Sterility Assurance with Filtration

Taking Bioburden, Membrane Integrity, and Process Conditions into Account

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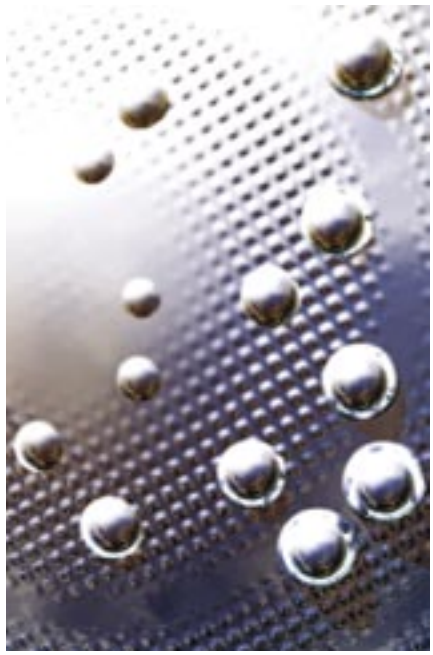

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Under ideal conditions, the result of a sterility test reflects the presence or absence of viable organisms in the samples analyzed. However, it has long been recognized that inferences regarding sterility (whether for a batch of in-process material or a finished dosage form) are subject to statistical limitations. The probability (P) of detecting contamination in a given batch of finished product by performing a 20-unit sterility test can be estimated using the equation $P = (1 - fc)^{20}$, where fc is the fraction of units contaminated. Thus, a 1:1000 rate of contamination would pass undetected in 98% of batches. Because of this statistical limitation, the validation of sterilization processes plays as important a role as passing the test.

Validation of Sterility Assurance

For terminal sterilization by moist heat, a realistic target of <1 contaminated unit for every million units produced has been set (**1**). Under pressure from the FDA, manufacturers of injectable products have learned how factors such as the quantitative and qualitative aspects of bioburden, the temperature profile in a given loading pattern, and exposure times affect achievement of this target. In a "bioburden" approach to validation, the nature of an organism is established from historical data or a monitoring program, in particular its heat resistance and the worst-case viable count in each unit of filled and sealed product. Cycles are designed such that at the coldest spot in an autoclave the probability of that organism's survival is no greater than 10^{-6}.

Alternatively, a cycle can be designed on two assumptions: that the bioburden of each unit to be sterilized will not exceed 10^6 cfu (colony-forming units), and that an organism's heat resistance is no greater than defined by D- and z-values of 1 minute and 10 °C, respectively. An "overkill" cycle based on these assumptions is adjusted such that exposure at the coldest spot of the loading pattern in question is equivalent to 12 minutes at 121 °C or an Fo value of 12 minutes. The risk of exceeding conditions that must be met for an overkill calculation to be valid is minimal, and an Fo value of 12 minutes has practically become synonymous to achieving a 10^{-6} safety margin in moist-heat sterilization cycles of finished product as well as for rubber stoppers, filling equipment, holding tanks, filters, utensils, and other items required in aseptic processing.

In aseptic processing, however, a totally different set of variables comes into play. The sterility of equipment and components can be readily validated as above, but factors such as the environmental quality of a critical processing area and the possibility of contamination by operators are more difficult to evaluate and control. Aspects pertaining to environmental monitoring and control — as well as validation of aseptic operations using so-called "media fills" — are important topics described extensively in various technical reports and general literature. Suffice it to say here that after decades of improvements (in facility design, work flow, environmental controls, gowning procedures, access controls, and other measures), achieving the contamination target of <1 per 1000 units produced aseptically has become common. In fact, it is a regulatory requirement. To prevent "artifacts," the medium used in a media fill is sterilized by conventional methods that are validated. But the sterility assurance of an actual bulk solution to be processed further must be addressed and validated independently.

Product Focus: Temperature-sensitive parenteral drugs

Process Focus: Downstream processing

Who Should Read: QA/QC, validation, process development, and manufacturing staff

Keywords: Aseptic processing, membrane filtration, validation, sterility, challenge testing, bioburden

Level: Intermediate

Sterilization of Bulk Solutions

For about half a century, membrane filters have been the tool of choice for removing bacteria from solutions that for stability reasons cannot be sterilized by any other method. Major applications include formulations intended for parenteral administration, including intermediate processes such as aseptic crystallization. Membranes are also used to control total microorganism count even when the ultimate use of a heat-labile substance is an oral or a topical dosage form — and for products such as substrates for in-vitro diagnostic kits and others that may be vulnerable to general attack by microorganisms.

