart® ATMP Fungi with compendial sterility test

Species	Microsart® ATMP Bacteria			Compendial culture method (External Lab)		
	Results available within 3 hours			Results available after 14 days		
	2 x LOD_{95}	LOD_{95}	½ x LOD_{95}	2 x LOD_{95}	LOD_{95}	½ x LOD_{95}
Bacillus subtilis	33.16	34.23	35.47	Positive	Positive	Positive
	33.23	34.32	34.38			
Staphylococcus aureus	35.42	35.77	36.56	Positive	Positive	Positive
	34.13	35.67	39.90			
Clostridium sporogenes	34.20	34.87	35.45	Positive	Positive	Positive
	34.10	33.43	35.61			
Pseudomonas aeruginosa	36.40	36.74	**37.22**	Positive	Positive	**Negative**
	36.22	37.96	**No Cq**			
Streptococcus pyogenes	34.89	35.53	36.55	Positive	Positive	Positive
	35.09	35.93	35.88			
Pseudomonas protegens	34.14	34.38	36.52	Positive	Positive	Positive
	33.28	34.51	35.61			
Species	**Microsart® ATMP Fungi**			**Compendial culture method (External Lab)**		
	2 x LOD_{95}	LOD_{95}	½ x LOD_{95}	2 x LOD_{95}	LOD_{95}	½ x LOD_{95}
Candida albicans	32.25	32.27	**33.96**	Positive	Positive	**Negative**
	31.94	32.12	**32.96**			
Aspergillus brasiliensis	34.38	37.06	34.94	Positive	Positive	Positive
	32.40	33.17	34.20			

Table 3: Results of bacterial and fungal detection using Microsart® ATMP Bacteria and Microsart® ATMP Fungi in comparison to the compendial culture method according to Ph. Eur. 2.6.1, JP 4.06 and USP <71>

Discussion

In this study, we compared the bacterial and fungal detection performance of the Microsart® ATMP Bacteria and Microsart® ATMP Fungi detection kits and the compendial method. We simultaneously tested spiked samples using rapid real-time PCR based detection and the compendial testing (performed at an external contract lab).

Our results, summarized in Table 3, show that the Microsart® ATMP Bacteria and Microsart® ATMP Fungi detection kits give equivalent results to the growth-based, compendial method. Only one sample containing *P. aeruginosa* at a concentration of 2.5 CFU/mL gave negative results using both methods. During validation of the Microsart® ATMP Bacteria kit, we determined an LOD_{95} of 5 CFU/ml for *P. aeruginosa*. However, 2.5 CFU/ml are still detectable with a probability of 83% and we detected one out of two. Of note, the lowest tested concentration of *C. albicans* was detected only with the Microsart® ATMP Fungi kit, while the traditional growth-based method failed to detect the contaminant.

The Microsart® ATMP Bacteria and Fungi assays enable simultaneous testing for bacteria and fungi within the same PCR, and give results in 3 hours instead of weeks. A rapid detection of such contaminants in short shelf-life cellular therapeutics, especially autologous cell therapies, is urgently needed prior to administration to terminally ill patients.

Our results emphasize that, in addition to the classic sterility testing, rapid real-time PCR-based detection of microbial contaminations contribute to a risk reduction, as they facilitate the availability of results prior treatment and, therefore, contribute to patient safety.

References

- European Pharmacopoeia 8th edition, Strasbourg, FR; European Directorate for the Quality of Medicines, 5.1.6
- European Pharmacopoeia 8th edition, Strasbourg, FR; European Directorate for the Quality of Medicines, 2.6.1
- Japanese Pharmacopoeia (JP): 4.06 Sterility Test, JP 16th edition, 2011, by the Ministry of Health, Labour and Welfare Ministerial Notification No. 65
- United States Pharmacopeia (USP): <71> Sterility Tests, revision 2009
- Instructions for use: EZ-CFU™ Microorganisms, Microbiologics, 2014, PI.046.ENG Rev B
- Instructions for use: EZ-CFU™ Microorganisms, Microbiologics, Ver. 07 | 2014
- Instructions for use: Microsart® ATMP Extraction, Sartorius, Prod. No. SMB95-2001, Ver. 06 | 2018
- Instructions for use: Microsart® ATMP Bacteria, Sartorius, Prod. No. SMB95-1008, Ver. 06 | 2018
- Instructions for use: Microsart® ATMP Fungi, Sartorius, Prod. No. SMB95-1012, Ver. 11 | 2019

