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Irradiation of advanced health care products – Tissues and biologics

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HIGHLIGHTS

- MPN testing can provide good bioburden results for tissue/biologics.
- There are appropriate situations to pool products for bioburden testing.
- Options on dealing with bioburden results of "less-than" the limit of detection.
- Underestimation and overestimation of bioburden and the dangers of both.

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ABSTRACT

Radiation sterilization of tissues and biologics has become more common in recent years. As a result it has become critical to understand how to adapt the typical test methods and validation approaches to a tissue or biological product scenario. Also data evaluation sometimes becomes more critical than with traditional medical devices because for many tissues and biologics a low radiation dose is required. It is the intent behind this paper to provide information on adapting bioburden tests used in radiation validations such that the data can be most effectively used on tissues and biologics. In addition challenges with data evaluation are discussed, particularly the use of less-than values for bioburden results in radiation validation studies.

1. Introduction

As the microbiological requirements and expectations for tissue and biological products are becoming more stringent, causing many of the manufacturers to consider terminal sterilization using radiation. Naturally the tissue and biological product industries look to the International Organization for Standardization (ISO) methods for guidance regarding how to accomplish radiation sterilization. It has been found that, although the same concepts employed for medical devices do apply to tissues and biologics, sometimes the methods or approaches must be modified. Bioburden tests and tests of sterility are among those methods that are frequently altered for use with these products. Often the biggest reason for the alterations is that a low sterilization dose is required for the product in question.

In review of the changes in approach that are necessary with tissues and biologics, there are a few which are higher in importance. Use of more sensitive bioburden test methods such as most probable number (MPN) or sampling pooling are valuable in facilitating the validation of a low sterilization dose. Also the method of interpretation of bioburden data is important; especially regarding the applicability of using "less-than" numbers in establishing radiation doses. These topics are the primary focus of this paper.

2. Bioburden test methods: MPN

The MPN method has been used in other industries as a way to determine bioburden counts. It sometimes is described as not being applicable to medical devices, tissues or biologics, but it is the experience of the author and others who are heavily involved in testing that, under the right circumstances, it can provide reliable and sensitive data. An MPN test can be invaluable in situations where the tissue or biologic cannot be extracted using normal bioburden test methods or where extractions of the product cause insoluble materials to be deposited on the surface of the membrane filter if extraction and filtration are attempted.

One of the primary benefits of MPN is that the bioburden results can be tabulated to less than one CFU. For example if 10 samples are tested, the limit of detection is 0.1 CFU and if 20 samples are tested the limit of detection is 0.05 CFU.

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In order to use the MPN method certain criteria should be in place as follows:

1. The product should not be water soluble. If the product is water soluble then the first choice is to use membrane filtration with the filter placed onto agar for incubation.
2. The bioburden should be uniformly distributed on the individual products. The reason the MPN test is applicable in other industries is because the product and manufacturing processes are such that the microorganisms are deposited on or are present in a uniform fashion. If the microorganisms are present on the individual products with isolated high bioburden areas the MPN test will not function correctly.
3. There should be no bioburden "spikes" within a batch of product. The bioburden should be randomly distributed among the samples in a batch of product.

It is sometimes assumed that the product bioburden must be low in order to use MPN but that is not the case. Sample item portions (SIPs) of the products can be used for an MPN under the right circumstances.

Functionally an MPN test is a test of sterility performed on non-sterilized product. The concept and equation are taken from the Stumbo–Murphy–Cochran Fraction Negative *D*-value calculation (provided in ISO 14161, Clause C.3.3). The equation is as follows:

$$\mathrm{MPN} = \ln\left(\frac{\#\ \text{tested}}{\#\ \text{negative}}\right)$$

where # tested is the quantity of samples tested for MPN and # negative is the quantity of samples tested for MPN which were negative for growth after incubation.

For example, if 10 samples were tested for MPN and three were positive for growth (seven were negative for growth) the equation would be

$$\mathrm{MPN} = \ln\left(\frac{10}{7}\right) = 0.36\ \mathrm{CFU}$$

If an SIP of the product was tested, or if multiple products were pooled into each test container, then the resulting number must be adjusted accordingly. If all 10 samples resulted in being positive for growth, a smaller portion of the product must be tested until fractional growth is obtained (i.e. at least one of the test samples is negative for growth).

If all 10 samples are negative for growth the calculation can be performed assuming one positive (nine negatives) in order to make the calculation work. In this case the resulting MPN value must be considered a maximum CFU because the actual CFU count is lower. Alternatively multiple samples can be placed into each test container in an effort to obtain at least one test container which is positive for growth. This concept of testing multiple samples in a single container for an MPN test is essentially a reverse to the traditional MPN test which includes performing dilutions on the test sample.

3. Bioburden test methods: pooling

Pooling of multiple samples into one test is especially helpful when the bioburden count on individual products is known to be low and when a better limit of detection is desired. This approach can be used with either extraction bioburden methods or MPN. The disadvantage is that this approach will mask bioburden "spikes". For example in a bioburden test where 10 samples are pooled into a single container and the result on the membrane filter was 35 CFU, it is unknown whether the 35 CFU were extracted from a single sample or whether approximately 3 CFU were present on each of the 10 samples tested. This means that prior to employing pooling for bioburden testing, data should be available which demonstrates consistent bioburden counts across the samples tested. In this example, the final result of 35 CFU would be divided by 10 to determine the average CFU per sample tested (3.5 CFU).

Table 1 contains example of bioburden data where use of a different media type was employed for aerobic bacteria and fungi. When zero CFU were recovered on the membrane filter after incubation the limit of detection was used as the bioburden count with a "<" symbol. Under the circumstances provided in Table 1 it is appropriate to pool samples together, if desired, in order to obtain a more accurate view of the actual bioburden count. Note however that a sample size of 10 (as shown below) from a single batch might not be sufficient to justify pooling samples in the future.

Table 2 contains example of bioburden data where the test was performed as described above. Under these circumstances it would not be appropriate to pool samples together due to the higher individual sample counts which would be masked by pooling.

in situations where the bioburden is very low, a pooled bioburden result can result in a number as low as 0.1 CFU per sample or even lower. This is beneficial because many of the dose establishment tables (i.e. Method 1 and some VDmax tables) allow for low sterilization doses when the bioburden can be shown to be 0.1 CFU per sample.

4. Bioburden data evaluation: use of "less-than" values

When performing bioburden testing on tissue and biologics it is common to test for anaerobes. This often increases the factor used

Table 1
Bioburden data where pooling would be appropriate.

Sample	Aerobic bacteria	Fungi	Total bioburden
1	< 4	< 4	< 8
2	4	< 4	< 8[a]
3	< 4	< 4	< 8
4	4	4	8
5	8	< 4	< 12
6	< 4	< 4	< 8
7	4	< 4	< 8
8	< 4	4	< 8
9	4	4	8
10	< 4	< 4	< 8
Average	< 4.4	< 4.0	< 8.4

[a] For purposes of this table, when either the aerobic bacteria or fungi result is a less than number for a particular sample, the less than symbol is carried over to the total bioburden for that sample to show that there is uncertainty in the number.

Table 2
Bioburden data where pooling would not be appropriate.

Sample	Aerobic bacteria	Fungi	Total bioburden
1	< 4	< 4	< 8
2	4	< 4	< 8
3	24	16	40
4	< 4	4	< 8
5	56	8	64
6	< 4	< 4	< 8
7	4	< 4	< 8
8	< 4	4	< 8
9	120	32	152
10	< 4	< 4	< 8
Average	< 22.8	< 8.4	< 31.2