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Continuous Microbial Air Monitoring in Clean Room Environments

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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.

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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.

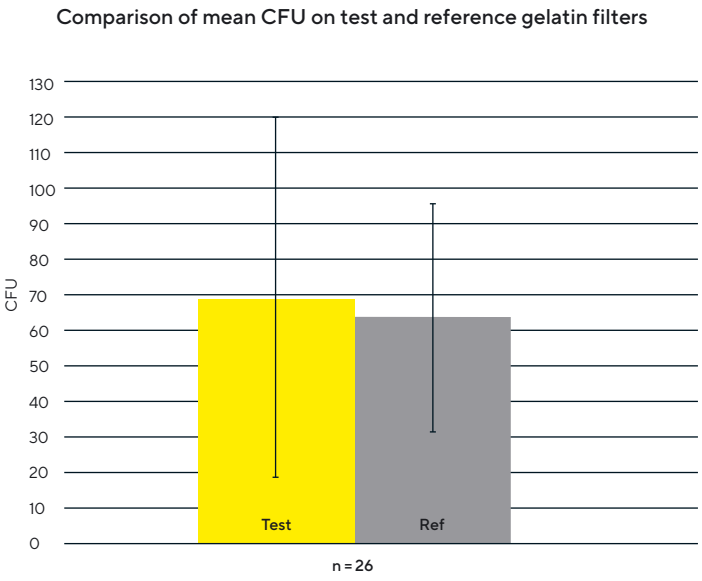


Figure 1: Comparison of mean CFU on test and reference gelatin filters.

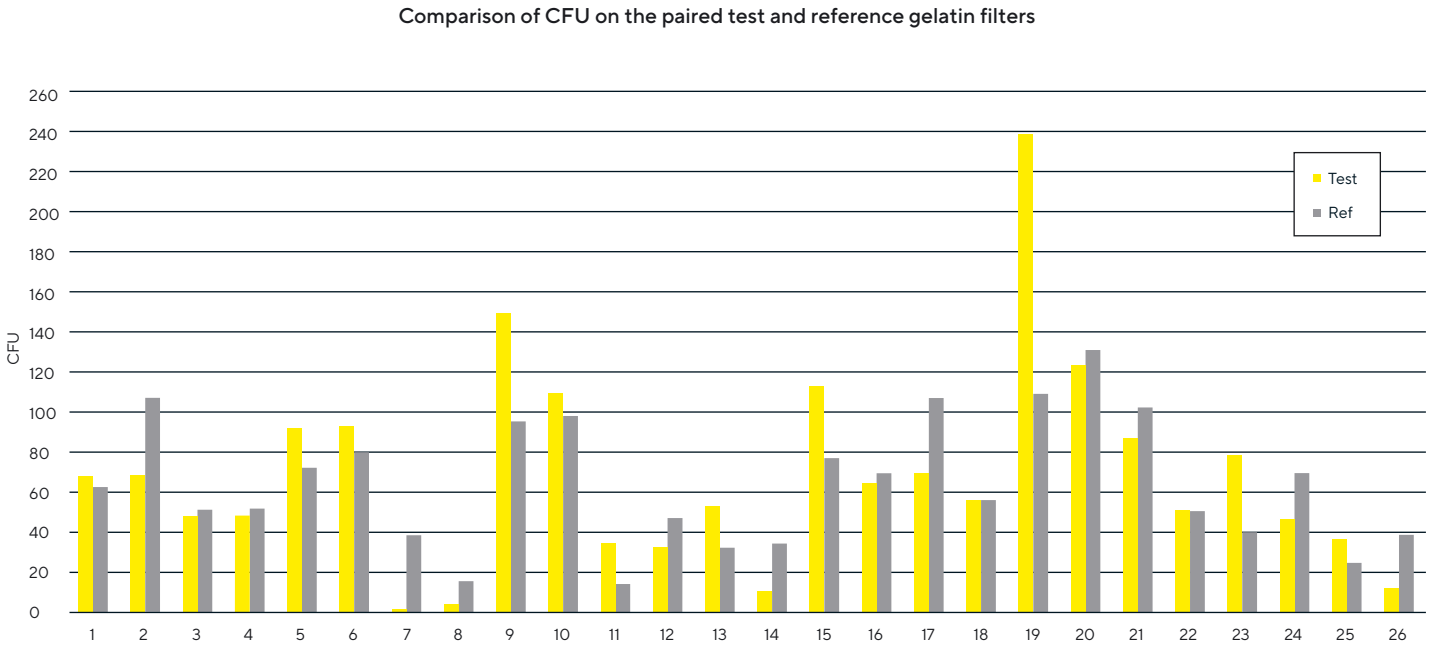


Figure 2: Comparison of CFU on the paired test and reference gelatin filters.