Sales and Service Contacts

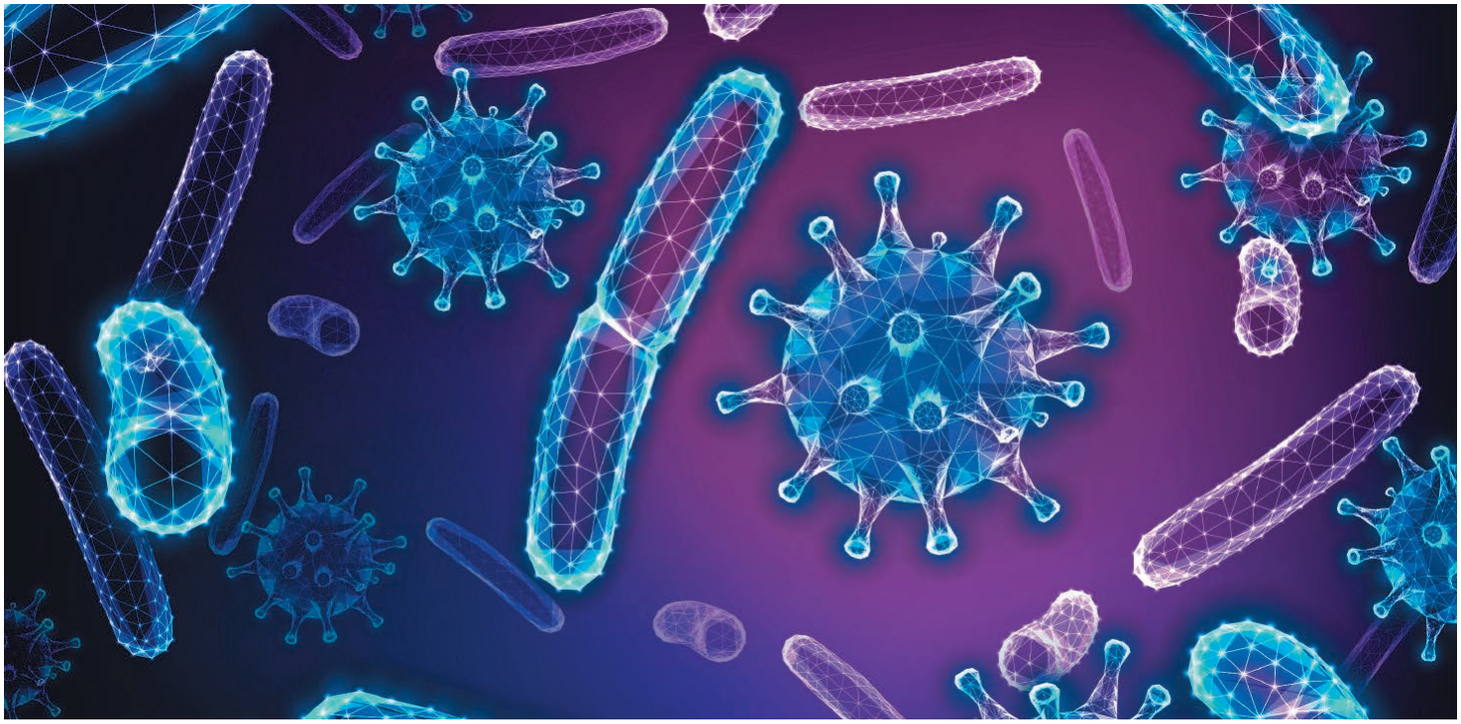
For further contacts, visit sartorius.com

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

Version 1: 02 | 2020

Authors: **Kim van Boxtel and Arjan Langen (GE Healthcare)**



Evaluation of rapid sterility test methods for radiopharmaceuticals

Radiopharmaceuticals are radioisotopes bound to biological molecules that can be used to diagnose patients.[1] The shelf life of radiopharmaceuticals depends on the decay time of the radioisotope and therefore it is important to administer these products to the patient on time.[2] The sterility of the product is assured by using terminal sterilisation or aseptic processing principles. The final product is tested for sterility according to the European Pharmacopoeia (Ph. Eur.) and is carried out retrospectively: due to the short half-life of the radioisotopes, the product needs to be injected shortly after manufacturing and before the results of the sterility test are known. The aim of the evaluation of rapid sterility test methods is to assess if a rapid, non-compendial sterility test can be performed prospectively.

