TECHTIP

Microbiology Testing FAQ

Q1: What actions need to be performed if bioburden test results come back significantly elevated compared to the product's historical/ trend data?

Answer: Routine monitoring of product-specific bioburden levels should be followed by trending and statistical analysis of bioburden data. It is important to establish alert and action levels for product bioburden levels based on evaluation of the product's historical bioburden data.

ISO 11737-1 section 8.0 and appendix A.8 gives detailed guidance on how this is to be achieved successfully. Per ISO 11737-1 section 8.5: A bioburden spike is *"a test result that is significantly greater than other values."* A bioburden spike or any bioburden data identified as atypical of the expected trend should be investigated to decide if the observed bioburden value is a normal and consistent part of bioburden distribution over time and to evaluate the impact, where applicable.

If the established alert and/or action levels are exceeded, a predetermined course of action should be taken. This could include, but is not limited to:

- Review of the product-specific environmental monitoring data
- Implement/review cleaning and disinfection processes in the product manufacturing area
- Review of raw materials supplier used in the product's manufacturing
- Review of the bioburden sampling plan in terms of number of samples or frequency of bioburden determinations
- Review of bioburden and/or environmental microbial characterization to assess the microbial species in order to determine a potential root cause of contamination or potential impacts

For more information on bioburden testing, click here.

Q2: If a product's bioburden test results are high, is there a correlation with endotoxin test results (ie. high endotoxin test levels)?

Answer: High product bioburden test results do not necessarily reflect a direct correlation with endotoxin test results, as an increase in bioburden counts may not lead to an increase in endotoxin levels).

Bacterial endotoxins as detailed in ISO 11737-3 are *"components of the cell walls of Gram-negative bacteria."* This means gram-negative bacteria specifically need to be looked for in the sample to address any concerns about endotoxins. Therefore, raw bioburden counts alone are not enough to indicate that endotoxins are present or increasing in a sample.

Using an identification process, such as Gram Stain or Maldi-ToF, will determine the type of microorganisms present. To further investigate, a test can be performed (i.e., bacterial endotoxin test (BET)), to quantify the levels of endotoxin present in the sample. When performing testing on products, an endotoxin method suitability study should be completed to validate the test method. Once a test method is validated for monitoring and control purposes, the product should be tested at frequencies determined as part of a sampling plan.

For more information on bacterial endotoxin testing, click here.

Q3: Is it possible to determine the exact microorganism from colony morphology alone?

Answer: Unfortunately, it is not possible to reliably identify microorganisms from physical colony morphology alone.

Colony morphology is the most basic level of microbial identifications. It gives a visual description of the colony's growth characteristics such as color, shape, consistence, and edge. This visual description helps classify observed phenotypes when coupled with further identification processes. Colony morphology can offer clues as to the identity of the microorganism, but it cannot provide a reliable identification.

Microorganism morphology can vary significantly between different species within a genus and even between different microorganisms within a species. In addition, the physical characteristics of microorganisms could also be quite similar between different species, making it difficult to distinguish.

Biochemical tests or the use of selective agars, when included as a part of the analysis, may provide more information towards identification, however these tests are labor intensive and their interpretation can be subjective. There is the potential

FOR MORE INFORMATION

STERIS Applied Sterilization Technologies
www.steris-ast.com
Email: ast_info@steris.com
(EMEA-APAC) is +44 330 236 8344
(Americas) +1 877.783.7479

STERIS

TECHTIP

Microbiology Testing FAQ

that another microorganism could have the same physical morphology and response to these types of tests and, therefore, the identity assigned to the microorganism under investigation may be incorrect.

Use of less subjective processes, such as MALDI-ToF or bacterial DNA sequencing, can give a more conclusive identification to genus or species level.

Physical colony morphology can be utilized to distinguish between different types of microorganisms on a sample. This is useful as it allows for fewer identifications to be performed, keeping costs low. Trained personnel can categorize the number of colony types present on a sample and provide information about which colony types are most numerous on the sample. This information can then be used to determine the overall number of isolates to undergo a further identification process.

For more information on microbial identification testing, click here.

Q4: Question: During a periodic dose audit, is bioburden required on 10 items from one lot only or individually from three lots?