Other aspects of filtration are important and need to be addressed in a validation program: e.g., particle retention and shedding, the toxicity of extractables, general compatibility and durability, and loss of potency through adsorption of active ingredients. However, the focus here is on bacterial retention.

When passage of a contaminant is observed, a reasonable conclusion is that an oversized pore or a defect large enough to allow such passage must be present on the filter used. Although retention is obviously affected by the characteristics of the filter, other factors are also important: e.g., the nature of contaminants, physicochemical properties of the carrier vehicle, and associated hydrodynamic conditions such as flow rate and pressure pulses incurred during a process. Because other factors can be at play in addition to pore size compared with the size of contaminants, retention capability must be evaluated empirically rather than surmised from physical size measurements alone, as was suggested by Einstein and Muhsam (**2**). However, if all the "variables" that affect retention are fixed in a set of challenge studies, the impact of mechanism will no longer be an issue, so the retention observed will be strictly a function of the integrity of the filter.

Retention Capability of Sterilizing Grade Membranes

By definition and accepted industry practice, sterilizing-grade membranes are expected to render sterile a carrier liquid that contains a bioburden high enough to provide a challenge level of $\geq 10^7$ cfu/cm^2 filter area under a differential pressure of 30 psig. Data demonstrating that a given filter membrane type qualifies as "sterilizing grade" generally come from challenge tests conducted by its manufacturer. Such approaches have amply been described in technical literature and by various professional and trade organizations (**3–6**). Bacterial challenge tests are destructive by nature, so their resulting retention data must be correlated to nondestructive physical characterization tests. Such tests indicate filter "integrity" in terms of absence of oversized pores and/or assembly defects that could allow passage of contaminants into the filter effluent. Theory and practical approaches to integrity testing have been described in detail (**7–10**).

Safety Margins for Integral Sterilizing Membranes: Invariably, the data presented by membrane manufacturers support the claim that their sterilizing-grade membranes are capable of quantitatively retaining challenge levels as high as 10^7 cfu/cm^2. Although such claims can no doubt be met, "10^7" cannot be regarded as an absolute safety margin. Instead, passage of organisms must be calculated from the product of bioburden and validated retention capability as in Equation 1.

For example, if a batch of product has a total volume of 100 L and a microbial count of 100 cfu/mL, its total bioburden is 10^7 cfu. If that lot is filtered through a capsule with an effective area of 1000 cm^2, then the passage will be $<(10^7/1000) \times (1/10^7)$ or less than 10^{-3} cfu.

That fractional passage can be interpreted as a probability of passing a single cfu in a given filtration event or as the passage of no more than 1 cfu in every thousand such events. Clearly, the safety margin could conceivably be boosted by an additional factor of 10 by filtering the batch through a 10,000 cm^2 cartridge — or by a factor of 100 if bioburden is reduced to 1 cfu/mL by an additional bacteria-retentive prefiltration step.

Terminology

bioburden: quantitative and qualitative aspects of microorganisms present in a product, mainly before efforts to reduce them

bubble-point value: integrity parameter that indicates the largest pores present on a membrane sample tested for integrity by the "bubble point test"

D-value: measures an organism's heat resistance (by the time it takes to reduce the population by a factor of 10 (1 log reduction) at a stated temperature (e.g., 121 °C)

filterability: the ease or difficulty with which a given microorganism is removed from a product solution by means of a membrane filter, particularly when compared with a model such as *Brevundimonas diminuta*

filter integrity: a combination of the correct pore size and absence of defects of an assembled filter device or system (true pore size is not the same as "pore-size rating"; most "0.2-µm absolute" membranes have pores larger than 0.5 µm)

filter retention mechanism: modality by which contaminants are filtered from a process stream, mainly through sieving and/or adsorptive phenomena

Fo: the exposure time corrected to be an equivalent at 121°C, for a z-value of 10 °C

z-value: measures how D-value is affected by the temperature selected for a sterilization process

