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RESEARCH

A Risk-based Approach to Setting Sterile Filtration Bioburden Limits

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ABSTRACT: Microbial control during the drug substance and drug product manufacturing process is critical for ensuring product quality and safety. For sterile biological drug products (finished dosage forms) typically manufactured by sterile filtration followed by aseptic processing, control of the microbial load at the sterile filtration step is an important component of the overall microbial control strategy. Both FDA and EMA regulatory guidelines stipulate that a maximum acceptable bioburden level, which is referred to as a *pre-filtration bioburden level* in this paper, should be stated at the point immediately prior to the sterile filtration step. The EMA guideline further states that a bioburden limit of no more than 10 colony-forming units (CFU) per 100 mL will be considered acceptable in most situations. The EMA guideline also states that a pre-filtration sample volume of less than 100 mL may be tested if justified. This paper introduces a risk-based method to establish pre-filtration bioburden acceptance levels and alternative test volumes. The relationship between bioburden risk, pre-filtration bioburden test limits, and sterile filtration process parameters, such as filtration volume, filter surface area, and microbial retention capacity of the sterilizing filter, was statistically determined. Taking into account the batch filtration volume, it is shown that pre-filtration bioburden test volumes and acceptance limits other than 10 CFU/100 mL may be justified, without compromise to sterility assurance.

KEYWORDS: Batch size, Negative binomial distribution, Poisson distribution, Pre-filtration bioburden, Risk-based approach, Sterilizing grade filter

LAY ABSTRACT: In the manufacturing of sterile medicinal products, good manufacturing practice requires that bioburden be monitored before the final sterilization filtration step. High bioburden increases the challenge to the sterilizing filter and may also lead to other quality issues. Therefore a pre-filtration bioburden limit should be established. This paper introduces a risk-based method to establish such limit which may be different from what is recommended in regulatory guidelines.

1. Introduction

Manufacture of sterile biological drug products is a complex process requiring a stringent microbial control strategy. Because drug substance manufacture is typically not sterile, low bioburden targets are part of the microbial control strategy for the overall manufacturing process to provide to the finished dosage form, which is sterile. There is a risk that bioburden may be introduced throughout the manufacturing process either through the processing environment, equipment, raw materials, or processing manipulations. In addition, bioburden can contribute endotoxins and other impurities to the drug product, thus compromising product quality. To mitigate the risk, various filtration steps can be employed to remove bioburden, in addition to minimizing manufacturing hold times between processing steps and utilizing refrigerated storage for process intermediates to minimize potential microbial growth. For the final sterile filtration, virtually all biological manufacturing processes utilize sterilizing-grade filters. Such filters, typically microporous membranes, have a nominally designated pore size of 0.2 μm or smaller (1) and can reliably retain particles larger than their designated size ratings. However, when the amount of bioburden in the drug solution to be sterile-filtered is too large for a given filter surface area, the

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sterilizing-grade filter might become overburdened, compromising microbial retention capabilities and restricting sufficient flow of solution across the filter (2). The issue can be resolved by either using a sterilizing-grade filter of larger surface area or by employing a pre-filter to reduce the bioburden to a sufficiently low level before sterile filtration.

2. Regulatory Guidelines

Minimizing and removing bioburden before final sterile filtration plays a critical role in the overall microbial control strategy and risk mitigation of the manufacturing process. As part of this control strategy, the pre-filtration bioburden test is both a critical manufacturing in-process control measure and an important regulatory requirement. U.S. Food and Drug Administration (FDA) guidelines state, "Manufacturing process controls should be designed to minimize the bioburden in the unfiltered product. . .. A prefiltration bioburden limit should be established" (3). However, the FDA guideline does not prescribe specific methods to establish an appropriate pre-filtration bioburden limit.