THE CURRENT METHOD used for sterility testing of radiopharmaceuticals is the one described in Ph. Eur. 2.6.1.[3] Hereby the test may be carried out using membrane filtration or by direct inoculation of the culture media with the product to be examined. A sample is inoculated using two different fluid media, Trypticase Soya-bean Broth medium (TSB) and Thioglycolate medium (FTM). Afterwards, the media are incubated for 14 days at two different temperatures (20-25°C and 30-35°C). If no turbidity occurs, the test complies with the requirements and no contamination is present.

To replace the current sterility test – which is based on the detection of microbial growth – with an alternative rapid method, several regulatory and user requirements need to be fulfilled. One of these requirements is that the Limit of Detection (LoD) of the alternative method should be equivalent to the compendial method.[4] Furthermore, it should be possible to perform an identification after a positive test. Additionally, the new method should have a low chance of getting false-positive test results due to secondary contamination. Finally, non-human verification of the sterility test result is preferred because of data integrity requirements.[5]

To assess if alternative methods would be suitable for a rapid sterility test, multiple available techniques and methods in development for sterility testing have been evaluated. These novel techniques are based on different principles such as solid-phase cytometry (direct measurement), polymerase chain reaction (PCR, cell component analysis), and growth-based methods such as ATP bioluminescence and detection of CO_2 produced by growing microorganisms.[4] With solid-phase cytometry, a sample is filtered through a membrane and subsequently labelled to produce fluorescence. PCR relies on the amplification and detection of DNA, but it is an insurmountable challenge to reach an LoD of 1 cell, due to the use of universal primers and probes. ATP bioluminescence is based on the release of ATP by viable microorganisms. Another technique uses the detection of carbon dioxide produced by growing microorganisms. An increase in CO_2 results in a change in pH, which can cause an indicator to change colour. These last two methods are growth-dependent and therefore have a time to result of several days.

When selecting a rapid sterility test, the specific properties of the radiopharmaceutical products must be considered. Because of the short shelf-life of radiopharmaceuticals, the time to result must be as short as possible. To reach this aim, it's best to have a method that is growth-independent. Therefore, direct detection methods using solid-phase cytometry currently seem to be the only suitable methods for sterility testing that can provide results before the radioactive product is administered to the patient. Techniques based on solid-phase cytometry can be used for testing radiopharmaceuticals to acquire short time to

ScanRDI®

Red One™

results. The following systems were part of our evaluation: MuScan™ (Innosieve Diagnostics), ScanRDI® (bioMérieux) and Red One™ (Redberry).

The ScanRDI system utilises a viability dye that stains microorganisms that are retained on a polyester track-etched membrane (TEM).[6] Viable cells cleave the non-fluorescent viability substrate by an enzymatic reaction in the presence of esterases. These enzymes are present in every metabolically active microorganism. Within this reaction, free fluorochrome is released. The cell membrane holds this light-emitting fluorochrome, which can be detected by the ScanRDI. The ScanRDI has a laser that scans the entire surface of the membrane where emitted light can be detected. The ScanRDI uses advanced discrimination parameters to differentiate between labelled microorganisms and background noise. Afterwards, all positive test results need to be confirmed by an analyst using a fluorescence microscope. The results of this method can be achieved within three hours with an LoD of 1 cell.[7] After scanning, post-identification could be possible for most microorganisms. However, to enable identification of Gram negative bacteria, further optimisation is still needed.[8]

The MuScan is a method developed by Innosieve Diagnostics where filtration is performed through a dedicated silicon nitride membrane.[9] Retained microorganisms are then stained with the Sieve-ID kit. When the cell is metabolically active, the dye will be converted into a fluorescent group and a residual product within the cell. This fluorescent group remains inside the cell and will cause the cell to emit fluorescence. The membrane is subsequently scanned, resulting in high-quality images. For these images, a patented optical scanning regime is used with optimal background reduction. The scanner provides and processes many images of the membrane and returns the ones in which fluorescent cells have been detected as a result of strict image analysis parameters. After detection, the analyst must confirm the positive results. Dependent on the protocol used, the MuScan can obtain results within one to two hours with a limit of detection of at least 1-5 fluorescent forming units (FFU).[10] These FFU are composed of both colony-forming units (CFU) counts and non-CFU (eg, viable but non-culturable microorganisms) counts.[11] Currently, identification of recovered microorganisms is under development.

