EBE Position Paper

A Risk-Based Approach to Setting Sterile Filtration Bioburden Limits

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Executive Summary

The rationale behind the EMA recommended bioburden limit before sterile filtration of NMT 10 CFU / 100 ml (EMA Guidelines of 1996 (1), 2012 (2) and Draft EMA Guideline 2016 (3)) has no clear origin. The limit has been taken from the pharmacopoeial specification for 'water for injection to produce bulk', but has no scientific basis when applied to drug product. Overall, we would instead propose a "Risk-based Approach" to bioburden control which justifies alternative bioburden specifications that take the product manufacturing process into consideration, in contrast to defaulting to the historical 10 CFU / 100 ml limit.

This risk-based approach considers the two main risks at the sterile filtration step of drug product manufacture: (a) capability of the microbiological bioburden method and, (b) microbial breakthrough during sterile filtration. These risks have been assessed for their interaction by means of statistics and possible risk-mitigating measures are described.

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1. Risk: The bioburden determination method

As Jornitz et al. (5) described, it is impossible due to the inherent variability of microbiological analyses, to determine an exact bioburden in the order of 10 CFU / 100 ml. The true bioburden could likely be below or above and thus the batch incorrectly accepted or falsely rejected. It is therefore more accurate to specify the acceptable limit of 10 CFU plus a margin of error. This inaccuracy in microbiological analyses is taken into account in USP Chapter <61> and also in the Ph.Eur. (Chapters 2.6.12 and 5.1.4): an acceptance criterion for microbiological quality of 10 CFU would therefore be corresponding to a maximum acceptable count of 20 CFU when the variability of analysis is considered.

We have modeled the sensitivity of the Ph.Eur. 2.6.12. bioburden assay method for an acceptance criterion of 10 CFU / 100 ml (6) while varying the test sample volume. The Poisson model assumes a uniform distribution of the bacteria in the solution prior to sterile filtration and is therefore not considered a suitable model. Bacteria can clump together to produce a non-uniform dispersion which leads to either overestimation or underestimation of detected bioburden when determined by the number of counts in the solution. This over-dispersion can be described by a negative binomial distribution with a corresponding probability density function and an over-dispersion factor.

Based on a negative binomial model with an over-dispersion factor of 2, it can be estimated that using a limit of 10 CFU / 100 ml, a solution with a true bioburden level of 10 CFU / 100 ml would have a 41.2% probability of batch rejection, although the specification would still be met. Conversely, a true bioburden of 11 CFU / 100 ml would still lead to a probability of batch acceptance of 50% (see figure 2, Addendum).

The probability curve also demonstrates that - given an acceptable risk bound of 5% - a batch passing with a measured bioburden of not greater than 10 CFU/100 mL could reveal a true bioburden of up to 20 CFU/100 mL (for a 100 mL test sampling plan). The probability curve shifts to the right as the volume tested decreases such that a 10 mL test volume may have up to 63 CFU/100 mL actual bioburden for the same 5% probability of false negatively passing and the same control limit. This increase in acceptable actual bioburden can be compensated by adjusting parameters for Risk 2, as described in the next section.

We conclude that the specification of the bioburden at the level 10 CFU / 100 ml is too tight, leading to unreasonably high rejection probability of acceptable batches. Furthermore, the microbiological quality of the solution prior to sterile filtration should be considered holistically and the total risk composed of many individual risks should be considered in order to avoid falsely accepting unacceptable batches due to the limited sensitivity of the test.

2. Risk: Bacteria breakthrough during sterile filtration

According to the FDA guideline (4) and industry standards, filters used for the final filtration should be validated to reproducibly remove microorganisms from a carrier solution containing bioburden of a high concentration of at least 10^7 CFU/cm^2 of effective filter area (EFA). The validation should be conducted under the worst-case production conditions for the material to be filtered, and challenge experiments should result in no passage of the challenge microorganism. Thus the retention capacity of a validated sterilizing-grade filter with an EFA of A (cm^2) is at least $A \times 10^7$ CFU. However, the currently used 0.22 µm sterile filter membranes can withhold much higher microbial challenges (unpublished company results for 0.22 µm polyvinylidene fluoride membranes a bacterial challenge concentration as high as 10^9 CFU/cm^2 can be validated).