Answer: ISO 11137-2 section 10.0 states "*once the sterilization dose has been established; periodic sterilization dose audits shall be carried out to confirm the continued appropriateness of the sterilization dose."* A dose audit confirms that bioburden is under control and that the sterilization dose continues to achieve the required sterility assurance level. Dose audits include bioburden and sterility testing.

As defined by section 10.0 of ISO 11137-2 for periodic dose audits, activities samples should be taken from the same batch.

Reference section 10.2.2 for Method 1, Method 2A, or Method 2B states *"select 110 product items from a single batch of product, in accordance with 5.1, 5.2 (if applicable) and 5.3."* This indicates, 10 samples for bioburden testing and 100 samples for sterility testing.

Reference section 10.3.2 of ISO 11137-2 for Method VD_{max}^{25} or Method VD_{max}^{15} and section 7.3.3.2 of ISO 13004 for Method VD_{max}^{SD} states "*select 20 product items from a single batch of product, in accordance with section 5.1, 5.2 (if applicable) and 5.3."* This indicates, 10 samples for bioburden testing and 10 samples for sterility testing.

Note: A batch is a *"defined quantity of product, intended or purported to be uniform in character and quality, which has been produced during a defined cycle of manufacture."*(ISO 11137-2 Section 3.1.1)

For more information on irradiation dose audits, click here.

Q5: What are the criteria for the numbers of samples selected for EO residual testing and the timeframes for taking these samples post the EO sterilization cycle?

Answer: The ISO 10993-7 standard does not have requirements around the number of samples to be tested for EO residuals. Samples shall be selected in a manner as to be truly representative of the product. STERIS can analyze for ethylene oxide and ethylene chlorohydrin residuals using a single sample. It is typical for Customers to test one sample at a particular timepoint and choose between three – five timepoints. A non-processed "blank" sample should also be sent for EO residual analysis to ensure no other sample matrix components interfere with any of the EO residues present. This blank sample will be analyzed using the same procedure as an EO sterilized sample.

The timepoints at which samples are required to be removed should be documented. ISO 10993-7 states that the residual analysis should be initiated as soon as possible after removal of the sample from the load, or if that is not possible the sample may be frozen for extraction later. However, the standard does not state an exact timeframe.

With regards to determining the minimum aeration time, Customers have the option to use a dissipation curve. When a dissipation curve is used, data should be collated from a minimum of three different sterilization batches with data at different timepoints (minimum of one sample per batch). Seasonal changes may also need to be considered.

For more information on EO residual testing, click here.

FOR MORE INFORMATION

STERIS Applied Sterilization Technologies
www.steris-ast.com
Email: ast_info@steris.com
(EMEA-APAC) is +44 330 236 8344
(Americas) +1 877.783.7479

STERIS

TECHTIP

Microbiology Testing FAQ

Q6: Is there a preferred recommendation for a bioburden validation (inoculation or native repetitive)?

Answer: Inoculation and native repetitive bioburden validation methods have inherent advantages and disadvantages. For products where the bioburden is expected to be high, ISO 11737-1 Annex A (Section A.7 Validation of the Method for Determining Bioburden) recommends that repetitive rinsing of the product's natural bioburden is performed. This method also provides information for organisms that are naturally found on the product through the manufacturing process prior to routine bioburden testing.

Inoculation using bacterial spores is typically used if a product is expected to have a low bioburden. This method is useful for products that have been manufactured in aseptic conditions, as these would usually be typically lower in bioburden. Samples that have been through a bioburden reduction step might also be used for inoculated bioburden method validation testing.

The inoculation method may also provide some insight into a product's inhibitory properties, if inhibitory screening is not carried out as a separate test, but only in terms of the organism selected for inoculation (typically a *Bacillus* species).

For more information on bioburden testing, click here.

Q7: In EO residual testing, is there a need to conduct multiple exhaustive extractions to demonstrate a reduction of 10% if the first 24-hour extraction met limits?

ISO 10997-3 defines an exhaustive extraction as *"extraction until the amount of EO or ECH in a subsequent extraction is less than 10% of that detected in the first extraction, or until there is no analytically significant increase in the cumulative residue levels detected."* Per the definition, **a minimum of two extractions must** be performed to be considered exhaustive.

For more information on EO residual testing click here.