With the Red One microorganisms are also captured and fixed on a polyester track-etched membrane after filtration.[12] Hereafter, the microorganisms are labelled with a cell viability staining agent which reacts to the presence of esterases. Fluorescence that is emitted by the microorganisms is measured with the Red One.

MuScan™

Find out more: www.sartorius.com

The staining of these microorganisms is performed fully automatically. During the measurement, high-resolution images are taken before, during and after the injection of the staining agent. With this measuring principle, the change in fluorescence can be recorded over time. This makes it possible to differentiate viable cells from inert particles. For testing sterility, the addition of either a reactivation step or an enrichment phase on the membrane is suggested. These steps consist of an incubation step from five hours (reactivation) to 48 hours (enrichment) to ensure an LoD of 1 CFU for all microorganisms. With the Red One, and using a specified workflow, no human verification of the final result is expected to be needed. Identification of recovered microorganisms is possible when using the enrichment step.

Most direct detection techniques are very promising but still in development for further optimisation. This involves various matters such as the possibility to do an identification afterwards, but also testing larger product volumes and optimising a fully automated process of generating results; preferably without the need to introduce a human verification. Before an alternative sterility test method can be implemented as a routine test, a full method validation needs to be performed. Additionally, it is recommended to set up a business case to financially justify the use of rapid methods.[13] For working with radiopharmaceuticals, additional factors must also be considered such as the safety of the analyst. For this purpose, sample handling steps should be adequately shielded to avoid safety concerns. A reduction of sample preparation steps would be beneficial in reducing the risk of having false-positive test results, as well as for safety reasons.

Based on the evaluation performed, several solid-phase cytometric methods seem to be suitable for sterility testing of radiopharmaceuticals. However, further feasibility studies are necessary to optimise the time to result, evaluate the LoD for a broad range of microorganisms, and to reduce the risk of test contamination so that a reliable sterility test result can be achieved before the product will be injected into the patient.

References

1. Diagnostic radiopharmaceuticals [Internet]. International Atomic Energy Agency. Available from: https://www.iaea.org/topics/diagnostic-radiopharmaceuticals
2. Annex 3 Guidelines on Good Manufacturing Practices for radiopharmaceutical products.
3. 2.6.1. sterility EUROPEAN PHARMACOPOEIA 6.0.
4. 5.1.6. Alternative methods for control of microbiological quality EUROPEAN PHARMACOPOEIA 6.0.
5. Medicines & Healthcare products Regulatory Agency (MHRA) "GXP" Data Integrity Guidance and Definitions. 2018.
6. Rapid Microbiology ScanRDI [Internet]. Available from: https://focus-lab.com/usp_71_rapid_microbiology_scanrdi.html
7. SCANRDI® | bioMérieux [Internet]. Available from: https://www.biomerieux-usa.com/industry/scan-rdi
8. Grosselin J, Palazuelo M, Girard C, Monnin V, Saccomani M-C, Wittemberg V, et al. Easy post-scan growth method provides combination of rapid industry relevant microorganisms quantification and identification with ScanRDI® and VITEK® MS [Internet]. 2015. Available from: https://www.researchgate.net/publication/306291896_Easy_post-scan_growth_method_provides_combination_of_rapid_industry_relevant_microorganisms_quantification_and_identification_with_ScanRDIR_and_VITEKR_MS
9. Saiz Balbastre S. Development and application of analytical techniques for quality control of biologics and sterile pharmaceutical products.
10. INNOSIEVE DIAGNOSTICS [Internet]. Available from: https://www.innosieve.com/rapid.html
11. Claassen-willemse M, Ijzerman-Boon P. RAPID DETECTION OF MICRO- ORGANISMS BY DIRECT DETECTION : A SOLUTION FOR TIME CRITICAL IN-PROCESS MONITORING. In 2018.
12. Automated and instantaneous enumeration of viable microorganisms with Red OneTM, solid-hase cytometry platform [Internet]. Available from: https://www.a3p.org/enumeration-viable-microorganisms/
13. Alternative and rapid micro methods (ARMM): A framework for the evaluation, validation and implementation of alternative and rapid microbiological testing methods – BioPhorum [Internet]. Available from: https://www.biophorum.com/download/alternative-and-rapid-micro-methods-armm-a-framework-for-the-evaluation-validation-and-implementation-of-alternative-and-rapid-microbiological-testing-methods/

Kim van Boxtel

Kim is a student at the Fontys University of Applied Sciences in The Netherlands. Currently she runs an internship at the Sterility Assurance department of GE Healthcare where she evaluates methods for rapid sterility testing of radiopharmaceuticals.