By contrast, the European Medicines Agency (EMA) guidelines are more specific. In both EMA Committee for Proprietary Medicinal Products (CPMP) *Notes for Guidance on Manufacture of Finished Dosage Form* and EMA *Guideline on the Requirements for Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trials* (4, 5), it is stated, "For sterilisation by filtration the maximum acceptable bioburden prior to the filtration must be stated in the application. In most situations NMT [not more than] 10 CFU/100 ml will be acceptable, depending on the volume to be filtered in relation to the diameter of the filter. If this requirement is not met, it is necessary to use a pre-filtration through a bacteria-retaining filter to obtain a sufficiently low bioburden." However, realizing that the batch size is likely small at an early stage of drug development, the EMA guideline further states, "Due to limited availability of the formulated medicinal product, a pre-/filtration volume of less than 100 ml may be tested if justified." (5).

3. Potential Limitations of the 10 CFU/100 mL Bioburden Limit

Although the EMA guidelines recommend a pre-filtration bioburden limit of 10 CFU/100 mL, the rationale behind this recommendation does not appear to be clear. Jornitz et al. (6) point out that due to inherent variability of microbiological analyses it is impossible to accurately enumerate bioburden at a level of 10 CFU/100 mL. While the true bioburden may be below 10 CFU/100 mL, the test outcome may exceed the limit. Given the sensitivity limitations of microbiological analyses, it is more reasonable to express the acceptance limit as 10 CFU plus a margin of error. Akers (7) also argues that given large variability of microbiological analysis and considering that microorganisms are not homogeneously distributed in most environments or materials, it is not appropriate to set an acceptance limit without taking into account the inaccuracy of such assays. It is also true that a single CFU may correspond to a single or multiple viable microorganisms because of cell clumping. The variability and limited sensitivity of microbiological analyses, together with the large variation of microorganisms, constrain the precision of the pre-filtration bioburden test result. As such, there is always a risk of rejecting a drug solution (due to failing test result) with a true bioburden level below any prescribed acceptance limit, or likewise of accepting a drug solution with an actual bioburden level above the limit. In fact, in Section 4 we demonstrate through modeling both assay variability and microorganism heterogeneity that if the true bioburden level is 9 CFU/100 mL, there is 33.4% chance to reject the batch per the above acceptance limit. On the other hand, if the true bioburden level is 11 CFU/100 mL, the chance to accept the batch is 50%. Therefore meeting or failing the 10 CFU/100 mL acceptance limit may not provide adequate assurance that the true bioburden level is below or above 10 CFU/100 mL. Most recently, recognizing the inexactness of microbiological analysis, USP Chapters <61>, <62>, and <1111> (8–10) concerning microbial limit tests state when an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows: in case of an established level of 10 CFU, the maximum acceptable count should be 20 CFU. Here the 20 CFU limit accounts for a bioburden level 10 CFU, which is deemed acceptable, and microbiological analysis variability. In light of the above observations, the pre-filtration acceptable limit should be chosen to ensure that batches that pass the acceptance criterion would have bioburden levels that would not exceed the retention capabilities of the final sterilizing-grade

filter. It is of great interest to determine such limit with a pre-specified probability of assurance.