In practice, accumulation of bioburden on the final filter might even cause partial clogging. As a result, a

bioburden CFU that reaches the filter early in the process may have a higher probability to penetrate the filter than those that reach a partly clogged filter later in the process. Therefore it is conservative to assume that all bioburden CFUs in the solution have the same probability to go through the final filter.

Similar to terminal sterilization methods, for sterile filtration a Sterility Assurance Level (SAL = degree of assurance with which the process renders a population of items sterile) can be calculated based on the bacteria retention capacity of the filter. Here, worst-case assumptions have been made, for example during the validation using a small microorganism (typically Brevundimonas *diminuta*).

Since the pre-sterile filtration bioburden is determined for each lot and not only as part of a validation procedure, the SAL can also be calculated as a LOT-SPECIFIC parameter from the actual, measured bioburden load (with a factor included for variability), the applied total filter area and the validated effective bacteria retention capability. Thus, the decisive factor of pre-filtration bioburden is known (within assay variability) and not assumed.

For products subjected to terminal sterilization, the true bioburden is not determined (the Draft EMA Guideline on the Sterilization of the Medicinal Product, Active Substance, Excipient and Primary Container suggests a maximum bioburden limit of 100 CFU / 100 ml or 100 g before) as the manufacturing process does not take place under aseptic conditions, and this must be compensated by incorporating appropriate safety factors. Since the filling process is run under class C conditions, a relatively high bioburden must be assumed which then has to be killed by the terminal sterilization procedure.

In contrast, the sterile filtration process prior to aseptic filling can be considered a closed system, followed by manufacture under class A conditions. Finally, the bacteria are mechanically retained instead of being inactivated, and therefore do not enter the final product.

In summary, for sterilization of product through sterile filters SAL values lower than used for terminal sterilization ($<10^{-6}$) should be acceptable e.g. 10^{-4} or 10^{-5}.

3. Combination of both main risks

The two types of main risks associated with the sterile filtration process can be statistically described (6):

- Risk 1 (“pre-sterile filtration risk”): **risk due to bioburden test method insensitivity** (risk of false negative = passing a batch with unacceptable bioburden), i.e. the drug solution with an unacceptable bioburden level before sterile filtration passes the pre-sterile filtration bioburden test, either due to inherent test method variability or a sampling method that does not have sufficient statistical power to detect drug solutions with unacceptable levels of bioburden
- Risk 2 (“post-sterile filtration risk”): **risk of breakthrough of bioburden through the final sterile filter** with ≥ 1 CFU entering the sterile filtered solution due to an inappropriate process, i.e. process-related risks and microbial breach across sterile filter

These two risks are inter-dependent as a high pre-sterile filtration risk would require a more stringent control of the post-sterile filtration risk and vice versa. Therefore an effective overall control strategy should take into account this inter-correlation.

Yang et al (6) linked maximum batch size, sample volumes of 10 ml, 30 ml and 100 ml (negative binomial model, specification limit 10 CFU / 100 mL and acceptance limit of pre-filtration bioburden 1 CFU/10 mL, 3 CFU/30 mL, 10 CFU/100 mL) to the Risk 1 probability of incorrectly accepting a batch (5%, 1% and 0.1%) and the Risk 2 probability of a breakthrough of ≥ 1 CFU in one of 10,000 cases (0.01% probability,

10^{-4}) or 1 of 100,000 cases (0.001% probability, 10^{-5}). A validated bacterial retention capability of the sterile filter of 10^7 CFU per cm^2 was used, for a 1000 cm^2 filter, to calculate the maximum volume to be sterile filtered. A modified version of the risks-linking table is attached to this position paper (see table 1, Addendum). By example, a 10 mL sample size using an action limit of 1 CFU/10 mL, with a 5% risk of passing a batch through a false negative result and a filter breakthrough risk of 10^{-4}, allows a maximum filtration batch volume of 424 L when using a 1000 cm^2 (0.1 m^2) filter.

