QUALIMETRIX
Analytical Laboratories - Quality Control

Filter validation

by QUALIMETRIX

Contents

About us

Qualimetrix is a customer-driven Testing Laboratory that employs a set of quality management tools in order to design and implement optimized processes with the aim of transforming customer inputs and requirements into “customer value”. As such, the first and probably the most critical factor for a successful project is its proper definition in terms of both customer and technical requirements.

To this end, a comprehensive study request form is provided to the customer with the following objectives:

Definition of the type and scope of the study

Provision of critical product information
and Review of Request

Design of the most suitable, expedient
and cost-effective approach

Direct, consistent and effective client
communication

Scientific support and complementary studies
for supporting responses to authorities

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Introduction

Sterilizing filtration is the process of removing microorganisms from a fluid stream without adversely affecting product quality. Filters are widely believed to work by permitting fluid passage through their pores, retaining particles too large to fit through these apertures. This mechanism of particle arrest, or capture, is known by a variety of terms, including sieve retention, physical capture, direct interception and size exclusion. This view is based on the axiom of solid geometry that a particle too large to fit into a pore is incapable of passing through it. Size exclusion is a combination of surface screening and entrapment within the filter matrix.

If each particle challenging the filter is too large to pass through the pores, the number of particles does not matter – none will pass the filter. Filter efficiency is independent of the applied differential pressure, as long as the pressure does not deform the particles of the pore, thus causing a failure in sieve retention.

Another mechanism of particle removal is adsorptive sequestration. Particles small enough to enter the filter pores, but that still may be captured by the filter, indicate that partice retention can depend upon other operating conditions governing the filtration. These effects are important if bacteria smaller than the pore size are present.

The effectiveness of adsorptive sequestration depends upon the surface chemistry of the filter and the type of particle or microorganism under the applied filtration conditions. Many different operational conditions govern a filter's adsorptive removal of particles, including applied differential pressure, flow rate, number of particles present and the liquid vehicle's makeup in terms of its surface tension, pH and ionic strength. All of these conditions should be considered and understood in filter validation.

The **Parenteral Drug Association** (PDA) published the authoritative summary of best practices in sterile filtration and validation of sterile filtration in its 1998 technical report, "Sterilizing Filtration of Liquids". It highlights the history of sterile filtration, explains how filters work, details selection criteria, and explains validation considerations and integrity testing methods. Furthemore, a revised Technical Report (TR) No 26 was publised in 2008. PDA's Technical Report No. 26 summarizes the principles and best practices of sterile filtration and its validation. TR26 recommends that the pharmaceutical manufacturer perform a process-specific validation which includes:

- establishing an integrity test methodology and demonstrating integrity of the sterilizing filter
- performing bacterial retention studies
- having a correlation between bacterial retention and the integrity test method
- verifying chemical compatibility
- performing extractables testing
- evaluating the effects of sterilization on filter integrity.

In addition to PDA TR 26, EN ISO 13408 - Aseptic processing of health care products – Part 2: Sterilizing filtration, is considered as guidance in other products, such as medical devices or other health care products.

Sterilizing Grade Filters

Since the classification of a sterilizing-grade filter by pore size has limited value, this measurement has been replaced by defining the filter in terms of its bacterial retention. Classically, a sterilizing-grade filter has been defined as a filter that will retain 10^7 cfu (colony forming units) of Brevundimonas diminuta (ATCC 19146) per cm^2 of effective filter surface area under specified process conditions.

Sterilizing grade filters are available in a variety of sizes, configurations (e.g., flat sheets, capsules, cartridges) and membrane chemistries so that the user can select the most appropriate one for a specific application. Commonly used membrane components include polyvinylidene fluoride (PVDF), polysulfone, polyethersulfone (PES), nylon, cellulose esters, polytetrafluorethylene (PTFE), polyester and polypropylene. Not only do different chemistries impact differences in flow characteristics and filtration performance, but they also affect: the levels of filter extractables and leachables; the thermal and physical properties of the filter and the interactions with the process stream.

Once the membrane is manufactured into its final format, it is evaluated with respect to effective filtration area, operating limits of temperature and pressure, extractables and compatibility with the product stream to be filtered.

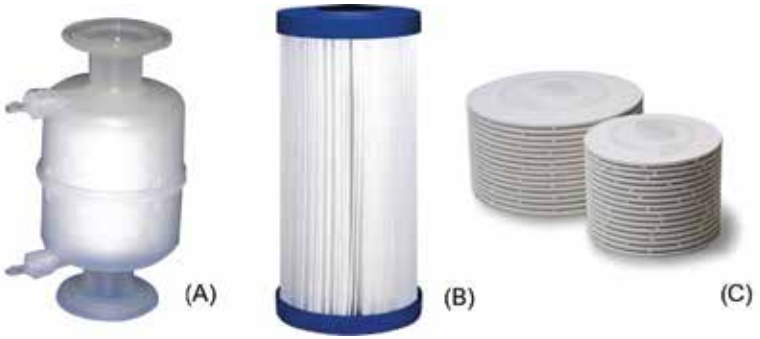

Figure 1: Common filter configurations. A) capsule, B) cartridge, C) flat sheets

Membrane polymer	Advantages	Disadvantages
Cellulose acetate (CA)	Extremely low adsorption High flow rates	Limited pH compatibility
Cellulose nitrate (CN)	Good flow rate	High adsorption Limited pH compatibility
Regenerated cellulose (RC)	Extremely low adsorption Extremely high flow rates	Limited pH compatibility
Polyamide (Nylon)	Good solvent compatibility Good mechanical strength Broad pH compatibility	High protein adsorption Moderate flow rates
Polyethylene sulfonate (PES)	Extremely low adsorption Good solvent compatibility Broad pH compatibility	Moderate flow rate
Polyvinylidene difluoride (PVDF)	Low adsorption Good solvent compatibility	Moderate flow rate

Table 1: Common membrane materials and their main characteristics

In validating and performing sterile filtration, it is essential to identify the bioburden or endemic microorganism(s) in a given process, to use the grade of filter that quantitatively removes the microorganism(s), and to demonstrate quantitative removal by test before using the filter in production. This is the essence of filter validation.