4. A Risk-Based Approach

Traditional drug manufacturing process development was compliance-driven, aimed at demonstrating that the process and product are capable of meeting regulatory requirements through testing, such as having a pre-filtration bioburden level of no more than 10 CFU/100 mL. However, a single bioburden test does not provide adequate sterility assurance in and by itself, but rather is one integral piece of the overall microbial control strategy. Multiple bioburden and sterility testing points throughout the manufacturing process (with appropriate acceptance limits) build confidence in the sterility assurance of the final drug product. Conversely, failing a pre-filtration test may not necessarily confirm a drug product quality or safety issue, especially when the sterile filtration process has been demonstrated and validated to be effective for microbial retention even at high levels of bioburden such as 10^7 CFU/cm^2 of filter surface area as required in the FDA guideline (3). In recent years, there has been a shift in regulatory thinking towards a risk-based process development paradigm that yields high product quality through increased scientific understanding of the process, establishment of a "design space", and implementation of control strategies appropriate to the stage of process development. The development of an appropriate bioburden control and pre-filtration testing strategy, including justification of an appropriate test sample volume and acceptance limit, should be driven by product and process knowledge. For example, for an antimicrobial product, the risk of microbial survival or proliferation may be low, even if there is breakthrough of bioburden. Likewise, the risk may be low because the overall quality systems have been shown to be effective in controlling bioburden throughout the manufacturing process. As such, the EMA pre-filtration bioburden limit of 10 CFU/100 mL may not be necessary when the overall bioburden control strategy, drug product attributes, and manufacturing process capabilities are considered together. A risk-based approach is consistent with the new FDA initiative, *Pharmaceutical Current Good Manufacturing Practices (cGMPs) for the Twenty-first Century* (11), intended to enhance and modernize pharmaceutical manufacturing and product quality. It is also in accordance with the quality by design (QbD) principles in ICH Q8 (12) that enable manufacturers to define a manufacturing process design space that consistently produces high-quality drug products through increased product and process knowledge.

4.1. Definition of Risk

In this section, through a risk-based, statistical analysis, a relationship is established between manufacturing process parameters and the risk of bioburden penetrating through the final filter. There are two types of risk associated with the sterile filtration process: (1) the drug solution to be sterile-filtered contains an unacceptable bioburden level, but it passes the pre-filtration bioburden test due to inherent test method variability (i.e., test sensitivity) and (2) breakthrough of bioburden through the final sterile filter. The risks are hereby referred to as the pre-filtration and final post-filtration risks and denoted as R_{pf} and R_{ff}, respectively. These risks are coupled to each other in the sense that a high pre-filtration bioburden risk increases the final post-filtration risk. Both types of risk are defined by their probabilities of occurrence. Specifically, R_{pf} is the probability for the unfiltered drug solution with an unacceptable level of bioburden to pass the pre-filtration test, and R_{ff} is the probability for at least 1 CFU of bioburden to pass through the final sterilizing-grade filter given the drug solution passed the pre-filtration test. To facilitate the method development, we introduce the following notations:

V—Pre-filtration test sample volume (mL)

D—Bioburden level in the unfiltered drug solution (CFU/100 mL)

D_0—Maximum acceptable level of bioburden in the unfiltered solution (CFU/100 mL)

AL—Acceptance limit of pre-filtration bioburden (CFU/V mL)

S—Batch size (mL) of unfiltered solution

A—Effective filter surface area (cm^2)

RC—Retention capacity of filter (CFU)

Pr—Probability of an event

X—Number of CFU in an unfiltered test sample of volume V

Y—Number of CFU in the drug solution after the final filtration

By definitions of R_{pf} and R_{ff}, we have

$$R_{pf} = \Pr[X \leq AL | D > D_0] \quad (1)$$

and

$$R_{ff} = \Pr[Y \geq 1 | X \leq AL]. \quad (2)$$

It is worth noting that R_{pf} is not only dependent on sample volume V and acceptance limit AL of the pre-filtration acceptance testing, but also the unknown bioburden level D. The pre-filtration bioburden evaluation is a form of acceptance testing devised to ensure the bioburden level in the drug solution before the final filtration is below an acceptance limit. Because 100% bioburden testing of a batch of product is impractical, there is a risk of either accepting a batch of a bioburden level exceeding the acceptance limit or rejecting a batch of a bioburden level below the acceptance limit, based on the test result of a sample from the batch. The former is known as *consumer's risk* and the latter *producer's risk* (13). Potential measures to control these risks will be discussed in detail in a later section.