Note that when e.g. δ_0 = 5% (pre-filtration risk, i.e. risk of bioburden testing insensitivity) and $\delta = 10^{-4}$ (post-filtration risk, i.e. breakthrough risk of bioburden through the sterile filter), there is a 95% probability that the pre-sterile filtration bioburden test (for any given test scheme) will successfully detect unacceptable levels of bioburden and only 1 batch out of 10,000 would have bioburden breakthrough (see Table 1). This implies that in a facility producing 100 batches a year, this event would only occur every 100 years. By limiting the maximum filtered batch size, accepted post-sterile filtration risk levels can achieve the same level of sterility quality assurance, for any given post-filtration risk (δ), as the bioburden testing with 100 mL samples and a 10 CFU/100 ml acceptance limit. Indeed, it is proposed that a Risk 1 probability of ≤ 5% should be acceptable.

Alternatively, to manipulating the filtration batch size, other parameters may also be varied to reduce risk, e.g. increasing filter area or validated maximum CFU. The relationship between sample volume and the batch size varies according to filter area, as illustrated in Figure 3 of the Addendum. A 10 mL sample size using a 1000 cm^2 filter allows up to 424 L to be filtered to retain a ≤ 5% probability of passing a batch with bioburden exceeding the acceptance limit and filter penetration of less than 1 in 10,000 batches. For a 2000 cm^2 filter the filtered volume could be increased to 849 L at the same risk level (see table 1). The relationship between filtration batch size and filter area is tabulated and provides the Sponsor with the opportunity to select the sterile filtration, minimum filter area according to filtration batch size to provide a desired level for Risk 1 (0.1% to 5%) and Risk 2 (10^{-4} to 10^{-5}).

Therefore, for a given risk bound, the pre-filtration risk can be controlled with a properly selected batch size or the post-filtration risk can be controlled through filter area and filter validation. The bioburden level D_0 may be even greater than 10 CFU/100 ml since its impact on the final post-filtration risk can be controlled through placing a limit on the size of a batch for the sterile filtration S_0 or adjusting the filter area or the validated range so as to ensure the total bioburden in the batch would not exceed the retention capacity of the final sterilizing filter.

The presented calculations support use of bioburden test sample volumes ≥ 10 ml using a filter area of ≥ 1000 cm^2, a maximum filtration batch size of 400 L and an action limit of up to 2 CFU/10 ml that includes a 3-fold safety factor for the bioburden determination precision (calculated from 63 CFU/100 ml). These limits ensure a pre-filtration bioburden risk of 5% and a post-filtration risk of 10^{-4}. Further risk mitigation is built into the product manufacturing process as summarized in the next section.

4. The holistic view considering a portfolio of risk-mitigating measures

Mitigating risk factors are essential for the final bioburden risk assessment of the sterile filtration process. This includes examination of the related process steps and understanding of the product characteristics and potential product related risk factors like viscosity, pH or growth promoting properties of the formulation.
For example, if the formulated product has growth promoting properties, this constitutes a risk factor per se.

It is also important to evaluate available bioburden data, including the manufacturing facility historical

bioburden detection trends for the product and across products when a multi-product site is used. Historical bioburden trend data are often indicative of the effectiveness of the overall bioburden control, and need to be considered in identification of risk factors.

The focus of this paper is on parenteral biotech drugs that cannot be terminally sterilized in the final container because they are thermo-labile. For such products, however, the testing of the bioburden prior to sterile filtration and the subsequent sterile filtration are not the only measures to control the microbiological status of the solutions for container filling (see figure 2, addendum):