As per Annex 1, Volume 4 EU GMP (Aug-2022), if the product cannot be sterilized in its final container (following the steps described in the relevant Decision tree of the EMA Guideline, EMA/CHMP/CVMP/QWP/850374/2015, illustrated in Figure 2), the sterilization procedure should be performed by filtration through a sterile sterilizing grade filter with a nominal pore size of a maximum of 0.22 µm that has been appropriately validated to obtain a sterile filtrate.

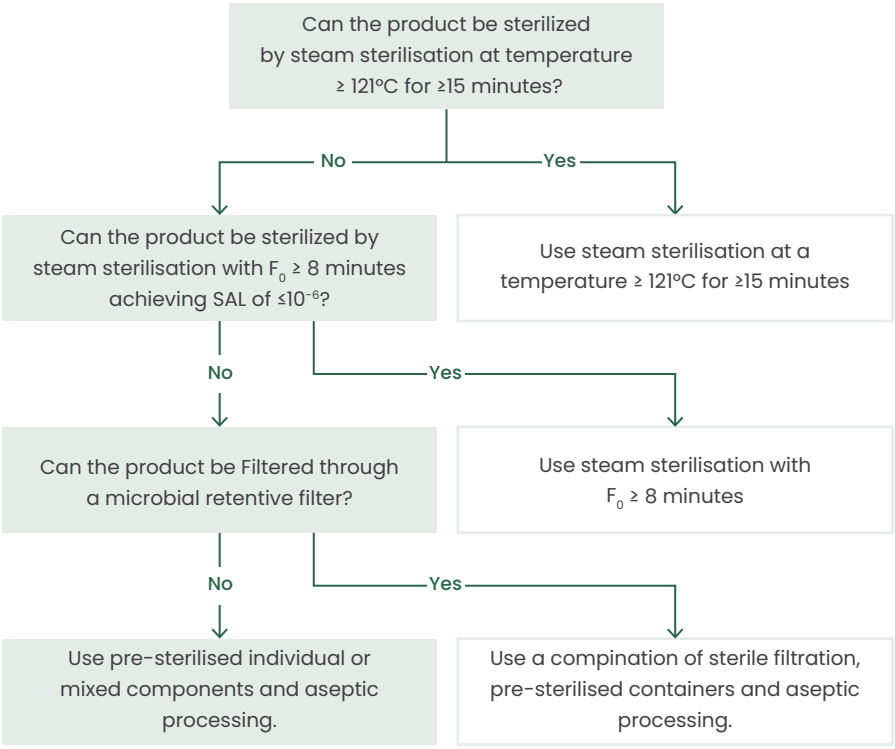

Figure 2: Decision tree for the choice of sterilization procedure

Once a filter is validated for use in a given process, **further validation** is required only when changes to the filtration procedure are applied.

Some changes that may require requalification include, but are not limited to:

- An increase in the volume to be filtered through a given filter area
- Product formulation changes, including product concentration, pH or conductivity
- Any change to the sterilizing filter or the pretreatment of the filter such as flushing or sterilization
- Any change to the process parameters
- Performing extractables testing
- Any change in filter manufacturing conditions as reported by the filter manufacturer with respect to their potential to affect the defined fluid and process parameters

A **risk assessment** should be performed to evaluate the potential impact of these changes. The quality unit should approve all changes that potentially impact the cGMP compliance of the system.

Regulatory requirements

The design of the validation studies is based on the requirements that have been set on sterilization validation according to the following guidelines, standards and compendial chapters. The execution of the validation studies is conducted at a GMP-certified laboratory following the cGMP and the Good Documentation Practices. Some of the major guidance references are presented below:

European Authorities:

European Commission, EUDRALEX Volume 4, Annex 1 **Manufacture of Sterile Medicinal Products**, 2022

- *"Before any sterilization process is adopted, its suitability for the product and equipment, and its efficacy in consistently achieving the desired sterilizing conditions ...should be validated notably by physical measurements and where appropriate by biological indicators"*

- *"The validity of the sterilizing process should be reviewed and verified at scheduled intervals based on risk"*

- *"The integrity of the sterilized filter should be verified by integrity testing before use (pre -use post sterilization integrity test or PUPSIT), to check for damage or loss of integrity caused by the filter preparation prior to used. A sterilizing grade filter that is used to sterilize a fluid should be subject to a non-destructive integrity test post-use prior to removal of the filter from its housing. The integrity test process should be validated and test results should correlate to the microbial retention capability of the filter established during validation. Examples of tests that are used include bubble point, diffusive flow, water intrusion or pressure hold test "*

European Guide to Good Manufacturing Practices (2010) Annex 13 - **Manufacture of investigational medicinal products**

> *"For sterile products, the validation of sterilizing processes should be of the same standard as for products authorized for marketing"*

European Commission, EUDRALEX Volume 4, "Good Manufacturing Practices, Medicinal Products for Human and Veterinary Use", Chapter 3, **"Premises and Equipment"**, 2014

> *"Production equipment shall not present any hazard to the products. The parts of the production equipment that come into contact with the product must not be reactive, additive or absorptive to such an extent that it will affect the quality of the product and thus present any hazard"*

Ph. Eur. 5.1.1 Methods of preparation of Sterile products – **Membrane filtratio**n, 2017