4.2. Acceptable Test Schemes

Because the pre-filtration bioburden test scheme, characterized by sample volume (V) and acceptance limit (AL), and final filtration impact synergistically on the risk, it will be shown that a pre-filtration test plan should be made in relation to risk factors including batch size and retention capacity of the sterilizing-grade filter. A test plan is considered to provide adequate sterility assurance if the sampling volume V and acceptance limit AL are chosen such that

$$R_{pf} \leq \delta_0 \quad (3)$$

and

$$R_{ff} \leq \delta \quad (4)$$

where δ_0 and δ are two pre-specified, small positive numbers serving as bounds on the respective risks. In acceptance sampling testing, δ_0 is often chosen to be either 5% or 1%.

On the other hand, selection of δ is conceivably product-specific and can be accomplished through a risk assessment. For example, if the product has antimicrobial properties, tolerance to the risk of having low level of bioburden in the filtered drug solution might be high. In such case, a relative larger value of δ can be used. Typically, because it is seldom that more than 100,000 or even 10,000 batches of a product are released during its lifetime, $\delta = 10^{-4}$ or 10^{-5} would be a reasonable choice. For instance, suppose that there are a total of no more than 10,000 batches of the product produced during the life cycle of the product. The expected number of batches which that have at least 1 CFU break through the final filter is given by $10{,}000R_{ff}$, which is no more than 1 because $R_{ff} \leq 10^{-4}$. Therefore for this particular product, $\delta = 10^{-4}$ is an appropriate risk bound.

4.3. Modeling Pre-Filtration Bioburden

4.3.1. Poisson Model: If the bacteria are uniformly distributed in the unfiltered drug solution, the number of CFUs, X, in a given volume V can be described through a Poisson distribution (14):

$$f(x|\lambda) = \frac{\lambda^x e^{-\lambda}}{x!} \quad (5)$$

where $\lambda = DV$ is the bioburden level or the average number of CFUs in a sample of test volume V.

4.3.2. Poisson Model with Overdispersion: Under the Poisson distribution, the bacteria are distributed uniformly throughout the bulk volume. However, in reality this is seldom true. The phenomenon that variability in the observed number of bacteria from a given sample is larger than under the Poisson assumption is called *overdispersion* and can be modeled using a negative binomial distribution (15) with density function given by

$$g(x|\lambda, k) = \frac{\Gamma(1/k + x)(k\lambda)^x}{x!\Gamma(1/k)(1 + k\lambda)^{1/k+x}} \quad (6)$$

where $\lambda > 0$ and $k > 0$, and k is an overdispersion factor. The distribution of X has a mean λ and variance $\lambda(1 + k\lambda)$. It is evident that the variance is greater than mean, whereas they are equal for the Poisson distribution. It is interesting to note that when $k \to 0$ $g(x|\lambda, k) \to \frac{\lambda^x e^{-\lambda}}{x!}$ (16). This implies that X approximately follows a Poisson distribution with mean microbial count of λ when the bioburden in the drug solution is approximately uniformly distributed.

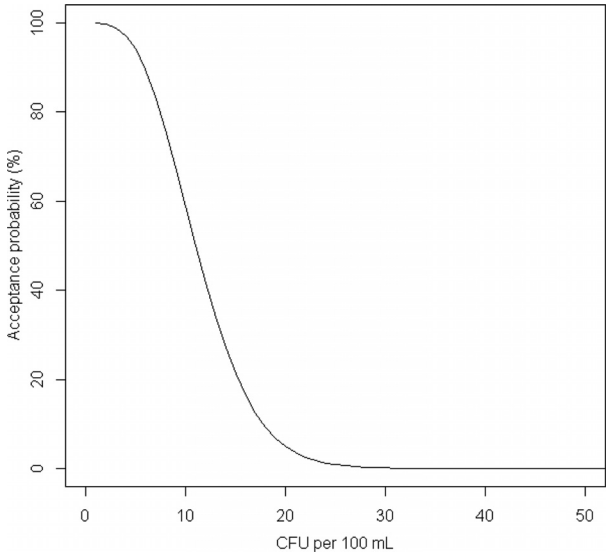

Figure 1

Performance characteristics of EMA-recommended test scheme, based on negative binomial distribution with a dispersion factor of 2.

