

# Food and beverage: Linking filter performance and product safety



Controlling levels of contamination in the food and beverage industries is a key role of filtration. Dr. Ralph Heusslein and Ursula Brendel-Thimmel of Pall Corporation, Scientific and Laboratory Services, explain the links between filter performance and microbiological product safety.

## Summary

Filtration is frequently used to control contamination levels in food and beverage applications. Filter performance can be expressed by a Logarithmic Reduction value (LRV) linked to titre reduction as established under various conditions used to challenge the filter. Ideally filter validations should be based on established standards with standard microorganisms, supplemented by more application oriented challenge tests with typical spoilage microorganisms in the respective fluid. The ability of a filter to reduce microorganisms from a process stream might be extremely high, but usually not 100% because of lifetime and throughput considerations. For example, a filter with a 99.9999% reduction would equal a LRV of 6. In food and beverage applications no established common standard exists for the definition of a microbiologically stable product, which sometimes is covered synonymously by the phrase 'commercially sterile'. The likelihood for a final product to be contaminated is directly linked to filter performance. Due to statistical aspects with sampling and testing of final product at low contamination levels, hardly any difference will be detected. Choice of the right filtration performance level in combination with regular controls on the process and the filter function are preferred means to establish microbiological product safety.

## Introduction

Brand protection, quality improvements and cost reduction are major drivers in the food and beverage industry. For these three reasons fine membrane filters are used, especially in beverage applications, to add a defined step for microbiological control to the overall process. This article reflects on ways to measure filter performance and the

statistical likelihood for microorganisms to contaminate a final product.

Microbiological performance of filters is typically evaluated under defined laboratory conditions [1, 2, 3]. This is done by challenging the filter under reproducible conditions with certain microorganisms suspended in a defined volume, at a defined concentration with a suitable flow of the

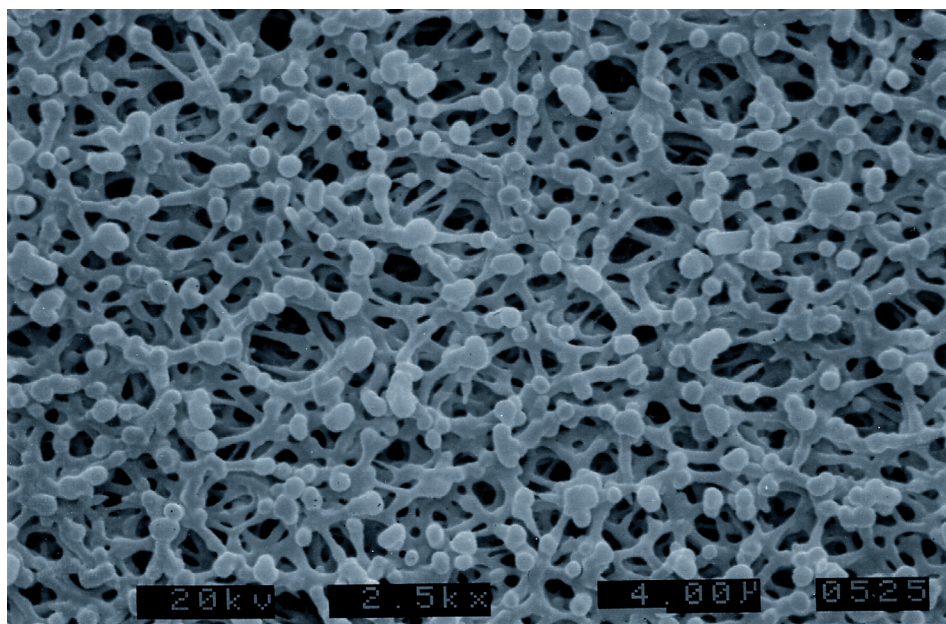


Figure 1: Sponge-like structure of a 0.2 µm membrane (Scanning Electron Microscopic Image, second bar from right represents 4 µm).

suspension through the filter. The relationship between upstream and downstream concentration of microorganisms is measured and used to calculate the titre reduction. For convenience these usually very high numbers are expressed by indicating the Logarithmic Reduction Value (LRV), representing the common logarithm of the titre reduction.

$$\text{LRV} = \log \frac{\text{Total number of microorganisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

Microbiological challenge tests are used to validate filters along standardised methods with standard microorganisms like *Brevundimonas diminuta*, *Serratia marcescens* or *Escherichia coli*.

Depending on the microorganism chosen for the test work, the filter is positioned as 0.2, 0.45 or 0.65 µm rated filter. This rating in combination with the ability to retain those microorganisms (expressed through the titre reduction) is an indication of the microbiological performance level of a filter, but not more. The ability to compare filter performance data is limited due to various factors of influence that have an impact on the reported result. The situation is even more complex if other microorganisms like typical spoilage organisms are used and the challenge is performed with the respective beverages instead of buffer. This article reflects on some of these aspects.