Arjan Langen

Arjan is a Dutch microbiologist and currently works as Sterility Assurance Director at GE Healthcare Pharmaceutical Diagnostics. He is an IRCA/QCI-qualified auditor and Green Belt certified. He is also a member of the ECA Annex 1 task force that works on the revision of Annex 1 for sterile manufacturing.

SARTORIUS

Application Note

January 6, 2020

Keywords or phrases:
sterility testing, septum sampling

Sterisart®

The Sterisart® Septum Enables Reliable Sampling from a Closed System Sterility Testing Unit

Jahnavi Ambekal Puttana[1], Arjun Simha Jayanagar Prahlada[1], Eric C. Arakel[2], Elke Rüngeling[1,3]

1. Research and Development Department, Sartorius Stedim Biotech, Bangalore, India
2. Product Management, Lab Essentials Microbiology, Sartorius Lab Instruments, Göttingen, Germany
3. Research and Development Department, Sartorius Stedim Biotech, Göttingen, Germany

Correspondence:
E-Mail: eric.arakel@sartorius.com

Abstract

In this study, we evaluated the Sterisart® closed system sterility testing device, with a septum, for the recurrent sterile extraction of samples. The results demonstrate that even after more than 100 repeated septum sampling events, which far exceeds any foreseeable sampling requirements, the septum remains intact and the growth media contained in these canisters remains sterile.

The Sterisart® septum allows easy inoculation and sampling, and enables the coupling of the conventional closed system sterility testing with rapid detection methods.

Introduction

Pharmaceutical products are routinely manufactured under strict GMP guidelines. Despite these strict codes, as a fail-safe prior to batch release, all pharmaceutical products undergo stringent sterility testing to identify the potential presence of viable microorganisms. It is crucial that pathogenic microbes, such as bacteria, viruses and fungi, are detected in contaminated products before patients come in contact with them. There have been rare instances where compromised drugs have been released to the market with devastating consequences, for the patients and also the pharmaceutical companies.

Sterility tests are performed in accordance with the regulatory requirements defined by the International Pharmacopeia (USP <71>, EP 2.6.1, JP 4:06). Sterility testing can be performed either by direct inoculation | transfer, or membrane filtration, which is the method of choice. Products are tested for sterility by direct inoculation only when the properties of the product do not permit membrane filtration. The membrane filtration approach typically relies on a closed filtration unit containing a membrane with a pore size not greater than 0.45 µm and that has reliably demonstrated the retention of microorganisms. Other components of the system include a suitable pressure supply (such as a peristaltic pump) that drives the sample across the membrane filter, an appropriate membrane rinsing solution, and growth | culture media. This closed setup is conventionally cleanroom compliant to eliminate any contamination risks and consequent false positives. Once sample filtration is complete, the closed system is incubated, typically for 14 days, and screened for turbidity as an indicator of microbial contamination.

Sterisart® canisters are a closed system for sterility testing based on the membrane filtration method. This closed system excludes the need for physically manipulating membrane filters and thereby mitigates the risk of secondary contamination and false positives. However, sample extraction is a prerequisite, when the growth media is rendered turbid by microbial growth, following the prescribed 14 days of incubation. If microbial growth is detected, the identity of the microorganism and the source of the contamination is determined, and the sterility test is declared invalid and then repeated. Aseptic sample withdrawal or aseptic enzyme supplementation, for instance to deactivate antibiotics that might result in false negatives, may also be required after filtration or during incubation.

Precipitation of the filtered test sample, or an adverse color change due to the inherent properties of the compound, can also render the growth media turbid, even prior to incubation at the prescribed temperatures. This convolutes the interpretation of the sterility test and the certification of the batch for release; the batch may require additional testing by sample extraction from the canister and subsequent sub-culturing.

Sample extraction in conventional sterility test systems involves puncturing or cutting the tubing leading to the inlet of the canister and then attempting to carefully extract a sample, without compromising the integrity of the canister or its contents. Sample extraction by cutting the tubing precludes repeated sampling. Multiple sampling using other approaches can increase the risk of contamination by compromising the closed system.