> *"Filtration effectiveness*
> *Microbial challenge tests with a suitable model system shall demonstrate the effectiveness of the filtration process"*

European Medicines Agency (EMA), **Guideline on the sterilization of the medicinal product, active substance, excipient and primary container**, 2019

- *"The integrity of the sterilised filter should be verified by testing before use unless specifically justified and validated, and should be verified by on line testing immediately after use"*

- *"All filters used in the manufacture of the finished product that come in contact with the finished product, or with any component (substance or intermediate product) incorporated in the finished product should be described and the information stated in Table 3, section 4.1.5 should be provided in the quality dossier"*

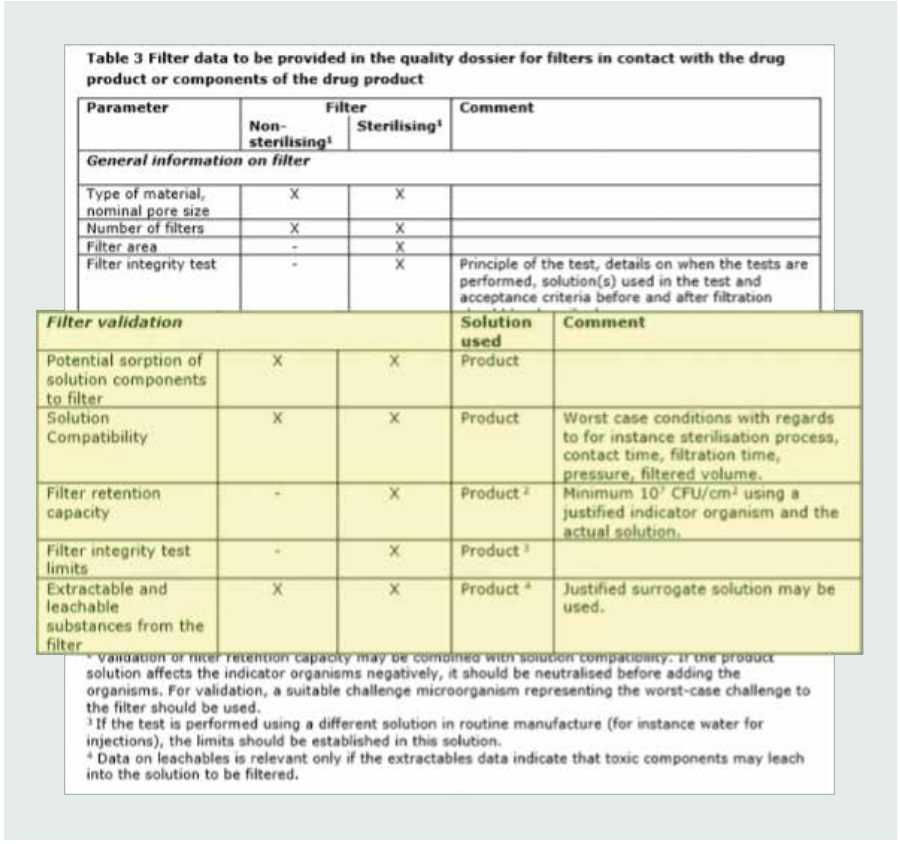

Table 3 Filter data to be provided in the quality dossier for filters in contact with the drug product or components of the drug product

Parameter	Filter		Comment
	Non-sterilising[1]	Sterilising[1]	
General information on filter			
Type of material, nominal pore size	X	X	
Number of filters	X	X	
Filter area	-	X	
Filter integrity test	-	X	Principle of the test, details on when the tests are performed, solution(s) used in the test and acceptance criteria before and after filtration
Filter validation			**Solution used** / **Comment**
Potential sorption of solution components to filter	X	X	Product
Solution Compatibility	X	X	Product — Worst case conditions with regards to for instance sterilisation process, contact time, filtration time, pressure, filtered volume.
Filter retention capacity	-	X	Product [2] — Minimum 10^7 CFU/cm² using a justified indicator organism and the actual solution.
Filter integrity test limits	-	X	Product [3]
Extractable and leachable substances from the filter	X	X	Product [4] — Justified surrogate solution may be used.

[2] Validation of filter retention capacity may be combined with solution compatibility. If the product solution affects the indicator organisms negatively, it should be neutralised before adding the organisms. For validation, a suitable challenge microorganism representing the worst-case challenge to the filter should be used.
[3] If the test is performed using a different solution in routine manufacture (for instance water for injections), the limits should be established in this solution.
[4] Data on leachables is relevant only if the extractables data indicate that toxic components may leach into the solution to be filtered.

Figure 3: Table 3 extracted from EMA's guideline on the sterilization of the medicinal product, active substance, excipient and primary container

Global Authorities:

FDA Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing – **Current Good Manufacturing Practice**, 2004

- *"Filter validation should be conducted using the worst-case conditions, such as maximum filter use time and pressure"*
- *"The specific type of filter membrane used in commercial production should be evaluated in filter validation studies"*

WHO Annex 6: **Good Manufacturing Practices for Sterile Pharmaceutical Products section 5.4**

- *"All Sterilization Processes Should be Validated"*

2004 FDA Guidance for Industry Sterile Drug Products Produced by Aseptic Processing

- *"A sterilizing grade filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent"*

FDA **Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products**, November 1994

- *"A summary should be provided containing information and data concerning the validation of the retention of microbes and compatibility of the filter used for the specific product"*

FDA, Code of Federal Regulations, Part 211, **"Current Good Manufacturing Practice for Finished Pharmaceuticals"**, Part 211.65, **"Equipment Construction"**, 2005

- *"Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements"*

PDA Technical report Nº26, 2008

- *"Chemical compatibility testing should encompass the entire device and depends on the fluid, filtration temperature and contact time"*

EN ISO 13408-2:2018 Aseptic processing of healthcare products

> *"Where validation establishes a reproducible relationship between the product-specific bacterial retention capability of a sterilizing grade filter and the physical integrity of that filter, then suitable non-destructive pre-use and post-use filter integrity tests are used to determine whether a full-scale sterilizing filtration process has been conducted successfully"*

The Figure below summarizes the overall filter validation approach as described in the major available guidelines and highlights the differences among them.