### Microbiological filter validation

Fine membrane filters (see Figure 1) can be characterised by various physicochemical parameters (for example, membrane thickness, surface, membrane chemistry, pore size distribution based on various methods, pore volume, flow/delta P, diffusional air flow, bubble point). A direct correlation to their ability to reduce certain microorganisms from a process stream is usually impossible or at least very limited. Laboratory scale microbiological validation of filters under defined conditions is the method of choice to establish filter performance data. Microbiological filter validation needs to reflect on a variety of fundamental aspects including:

- the appropriate choice of challenge level
- selection of challenge fluid
- total throughput or challenge volume
- volume per time (flow)
- the selection of the right microorganism cultured under appropriate conditions.

If the challenge level is too low, the small number of microorganisms downstream of the filter could limit the precise calculation of titre reduction. In this case, sterile effluent may be incorrectly interpreted as high performance. Alternatively, if the calculation is performed correctly with the theoretical limit of the method a false low performance may be the result. Typically one organism

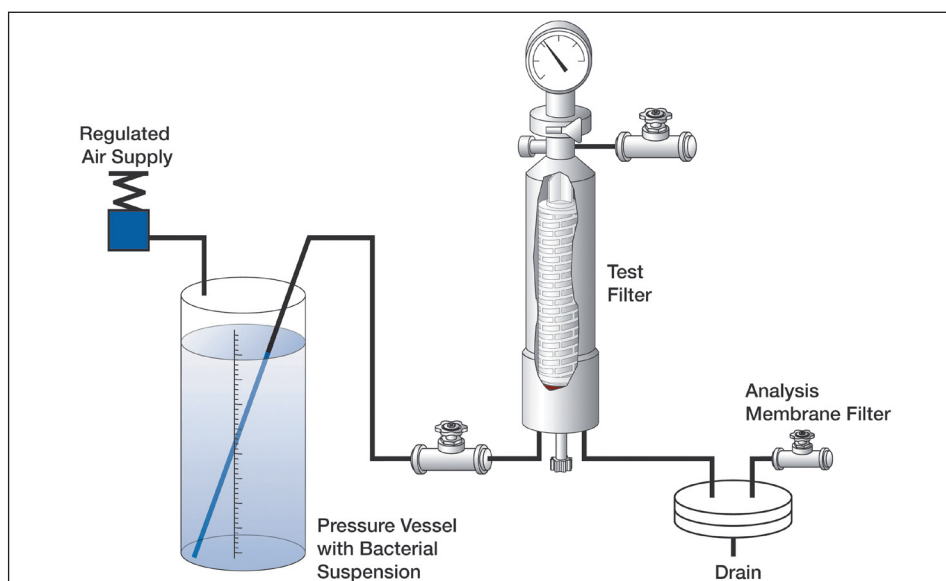


Figure 2: Set-up for a bacterial challenge test.

downstream is seen as the limit of the method. If the upstream challenge level is too high, the formation of a layer of retained microorganisms on the membrane may lead to an artificially high titre reduction value. In such cases filtration is not just done by the membrane alone, the retained layer may act as a kind of pre-filter.

For obvious reasons it is essential that the challenge is done in a controlled manner under defined conditions. The theoretical sensitivity of the individual set up should be considered, especially if filters are expected to provide sterile effluent. To detect low levels of organisms in the effluent, the membrane filter method is an elegant option to retain all organisms downstream of the test filter for subsequent culturing (see Figure 2). After filtration of the effluent through a very fine membrane, this is placed on a suitable nutrient with the microorganisms facing up. This way nutrient can permeate to the retained microorganisms while metabolic end product, which might have a growth retarding effect, do not accumulate and is diffusing through the membrane away from the organisms into the nutrient. This way of sampling microorganisms should be done on 100% of the effluent wherever feasible. Sampling of fractions representing smaller volumes and determination of colony count by plating or MPN (Most Probable Number) technique combined with subsequent extrapolation for the total volume is especially inaccurate when filters show high performance and therefore lead to very low levels of downstream microorganisms. These low levels wouldn't be detected by colony counting techniques other than a membrane filter technique.

Volume flow through the filter and the test fluid itself should ideally be selected based on the requirements of the filter application. Physical and chemical aspects linked to the composition of the fluids are defined

by parameters (for example, dissolved electrolytes, sugars, proteins, phenols and alcohols) that directly or indirectly affect performance through viscosity, surface tension, ionic strength and pH. These factors not only impact filtration mechanisms like electrostatic or hydrophobic or hydrophilic interactions of contaminant and media but also influence media properties mechanically or physicochemically. Therefore, for a filter validation these parameters are kept constant by using standardised challenge microorganisms suspended in a standardised buffer solution [4].