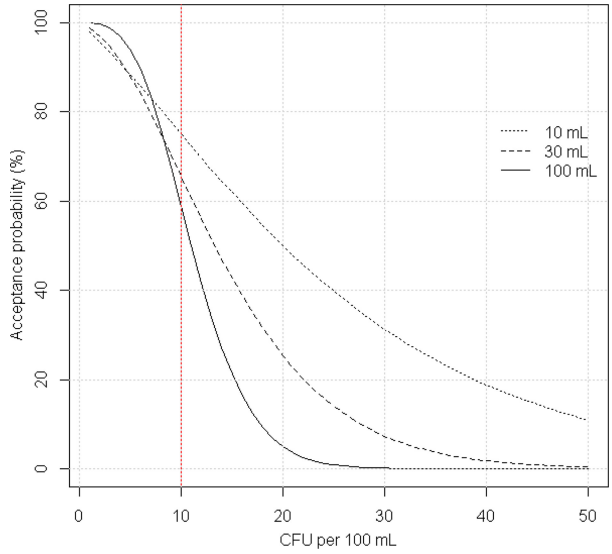

Figure 2

Performance characteristics of three different test schemes, based on negative binomial distribution with a dispersion factor of 2.

4.3.3. Performance Characteristics of Various Test Schemes: In the present paper, the negative binomial model is used because it is a more realistic model to describe bioburden data. It is of interest to understand the performance characteristics of the EMA-recommended test scheme consisting of a sample volume of 100 mL and acceptance limit of 10 CFU/100 mL. For the purpose of illustration, the over-dispersion parameter k is so chosen that the variance of the negative binomial distribution is twice as large as its mean. In practice, using the fact that mean and variance of the negative binomial distribution are λ and $\lambda(1 + k\lambda)$, respectively, k can be estimated by $(\hat{\sigma}^2 - \hat{\lambda})/\hat{\lambda}$ where $\hat{\sigma}^2$ and $\hat{\lambda}$ are sample mean and variance. Based on the acceptance limit 10 CFU/100 mL in the EMA guidelines, the probability of accepting a batch with bioburden level D is given by $\Pr[X \leq AL|D]$, which is depicted in Figure 1.

It can be calculated that the probabilities of accepting batches of bioburden levels of 9 CFU/100 mL, 10 CFU/100 mL, and 11 CFU/100 mL are 66.6%, 58.8%, and 50%, respectively. Therefore if the true bioburden level is 9 CFU/100 mL, there is 33.4% chance to reject the batch per the above acceptance limit. On the other hand, if the true bioburden level is 11 CFU/100 mL, the chance to accept the batch is 50%. The results suggest the EMA-recommended acceptance limits provides protection to neither the consumer's risk nor the producer's risk.

It is also of interest to evaluate performance characteristics of alternative test schemes. Two schemes which are often used in pre-filtration testing consist of $(V, AL) = (10\text{ mL}, 1\text{ CFU}/10\text{ mL})$ and $(30\text{ mL}, 3\text{ CFU}/30\text{ mL})$, respectively. We refer them as Schemes 1 and 2, and the EMA-suggested test plan as Scheme 3. Let (V_i, AL_i) $(i = 1, 2, 3)$ denote their respective sample volume and acceptance limit. The performance characteristic of Scheme i is the probability, P_i, that the sample of volume V_i passes the acceptance criterion of Scheme i given the bioburden level of the drug solution is D. That is,

$$P_i = \Pr[X \leq AL_i|D]. \quad (7)$$

where X is the test result, which follows a negative binomial distribution. The performance characteristic curves P_i of the above three test schemes are estimated and plotted over the range 0–50 CFU/100 mL of the bioburden level in Figure 2. It is interesting to note that when the bioburden level is less than 7 CFU/100 mL, Scheme 3 has slightly higher acceptance probability than Schemes 1 and 2, and vice versa when the bioburden level is greater than 7 CFU/100 mL. At bioburden level of 10 CFU/100 mL, the acceptance probabilities for Schemes 1, 2,

and 3 are 58.8%, 65.7%, and 75.0%, respectively. The biggest difference in acceptance probability between Scheme 1 and Scheme 2 (3) is observed at the bioburden level 10 CFU/100 mL. The difference becomes smaller as the bioburden level gets larger.