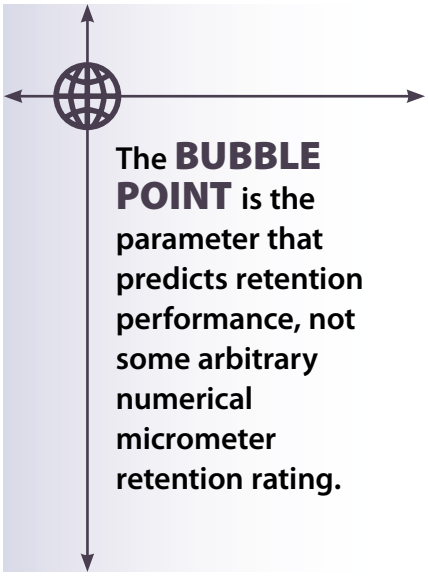
Equation 1

$$\text{Passage (cfu)} = \frac{\text{Total bioburden (cfu)}}{\text{area (cm}^2\text{)}} \times \frac{<1 \text{ cfu}}{10^7 \text{ cfu/cm}^2}$$

In view of such remarkable safety margins, which approach those obtained by moist-heat sterilization processes, it is not surprising that sterilizing filtration is sill perceived by many as an "absolute" process. However, safety margin can be adversely affected by differences between challenge tests and the actual production setting. The main areas of concern here are processing parameters, the nature and count of real bioburden, and the true integrity of a filter unit used.

Actual Product Properties and Processing Conditions

As discussed above, the retention capability of a filter depends on properties of the carrier liquid and



use parameters. To take any possible effect of those variables into consideration, it has become customary (as well as a regulatory requirement) to perform a challenge test mimicking actual-use conditions as best as feasible and using the actual product as a carrier (**11**). If that product is toxic, or if the process itself tends to reduce challenge levels, then suitable adjustments and suitable surrogates must be justified. Suggestions on challenge tests in actual products are further discussed by Montalvo (**12**) and PDA Technical Report No. 26 (**6**). Adherence to such suggestions minimizes the risk of passage that could be introduced as a consequence of differences between challenge-test and actual process conditions.

Membrane Integrity: The integrity parameter of choice in conjunction with challenge tests is the bubble-point test, which is a reflection of the size of the largest pores present on a membrane filter. To support their retention claims, filter manufacturers typically present massive amounts of challenge test data, e.g., the "Matrix Approach" and similar articles published by Levy et al (**13–15**). In these studies, hundreds of membrane samples were challenged with *Brevundimonas diminuta* suspended in a variety of product solutions covering a wide spectrum of physicochemical properties. Levy concludes that a 0.22-µm Durapore sterilizing-grade membrane is capable of retaining 10^7 cfu of *B. diminuta* for each cm^2 of membrane area over a wide spectrum of physicochemical properties (**15**). However, most membranes included in the study had a bubble point of 50 psig or higher. So the valid conclusion that can be supported by the data presented at the time is that the total retention claimed is readily achieved with a bubble point of 50 psig or higher. After all, the bubble point is the parameter that is indicative of retention performance rather than an arbitrary numerical micrometer retention rating. Data to support a minimum bubble point specification of 40 psig were not included in those studies.

Extrapolating retention results obtained with samples at a high bubble point to a filter can with a lower bubble point lead to integrity test specifications that are not indicative of expected retention performance. In terms of moist-heat sterilization, that would be equivalent to "validating" a 30-minute production autoclave cycle by performing 45-minute validation runs. To minimize the risk of establishing meaningless integrity test parameters, Montalvo (**12**) and TR No. 26 (**6**) both suggest that membrane samples used for product-specific challenge tests should not exceed minimum specifications by more than 5%.