Pore size is a major differentiator used to establish coarse performance categories for various media types. In the case of media, which is intended to retain microbes, pore size is measured indirectly by using certain model microorganisms like *B. diminuta* or *S. marcescens* (see Figure 3). When showing a certain performance level with these microorganisms, filters are classified as 0.2 µm or 0.45 µm rated filters. Microbiological retention determines pore size attributed to a fine membrane filter, not a given pore size guaranteeing a level of microbiological retention [2, 3]. In fact, the situation is more complex as the membrane microstructure, its chemistry, thickness, as well as the pore size distribution have a major impact on the microbiological performance. Therefore, a filter comparison based on an indicated pore size is very limited. Data involving relevant test organisms might provide more substantial information, although benchmarking based on these data is still limited due to the complexity of microbiological testing with multiple factors of influence and the difficulty of standardising such tests.

Test work performed under 'real life' or process conditions can help to further confirm filter function in terms of an application specific qualification. Such



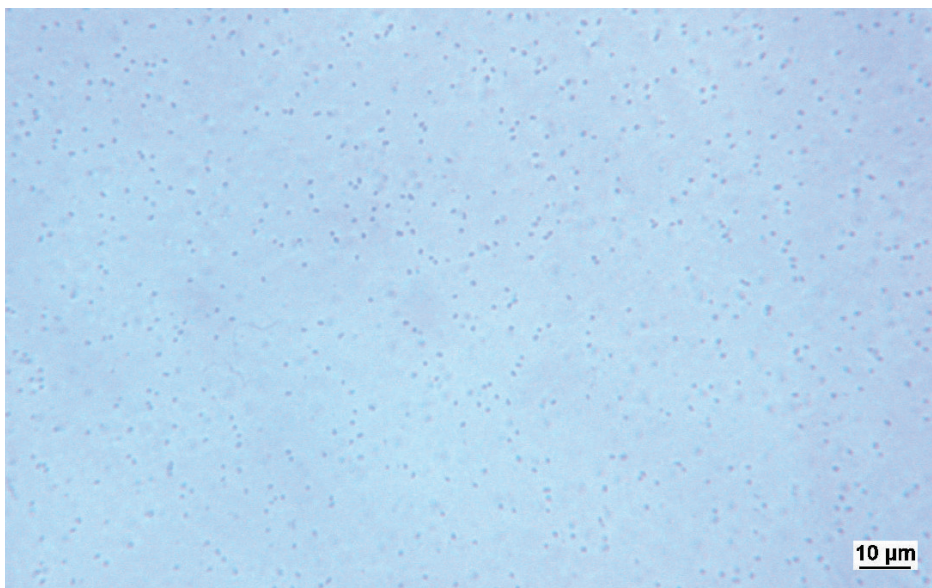


Figure 3: Standard Test Organism *Serratia marcescens*.

an approach is usually not suited to establishing a defined and reproducible titre reduction value due to limitations in precise determination of the usually heterogeneous upstream challenge and the number of microorganisms downstream. Sampling from high volume streams is usually limited to relatively small volume samples. This method can therefore lead to errors in both directions, false high or low values of overall titre reduction based on errors linked to limitations in both upstream and downstream sampling, high multipliers to reflect total process volume as well as other interfering factors like secondary contamination. Overall this approach has usually limited reproducibility and lags the ability to validate the method. Ideally a filter intended to reduce levels of microorganisms, for example from beer, should be tested with beer using the microorganisms typically contaminating beer like certain types of *Pediococcus* or *Lactobacillus* (Figures 4a and b). Other model microorganisms or model solutions might be

an appropriate alternative if sufficient test work has demonstrated equivalence of results. Unfortunately guidelines to standardise and harmonise such tests do not exist. In any case those tests would likely be done under controlled laboratory conditions.

Microbiological performance of filters is often expressed in terms which hardly allow comparisons amongst various products. In general, if filters are claimed to provide a sterile filtrate with a certain microorganism, the total challenge or preferably the specific challenge per cm<sup>2</sup> should be indicated. Ideally presenting individual performance data per tested product would allow a comparison of all data points and provide a better understanding of the statistical base on which such a statement is made. Nevertheless, the lag of standardisation and the difficulty in doing challenge tests fully standardised will still limit the ability for a direct comparison of data generated on different products by different laboratories.

Microbiological performance can be linked to a certain extent with physical parameters, allowing a quick and easy method to perform integrity tests of filters. The underlying physical principle is simple. A hydrophilic membrane will soak with water when getting rinsed. Exposed to a defined air pressure (usually applied from the upstream side) this water will get pushed out of the membrane. Due to surface tension and the hydrophilic nature of the membrane a thin film of water will remain within the pores. A wetted membrane will therefore not allow convective air flow through the pores, provided the test does not exceed a certain critical pressure. The ongoing diffusion of gas through the water film within the membrane leads to a far lower volume stream than convective flow allows. Using appropriate equipment this low diffusional flow of gas may be measured directly using constant pressure or indirectly by pressure decay or increase over a defined period in a defined volume (see Figure 5). A more sophisticated option is to measure diffusional flow through the wetted membrane using increasing differential pressure to establish a typical curve (a multiple point diffusion test plotting diffusion values against pressure) to provide more detailed information about membrane structure and pore size distribution[5].