Requirements	EU GMP ANnex 1	FDA	WHO Annex 6	PIC/S
Adsorption/ Extraction/ Leaching	Yes	Yes	Yes	Not Addressed
Filter Integrity	Yes	Yes	Yes	Yes
Bacterial challenge test	Yes	Yes	Yes	Yes
Grouping possible?	Yes	Not Addressed	Not Addressed	Yes
Bioburden limits?	Yes	Yes	Yes	Not Addressed
Multiple use of filter?	Yes	Yes	Yes	Not Addressed

Figure 4: Points to consider for sterilization by filtration according to the available guidance

All the required data illustrated in Figure 4 can be generated by means of the studies depicted in Figure 5, namely, Filter Compatibility study (filter integrity), filterability, filter extractables and filter retention study. In general, our filter validation scheme is based on the provisions of PDA's Technical Report No.26.

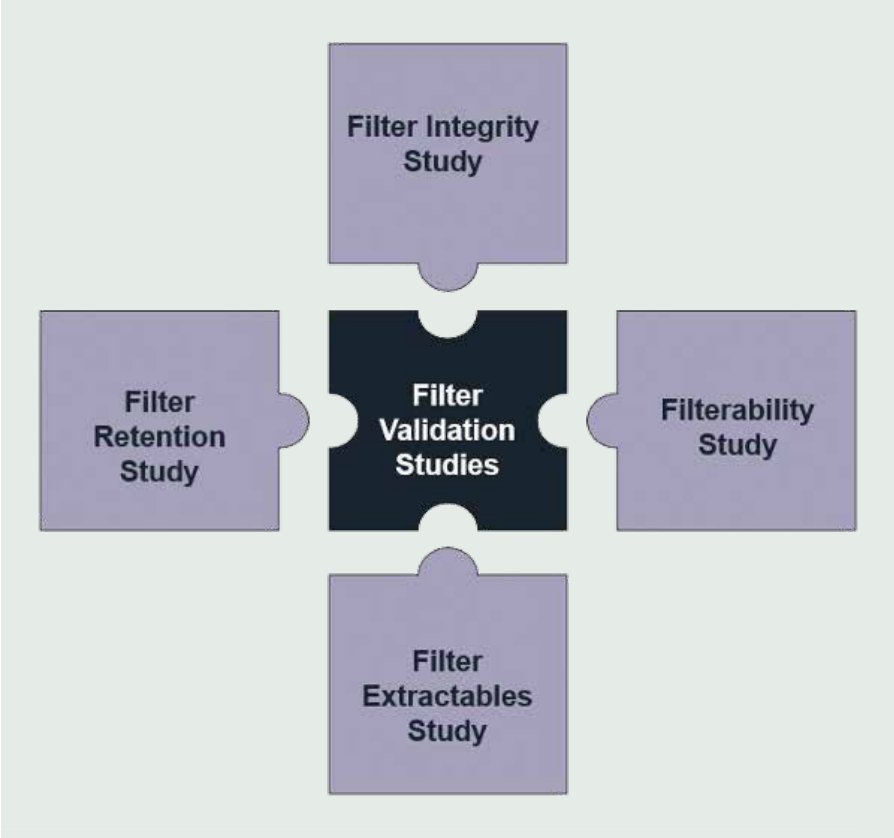

Figure 5: The "puzzle" of filter validation studies

Small - Scale device testing - Filterability

Typically, filter screening is performed using constant pressure or constant flow conditions with 47 mm disc or disc composites, installed in holders (Figure 6). These tests are also helpful to determine potential prefilter combinations to optimize the total throughput. Most filterability tests are performed with a lab-scale batch size under the simulated process parameters and measuring the decline in flow rate as a function of the filtered volume. The pressure/flow set point should mimic production pressure conditions.

Figure 6: Filter disc holders

Compatibility study

The chemical compatibility evaluation should include the entire device and depends on the fluid, the filtration temperature and contact time. The filter devices are constructed of polymeric materials such as thermoplastic polymers and elastomers, which may interact with formulation components from the product. Since there may be numerous chemical interactions between the filter device components and the process fluid or solvents, the typical chemical compatibility table provided by the filter manufacturer and literature data are commonly used as a starting point for further testing.

Integrity Testing

The main objective of a nondestructive physical integrity test is to determine the presence of defects that may compromise a filter's retention capability without destroying the filter. Additionally, the integrity test establishes the similarity of the test filter to validated bacterial retention-challenged filters under process-related conditions. Test results must correlate to bacterial retention.

For integrity tests, gas flow properties of wetted filter membranes are evaluated over a range of pressures. After completely wetting the entire filter membrane, gas (air or nitrogen) is introduced onto the upstream side of the membrane at a low pressure. Capillary forces keep the liquid from being expelled from the pores. When pressure is applied on the upstream side of the filter, the gas dissolves into the wetting liquid, diffuses across the wetted membrane, and is released on the downstream side, which is at atmospheric pressure. Diffusion increases as the pressure on the upstream side is increased. If the amount of gas that passes to the downstream is measured, the membrane characterization curve can be obtained for the given membrane filter. Figure 7 illustrates the gas flow through the membrane at the pressure where the wetting fluid is held in the pores by capillary forces, and at exceeding the bubble point, respectively. Plotting the gas flow (diffusive flow and subsequent bulk flow) against the pressure results in a typical membrane characterization curve, as presented in Figure 8.