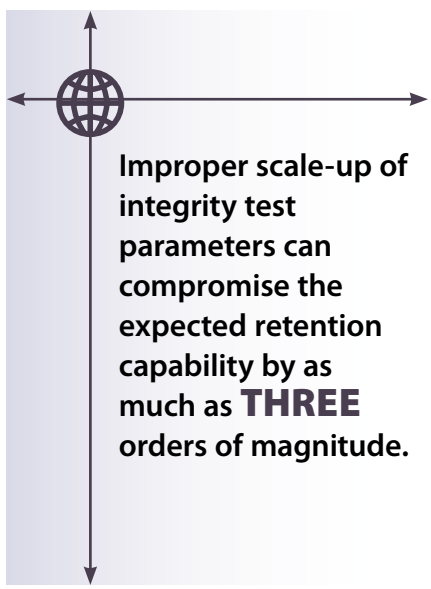
Suppliers typically say they cannot make filter membranes that close to specification, but they do not adjust their integrity specifications to reflect those manufacturing capabilities. Consequently, in most cases, passing a product-specific challenge test is interpreted simply by accepting that filters of a particular "type and rating" will sterilize the product under investigation — no matter what the actual bubble point of the sample challenged was. However, in the absence of other data, the only acceptable conclusion should be that "membranes as tight as or tighter than" the one used in the product-specific challenge need to be used in a production setting to ensure the desired retention.

The risk associated with accepting retention capability based on type and rating alone is magnified further when retention results of challenge data obtained using small disc samples are extrapolated to units of larger effective filtration area. In the "Matrix Approach" described above (**13–15**), the integrity test for the corresponding cartridges was to be conducted at 30 psig at the time. At that test pressure, a minimum bubble point of 40 psig cannot be substantiated.

In another article, Kirnbauer and Pall presented the retention capability of a 0.2-µm nylon membrane as a function of bubble point (**16**). Total retention of *B. diminuta* is reported at values above 49 psig. At a bubble point of 40 psig, observed retention was compromised by over three orders of magnitude. Clearly, an integrity test at 40 psig cannot assure total retention of the corresponding cartridges because the results obtained at a minimum bubble point of 49 psig cannot be substantiated at a test pressure of 40 psig.

As has also been derived elsewhere (**17, 18**), improperly scaled up integrity test parameters can compromise expected retention capabilities by as much as three orders of magnitude. In practice, such errors can readily account for reported filter failures even when integrity criteria specified by a filter manufacturer were met (**19–23**). As should be anticipated, bacterial passage becomes even more pronounced in longer-term challenges (**24, 25**).

Actual Bioburden: One limitation of a *B. diminuta* challenge, even when that microorganism is suspended in the actual product solution, is the fact that those present in unfiltered bulk product could exhibit different filtration characteristics. Factors such



as size, rigidity, and adsorptivity of the actual bioburden may not be reflected at all by an arbitrary microbial model. The belief that *B. diminuta* can be considered a "worst-case" has been dispelled in technical literature for years. Early reports by Duberstein and Howard showed passage of waterborne organisms through *B. diminuta*–retentive membrane cartridges (**26**). More recently, several articles and presentations by Sundaram et al. have shown similar results (**27–30**).

Depending on challenge conditions, retention of the organism *Hydrogenophaga pseudoflava* through membranes that retain *B. diminuta* to the 10^7 level is reduced by as much as six orders of magnitude. Such divergence from anticipated retention capability obviously would affect the safety margin of routine 0.2-µm sterilizing-grade membranes even more drastically than the three-log compromise in *B. diminuta* that could be anticipated from unsafe integrity parameter selection practices.

As mentioned, retention capability is a function of bubble point rather than of a somewhat arbitrarily assigned micrometer retention rating. Employed by a filter supplier at the time of the presentation (**30**), Sundaram pointed was quick to point out that its 0.1-µm rated and qualified membrane is indeed capable of retaining 10^7 levels of *H. pseudoflava*. But it must be kept in mind that the true integrity of a filter rather than its numerical retention rating that is accountable for its retention capability. The hidden but valuable message there was a call for filter users to become more aware of true filter retention capability in regards to their own real processing needs.

In view of the potential effects of the retention mechanism, the most meaningful capability data will be obtained from challenge studies that involve organisms present in an actual product. As proposed by Levy (**15**), a cross-flow filtration step of an actual product preparation to reach a concentration suitable for challenge tests may be most appropriate. Culturing isolates in media other than the actual product may lead to totally different "filterability" characteristics.