- The upstream and downstream processes are likely to have multiple 0.2 micron or nano-filtration steps for reduction of adventitious microbial, particulates or viral contamination.
- The fermentation itself takes place in closed systems subject to a CIP / SIP process and under the addition of filtered or HTST-treated or even sterile media.
- The final formulation steps are carried out in a Grade C environment and also use steam-decontaminated or sterilized containers into which the solution is being transferred via 0.2 micron filter.
- In the event of extended storage of the formulated solution, storage is usually at low temperatures, typically -20, -40 or -80 ° C. In the frozen state bacterial growth is extremely unlikely.
- Interim storage in the liquid state is time-limited for reasons of product stability alone and performed mostly also at reduced temperatures (2 – 8°C), further bacterial growth is hereby also limited. Storage and hold times of bulk after final formulation or thawing are evaluated in studies and eventually validated. Room temperature storage is kept at a minimum. Monitoring the container integrity during such interim storage is often done by superimposing pressure (nitrogen). Furthermore, interim bulk storage after thawing may be (re-)validated using growth-promoting media in order to uncover microbiological weaknesses (as part of the regular media fills).
- Increasingly, upstream, downstream and fill/finish processing use pre-sterilized single-use materials and aseptic connectors (“disposables processing components”). These are often sourced pre-assembled as manifolds, pre-sterilized and aseptically connected. In addition, these components represent closed systems, which reduce the risk of bioburden entering by non-aseptic connections. Through increasing automation of manufacturing processes, individual personnel errors are further mitigated.
- The commercially available sterile filters have a larger bacteria retention capacity than the underlying minimum expectation of 10^7 CFU / cm^2.
- For early clinical phase manufacture, the ratio between filter surface area and batch size is typically relatively high compared to commercial scale manufacture.
- Endotoxin content (in case e.g. pyrogens are produced by bioburden) and sterility testing is part of the Drug Product release process
- An analysis of data from the companies involved in this paper has shown that it is a very rare event to find bioburden over the limit set by the EMA before the sterile filtration. Often it is an artifact of sampling or in the analysis, but both are each difficult to prove with 100% certainty, so finally the batch is often still discarded which is very costly and may endanger the product development timelines. Each bioburden found, even if below the limit of 10 CFU / 100 mL may trigger identification of the microbial contaminant, so that regardless of a limit set, investigation and corrective measures are initiated.

For this reason, the authors are of the opinion that the processing of biotechnological active ingredients is typically under ample control for bioburden, which allows the determination and justification of lower sample volumes and wider pre-defined levels of bioburden before sterile filtration, using risk-based strategies.

5. Conclusions

Biotechnology-derived drug products are typically labile to methods of terminal sterilization such as irradiation, chemical treatment or autoclaving, and are manufactured through aseptic processing which typically employs sterile filtration to remove microbial contaminants. High bioburden in the drug solution prior to sterile filtration may increase the chance of breaching the sterilizing filter and cause product safety and quality issues. As a result, pre-filtration bioburden test is recommended by regulatory guidelines. The current EMA guidelines stipulate that a maximum acceptable bioburden level must be established before the sterile filtration step and that in general no more than 10 CFU/100 mL would be acceptable. However, a sample volume of 100 mL often represents a significant proportion of the batch and a high cost for biotech products, particularly taking into account that sufficient material would typically be drawn for replicate testing, assay controls, overage and reserve samples. Therefore it is desirable to explore alternate sample volumes and test methods that warrant the same or higher level of quality assurance as the EMA-recommended method. The guidelines allow the Sponsor to justify alternative sampling procedures though no guidance is provided on what might constitute an acceptable justification. Furthermore, the EMA guidelines do not provide scientific rationale for limit 10 CFU/100 mL, thereby compounding the difficulty to justify a test volume smaller than 100 mL or other limits.

In this paper, a risk-based method is proposed to provide a strategy and scientific methodology for justifying alternate (smaller) bioburden test volumes than 100 mL and alternate specifications (>10 CFU/100 mL or >1CFU/10 mL).. By modeling bioburden in the solution prior to sterile filtration, the relationship(s) between pre-sterile filtration risk (probability of detecting bioburden in the solution prior to sterile filtration) and post-sterile filtration risk (probability of bioburden breaching the filter), their combined risk as well as the associated risk factors such as test sample volume or batch size are established. Such relationships allow for quantitative evaluation of the impact of the risk factors on the development of effective risk-based control strategies, which include acceptable selection of sample test volume and maximum pre-filtration bioburden level. The risk-based method is in accordance with the quality by design (QbD) principles in ICH Q8 that enable manufacturers to define a manufacturing process design space that consistently produces high-quality products through increased understanding and knowledge of the product and process. It is also consistent with ICH quality initiative, Quality Risk Management, which achieves product greater quality assurance through risk identification, analysis, and control.

The risk-based approach also justifies use of action limits for control of the pre-sterile filtration bioburden limit through implementation of an effective Quality Management System (QMS) that describes the actions followed in the eventuality of any detected bioburden. The conditions that initiate investigation of bioburden, determination of likely root cause and any resulting CAPA should be available to the agency and may be verified through GMP inspection. Sponsors may also employ tighter internal criteria per the QMS. Ultimately, the agency should be assured that any batch with a significant bioburden breach potentially impacting product quality or safety would be rejected. The onus should remain with the Sponsor to determine the definition of ‘significant’.

