



Liquid and Aerosol Bacterial Challenge Testing in Sterile Air Filter Validation

Overview

Microbial retention is the single most important performance attribute of sterilizing-grade air and gas filters. If sterility of the air or gas critical to the success of a food and beverage industry application cannot be achieved, the primary goal of the filtration fails. There are, of course, other attributes of importance when selecting sterile air filters, which dictate their suitability for use¹.

Understanding how sterilizing-grade cartridge air filters are validated for microbial removal from air or gas assists the end user in effective filter selection². According to guidance from FDA³, the validation work carried out by filter manufacturers demonstrates the microbial retention and removal efficiency of hydrophobic sterilizing-grade cartridge air filters when challenged with a defined minimum concentration of model microorganisms, namely, *Brevundimonas diminuta* (*B. diminuta*) (ATCC 19146) at 10^7 cfu/cm² of effective filtration area, either from a liquid suspension ("liquid bacterial challenge") or from a gas ("aerosol bacterial challenge"), or both. In addition to these basic bacterial challenge tests, which sufficiently demonstrate the sterilizing ability of cartridge air filters, filter manufacturers may do more validation work demonstrating filter retention of bacterial spores, long-term aerosol bacterial challenges, and aerosol bacteriophage removal, to illustrate the filters' fit for purpose in various applications.

This article discusses the implications of filtration performance under liquid or aerosol conditions, and describes the methods used by filter manufacturers to validate microbial retention performance for these cases.

Liquid and Gas Removal Performance in Air Filtration

In air filtration, filters perform differently based on whether they are removing contaminants from a liquid or a gas. Filters have both a liquid and a gas removal rating. Due to the mechanisms at play in liquid or gas filtration⁴, it is more "difficult" to remove contaminants from liquids than from gases, *i.e.*, it requires a "tighter" filter to remove contaminants from a liquid than from a gas. For example, a 0.2 micron (μm) microbially validated liquid-rated filter will remove microbial contaminants in the size range equal to or larger than *B. diminuta* from a liquid, but the same filter removes these contaminants approximately five to ten times smaller from a dry gas. Further, a liquid-rated 0.2 μm filter, which removes the described contaminants from a liquid, is much tighter than a gas-rated 0.2 μm filter, which removes these contaminants from a gas.

Due to the filtration mechanism known as diffusional interception^{4,5}, air filters filtering dry gas can remove contaminants many times smaller than what would be expected based on their pore size rating. When those pores are wetted, the filters revert to their liquid removal performance. Figure 1 illustrates this principle conceptually.

Air filters should be hydrophobic in order to prevent them from wetting out due to steam condensate or exposure to moisture under normal conditions of production. By remaining dry, the hydrophobic filters provide the least possible restriction to airflow. However, in spite of being hydrophobic, depending on the filter media materials used and their degree of hydrophobicity, there are conditions in production in which the filter pores can wet out, partially or completely. These cases can occur during process upsets, and represent worst-case conditions. Under such conditions, the filters revert to their liquid, more "open" removal performance.

Figure 1a: Due to Brownian motion of contaminants in dry gas, contaminants many times smaller than the filter pore size impinge on the filter media and are intercepted.

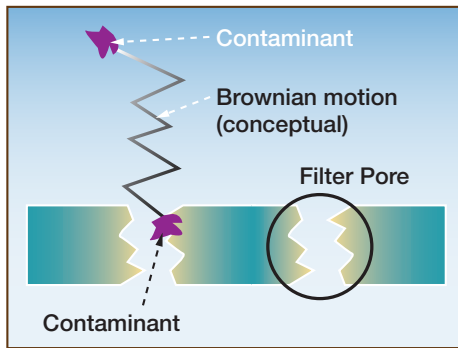
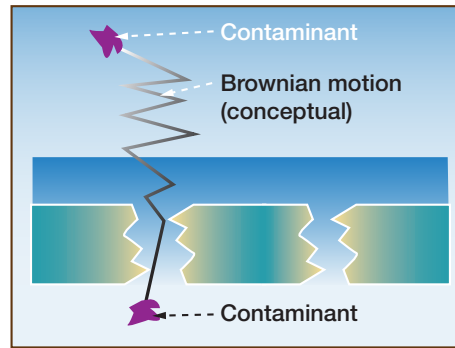


Figure 1b: Under moist conditions, Brownian motion can no longer occur. The contaminants follow the streams of liquid flow, and filter pores would need to be much smaller to retain them.



In process upset situations, even hydrophobic filters on compressed air or gas streams may allow moisture through. Examples for such situations are failure of upstream air dryers or pressure surges which overcome the bubble point of a filter, or hydrophilic spots (wetted pores) on the filters due to exposure to solvent chemicals or oils which reduce the surface tension of moisture in the air. Should any moisture pass the filter, aerosol-challenged filters would not maintain sterility in the filtered air, while liquid-challenged filters would.