The Sterisart® septum was designed to facilitate repeated sampling during incubation of the growth promotion test. In this report, we show that multiple sampling performed through the Sterisart® canister septum – over 100 times – exceeding any conceivable requirement for aseptic sampling, does not lead to the contamination of the system.

Reasons for septum usage:

a)	The growth media is rendered turbid by microbial growth, following incubation, and necessitates the identification of the micro-organism as part of a root cause analysis.
b)	The product renders the growth medium turbid, prior to incubation, and requires sub-culturing	dilution.
c)	Samples are drawn to test for microbial contamination by rapid detection methods.
d)	Samples are supplemented with agents to counteract anti-microbial components of the tested product.

Materials and Methods

Consumables
Tryptic soy broth (TSB) (Gila/BD), Fluid thioglycollate medium (FTM) (Gila/BD), TSB (Merck), FTM (Merck), Tryptic soy agar (TSA) (Merck), Glass reaction tubes, 30 ml (Borosil), Needle – 0.90 × 70 mm, 20G × 2 ¾ (Sterican – B. Braun), Syringe – F Luer (Omnifix – B. Braun).

Equipment
Sterisart® universal pump, Incubator (Sartorius Stedim Biotech GmbH), Combisart® 3-branch filtration manifold (Sartorius Stedim Biotech GmbH), e.jet Pump (Sartorius Stedim Biotech GmbH).

Sterisart® NF sterility testing system
16467--------GSD, 16475--------GSD, 16466--------GSD

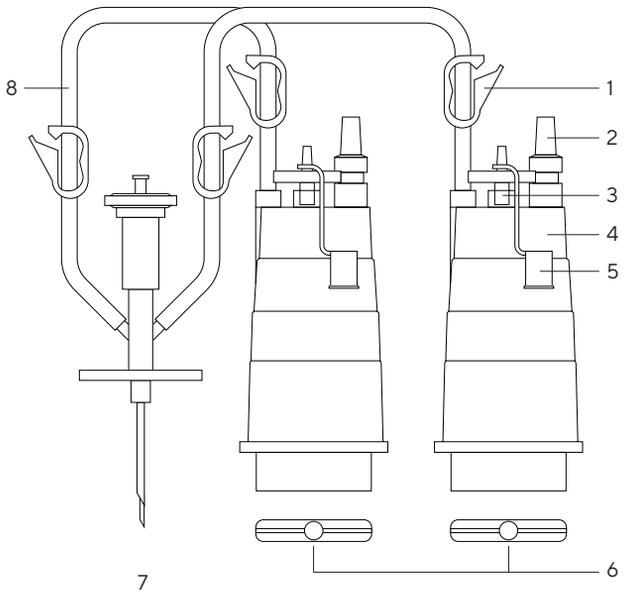
Membrane filtration:
Ten individual Sterisart® canisters from the three types of septum variants (30 in total) were analyzed in the septum sampling tests. One of the ten canisters (from each Sterisart® canister type) served as a negative control (i.e. samples were not extracted from this canister until day 24 of the test).

The Sterisart® canisters were filled with growth media under aseptic sterile conditions in a biosafety cabinet. The two Sterisart® canisters were positioned in the pump holder and the Sterisart® tubing system was thread through the pump head. The outlet of each Sterisart® canister was sealed using the enclosed wing nut plugs. The two sterile vent filters were left uncapped. The yellow tube clamp at the outlet of the Y-distributor was opened and the adjacent white tube clamp closed. The dual-needle metal spike was inserted into a bottle containing FTM and the Sterisart® Universal pump was switched on. The pump was switched off once a predefined volume (75 ml) of medium was transferred into the first canister. The white tube clamp at the outlet of Y-distributor was opened and the adjacent yellow clamp closed. The dual-needle metal spike was inserted into a bottle containing TSB and the Sterisart® Universal pump was switched on. A similar volume (75 ml) of medium was transferred into the second canister. The tubing was sealed off using the two clamps above the inlets of the canisters and the tubing was cut off. Please refer to our **Sterisart® NF gamma user manual** for a pictorial depiction of the described process.

Sterisart® canisters containing TSB, the recommended growth media used in the detection of low incidence fungi and aerobic bacteria, were incubated at 22.5° C for 24 days. Sterisart® canisters containing FTM, the recommended growth media for cultivating aerobic, microaerophilic, and anaerobic microorganisms were incubated at 32.5° C for 24 days.