4.4. Modeling Post-Filtration Bioburden

According to the FDA guideline 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) (3). The validation should be conducted under the worst-case production conditions for the material to be filtered, and the 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. In practice, accumulation of bioburden on the final filter might 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 the filter later. Therefore it is conservative to assume that all bioburden CFUs in the solution have the same probability to go through the final filter as a single CFU, when it is the only CFU in a solution passing through the final filter. We denote this probability for a single CFU to penetrate the final filter as p_0.

Let n be the total number of CFUs in the drug solution which passes the pre-filtration test, and thus is viewed as having a bioburden level D no greater than D_0. Hence

$$n \le (S - V) \times D_0 \tag{8}$$

where $S - V$ is the difference between the batch size S and the sample volume V used for the pre-filtration bioburden test. Therefore the total number of CFUs, Y, in the drug solution after the final filtration can be modeled using a binomial distribution:

$$\Pr[Y = y | X \le AL] = \frac{n!}{(n-y)!y!} p_0^y (1-p_0)^{n-y} \tag{9}$$

where $0 \le y \le n$.

4.5. Risk Control

4.5.1. Pre-Filtration Risk Control: As shown in Figure 2, when the total number of pre-filtration bioburden CFUs X is modeled using negative binomial distribution, $f(D) = \Pr[X \le AL|D]$ is a decreasing function of the pre-filtration bioburden level D. For a pre-selected δ_0 ($0 < \delta_0 < 1$), we choose D_0 such that

$$f(D_0) = \Pr[X \le AL|D_0] = \delta_0. \tag{10}$$

Hence

$$R_{pf} = \Pr[X \le AL | D > D_0] = f(D) \le f(D_0) = \delta_0. \tag{11}$$

In other words, for a given bound of risk bound, the pre-filtration risk R_{pf} can be controlled with a properly selected bioburden level D_0. It is true that D_0 may be greater than 10 CFU/100 mL. However, its impact on the final post-filtration risk will be mitigated through placing a limit on the size of a batch for the final filtration so as to ensure the total bioburden in the batch would not exceed the retention capacity of the final sterilizing filter.

4.5.2. Final Post-Filtration Risk Control: We let N be the total number of CFUs used in the filter validation study, and n_1 be the number of CFUs that penetrate the filter. Because the FDA guidance requires that a challenge concentration of at least 10^7 CFU/cm^2 be used, resulting in no passage of the challenge microorganism, we have

$$N \ge A \times 10^7 \text{ and } n_1 = 0 \tag{12}$$

where A is the area of the filter. As a result, a one-sided $(1 - \alpha)100\%$ exact confidence interval of p_0 can be constructed, using the Clopper-Pearson method (17):

$$p_0 \le \left[1 + \frac{N - n_1}{(n_1 + 1)F(\alpha, 2(n_1+1), 2(N-n_1))}\right]^{-1}$$

$$= \frac{F(\alpha, 2, N)}{N + F(\alpha, 2, N)} \equiv p_1 \tag{13}$$

where $F(\alpha, 2, N)$ is the $100(1 - \alpha)^{th}$ percentile of an F distribution with degrees of freedom of 2 and N. By eqs 2 and 9 and 13, we obtain the risk of having at least one CFU in the filtered drug solution given the drug solution passed the pre-filtration acceptance test:

$$R_{ff} = \Pr[Y \geq 1 | X \leq AL].$$

$$= 1 - \Pr ob[Y = 0 | X \leq AL]$$

$$= 1 - (1 - p_0)^{(S - V) \times D}. \quad (14)$$

Let

$$R_{ff}^0 = 1 - (1 - p_1)^{(S - V) \times D_0}. \quad (15)$$

Note that R_{ff} is an increasing function of p_0 and D. Because $X \leq AL$, the bioburden level D is deemed to be no greater D_0. The fact that $D \leq D_0$, coupled with eqs 13–15, makes it apparent that R_{ff}^0 is an upper bound of R_{ff} with a confidence level greater than or equal to $(1 - \alpha)100\%$:

$$R_{ff} \leq R_{ff}^0. \quad (16)$$

For a given safety margin δ $(0 < \delta < 1)$, in the following we demonstrate that R_{ff}^0 can be bounded by choosing batch size S_0 such that

$$1 - (1 - p_0)^{(S_0 - V) \times D_0} = \delta. \quad (17)$$

Solving eq 17 for S_0, we obtain

$$S_0 = V + \frac{\ln(1 - \delta)}{D_0 \ln(1 - p_1)}. \quad (18)$$

Combining eqs 16–18 and noting R_{ff}^0 in eq 15 is an increasing function of S, for $S \leq S_0$ we have

$$R_{ff} \leq R_{ff}^0 = 1 - (1 - p_1)^{(S - V) \times D_0} \leq 1 - (1 - p_0)^{(S_0 - V) \times D_0} = \delta. \quad (19)$$

In other words, the bioburden breakthrough risk can be controlled by placing a limit on the batch size.

Inequality eq 16 establishes a relationship between the upper limit R_{ff}^0 of the risk, R_{ff}, of bioburden passage through the final filter and pre-filtration test sample volume V, acceptance limit AL, maximum acceptable bioburden level D_0 in the unfiltered drug solution, batch size S, and effective filter area A. Because the influence of the batch size and pre-filtration bioburden level is exerted on R_{ff}^0 through the maximum total bioburden $n = (S - V) \times D_0$ in the unfiltered drug solution, different combinations of batch size S and bioburden level D_0 may result in the same effect on R_{ff}^0. For example, two solutions with batch size and bioburden level $(S + V)$, $D_0/2$) and $((S/2 + V)$, $D_0)$ essentially have the same R_{ff}^0. The above observation renders manufactures some flexibility in setting up pre-filtration bioburden acceptance limits.

5. Evaluation of Pre-Filtration Test Schemes

In this section, we apply the risk-based approach developed in Section 4 to assess the feasibility of using the alternative test schemes, namely, Scheme 1 and Scheme 2 corresponding to (V, AL) = (10 mL, 1 CFU/10 mL) and (30 mL, 3 CFU/30 mL), respectively, in lieu of the EMA-recommended scheme corresponding to (V, AL) = (100 mL, 10 CFU/100 mL). The maximum acceptable bioburden level D_0 is determined for $\delta_0 = 5\%$, 1%, and 0.1%, using the negative binomial distribution whose variance is twice as large as its mean. The final filtration filter is assumed to be of sterilizing grade, with an effective filter area of 1000 cm^2 and a microbial retention capacity of $\geq 10^7$ CFU/cm^2. The maximum allowable batch size S_0 is calculated for $\delta = 10^{-4}$ and 10^{-5} in combination with various values of δ_0 and test schemes. Again, for the purpose of illustration, we choose the overdispersion parameter k in the negative binomial model in eq 1 to render the variance of the distribution to be twice as large as its mean. In practice, k needs to be empirically determined based on bioburden data from a specific drug. The results are presented in Table I.