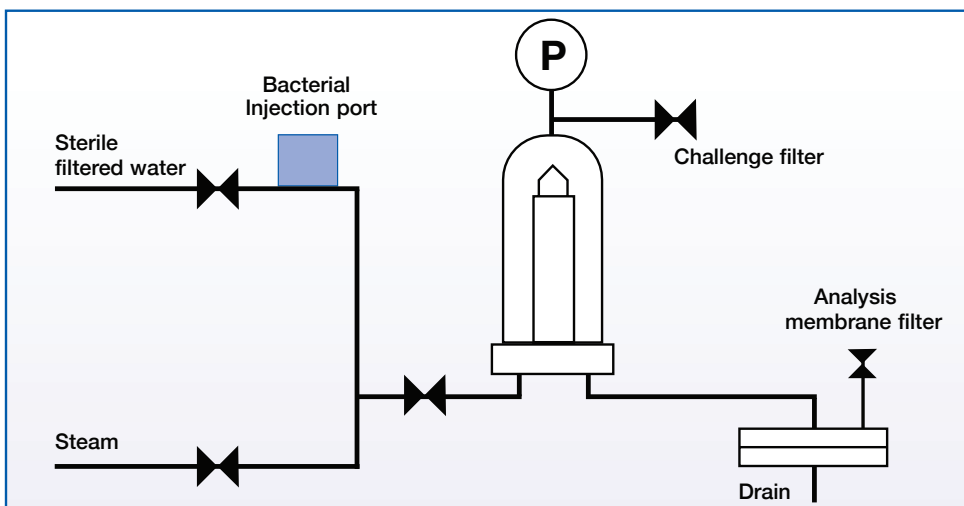
Bacterial challenge testing is carried out under conditions that represent normal (dry gas) and worst-case (liquid) production conditions.

Liquid Bacterial Challenge Validation Testing

Liquid bacterial challenge demonstrates the filter's microbial retention and removal efficiency performance when challenged with a *liquid* suspension inoculated with the challenge microorganism.

Figure 2 illustrates a typical liquid bacterial challenge test set-up, based on guidance from ASTM F838-83⁶ and updated versions thereof. The test filter is initially integrity tested, steam-sterilized and cooled. As an air filter is hydrophobic, it must be wetted with a fluid of low surface tension such as an alcohol solution, to enable passage of liquid through its pores. The alcohol solution is flushed out by sterile water or buffer. An aqueous suspension of challenge microorganism (the 'influent') at the required area challenge level of $>10^7/\text{cm}^2$ is introduced to the test filter at a given flow rate for a period of time. The entire volume of test liquid passes through the filter (the 'effluent'). It is then passed through downstream 0.2 μm analysis membrane filter discs which retain any microorganisms that may have penetrated the filter. The analysis discs are incubated to check for growth of any viable microorganisms. If none are found, sterile effluent has been achieved. If even one viable microorganism is found, the filter has failed the validation test. Another filter integrity test is carried out to reconfirm filter integrity after the challenge test is completed.

Fig. 2: Schematic of liquid bacterial challenge test set-up



Aerosol Bacterial Challenge Validation Testing

Aerosol bacterial challenge demonstrates the filter's microbial retention and removal efficiency performance when challenged in a gas with the nebulized challenge microorganism.

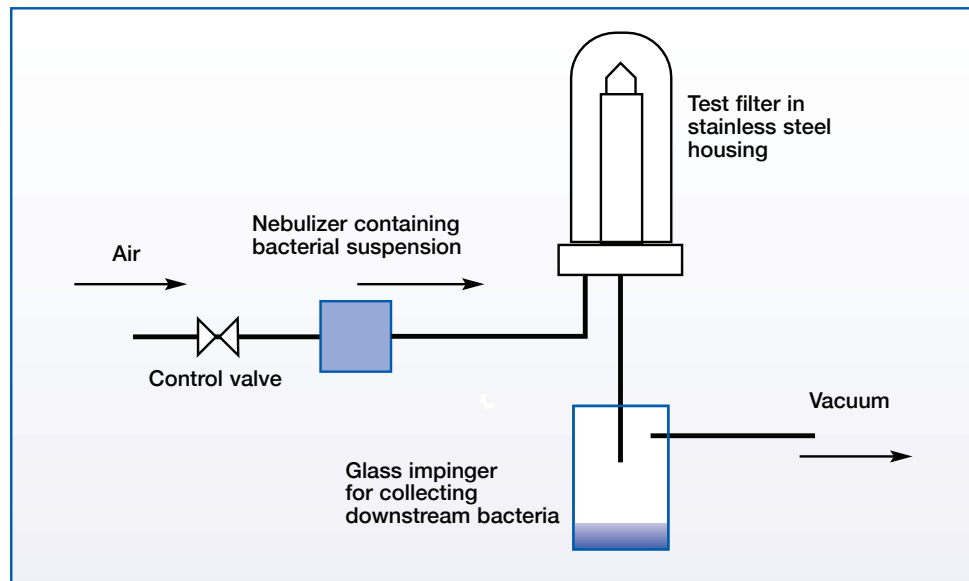
Figure 3 illustrates a typical aerosol bacterial challenge set-up. There are different approaches available to conduct this test; it is important that the test design matches the purpose for which the filter is being tested. A basic description follows.