Key findings of the paper include:

- Pre-filtration and post-filtration risks are inter-correlated. A holistic approach needs to be taken in development of risk mitigation strategies so that a seemingly high risk in one process step can be mitigated through controlling risk factors in other step.
- Risk factors are inter-dependent and should be considered jointly when evaluating their impact of the pre-filtration and post filtration risks. This includes selection of sample test volume, acceptable bioburden limits, sterilizing filter area, in an integrated assessment.
- A bioburden level higher than 10 CFU/ 100 mL in the unfiltered drug solution may not incur unnecessary risk as its impact can be mitigated through effective sterilizing filtration.
- Sample volumes less than 100 mL and acceptance limits different from 10 CFU/100 mL can be justified, through controlling other risk factors such as batch size or filter area, without increasing the risk of bioburden breakthrough in the final filtration.
- Testing of bioburden prior to final filtration and the sterile filtration are not the only measures to control the microbial status and to generate sterile product. Holistic concepts may be established that reduce risks prior to the final bioburden testing and the sterile filtration.
- Enhanced understanding of the manufacturing process and product attributes is key to successful bioburden risk management.

6. References

1. EMA (1996). CPMP Notes for Guidance on Manufacture of Finished Dosage Form.
2. EMA (2012). EMA Guideline on the Requirements for Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trials. (EMA/CHMP/BWP/534898/2008), currently under revision
3. Draft Guideline of EMA on the sterilization of the medicinal product, active substance, excipient and primary container, April 11, 2016
4. U.S. FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice, 2004.
5. Jornitz MW, Akers JE, Agalloco JP, Madsen RE, and Melzer TH (2003). Considerations in sterile filtration. Part II: the sterilizing filter and its organism challenge: a critique of regulatory standards. PDA Journal of Pharmaceutical Science and Technology. March/April. Vol. 57, No. 2, p 88 – 96.
6. Yang H, Li N and Chang S (2013). A risk-based approach to setting sterile filtration bioburden limits. PDA J. of Pharm. Science and Technology. Vol. 67: 601-609

7. Addendum

Manufacture of drug products (chemical synthesis or from cell/microbial cell cultures) generally follow a similar set of process steps illustrated in Figure 1. Each of the nine steps includes control of either bioburden or sterility. They also include several filtration and chromatographic steps that may deplete any adventitious bioburden from the product. The product contact process materials are cleaned and validated to minimize risk on contamination.