Such test pressures and flow rates would typically get defined for a filter type as part of the filter validation work and correlated against its microbiological performance. A membrane during use might be impacted by, for example, mechanical forces leading to a small defect. When wetted and exposed to air pressure during integrity testing, these areas will have a less stable water film. This defect will allow the test gas to stream (convective flow, Fick's law) through the dewetted defect at far higher rates compared to the diffusional flow through the water film over an intact membrane. This difference can be picked up as an increased forward flow at defined test pressures by

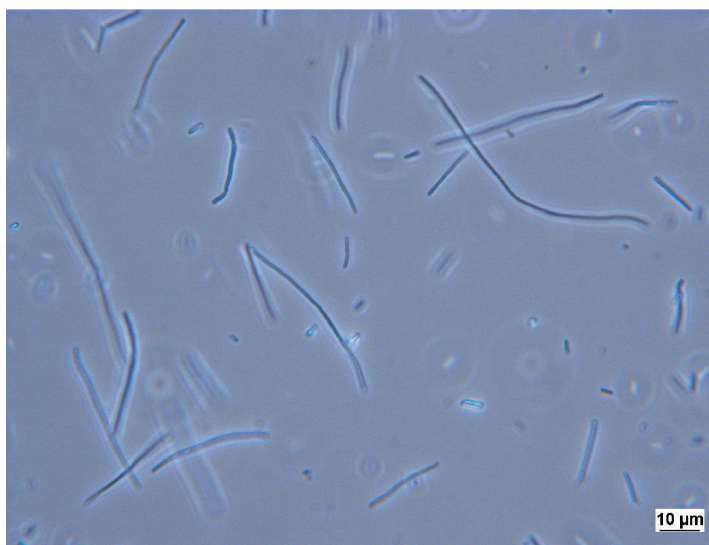


Figure 4a: *Lactobacillus brevis*.

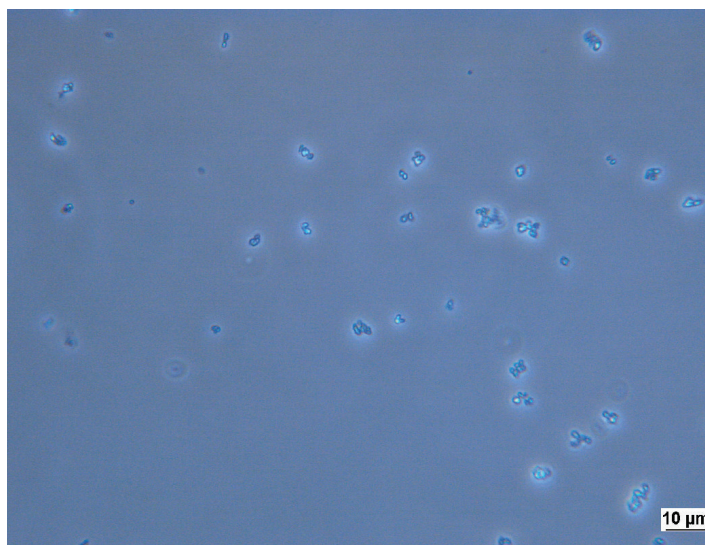


Figure 4b: *Pediococcus damnosus*.

appropriate equipment and indicates a filter out of specification. This principle is used in filter production to screen filters as part of standard Quality Control acceptance testing as well as by filter users to ensure installed filters are intact. Test conditions are critical to obtain reliable results with this sensitive measurement. Besides device calibration, all factors influencing filter wettability or water surface tension may have an effect on the result. Additionally, any parameters leading to unstable pressure conditions like changes in temperature or volume during the test procedure may invalidate the test. Besides variations in the individual test as mentioned, aspects like the total surface involved, variations in the forward flow values of each set of filters, tolerance of test parameters to cover those variations and housing volume should be considered. All those factors are limiting sensitivity and precision.

### Impact of filter performance on microbiological safety

Sterility in food and beverage applications is not a generically defined term as it is within pharmaceutical applications, where the definition of a sterile product is given as one with a maximum 'contamination' level of 1 CFU/10<sup>6</sup> sterilised units based upon a limited number of CFU/ml upstream of the filter [6]. The final unit can be of different volume. Practically this may lead to a maximum of 1 CFU in 1000 litres if the unit is a 1 ml ampoule; or 1 CFU in 500,000 litres if the unit is a bag containing 0.5 litre solution. For a filter this results in very clear performance definitions and requirements for performance validation [published in 1982 by the Health Industry Manufacturer's Association [1], updated and repositioned by the FDA, (Guideline on Sterile Drug Products Produced by Aseptic Processing) [7] or within the EU (Guideline of Good Manufacturing Practice for Pharmaceutical Products [8]). The up-to-date position on sterilising filtration of liquids and the use in the pharmaceutical industry is outlined by the PDA Technical Report No. 26[9].

