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Sterilization of medical devices — Microbiological methods —

Part 3: Guidance on evaluation and interpretation of bioburden data

Stérilisation des dispositifs médicaux — Méthodes microbiologiques —

Partie 3: Lignes directrices sur l'évaluation et l'interprétation de données de charge biologique

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 11737-3 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

ISO 11737 consists of the following parts, under the general title *Sterilization of medical devices — Microbiological methods*:

— *Part 1: Estimation of population of microorganisms on products*

— *Part 2: Tests of sterility performed in the validation of a sterilization process*

— *Part 3: Guidance on evaluation and interpretation of bioburden data*

Introduction

International standards for the validation and routine control of sterilization processes have been published (ISO 11134, ISO 11135, ISO 11137, ISO 14160 and ISO 14937). These standards specify that the bioburden, i.e. the population of microorganisms present on product, be estimated during validation and that the routine control of the sterilization process include a programme of bioburden monitoring. These requirements are specified because it is important that the microbiological quality of product presented for sterilization is consistent over time, and that the level of microbiological contamination is as low as practicable taking into account the nature of the raw materials, the product itself and the processes involved in manufacture. ISO 11737-1 specifies requirements for the estimation of bioburden.

The natural microbial population on and/or in product items is the challenge to the sterilization process. Bioburden estimations, performed as part of validation, provide information about this challenge. The results of performing bioburden estimations may be used in the determination of the extent of treatment to be applied in the sterilization process (see, for example, ISO 11137). However, such application of bioburden data is particular to the method of sterilization and, therefore, is not considered in this part of ISO 11737.

The estimations performed as part of routine control are intended to detect changes in bioburden in terms of the number of contaminating microorganisms and/or the types of microorganisms present. Bioburden data are an element of the system of monitoring the effectiveness of controls applied to manufacturing processes and to the environment in which medical devices are manufactured; as such, bioburden data are part of the quality records within the quality management system (see ISO 13485). In the context of a quality management system, bioburden estimations may be an element of one or more of the following:

— an overall environmental monitoring system;

— an assessment of the effectiveness of a cleaning process in removing microorganisms;

— a programme for monitoring a manufacturing process for product supplied non-sterile but for which a level of microbiological cleanliness is specified;

— a programme of monitoring the microbiological quality of raw materials, components or packaging.

This part of ISO 11737 is intended to provide guidance only on the evaluation and interpretation of bioburden data in routine control and monitoring. The guidance given here is additional to that provided in ISO 11737-1:1995, Annex A.

Sterilization of medical devices — Microbiological methods —

Part 3:
Guidance on evaluation and interpretation of bioburden data

1 Scope

This part of ISO 11737 provides guidance on evaluating and interpreting the data generated during routine monitoring of the microbiological quality of medical devices.

This part of ISO 11737 is not applicable to the use of bioburden data generated for establishing the extent of treatment to be applied in a sterilization process.

This part of ISO 11737 is not applicable to microbiological data generated from sampling the environment in manufacturing areas.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11737-1:1995, *Sterilization of medical devices — Microbiological methods — Part 1: Estimation of population of microorganisms on products*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11737-1 apply.

NOTE The term bioburden as used in this document may include pre-sterilization count, viable count, or bioburden estimate.

4 Origins of bioburden and bioburden control

4.1 Origins of bioburden

4.1.1 Contributors to bioburden include

— raw materials (of synthetic or natural origin),

— manufacturing of components (e.g. moulding, casting or hand cutting),

— assembly process (manual, automated or a combination of these),

— manufacturing environment,

— assembly/manufacturing aids (e.g. compressed air, water, lubricants),

— cleaning process, and

— packaging of finished products (manual or automated).

4.1.2 In some cases, the most significant contributor to product or component bioburden is a raw material. Raw materials of natural origin often have a high bioburden exhibiting a great deal of variability. A raw material of natural origin can also introduce microorganisms that generally are not present on medical devices. Synthetic raw materials usually have lower and less variable bioburden. If the raw material can support microbial growth, this can significantly influence the bioburden.

4.1.3 The method of manufacture of components can have an impact on their bioburden. The hand cutting of material into components or the manual handling of materials contributes to bioburden and may increase variability. Moulding under high temperature and pressure on the other hand can produce components that have low bioburden. Bioburden on such moulded components is usually the result of handling or exposure to the environment. If this is controlled, the bioburden will remain low with little variability.

4.1.4 Product and component handling during assembly and other production activities, such as inspection, have been identified as being a major contributor to product bioburden. Automated assembly processes have been shown to produce products with lower bioburden, and less variable bioburden than manual assembly processes. Manual assembly results not only in higher bioburden but also in greater variability. If the assembly process is complex, the bioburden can increase, due to the length of exposure to the manufacturing environment and the number of steps in which bioburden could be introduced.