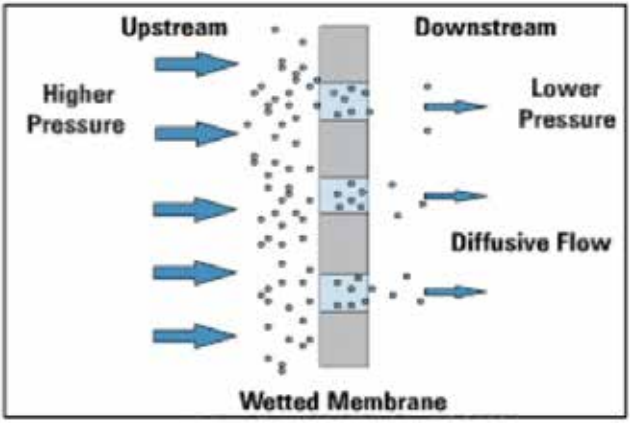

Figure 7: Gas Diffusion through a wetted membrane

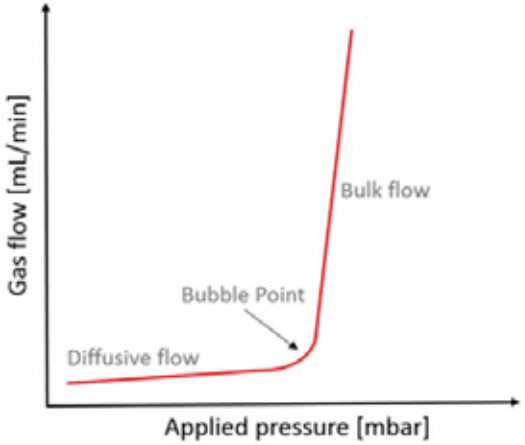

Figure 8: Typical membrane characterization curve

The method for integrity testing of production filters should be chosen to provide reliable results based on the nature of the filter and processing conditions. Bubble point, multiple point and single point diffusive/forward flow and pressure hold tests can be used, recognizing that each has strengths and limitations that must be evaluated in terms of the particular circumstances of the test.

Product - Wetted integrity testing

The method for product-wetted integrity test determines the forward flow and bubble point test limits for a membrane filter that is wetted with process fluid. The usage of product-wetted integrity test parameters eliminate the need to flush with fluids other than the product solution in order to meet the requirements of the new GMP, Annex I and perform “on site” post use integrity test.

Figure 9: Integrity testing equipment

The Integritest 4 instrument (Figure 9) supports all traditional tests such as bubble point and diffusion flow. The accuracy of this algorithm is key to proving that the filter is integral. The Integritest 4N software meets the technical requirements of FDA regulation 21 CFR Part 11 for electronic records and electronic signatures. The Integritest 4 instrument was also developed and validated according to the GAMP Guide for Validation of Automated Systems.

Filter extractables

Filter Extractables are chemical compounds that can be extracted from product contacting surfaces when exposed to an appropriate solvent under exaggerated conditions. Potential filter extractables include oligomers, mold release agents, antioxidants, wetting agents, manufacturing debris and plasticizers, deriving from the membrane itself or the cartridge body and O-ring material. Extractables themselves, or substances derived from extractables, have the potential to leach into a drug product under normal conditions of storage and use and thus become leachables.

The first step of the approach is to perform an extraction study with a suitable model solvent simulating worst-case conditions. Non-specific methods, such as non-volatile residue (NVR) and Fourier-transform infrared spectroscopy (FTIR) are employed to provide general quantitation and qualification of potential leachables. This represents the essential and simplified approach. However, it should be noted that -although some authorities may still accept the simplified approach- these methods (NVR, FTIR) are used to give general quantitation and qualification of extractables by comparison to the manufacturer's established specification on the non-volatile residue.

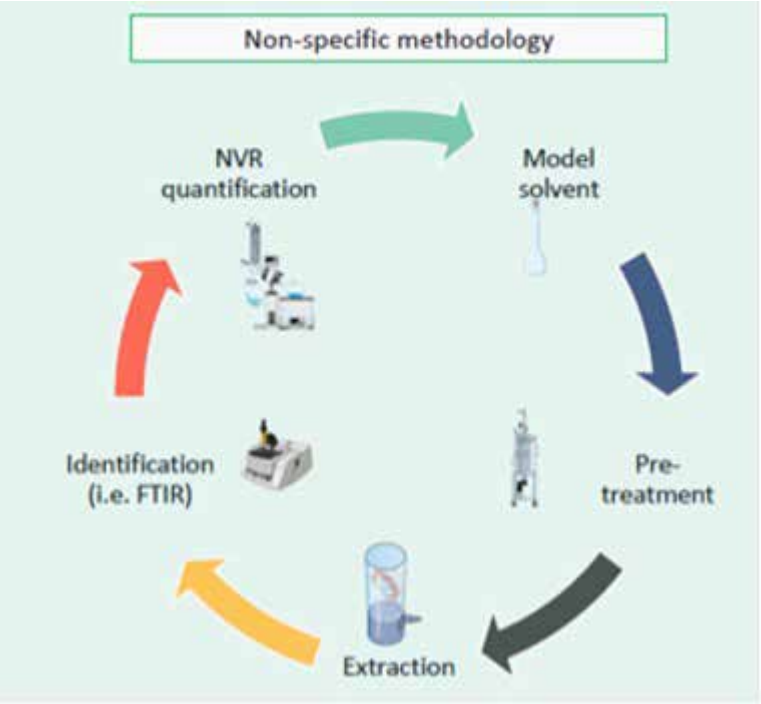

Figure 10: Filter extractables- non specific methodology

Nowadays, the authorities expect a more thorough and extensive extractable species profile assessment. EMA's guideline on the sterilisation of the medicinal product, active substance, excipient and primary container, notes that data on leachables is relevant only if the extractables data indicate that toxic components may leach into the solution to be filtered. The two main aspects determining toxicity are the detected species identity, since their chemical structure is linked with biological activity and concentration, as the magnitude of any biological effect is directly related to the patient's exposure. To this end, a suitable study design should involve **extraction by various media**, to cover and extract the total pool of potential leachables, and by employing **specific orthogonal techniques** (LC-MS, GC-MS, Headspace GC-MS, ICP-MS) to address their chemical diversity and provide information with respect to both their identity and concentration levels.