The situation in the food and beverage industry is less defined. 'Sterility' is usually used in a very pragmatic way and implies a microbiologically stable product and not one free of all microbes. The Codex Alimentarius Commission (WHO/FAO) CAC/RCP 40-1993 [10] defines the term commercial sterility for low-acid food: *Commercial sterility means the absence of microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during manufacture, distribution and storage.*

The term 'sterility' in food and beverage applications refers to a product which is microbiologically stable under foreseeable conditions over the entire shelf life. A step in production leading to sterility using this

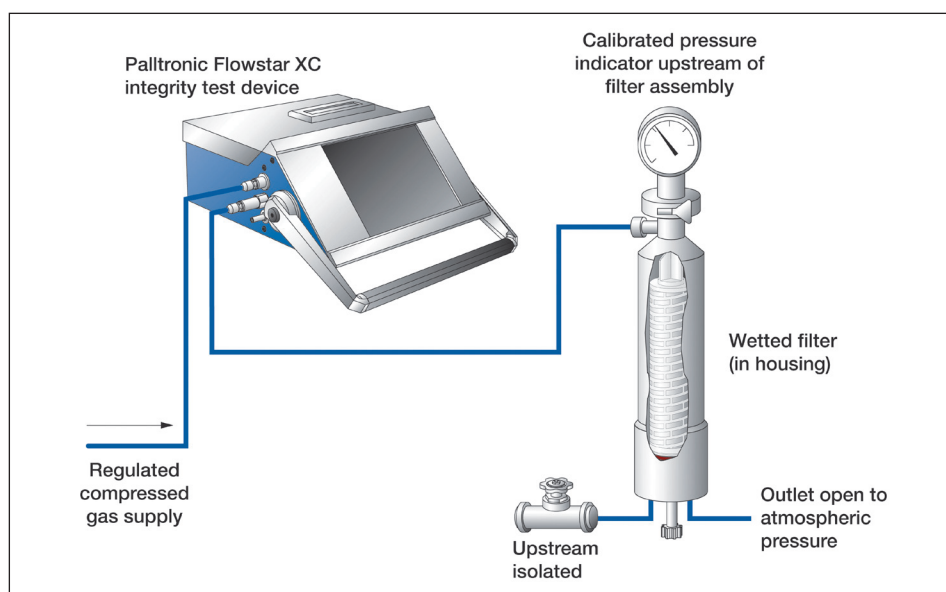


Figure 5: Scheme of automated Forward Flow testing.

logic should not be considered as sterilization. 'Sterility' is achieved by the combination of process steps including all aspects of hygienic processing to control the microbiological status and to avoid secondary contamination. These aspects are often summarised under good manufacturing practices used to produce a stable product.

Whether heat treatment or filtration is utilised, the likelihood of a microorganism being in the final product is not zero. Major factors of influence when using heat include the time and temperature of heat exposure (linked with volume throughput), the initial number and type of microorganisms and their heat-resistance. With filtration the likelihood of a microorganism ending up in a unit of final product is a matter of the volume of this final product, the level of contamination upstream and the efficiency of the membrane to retain organisms in the process stream. The total volume processed, equivalent to the numbers of units produced, has direct influence on the total number of contamination events. All these considerations indicate that it might be misleading to use the term sterile too generically. Instead it is more appropriate to reflect on the residual risk for a final product to be contaminated or even spoiled and to define a residual maximum level of microbiological contamination leading to a stable product. Ideally major aspects should get validated or covered in a kind of risk analysis. A continuous system and maintenance control is another important aspect in order to ensure higher security and lower risk for the process. A regular integrity test by automated test apparatus, adequate documentation and trend analysis of the integrity test values, as well as records of process parameters like temperatures and pressure during sterilisation of filters during filtration, should ideally be part of such a surveillance system [11].

Microorganisms like bacteria and yeast, in beer or wine for example, should usually be avoided in the end product. Both types of microbes are retained mechanically by filtration, but not to a level of 100%. Lifetime and cost issues require a fine balance between throughput and retention capabilities (to a certain extent these parameters go in opposite directions with respect to physical filter parameters). Because of their larger size, it is easier to remove yeast and by appropriate fine filtration yeast cells might get fully retained. Control of bacteria is more challenging due to their cell size, motility and replication time. After clarification and before final filtration the maximum acceptable levels of bacteria in wine and beer are typically less than 200 CFU/ml and 10,000 CFU/ml. In the case of beer there is an emphasis on enumeration of specific beer spoiling organisms like *Lactobacillus lindneri* or *Pediococcus damnosus*. Depending on the source of information the resulting acceptable level of bacteria in a volume of 1 litre of finished product is between 1 (beer) and 25 (wine) CFU.

Filter removal ratings, if expressed as LRV or titre reduction, describe the likelihood of a particle or cell to pass through the membrane. The risk or likelihood of having contamination in a batch of final units (for example bottles of beer) is given by:

- the titre reduction (upstream contamination level divided by downstream contamination level) including all factors affecting filter performance;
- the upstream contamination per total volume;
- the final product volume.