Table 1: Expected maximum passage (cfu) as a function of the challenge level, effective filter area, and true retention capability of membrane filters

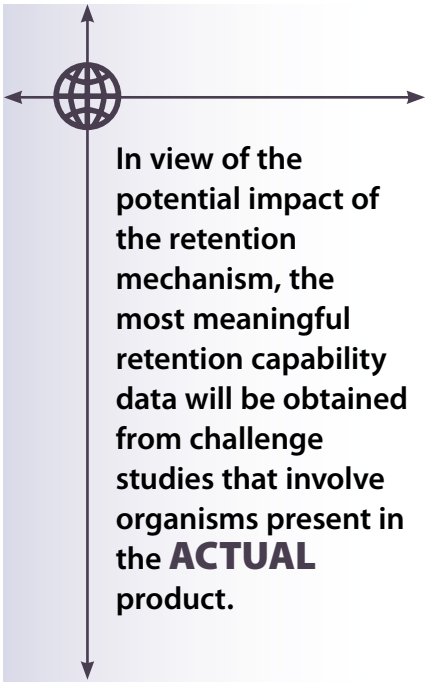
Challenge (cfu/cm^2)	EFA (cm^2)	Total Bioburden (cfu/batch)	True 10^7 cfu/cm^2 Retention	2-Log Compromise	4-Log Compromise
10^7	1	10^7	10^{-7}	100	10^4
	10	10^8	10^{-8}	1,000	10^5
	100	10^9	10^{-9}	10,000	10^6
	1,000	10^{10}	10^{-10}	100,000	10^7
	10,000	10^{11}	10^{-11}	1,000,000	10^8
10^5	1	10^5		1	100
	10	10^6	100× lower	10	1,000
	100	10^7	than above,	100	10,000
	1,000	10^8	essentially zero	1000	100,000
	10,000	10^9		10,000	1,000,000
10^3	1	10^3		0.01	1
	10	10^4		0.1	10
	100	10^5		1	100
	1,000	10^6		10	1000
	10,000	10^7		100	10,000
10	1	10		0.0001	0.01
	10	10^2		0.001	0.1
	100	10^3		0.01	1
	1,000	10^4		0.1	10
	10,000	10^5		1	100

Comparing True and Anticipated Retention Capabilities

As indicated above, the retention capability that can be anticipated for truly retentive membrane filters is remarkably high. By definition, a 1-cm^2 sample challenged with 10^7 cfu must result in zero passage — with a probability of passage at 10^{-7}. Such results are expected also for integral sterilizing membrane devices with larger effective areas. For larger filter units, the probability of passage will now be reduced further because the "<1" cfu is related to proportionally greater total challenges. As illustrated, even at moderate to relatively high bioburden levels, theoretical passage expected is in the order of 1 cfu in literally thousands of filtration events for totally retentive filter units. Nonetheless, prudence as well as regulations encourage filter users to establish counts as well as the nature of prefiltration bioburden — and to minimize it rather than dare to push a filter to such extreme challenge levels in an actual production setting.

If the integrity test specifications are misleading for any of the reasons discussed above, the anticipated "perfect" retention capability will not be met. Instead, the probability of passage will increase rapidly due to the the presence of oversized pores or uniformly distributed defects that allow passage of 1 or more cfu/cm^2, which will result in passage that is directly proportional to the effective filter area. For instance, if at a challenge level of 10^7 cfu/cm^2 passage of 1000 cfu is observed through a 10-cm^2 sample, then passage through a 1000-cm^2 capsule could be as high as 100,000 cfu. On the other hand, if the challenge level is reduced from 10^7 cfu/cm^2 to only 10^3, then a 10,000-fold reduction in passage through the same defects should be observed. Thus, at a challenge level of 10^3 cfu/cm^2, a 2-log compromised 1000-cm^2 effective filter area would show passage of only 100,000:10^4 = 10 cfu.

Theoretically expected maximum passage through filters that are not totally retentive can readily be calculated as above. For quick reference, results are presented in Table 1 for filters that are truly retentive to the 10^7 challenge level as well as those that show 2- and 4-log compromises in actual retention performance. For simplicity, arbitrarily effective filtration areas of 1, 10, 100, 1000 and 10,000 cm^2 were chosen. Within reason, they respectively correspond to the area of 13-, 47-, and 142-mm discs, a disposable capsule, and a 10-in. cartridge. Note that the anticipated



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probability of passage refers to *B. diminuta* or any other model organism chosen as a reference. Mathematically, the reason for anticipated passage does not matter. The numbers calculated for each level of compromise could be the result of oversized pores or uniformly distributed defects that go undetected by misleading integrity test parameters, by the presence of organisms that are inherently more difficult to retain than those used as a model, or by a combination of both effects.