It is evident that the maximum batch size is dependent on test scheme after the bounds on risks (i.e., risk tolerance) are selected. For example, when $\delta_0 = 5\%$ and $\delta = 10^{-4}$, the maximum batch sizes are 521, 1016, and 1666 L for the test schemes (V, AL) = (10 mL, 1 CFU/10 mL), (30 mL, 3 CFU/30 mL), and (100 mL, 10 CFU/100 mL), respectively. In general, the maximum allowable batch size decreases as the risk decreases. As described previously, the risk bounds, δ_0 and δ, are upper limits of the probability for a solution of unacceptable level of bioburden to pass the pre-filtration test, and the probability for at least 1 CFU to break through the final sterilizing filter, respectively. Smaller probability values offer a higher degree of confidence, or equally, a lower tolerance for risk. Note that when $\delta_0 = 5\%$ and $\delta = 10^{-4}$, there is a 95% probability that the pre-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. By decreasing the maximum batch

Table I
Maximum Batch Sizes Based on Risks and Pre-Filtration Test Schemes

Risk Bound		Pre-filtration Test Scheme		Maximum Bioburden D_0 (CFU/100 mL)	Maximum Batch Size S_0 (L)
Pre-filtration δ_0	Final Post Filtration δ	Sample Volume V (mL)	Acceptance Limit AL (CFU/V)		
5%	10^{-4}	10	1	63	521
		30	3	32	1016
		100	10	20	1666
	10^{-5}	10	1	63	52
		30	3	32	101
		100	10	20	166
1%	10^{-4}	10	1	91	365
		30	3	43	762
		100	10	24	1360
	10^{-5}	10	1	91	36
		30	3	43	76
		100	10	24	136
0.1%	10^{-4}	10	1	128	259
		30	3	58	572
		100	10	30	1103
	10^{-5}	10	1	128	25
		30	3	58	57
		100	10	30	110

size, Schemes 1 and 2 can achieve the same level of sterility quality assurance for any given δ as the EMA-recommended test plan.

6. Discussion

Through statistical modeling, we introduce a risk-based approach to determining pre-filtration bioburden testing schemes and establishing acceptance limits. Risks associated with realistic test schemes are evaluated along with the sample test plan stated in the EMA guidelines. It is shown that by limiting batch size, it is possible to use test volumes and acceptance limits that differ from 10 CFU/100 mL, without compromising sterility assurance. The maximum allowable batch size is calculated to ensure that the upper confidence limit of the risk associated with bioburden passing through the final filter is capped by an acceptable bound. Therefore, the determination of an appropriate risk bound at a desired level of confidence enables selection of a justifiable bioburden test volume and acceptance criterion. Such risk bound can be obtained based on product and process understanding.

There are several other factors that can be controlled to further mitigate bioburden risk or to maintain the same risk. For example, increasing the filter surface area will decrease the risk at the same batch size. Alternatively, by doubling the filter area from 1000 to 2000 cm^2, the maximum batch size of the test scheme corresponding to (V, AL) = (30 mL, 3 CFU/10 mL) increases from 101 L to 202 L without exceeding the risk bounds $\delta_0 = 5\%$ and $\delta = 10^{-4}$. Likewise, utilization of pre-filters or redundant sterile filters can also be used to further decrease the risk. The proposed method establishes the linkage between bioburden risk and sterile filtration process parameters, such as filter area. Therefore, it can be potentially used to establish a "design space" that defines the range for process parameters, with the assurance that change of the parameters within the range will not cause bioburden safety concerns. Such a "design space" (quality by design, QbD) concept, coupled with quality risk management (QRM), is consistent with regulatory thinking and expectations. Application of QbD and QRM requires thorough scientific understanding of the manufacturing process and associated process risks but sup-

ports increased manufacturing robustness and agility, while maintaining product quality standards.

Lastly, overdispersion is a common phenomenon in Poisson modeling. In this paper we use negative binomial model to account for possible overdispersion, In practice, testing approaches such as the Wald test, likelihood ratio test, and quasi-likelihood test for overdispersion are available, and overdispersion factor k can also be estimated based on historical data (14, 15, 18). After a proper model is validated, the calculations done in Section 4 can be repeated to determine the maximum allowable batch size for the drug solution.

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