These parameters can be put into a simple formula, where the likelihood ( $L_{Unit}$ ) is the probability to find a microbiologically unstable product. It is calculated by multiplying the unit volume (ml) with the upstream contamination level (CFU/ml)



divided by the titre reduction provided by the filter.

$$L_{\text{Unit}} = \frac{\text{Unit vol [ml]} \times \text{upstream contamination level [CFU/ml]}}{\text{Titre reduction}}$$

The risk for the individual product to be spoiled – provided this is about spoilage organisms – is directly linked to the product volume and the upstream contamination level.

The likelihood to have contaminated product in a batch  $L_{\text{Batch}}$  is simply a result of the number of units produced with this  $L_{\text{Unit}}$ :

$$L_{\text{Batch}} = \text{Total number of units} \times L_{\text{Unit}}$$

In a simple example, a filter with a titre reduction of 1,000,000/1 or LRV 6 is used to fill into 100 half litre bottles. At a contamination of 20 CFU/ml coming from the process this will lead to a likelihood of a specific unit to be spoiled ( $L_{\text{Unit}}$ ) of 0.01 or 1% (= 1 bottle out of 100). The likelihood ( $L_{\text{Batch}}$ ) that at least one product in that batch is contaminated is 1 or 100%.

In any case there is a direct correlation between filter performance and risk of

contamination, provided the process is stable. A titre reduction that is improved 10 fold by changing to a tighter filter results in a contamination risk which is 10 fold reduced. If fine filters for final product filtration are selected, it should be considered that a LRV of 8 compared to a LRV 6 indicates an improvement by a factor of 100, resulting in a 100 fold reduced likelihood or risk for microorganisms to contaminate the final product (Figure 6). In many cases filter performance is a highly influential factor for final product safety. Whereas filters are available in a wide performance range, it is usually a less realistic option, typically linked to higher efforts and investments, to reduce contamination by a factor of 100, for example, through other means like process changes.

In order to define the right filter in terms of microbiological performance in a theoretical example, we assume it is intended to reduce from an upstream level of 10,000 CFU/ml ( $10^4/\text{ml} = 10^7/\text{L}$ ) for a certain type of microorganism in beer to 1 CFU/L ( $10^0/\text{L}$ ). Would a filter providing a reduction by a factor of 10,000,000 (equals

LRV 7) be sufficient? Provided the filter has a specified LRV of at least 7 (ideally measured with this microorganism in beer), putting it into the process would result in an average contamination of 1 CFU per litre of beer. To express it differently, if this beer is filled in 0.5 litre bottles 50% of the batch will be likely to be contaminated. With another filter providing a LRV of 9 in the same situation the percentage of contaminated product will drop to 0.5%, with a LRV of 11 it would be 0.005%. Apart from this mathematical exercise, what level is acceptable or seen as a risk is dependent on the individual risk assessment (reflecting on product, storage conditions, shelf life and other factors). Each application may have a typical, acceptable or critical level of contamination. For example, in the case of a spoilage organism with high viability in the final product the critical level may be just 1 CFU/bottle.

### Measuring contamination

In practice, assessing contamination of final product requires testing of final product, where it is not the number of products tested but the total volume represented by those units which is key to define the acceptable quality level and in the end filtration performance requirements. Most established methods for this are membrane filter techniques and plate count techniques. Rapid detection techniques like ATP measurement or use of PCR are of potential interest but not yet widely spread.

Using the plating technique for a colony count, a sample of the liquid is spread out on nutrient agar plates and colonies (CFU) are counted, that arise from individual bacterial cells after incubation. The concentration of bacteria in the product (CFU/ml) is calculated by dividing the CFU by the volume spread on the agar plate. To achieve a countable number (from 1 to about 300 CFU) the liquid may be diluted. In this case the CFU/ml is multiplied by the dilution factor to estimate the bacterial concentration accurately. This method is time consuming and labour intensive and used for high colony counts. To qualify a process with respect to its likelihood to provide microbiologically stable product a sample plan would be based on statistical considerations taking into account the acceptable rate of contaminated, defect product and the probability to detect the defect.

One of the most widely used quality control tools is the attribute acceptance sampling plan [12, 13], which can be applied in a variety of ways. For example, in the context of manufacturing, it can be used to ensure that the quality of finished products meets the customer's specifications before they are shipped. Each attribute sampling plan has three parameters (N, n, c) – lot size, sample size, and acceptance number, respectively. The operation of an attribute sampling plan