The effect of passage on the fraction of units contaminated in a given aseptic operation can be calculated from the bioburden of the batch to be filtered (cfu/mL), the batch size, and the fill volume. Considering that each cfu in filter effluent will contaminate at least one unit of finished product, the sterility assurance of filtered bulk is obviously a limiting factor of sterility assurance achieved by an overall process. Subsequent aseptic handling downstream of the final filtration can only increase the microbial count and, hence, the number of units contaminated. Further, if retention of organisms is not complete, proliferation can take place in filtered bulk, particularly if a solution is only marginally or not at all protected by a preservative system. In such a case, the number of contaminated units will be higher than predicted from passage alone.

For example, consider a 100-L batch that has a bioburden of 10 cfu/mL. Its total bioburden is 10^6 cfu — or 10^3 cfu/cm^2 if it is filtered through a 1000-cm^2 capsule. The anticipated probability of passage would be $<(10^{-10}) \times (10^{-4})$ or $<10^{-14}$ cfu, essentially nil. If the fill volume is 10 mL, the fraction contaminated by filter passage of organisms will be a low 10^{-10}, as calculated from (10^{-14} cfu per batch) × (1 unit contaminated per cfu) / (10^4 units per batch).

However, if in reality that filter capsule has a two-log compromise, then bioburden passage would increase to 10 cfu per batch, resulting in a 1:1000 contamination rate for the finished product. At this contamination level, the lot in question would pass the sterility tet 98% of the time, and even passage of 340 cfu would result in sterility test acceptance probability of 50%. Obviously, a 50% probability of such a false negative is not an acceptable target by any means, but it illustrates how "reliable" the sterile filtration process can be if a sterility test is the sole judge of its capability.

Comparing Moist Heat and Sterile Filtration

Unlike other sterilization methods, sterile filtration does not kill but rather simply removes bacteria from processed product. Thus a direct comparison between steam sterilization and filtration is likely to be questioned, but some parallels can be drawn. In both processes, a higher bioburden will increase the probability of survival and passage. Cold spots in autoclaves are comparable to defects or oversized pores, as each can also lead to an increased probability of survival and failure to retain. Organisms that are not retained as effectively by a given membrane are equivalent to those having higher than anticipated heat resistance.

One major difference between these two processes is that the impact of variables and the selection of control parameters for moist heat sterilization processes are better understood through validation studies performed by autoclave users. Before the advent of concerted efforts to validate such processes, the associated science was controlled by autoclave manufacturers. Although regulations hold filter users responsible for the validity of retention validation reports presented by filter suppliers, sterile filtration has not been subjected to equivalent process analysis by its users. For practical purposes, users continue to rely on science handed down by filter manufacturers.

The limited regulatory pressure for filter users to become more involved in validation of such a crucial processing step is a reflection of the proven reliability of sterile filtration under normal circumstances. In fact, its perceived reliability is so high that in the absense of test error, investigations into sterility failure are almost automatically traced to "the usual suspects": human error and environmental control problems.

The lack of sterility in a bulk solution is seldom investigated to any depth as a possible root cause. However, several unfavorable conditions can drastically change the picture of unquestionable reliability. Bypass of a feed solution through oversized pores and defects could be masked by improper selection of integrity test parameters. Bioburden increases will raise the probability of passage by the same factor. And anticipated retention capabilities may not apply when actual bioburden is not retained as effectively as the challenge organism used in retention validation studies.

Fortunately, sterility test failures are rare incidents. Considering the potential loss of product — and the cost of ensuing investigation — it is obviously an undesirable problem. Unfortunately, problems tend to recur unexpectedly if not all of their possible root causes are appropriately addressed and controlled. As long as the reliability of sterile filtration is taken for granted, there is no good reason to expect that sterility problems will disappear even if the traditional culprits in aseptic processing are taken out of the equation through advances such as isolator technology and robotics.

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