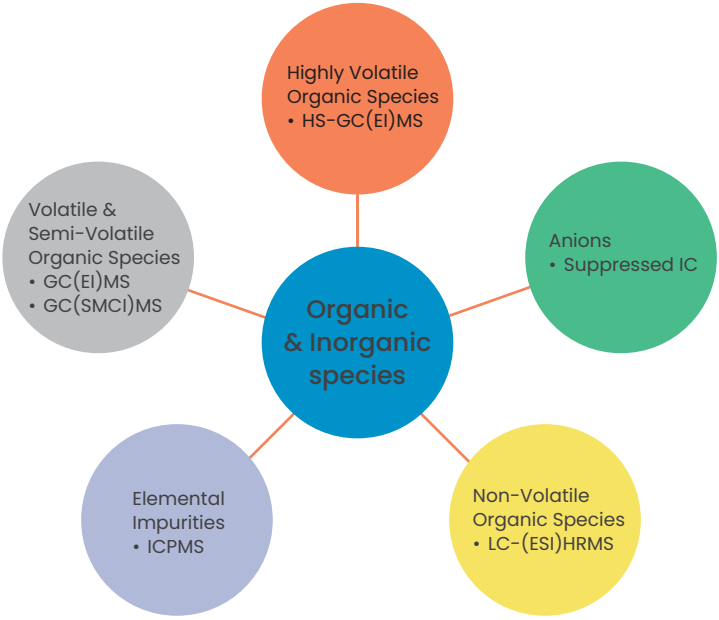

Figure 11: Filter extractables- specific methodology

The presence of extractables may be related to degradation of the filter components ultimately affecting its ability to perform as intended. Extraction studies may be conducted using methods such as static contact or recirculation where the extract is subsequently collected and tested for the presence of filter extractables.

Last but not least, the general tendency of the authorities is to scrutinize all safety-related studies especially when it comes to **Process - Equipment - Related - Leachables** (PERLs). Qualimetrix has extensive experience and expertise in performing extractables and leachables studies that are combined with cutting-edge analytical instrumentation (elemental impurities/ ICP-MS, volatile organic compounds & semi-volatile organic compounds/ GC-MS, non-volatile organic compounds/ LC-HRMS).

Bacterial challenge testing

The bacterial challenge test serves two major functions. The filter manufacturer employs this test to classify filters as sterilizing grade if the filter provides a sterile effluent when challenged with a minimum of 10^7 cells of the reference microorganism per cm^2 of effective filter surface area. It is generally accepted that in case smaller microorganisms have not been identified either in the bioburden of the unfiltered bulk solution or as environmental isolates in the manufacturing area, the microorganism Brevundimonas diminuta ATCC 19146 serves as an optimum choice for the bacterial challenge test.

Bacterial challenge tests are also required to validate the sterilizing filtration process of a specific product. The filter challenge test must be performed with the actual product or, where justified, with a suitable surrogate fluid.

Factors influencing bacterial retention

Those factors potentially affecting microbial retention include **filter type** (structure, base polymer, surface modification chemistry, pore size distribution, thickness), **fluid components** (formulation, surfactants, additives), **fluid properties** (pH, viscosity, osmolarity, ionic strength, surface tension), **process conditions** (temperature, pressure differential, flow rate, contact time) and the **specific characteristics of the actual bioburden in the product.**

One should also consider the potential of product formulations or process conditions to affect cell size or other physiological or morphological microorganism attributes which might allow membrane penetration. Sterilizing filtration process validation should be conducted under **"worst-case"** conditions, using the filter membrane or device selected for the product.

Challenge organism selection criteria

The challenge bacteria should be small enough to challenge the retentivity of the sterilizing grade filter and simulate the smallest microorganism that may occur in the production environment.

Neither US, nor EU GMP guidance recommends any single protocol or challenge organism for validating the sterilizing filtration process for a given product. The appropriateness of any bacterial retention test protocol used to validate product-specific sterilization processes using filtration is considered on an individual basis. Historically, P. diminuta, recently reclassified to Brevundimonas diminuta ATCC 19146, has been selected as the microorganism of choice due to its size and cell mobility.

If there is concern that the indigenous bioburden might include microorganisms that are smaller than Brevundimonas diminuta, or small enough to challenge the retention capability of the sterilizing grade filter, then a suitable challenge microorganism shall be used.

Where a 0.10 µm sterilizing filter is selected to prevent the passage of Mycoplasma or other organisms smaller than B. diminuta, the challenge organism may be Acholeplasma laidlawii (ATCC 23206) or similar.

Key considerations for the validation of **sterilizing filtration process** are:

___ product contact time

___ differential pressure

___ flow rate per unit area

___ temperature

___ bioburden

___ integrity test correlation

Bacterial challenge procedure

The viability of the test microorganism, B. diminuta, in the pharmaceutical product is compared to a control solution over the defined process time. The viability results are used to determine the effect of the product on the reference microorganism and the categorization of the product is performed according to the following definitions:

Non-Bactericidal:

A decrease in the viability of the micro-organism of less than one log over the process time is defined as a non-bactericidal product.