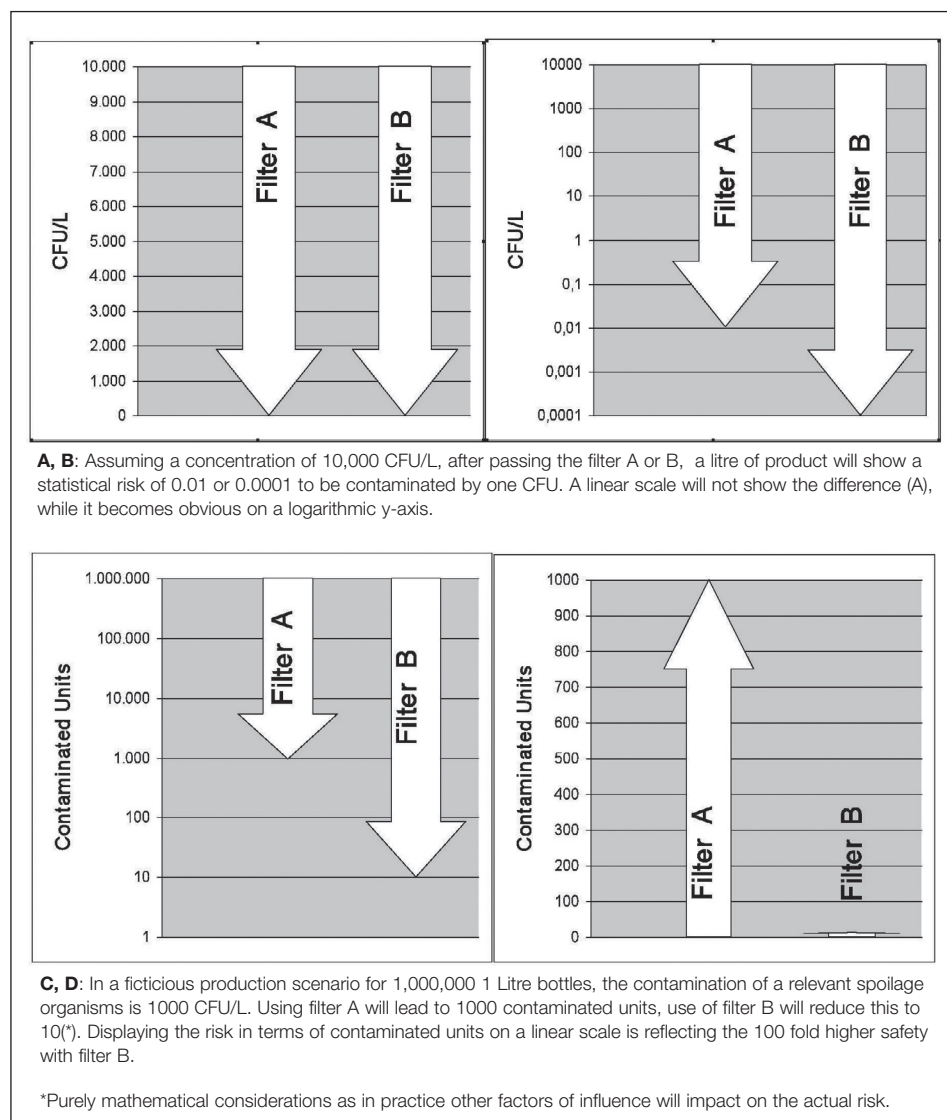
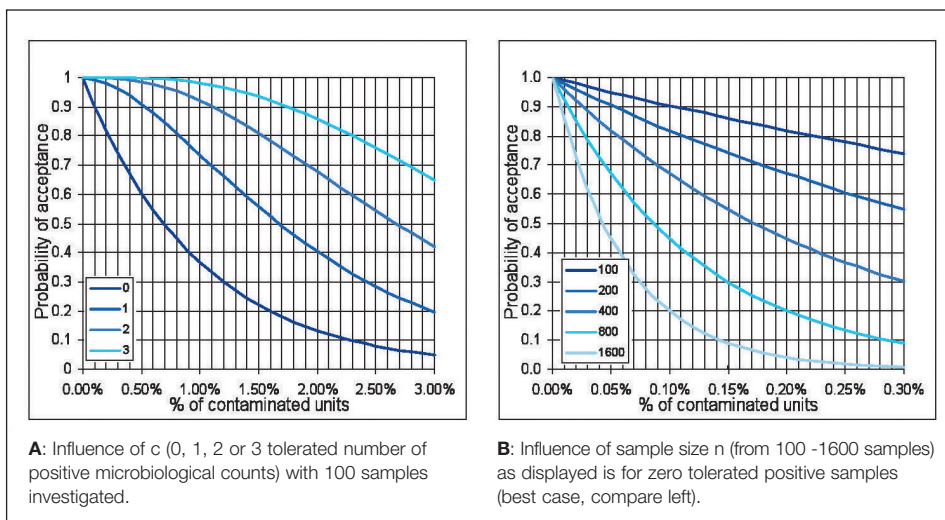


Figure 6 A-D: Filter performance and risk of contamination, theoretical example visualising the use of 2 different filters in the same production process; Filter A has LRV 6 vs. Filter B with LRV 8.



Figures 7 A, B: Probability of lot acceptance based on binomial approximation for different lot quality levels (% contaminated units).

is simple. A random sample of  $n$  units from the incoming lot of size  $N$  is selected and the number of contaminated units is established. If this number does not exceed the pre-determined  $c$ , the lot is accepted; otherwise the lot is rejected.

