TECHNICAL TIP

THE BASICS OF BIOBURDEN TESTING

What is bioburden?

Bioburden is the quantity and types of native bacterial and fungal flora present on or in a device, substrate, or chemical (test unit). Bioburden plays a large role in determining what is necessary to achieve sterility, and can impact the presence of toxins.

In a medical device or pharmaceutical manufacturing environment, the main contributors of bioburden contamination come from personnel, raw components, lack of environment control (poor cleaning practices, lack of positive pressure and particulate control of environment), or water used in the manufacturing process.

What is bioburden testing:

Bioburden testing is the activity required to determine the microbiological quality or cleanliness of a test unit. Not only is bioburden testing crucial to understanding the number of microbes present on a material, it can also give insight to the comparative resistance of bioburden found on the material.

Bioburden testing for terminally sterilized medical devices is performed according to ISO 11737-1.

Bioburden testing is primarily performed by cutting up, disassembling, or flushing the fluid path of the test unit using sterile tools to prepare the sample. When cutting up and disassembling the test unit, extensive manipulation may be necessary to allow for placement of the sample in a sterile test container (generally a jar or bag) in order to wash (extract) the microorganisms from the test unit. Extensive manipulation has the potential to increase contamination during testing because of the additional handling.

Bioburden testing of substates and chemicals can be quite complicated and will not be discussed in this TechTip. For testing of these categories, please contact your local STERIS laboratory.

The microbial extraction step in bioburden testing:

Once the test sample is placed in the test container, a measured volume of sterile rinsate solution (generally buffered water or buffered water with a surfactant) is added to the test container with the sample.

The test sample may then be agitated in a variety of methods or combination of methods, based on material rigidity and design, to remove microbes from the surface.

Extraction methods utilized include; but are not limited to:

1. Sonication (use of sonic energy to remove organisms from a surface)
2. Mechanical/hand shaking (use of vertical or horizontal agitation to remove organisms from a surface)
3. Vortexing (use of circular agitation with a mechanical vortexer to remove organisms from a surface)
4. Stomaching (use of compressive agitation to remove organisms from a non-rigid, usually absorbant, surface such as textiles)

After the extraction process, the extract fluid may be divided for culturing of different organism types and will generally be assayed by filtration or pour plating.

The plating step in bioburden testing:

Two types of “plating” are available for the bioburden testing.

Filtration plating:

For filtration, the extraction fluid is poured into a filter cup, with a membrane filter that is attached to a filtration manifold. A vacuum pump is used to pull a vacuum and a valve is opened to suction the test fluid through the filter, trapping the bacteria and fungi within the filter material. The filter is then “plated” by placing the filter onto a growth promoting agar and incubated for the incubation times and temperatures specified in the ISO 11737-1 standard.

Extended incubation can be requested if needed, if slow growing organisms are a concern.

FOR MORE INFORMATION

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Pour plating:

Pour plating is used if filtration is not an option due to product debris/particulate, gels, or other items that may clog or degrade the membrane filter. Pour plating is performed by pouring an appropriate amount of extraction fluid into a growth plate directly with tempered media, swirled to mix gently, then allowed to cool for solidification before incubation.

After incubation, the visible colonies on the filter or in the pour plate are counted. The count is then multiplied by any dilution factors, sample item portion (SIP) factor, and a correction factor from the test method validation.

Most Probable Number (MPN):

For products with very low and evenly distributed bioburden, MPN (Most Probable Number) may be another option to determine an estimate of the bioburden population. The test is performed by taking replicate samples and submitting them to tests of sterility. The samples are incubated and scored for presence/absence of growth. The number of samples that are positive for growth is used to calculate the bioburden estimate.

Bioburden data

Results may include indication of "TNTC" or Too Numerous To Count, indicating values outside a countable range. Results may also describe the presence of a spreader, which is a colony that grows over a considerable portion of the counting area and impacts the accuracy of counts.

Bioburden data is expected to be performed on a scheduled frequency determined by either the sterilization standard (ISO 11137) or the manufacturer. The frequency should be justified to ensure the manufacturing process is under microbiological control. The data from testing should be trended, alert and action limits established, and this data reviewed at regular intervals by the manufacturer for action or mitigation. Seasonal variations should also be considered in frequency and monitoring of bioburden data. The quantity as well as the resistance of the organism to the intended sterilization modality should be considered for trending.

The bioburden detection limit can be improved by reducing the parameters tested, if it is reasonable and appropriate to do so. Additionally, dual incubation of plates or pooling of samples can assist in improving bioburden detection. Pooling samples for extraction can affect the ability to see variation of bioburden from sample to sample.

Bioburden considerations:

If raw colony forming units (CFU) counts of greater than 300 CFU are expected, the testing laboratory should be notified in order to proactively plan for performing serial dilutions or aliquots to ensure a countable result.

It is important to communicate with the laboratory regarding portions of the device that are to be considered for testing and those that are not. It is industry expectation that all items located within the sterile barrier system are tested unless otherwise specified and justified. Items such as instruction for use (IFU) packets, packaging such as zip ties, paper ties, inner pouches, labels, carrier hoops, or packaging within the sterile barrier may be selected to be excluded from testing by the manufacturer. Documentation of the justification for excluding materials or items within the sterile barrier system need to be generated by the manufacturer, maintained, and be available for auditing.

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