The test set-up consists of two parallel streams, one with a test filter installed and one without. The test filter is initially integrity tested, dried, autoclave-sterilized, and cooled. A nebulized suspension of challenge microorganisms in dry air (an aerosol, the 'influent') up to the required area challenge level of $> 10^7/\text{cm}^2$ is passed through the test filter at a given flow rate for a period of time. The filtrate or effluent passes to downstream collection devices such as impingers with recovery buffer, which capture and suspend any microorganisms that may have penetrated the test filter. It is important to mention that in microbial aerosol challenge work, the entire filtered stream of effluent air (*i.e.*, not just a slip stream portion thereof) passes through the downstream collection devices, so that very low counts or even a single test organism that may have penetrated the test filter is recovered.

In the parallel stream, the aerosol is introduced directly through the test system without the test filter, and to a separate set of downstream impingers. A titer reduction is calculated by comparing the concentration of bacteria found at both sets of impingers, *i.e.*, by analyzing influent and effluent counts. The test filter achieves sterilization capability if sterile effluent, or no viable microorganisms are found in the recovery buffer.

Another filter integrity test is carried out to reconfirm filter integrity after the challenge test is completed.

Fig. 3: Schematic of aerosol bacterial challenge test set-up



Comparison of Liquid Bacterial Challenge and Aerosol Bacterial Challenge Validation

Both microbial validation tests demonstrate the air sterilizing capability of a filter according to the definition for sterilizing-grade cartridge air filters³, however under different conditions. Both methods provide extremely high sensitivity: by evaluating the totality of filter effluent, they are able to detect even very low numbers of viable bacterial cells which may have penetrated the filter.

Liquid bacterial challenge is the more stringent validation method. In effect, a sterilizing-grade cartridge air filter which is demonstrated to remove a defined liquid suspension of challenge microorganisms, yielding sterile effluent, will be effective under normal, dry gas *and* worst-case liquid conditions. A liquid challenge-validated filter will always pass an aerosol challenge test, but not vice-versa. A liquid challenge claim in a sterilizing-grade air filter will ensure the lowest possible risk to compromising sterility.

An aerosol challenge-validated filter will remove a defined aerosol suspension of challenge microorganisms from dry air or gas, but it will not necessarily be effective if filter pores wet out under upset conditions. Filter performance under these conditions assumes low risk for process upset conditions.

In critical sterilizing air and gas filtration applications, it is always preferable from a microbial retention perspective to use liquid-challenged filters.

Finally, a word of caution to the test methods used in microbial challenge work. Liquid challenge testing is standardized and replicated in filter manufacturers' laboratories on a routine basis. Conditions and parameters for testing are described in detail in the respective standards. Reproducibility and comparison of results is excellent.

Aerosol challenge testing is by far more difficult to realize. There is no official standard test method existing for aerosol challenge tests. There are several critical aspects that need to be properly validated like the nebulizing process, the size of droplets, microorganisms to be prevented from drying out during testing and last but not least the type of analytical device to collect the downstream microorganisms.

Summary

Sterilizing-grade air and gas filters exhibit their highest removal efficiency in dry gas filtration. In operation, there are conditions under which filters can wet out, partially or completely. In these cases, filters revert to their liquid removal performance; they would enable contaminants to pass through that would otherwise be retained in dry gas conditions. For critical sterilizing applications, users should select liquid-validated sterilizing-grade filters.

References

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- ³ Food and Drug Administration. *Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice*; CDER, CBER, ORA: Rockville, MD, September 2004. Accessed at <https://www.fda.gov/downloads/Drugs/Guidances/ucm070342.pdf>
- ⁴ Pall Corporation. *Mechanisms of Air Filtration*. 2020.
- ⁵ Parenteral Drug Association (PDA). *Sterilizing Filtration of Gases*; PDA Technical Report No. 40 (TR 40); *PDA J. Pharm. Sci. Technol.* 2005, 58 (No. S-1).
- ⁶ American Society for Testing and Materials (ASTM). *Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration*; ASTM Standard F838-83; Philadelphia, PA, 1983, Reapproved 1988, 1993 2005, 2015.



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