There are two types of risk associated with each attribute sampling plan. The producer's risk, if a good quality level is rejected, or the consumer's risk. The second type of risk menacing the consumer is that a lot with a bad quality level is accepted (bad quality would need individual definition, in the context of this article it would be a microbiologically unstable product.). Because of sampling, there is a possibility that the number of defectives found in the sample does not exceed  $c$ , which will lead to the decision that a bad quality level lot is accepted. When the lot size  $N$  is large compared with the sample size  $n$ , the sampling plan ( $N, n, c$ ) can be replaced by ( $n, c$ ) so the Binomial approximation suffices without affecting the result much. The acceptance number  $c$  has a much greater effect than the sample size  $n$  and the lot size  $N$  on the risk described.

The acceptance level is calculated for a sampling plan based on a maximum of 0-3 contaminated samples with 100 samples investigated out of a high number of units produced. The likelihood to accept a lot with a true contamination level of 1% based on zero microbiological findings in any of the 100 samples tested is 37%. If one positive sample is accepted this will already increase the probability for acceptance to 74%. A tolerance of 2 positive samples results in 92%, and 3 in 98% to accept the lot.

If a lot with a 10 fold lower contamination level of 0.1% is to be tested and should get rejected if only one sample is positive for microbiological count (highest sensitivity possible if 1 contaminated unit out of 1000 units produced is considered critical),

with a sample size of 100 this will result in about 90% probability for this lot passing the test. With an increase of the sample size to 800 samples this probability will drop to 45% (see Figure 7).

Spoilage rates below 1% would therefore need unrealistic numbers for testing, except where 100% sampling and testing would be possible [13]. As the residual contamination level downstream a filter and its fluctuations are hardly measurable, differences in filter performance will likely not get detected in practice.

To establish residual risk levels in production, it might be more appropriate to determine the level of contamination upstream of the filter and estimate the risk for a unit to get contaminated based on filter performance data, which ideally are based on application specific data tested with relevant spoilage organisms in a relevant test medium under defined conditions. In pharmaceutical applications, a product specific filter validation is used to confirm that sterility will be achieved. As explained, in most beverage applications, the intent is to have a microbiologically stable product, not a sterile product. This residual risk is therefore not a simple yes or no decision but more a fine balance to be established for each individual product under its individual process conditions. As the risk of having a spoiled product in a batch is directly correlated with filter performance, it can get estimated based on filter performance data. Process control combined with appropriate monitoring to ensure the upstream contamination stays within a defined range is key for overall product safety and would allow validation of the process, including the filtration step. Selection of the right filter combined with measures to maintain filter function (for example integrity testing) can be a decisive step to reliably provide a microbiologically stable product. ●

## References

1. HIMA (now Advamed), 'Microbiological Evaluation of Filters for Sterilizing Liquids', draft doc. No.3 Vol. 4, April, 1982 (obsolete)
2. American Society for Testing and Materials (ASTM), 'Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilised for Liquid Filtration', ASTM Standard F838-83, ASTM, Philadelphia, PA (1983, Revised 2005)
3. DIN 58355 Teil 1-6 Membranfilter und DIN 58356 Teil 1-12 Membranfilterelemente, Beuth Verlag, Berlin
4. FDA Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice (Section IX. Validation of Aseptic Processing and Sterilization), 2004
5. Pall Corp., 'Determination of product wet integrity test values for Pall filter cartridge', Publ. No. USTR 1471(1)
6. United States Pharmacopeia (USP23 – NF28, April 2010)
7. FDA Guideline on Sterile Drug Products Produced by Aseptic Processing, 1987
8. EU Guideline of Good Manufacturing Practice for Pharmaceutical Products, Annex 15, July 2001
9. PDA Journal of Pharmaceutical Science and Technology, Technical Report No. 26 Sterilizing Filtration of Liquids, 1998, Supplement Volume 52, Number S1
10. Codex Alimentarius Commission (WHO/FAO) CAC/RCP 40-1993, p.3, Code of Hygienic Practice for aseptically processed and packaged low-acid food
11. Chemie Ingenieur Technik 'Sterilfiltration von Flüssigkeiten und Gasen', U. Brendel-Thimmel et al., November 2006, 1655, CITEAH 78/11 1579-1750 (2006) ISSN 0009-286 X
12. ICMSF Microorganism in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications. 2<sup>nd</sup> ed. Toronto, Canada: University of Toronto Press, 1986
13. Wetherill, G. B. (1969). *Sampling Distribution and Quality Control*, Methuen and Co. Ltd.

## Contact:

Ursula Brendel-Thimmel  
Pall Corporation,  
Scientific and Laboratory Services  
Planiger Str. 137  
55543 Bad Kreuznach  
Germany  
Tel: +49 671 88 22 190  
Fax: +49 671 88 22 262  
Email:  
Ursula.Brendel-Thimmel@europe.pall.com