Septum sampling:
Samples were extracted from the Sterisart® canisters under sterile conditions in a biosafety cabinet. Three samples of 100 µl each were extracted twice a day from the top, middle, and bottom of the Sterisart® canister, over a period of 17 days (3 × 2 × 17 = 102 samples). The extracted samples were transferred into the glass reaction tubes containing the sterile liquid media, FTM and TSB. The vials containing TSB were incubated at 22.5° C for 14 days, and the vials containing FTM were incubated at 32.5° C for 14 days. The results were recorded by photographing each Sterisart® unit and the corresponding extracted sample.

Microbial enumeration:
A final inspection was performed using a black gridded membrane filter placed in a sterile Sartorius Combisart® filtration unit and connected to an e.jet pump. 60 – 70 ml of TSB (following the 24 day incubation period of the Sterisart® canisters) was filled into the funnel and filtered through the black membrane filter. The filter was transferred using sterile forceps onto a TSA plate, and the plate was incubated at 36° C for 3 – 5 days. These plates were then inspected for microbial contamination.



No.	Component
1.	Pre-installed tube clamp
2.	Connector with septum for sterile sampling
3.	Vent filter
4.	Sterisart® container
5.	Tethered filter cap
6.	Wing nut plug
7.	Dual-needle metal spike for closed containers (16466)
8.	Tubing

Results and Discussion

After 102 septum piercings and repeated sample withdrawals, it was established that all Sterisart® canisters (3 × 9 containing FTM, and 3 × 9 containing TSB; the 10th canister containing FTM and TSB serving as their respective controls) were sterile and showed no detectable microbial contamination after 24 days. (Figure 1)

Similarly, the extracted samples were likewise sterile and free of microbial growth demonstrating that the Sterisart® septum promotes efficient and highly reliable aseptic sampling. (Figure 2)

Figure 1: No microbial contamination after repeated sample extraction. Representative images of the Sterisart® 16466 GSD version filled with TSB (A and B) FTM (C and D) incubated for 24 days at 22.5° C and 32.5° C respectively. Negative controls are shown in A and C.
Canisters in B and D were pierced 102 times for sample extraction.

Figure 2: No microbial contamination in samples extracted from Sterisart® 16466 GSD after incubation in glass vials. Samples were inoculated into glass vials containing TSB (upper panel) and FTM (lower panel) were grown at 22.5° C and 32.5° C, respectively, for 14 days.

Coring can occur when a septum has been punctured multiple times or if an inappropriate needle type is used. Only after 36 piercings were small particles observed in some Sterisart® canisters. These particles were collected after 24 days on a black membrane filter and monitored for their ability to form colonies on TSA plates. These particles did not demonstrate any growth even after an incubation period of five days, suggesting that these particles are not biological in nature. Based on their morphology, we conclude that these inert particles are fragments of rubber that are sheared off the septum during repeated piercing with syringe needles. These fragments do not influence the efficacy of the sterility test and are barely visible in the growth medium.

We recommend that septum sampling be performed only after unplugging the sterile vent (i.e. uncapped) in a controlled environment.

Our results demonstrate that Sterisart® canisters remain a closed and sterile unit, even after successive sampling for a tested total of 102 extractions.

Figure 3: Representative image of the Sterisart® septum after 102 sample extractions.

Conclusion

In summary, we demonstrate that the Sterisart® septum is exceedingly robust and maintains an intact sterile environment even after more than a hundred sample extractions.

The presence of preservatives and anti-microbial agents, in products tested for sterility, have to a great extent impeded the adoption of rapid detection methods that rely on direct inoculation. Membrane filtration, and subsequent membrane rinsing, of such products curtails the risk of false negatives during sterility testing. By also facilitating the analysis of large volumes through membrane filtration and by enabling the extraction of samples, we afford our users the ability to integrate closed system sterility testing with rapid sterility testing methods.

However, some slow growing anaerobes can be difficult to detect using some rapid sterility methods. Given that septum sampling does not compromise the integrity of the closed system sterility test, we provide our customers with the potential to sample for rapid sterility testing, yet re-incubate the sterility tests for the stipulated 14-day period of incubation.


SARTORIUS
Manual
Prg. 1/ 1
SPEED
100
TIME
00:00
Speed
Menu
Esc
FRONT
STERISART® UNIVERSAL