Moderately Bactericidal:

A decrease in the viability of the micro-organism of less than one log over 60 minutes and of 1 log or more over the process time is defined as a moderately bactericidal product and may allow for an in-product bacterial challenge after filter preconditioning (i.e., product recirculation).

Bactericidal:

A decrease in the viability of the micro-organism of greater than one log over a minimum of 60 minutes duration is defined as a bactericidal product.
The design of the bacterial challenge test is performed according to the table below, taking into consideration the results obtained from the viability study.

Product	Challenge testing mode
Non Bactericidal	**Challenge Phase:** Direct inoculation of the challenge organism at a minimum challenge level of 10^7 CFU/cm^2 of filter surface area into the product solution. The product solution is recirculated through the filter membranes as described under simulated processing conditions of the product.
Moderately Bactericidal	**Recirculation phase:** The product solution is recirculated through the filter membranes, without the challenge organism, for the non-viable time recorded in the bacterial viability test and as described under simulated processing conditions of the product. **Challenge Phase:** The challenge organism is inoculated at a minimum challenge level of 10^7 CFU/cm^2 of filter surface area into the product solution for the maximum viable time recorded in the bacterial viability test. The recirculation of the product solution is maintained until the maximum processing time.
Bactericidal	**Recirculation phase:** The product solution is recirculated through the filter membranes, without the challenge organism, for the maximum processing time. **Filter Flushing:** After the dynamic exposure of the product, the filters are flushed with the appropriate flushing regime, according to the filter flushing tests. **Challenge Phase:** The challenge microorganism is inoculated at a minimum challenge level of 10^7 CFU/cm^2 of filter surface area into the altered product or an appropriate product simulant solution, as established during the Bacterial Viability study. The inoculated altered product or product simulant solution is recirculated through the filter membranes for a contact time equal to the max half of the process time, under simulated processing conditions.

The challenge procedure typically consists of cleaning and autoclaving the apparatus, integrity testing of the test filters, recirculating the product through the filters at the process flow rate or pressure and integrity testing of the filters at the end of the filtration procedure. The integrity testing of the membrane filters is performed according to the rationale of PUPSIT recommended in GMP/ Annex 1, while the post-use integrity test is performed on site immediately upon the completion of the challenge phase.

In case of a bactericidal product solution, the flushing of the membranes is performed upon completion of the recirculation phase and prior to the challenge phase in order to remove potential bactericidal residues. The proper flushing fluid and volume is defined in the "test filter & recovery filter flush studies" as described below.

Bacteria are introduced according to the results of the viability study. Before introducing the bacteria, recovery membrane filters are installed downstream of all test and control filters. Recovery membrane filters must have a pore size of at least 0.45 µm or tighter. Membranes that are 0.45 µm will retain 10^4–10^6 logs of B. diminuta, which is generally accepted as adequate for a valid test. The recovery membrane filters are transferred on the surface of petri dishes containing sterile Casein Soya Bean Digest Agar upon the completion of the test procedure. The petri dishes are incubated at 32.5 + 2.5 °C for 2-7 days and tested for growth.

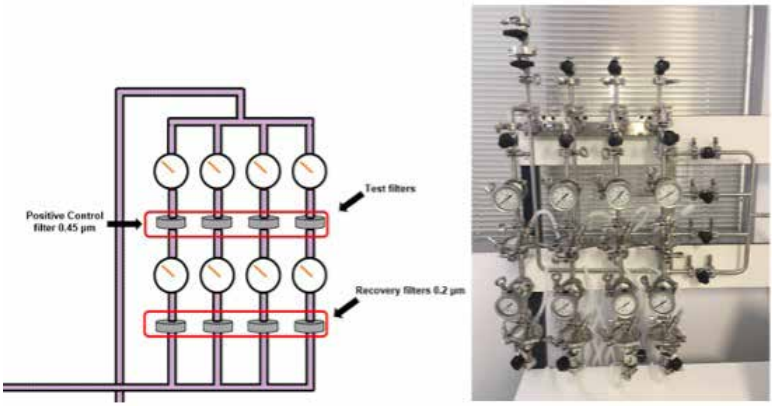

Figure 12: Bacterial challenge test filter apparatus

If B. diminuta is not viable in the liquid, the product fluid can be modified to ensure the viability of the challenge organism, the exposure time can be reduced to ensure that the challenge organism remains viable or a product simulant can be used instead. The ideal simulant solution would minimize adsorptive retention, ensuring that the sterilizing action of the filter under consideration is the consequence of sieve-retention. Moreover, it should match the product as closely as possible in terms of its physical and chemical characteristics (pH, osmolality, surface tension, viscosity), without adversely affecting the challenge micro-organism. Table 2 presents the product attributes that influence bacterial retention and their worst-case target values when developing a product simulant.

Moreover, a flush study must be conducted. The filter flush studies are performed in order to validate the appropriate flushing volumes to be applied during the bacterial challenge test, in order to remove any bactericidal residues from the filters, prior to challenging the altered product or simulant fluid with B. diminuta. The flush study consists of three distinct phases, the flushing of the test filter membranes, the collection of the last 10 mL of filtrate, passed through the test filter membranes and the flushing of the recovery membranes, that are assembled downstream of the test filter membranes, in order to recover any microorganism that might penetrate the filter membranes.

Characteristic	Effect	Worst-case value
Osmolarity	Size of micro-organism	Highest
Surface Tension	Retention mechanism	Lowest
Viscosity	Retention mechanism	Highest
pH	Organism viability	< 5 or > 9

Table 2: Characterization of properties of a simulant fluid

The flowchart below (Figure 13) presents the key steps to be considered when selecting the appropriate validation strategy for a specific filter and product/process combination (PDA Technical Report No. 26).