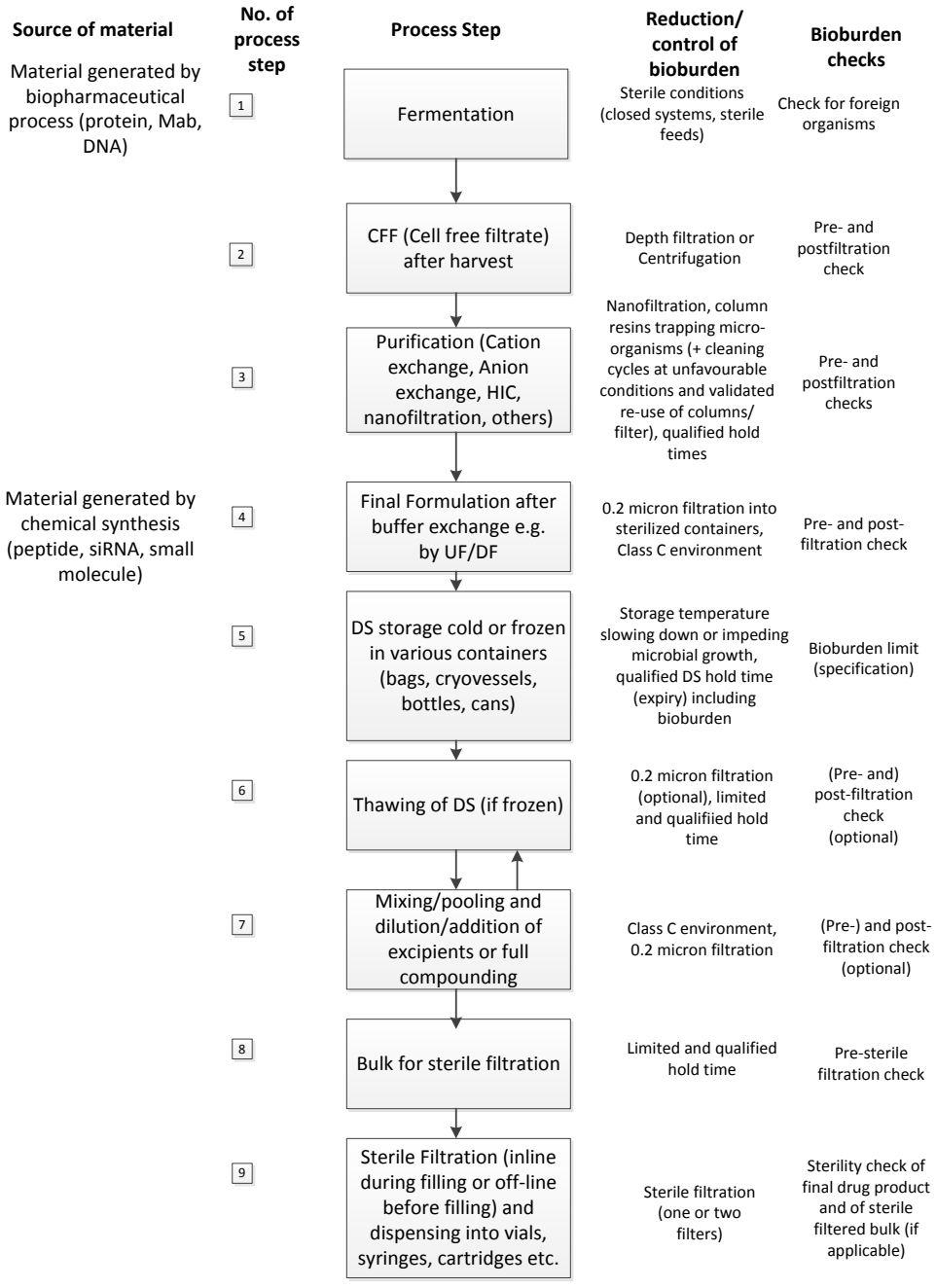
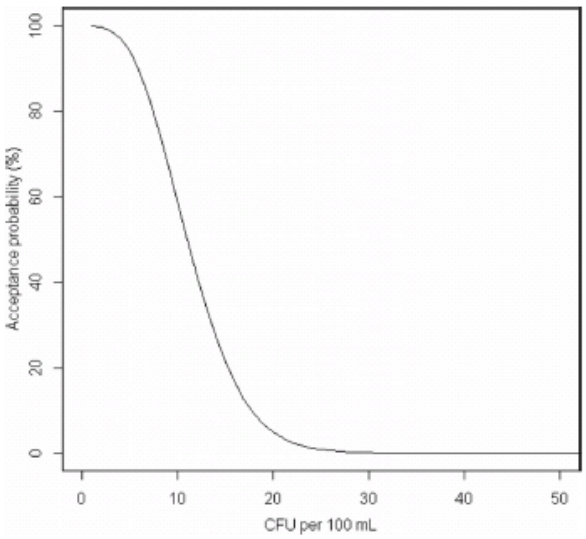


Figure 1. Typical process flow diagram for manufacture of liquid biological drug product sterilised by filtration.



Actual Bioburden (CFU/100 ml)	Probability (to pass bioburden test)
9	66.6%
10	58.8%
11	50.0%

Figure 2: Performance characteristics of bioburden testing using 100 ml samples and 10 CFU/100 ml acceptance limit, based on negative binomial distribution with a dispersion factor of 2 (6); dotted line: 5% acceptable risk bound.

The Ph.Eur test for bioburden does not have the capability to determine an accurate bioburden count in a solution as detection depends on the volume tested. The probability of passing a batch for bioburden is best determined using a negative binomial distribution to account for the clumping properties of microbes. Figure 2 illustrates such a probability curve relationship with the actual bioburden level when 100 ml is tested. There is a significant probability of failing and rejecting a 'good' batch or passing a 'bad' batch on the basis of any single bioburden test when considered in isolation and applying an acceptance criterion of 10 CFU/100 ml. A holistic approach to bioburden risk is recommended that accommodates the sterile filtration process and associated risk.