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.

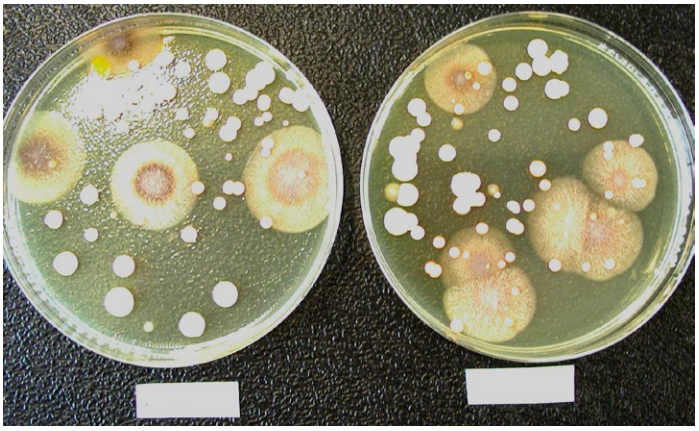


Figure 3: Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right). The composition of the microbiological population found on the test and reference filters is comparable. Representative soybean-casein-digest agar medium plates showing the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

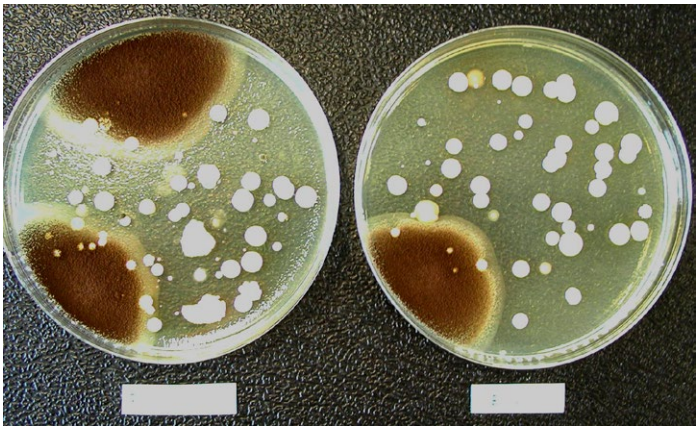
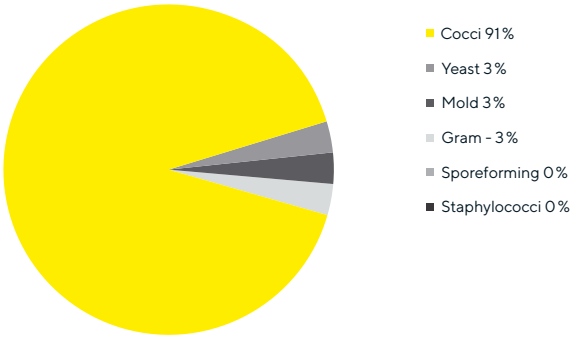


Figure 4: Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right). The composition of the microbiological population found on the test and reference filters is comparable. Representative soybean-casein-digest agar medium plates showing the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

A



B

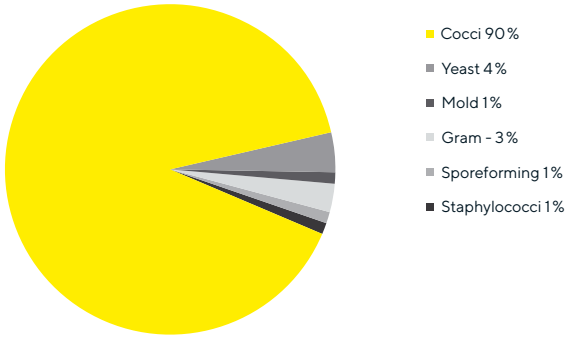


Figure 5: A. Composition of the microbiological population grown on the test gelatin filters. Almost all microbes grown on test filters is Cocci. This figure shows a breakdown of microbes grown on soybean-casein-digest agar medium plates from test filters. B. Composition of the microbiological population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

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