4.1.5 The manufacturing environment and the extent of control of that environment can have an impact on bioburden. Prolonged exposure of components or products to an uncontrolled environment can be a significant contributor to high levels of bioburden. If the manufacturing environment and practices do not provide barriers between the product and an uncontrolled environment, the product bioburden can show shifts in numbers and types (e.g. genus, and species or morphological state, such as vegetative versus spores) due to climatic and seasonal changes. Work surfaces in the manufacturing environment can also accumulate microorganisms that can be transferred to components and products during assembly.

4.1.6 Assembly aids, such as compressed gasses, water, lubricants, etc., can be a source of bioburden, and their use can result not only in increased levels but also in large variability. If these assembly aids support microbial growth, the level of bioburden and its variability can increase. A final cleaning step prior to packaging can reduce both the overall level of bioburden and the variability. If the cleaning process leaves a residue in/on the product, however, the opposite effect can occur, resulting in increased bioburden and variability.

4.1.7 Just as with automated assembly, automated packaging will contribute fewer organisms and there will be less variability than with manual packaging. Packaging components of plastics or nonwoven synthetic materials are generally not substantial contributors of bioburden. However, if paper products are part of the primary package, they may have a higher bioburden than the product being packaged.

4.2 Nature of bioburden data

4.2.1 The examination of estimates of bioburden derived from a wide range of products illustrates the variability of bioburden data. Estimates obtained from a group of items will vary within the group of items, and, therefore, analyses of bioburden data generally use means. Clearly, these means may take high, intermediate or low values, and mean values will vary over time. Furthermore, the types of microorganism that comprise the bioburden can also vary.

4.2.2 A commonly observed characteristic of the frequency distributions of bioburden data is that distributions are extremely skewed and frequently show extremely long tails. For low or intermediate bioburden data, the modal value is zero. In these circumstances, the bioburden estimate is generally low but there may be occasional high estimates, even though the control measures are effectively applied.

4.2.3 The extreme asymmetry of these skewed frequency distributions means that the established techniques of quality control based on symmetric distributions are not always appropriate. If statistical analysis of bioburden data is carried out, special statistical techniques may have to be developed for individual cases, either

a) using transformation techniques to make the distribution of the data symmetrical and applying standard techniques, or

b) developing a new technique specifically suited to a skewed distribution.

4.3 Monitoring of bioburden

4.3.1 A bioburden monitoring programme does not control the bioburden on raw materials, components or products. Generally, control is obtained through implementation of appropriate operational measures. A monitoring programme is a means of assessing the effectiveness of the control measures in place. In order to implement effective measures, it is necessary to know the bioburden contributors in a process. A bioburden monitoring programme that identifies the major contributors to bioburden can provide meaningful data on where to apply control measures. Once the control measures are in place, their continued effectiveness can be confirmed by periodic monitoring of products and/or components.

4.3.2 The monitoring programme should include product manufactured using established operational controls. The same types of sampling equipment and media as those identified during the validation of the bioburden determination should be used to provide consistency for the generation of data.

4.3.3 The monitoring programme should be defined in a standard procedure. In developing this procedure, the following should be included:

a) sample size and rationale for its choice;

b) sampling frequency based on time (e.g. monthly or quarterly) or on production schedule (e.g. every third batch); and

c) acceptable limits and the actions to be taken if a limit is exceeded.

5 Sample size

It is common practice to use a sample size of between 3 and 10 product items for routine monitoring of bioburden levels. A rational choice of sample size primarily depends upon two factors:

a) The magnitude of change in bioburden level to be detected.

This will depend upon the consequences associated with a change (either increase or decrease) in bioburden level and how the bioburden information is being applied. For early detection of a small change in the mean bioburden level, a larger sample size may be needed.

b) The variation in estimates of the number of viable microorganisms present on individual items.

The degree of this variability will determine the sample size necessary to detect a given change. Small item-to-item variation in such estimates will require a smaller sample size to detect a change than is required for large item-to-item variation.

Table 1, provided as an illustration only, demonstrates how sample size and variability of bioburden affect the ability to detect a given change in the magnitude of bioburden. Clearly, large sample sizes provide increased confidence in detecting significant changes.

It should be recognized that the use to which bioburden data is put could influence the desired level of confidence in detecting a change of a given magnitude. A rational choice of the magnitude of change to be detected and the probability of achieving that detection should be made.

Table 1 — Probability of a Shewhart Control Chart for bioburden data detecting a tenfold change in bioburden level with changing sample size and within-sample variability