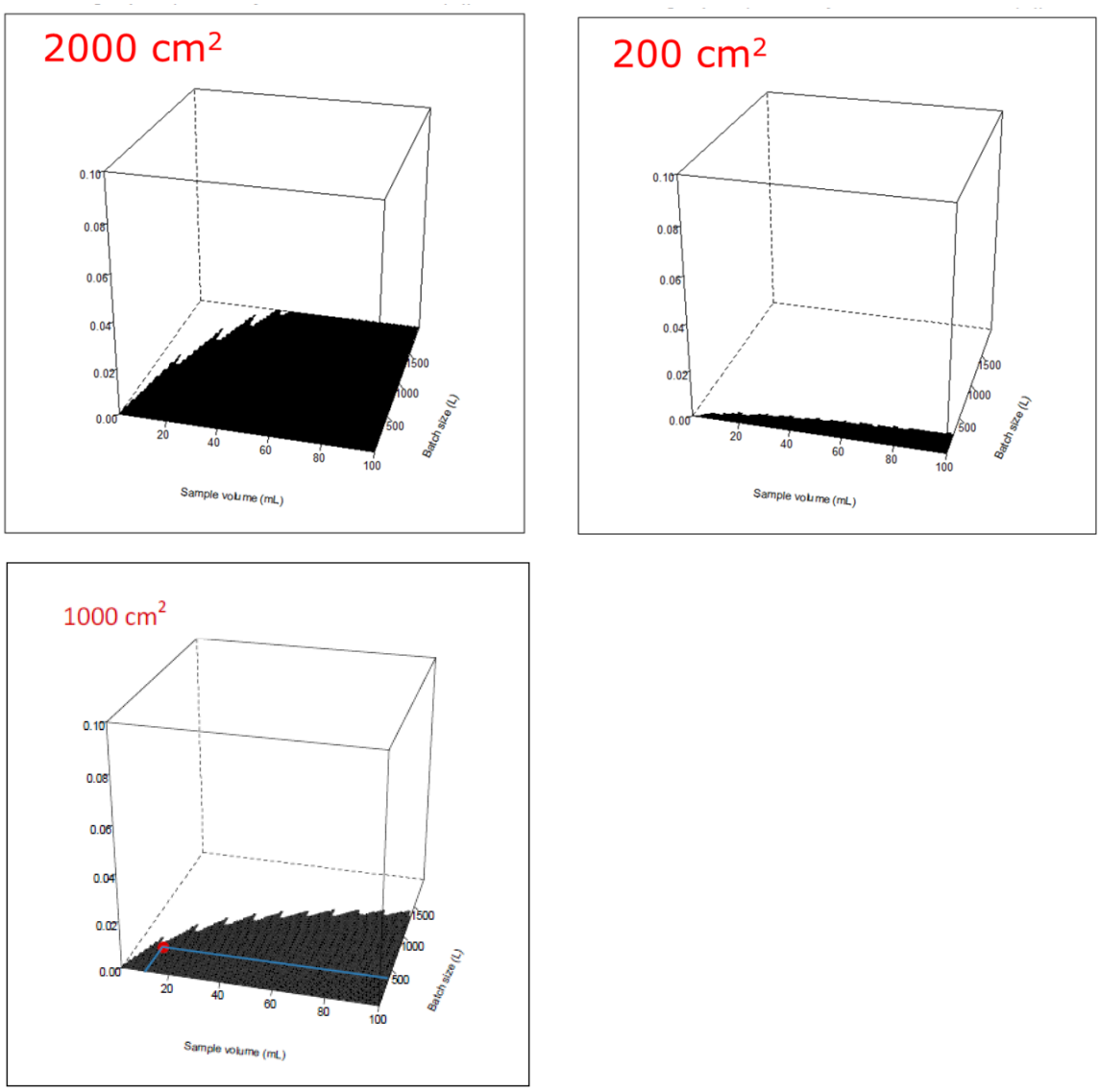


Figure 3: Design Space for bioburden test scheme (here: probability of at least 1 CFU < 10^{-4}) dependent on filtered volume and filter area following a risk-based approach

The relationships demonstrate the breakthrough risk following bioburden determination can be controlled through the batch volume filtered and/or filter area. The Sponsor may determine the bioburden control strategy that best fits the process and facility within the design space. A test sample volume of 10 ml from a 424 L filtration batch size is illustrated (red circle) as acceptable when using a 1000 cm^2 filter.