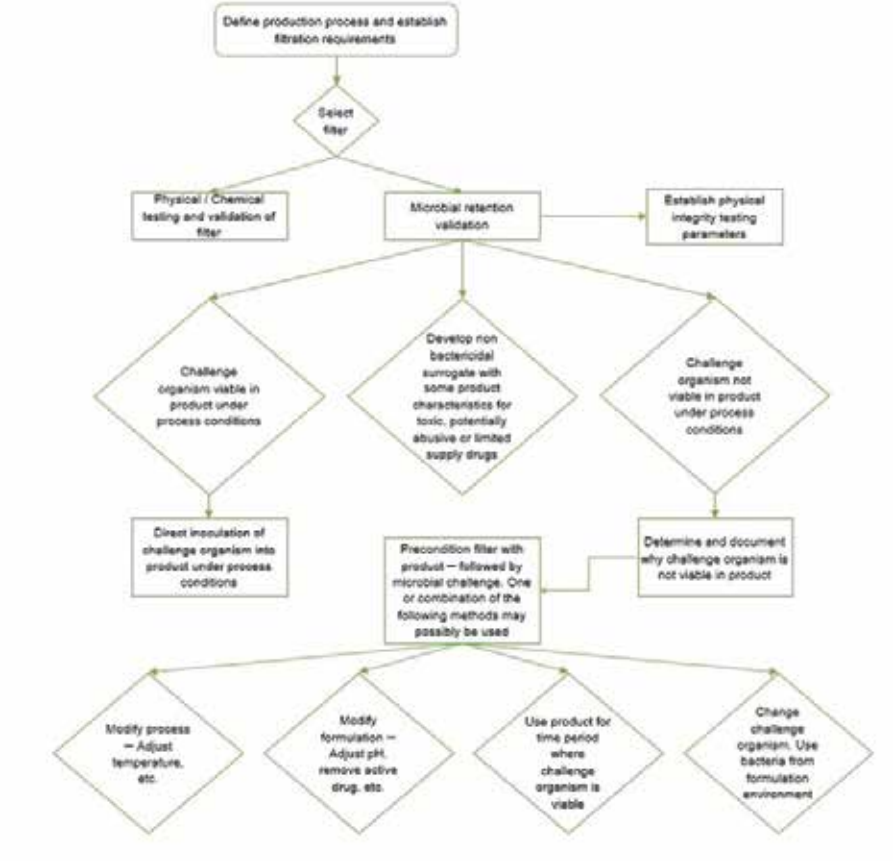

Figure 13: Key steps to be considered when selecting the appropriate validation strategy for a specific filter and product/process combination

Filter adsorption

Adsorption is a mechanism of product binding to the membrane which may affect the product composition and concentration. Adsorptive filter materials include membrane, hardware and support materials. Flow rate, product concentration, contact time, preservative concentration, temperature and pH are some of the factors that can affect the level of adsorption. For filtration processes in which the level of adsorption is tolerable but still relatively high, it may be helpful to pool the product prior to filling so that the mass of material adsorbed is minimal in comparison to the mass of the material in the upstream process volume. During process development, adsorption tests are typically performed at small scale and confirmed at large scale. These tests can also be used to establish potential pretreatment (e.g., buffer flush, soaking) options, operational parameters or membrane polymer choices.

Risk-based grouping

The experts of Qualimetrix can provide a cost-effective solution to the filter validation studies by grouping of different strengths or variations of a product on the basis a risk management approach and a scientifically sound rationale which will define a worst-case formulation with worst-case process parameters based on the variables that affect the effectiveness of microbial removal.

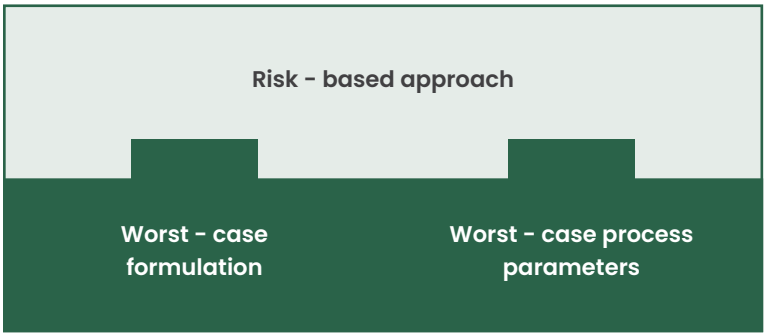

Figure 14: The rationale of a risk-based approach

Grouping is allowed by both the GMP Annex 1 and the PIC/S guidance.

"Sterile filtration of liquids should be validated in accordance with relevant Pharmacopeia requirements. Validation can be grouped by different strengths or variations of a product but should be done under worst-case conditions. The rationale for grouping should be justified and documented."

Apart from these documents, the relevant ISO on sterilizing filtration allows this approach under clause 8.2.2.2 referring to filter leachables and under clause 9.2.1.4 referring to bacterial challenge tests:

"For leachables, the identity and quantity of materials leachable from the filter shall be determined using the process fluid and the same filter type as that used for production. Where it is not possible to use the process fluid, a surrogate may be used. Fluids with similar properties may be grouped and a worst-case representative selected for testing. Where a surrogate fluid or grouping approach is used, the rationale shall be documented."

"Liquids with similar properties may be grouped and worst-case representatives used for bacteria retention studies. Justification for grouping fluids and selection of worst-case representatives shall be documented."

High risk	Factor	Lower risk
Higher levels, diminutive organisms	Bioburden	Lower levels, large organisms
Higher	Differential pressure	Lower
Higher	Flow rate	Lower
Growth - promoting	Product	Bactericidal or preserved
Ambient and higher	Temperatures	Refrigerated
Longer	Time	Shorter

Figure 15: Evaluation of the critical parameters from grouping

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