Sample size	Within-sample variability							
	Std. dev. = 0,3	Std. dev. = 0,4	Std. dev. = 0,5	Std. dev. = 0,6	Std. dev. = 0,7	Std. dev. = 0,8	Std. dev. = 0,9	Std. dev. = 1,0
3	0,997 23	0,908 26	0,678 71	0,454 92	0,299 57	0,201 88	0,141 08	0,102 41
4	0,999 88	0,977 25	0,841 34	0,630 56	0,443 20	0,308 54	0,218 35	0,158 66
5	1,000 00	0,995 20	0,929 51	0,766 32	0,577 06	0,418 82	0,303 11	0,222 45
6	1,000 00	0,999 11	0,971 22	0,860 48	0,691 21	0,524 66	0,390 37	0,290 98
7	1,000 00	0,999 85	0,989 03	0,920 67	0,782 20	0,620 65	0,475 97	0,361 58
8	1,000 00	0,999 98	0,996 06	0,956 74	0,850 97	0,703 86	0,556 74	0,431 89
9	1,000 00	1,000 00	0,998 65	0,977 25	0,900 73	0,773 37	0,630 56	0,500 00
10	1,000 00	1,000 00	0,999 56	0,988 41	0,935 43	0,829 67	0,696 25	0,564 46
11					0,958 90	0,874 06	0,753 37	0,624 24
12					0,974 34	0,908 26	0,802 06	0,678 71
13					0,984 25	0,934 09	0,842 83	0,727 59
14					0,990 49	0,953 24	0,876 44	0,770 85
15					0,994 34	0,967 21	0,903 77	0,808 66

NOTE Table 1 has been compiled based on the following:

— logarithmic transformation of bioburden estimates;

— use of standard shewhart control charts;

— transformed data follow a normal distribution;

— a tenfold increase in bioburden has occurred;

— the sample mean takes a value greater than the 3σ control limit.

6 Sampling frequency

In order to demonstrate that effective control of microbiological quality has been established and maintained, a programme of monitoring product and/or components should be developed. A rational basis should be chosen for frequency of monitoring, taking into account a variety of factors, including

a) the availability of historical data,

b) the purpose of generating the data,

c) the nature of the manufacturing process,

d) the production frequency for the product,

e) the criticality of detecting bioburden changes in a timely fashion, and

f) seasonal and environmental variations.

For a new product, in the absence of historical information on similar products, the first three batches manufactured should be evaluated to provide a basis for future comparisons.

7 Limit setting

7.1 Acceptable limits are stipulated for allowable bioburden levels in accordance with ISO 11737-1:1995, Clause 8, and a predetermined course of action (see Clause 9) should be followed when limits are exceeded. If corrective actions lead to changes in the process that affect the bioburden level, new bioburden data should be gathered and new limits established for the product when necessary.

7.2 The limits used for monitoring bioburden data are based upon historical data for a product. In the absence of such historical data, tentative limits can be set upon evaluating the first three batches of a given product. Based upon successive test results, these should be re-evaluated after a period of time to verify whether these original limits are appropriate.

7.3 Bioburden data collected for a product may not precisely follow a well-recognized mathematical distribution. In particular, some bioburden data exhibit many zero counts with a few high counts, and a histogram representing such bioburden data will exhibit a long tail to the right. If a mathematical distribution can be fitted to a given set of data, upper limits for the data can then be set accordingly. Thus, an upper probability limit can be chosen (perhaps the 95 % or 99 % probability limit) for the bioburden that should not be exceeded if the production process continues to operate in the same manner as that used to gather the historical data.

7.4 In the absence of a fit to a mathematical distribution, it is possible to apply the principle of control charting to bioburden data and set limits accordingly. The preparation of control charts does not strictly depend upon any underlying assumed distribution. The quantities that are plotted on control charts are the mean and the standard deviation (or range). Means will tend to have a more symmetrical distribution than individual values. Transformation of raw data may further improve the applicability of a standard Shewhart control chart. Experience gained in fitting empirical distributions to bioburden data sets suggests that the following two transformations of individual counts are appropriate.

a) For product with an average bioburden of less than 10 colony forming units (cfu) per unit, the suggested transformation to improve symmetry is:

$$Y = \sqrt{N} \log_{10} \left(\sqrt{x/N} + \sqrt{x/N + 1} \right)$$

where

Y is the transformed count;

x is the original untransformed count;

N is a scaling factor based upon variance, and is equal to mean^2/(variance − mean).

NOTE For those cases where the historical mean exceeds the historical (within-group) standard deviation, ignore the transformation since the constant N will be undefined.

b) For product with an average bioburden exceeding 10 cfu per unit, the distribution could again tend to be skewed with a long tail to the right. In practice, taking logarithms of the individual counts will make the data approximate a normal distribution. Since bioburden counts of zero may be observed, a positive constant of 0,1 should be added to all counts before taking logarithms. The suggested transformation then becomes:

$$Y = \log_{10} (x + 0{,}1)$$

where

Y is the transformed count;

x is the original untransformed count.

Other techniques used for transforming bioburden data might be acceptable.

7.5 Control limits for a standard Shewhart control chart of the average bioburden can be established after a sufficient number of historical counts have been collected for a product. (See ISO 8258 and ISO/TR 7871 for control charting procedures.) If a sufficient number of historical groups are not available for a given product, conditional control limits may be established with fewer values. The limits can be revised when more bioburden data become available for a product. Where appropriate, seasonal variation should be taken into account when setting limits. Any data identified as outliers should be discarded in setting the limits for bioburden monitoring.

Data identified as unusually large or small should be investigated (see Clause 9). If they are determined to be other than laboratory error or the occasional high values found in the manufacturing process, the data may be considered outliers and can be omitted from calculations in setting the limits for bioburden monitoring.