Q8: Can a bioburden test method validation run concurrently with product bioburden testing?

Answer: The validation of a bioburden test method is an important component of the routine bioburden process as it ensures that the test method to be employed will be a more accurate bioburden estimation.

In bioburden testing there are two components of test method validations that should be considered:

1. Recovery Efficiency Test Method Validation

Recovery efficiency test method validation validates the removal of microorganisms from the product to provide a more accurate measurement of bioburden. This test generates a correction factor that can be applied to bioburden raw data which adjusts the bioburden estimate for the efficiency of the method.

2. Adverse/Inhibitory Substances Screening Test Method Validation

Adverse/inhibitory substances screening test method validation validates the neutralization of substances that may be inhibitory or lethal to microbes during the extraction process to ensure the measurement of contamination on/in a product is not impeded/impacted by substances present in the test.

Note: Reference ISO 11737-1 Section 7.2 gives further guidance on the components of a bioburden method validation.

These test methods are typically performed in advance of performing any routine bioburden as they validate that the test method being employed, will efficiently remove organisms from the product, and will not impact their enumeration. Performing the routine testing concurrently may have the appearance of an advantage from a reduced timeline perspective,. However, if the validation test results demonstrate that the method being employed does not give a satisfactory recovery efficiency or that inhibition of the microorganisms present is occurring, then the routine testing would need to be repeated after changes to the method have shown an improvement in the validations. Therefore, performing the validation and routine test concurrently results in the risk of loss of time, additional cost (i.e. cost of testing the repeat routine bioburden), and the requirement for additional samples to perform the routine bioburden testing again if the validation work is unsuccessful.

For more information on bioburden method validation, click here.

FOR MORE INFORMATION

STERIS Applied Sterilization Technologies
www.steris-ast.com
Email: ast_info@steris.com
(EMEA-APAC) is +44 330 236 8344
(Americas) +1 877.783.7479

STERIS

TECHTIP

Microbiology Testing FAQ

Q9: How can the worst case of a product group be determined in bioburden testing?

Answer: The worst-case product of a product group is defined in the ISO standard 11137-2, as the *"Master product."* It is the product that will be used to represent a product family for performance of a verification dose experiment or sterilization dose audit.

As outlined ISO 11137-2 section 4.3 *"A member of a product family shall only be considered a master product if assessment indicates that the member presents a challenge that is greater than that of all other product family members."* However, a greater challenge may be presented, for example, on products in a family with the largest surface area (due to challenge for manipulation), or products in a family where microorganisms are more difficult to detach (due to materials or product design).

To identify a master product of a product family, section 4.3.1.3 of the standard states that the following points for consideration:

a) number of microorganisms comprising the bioburden;

b) types of microorganisms comprising the bioburden;

c) environment in which the microorganisms occur;

d) size of product;

e) number of components;

f) complexity of product;

g) degree of automation during manufacture;

h) manufacturing environment

Note: Data on bioburden quantity and type should be a leading factor when selecting the master product. ISO 11137-2 section 4.3.1.1 states *"The number and types of microorganisms on or in product shall be used as the basis for selecting product to represent a product family."*

For more information on bioburden testing, click here.

Q10: What method is most reliable for identifying organisms, genetic or MALDI-ToF?

Answer: Regulatory bodies recognize both genetic and MALDI-ToF analysis as appropriate and reliable methods for microorganism identification. Both methods can provide a genus and species level identification.

The main difference between these two identification methods is that genetic analysis utilizes the unique DNA sequence from the unknown microorganism, while Matrix Assisted Lasor Desorption Ionization Time-of-Flight (MALDI-ToF) analysis uses the ribosomal protein fingerprint of the microorganism. Both parameters are unique to the microorganism being tested, but they are simply a different component of the organism.

Once the unique sequence of the unknown test organism has been analyzed, that information is compared to data for known microorganisms in a qualified reference library. The software looks for an entry in the reference library database that matches the information for the unknown microorganism. If a high confidence match is found, an identification to genus or genus and species is provided. The criteria for a match are dependent on the validated system used for the analysis.

The main limitation for both genetic and MALDI-ToF methods is the reference library. If the reference library used for the analysis is not extensive, uncommon organisms may not be successfully identified and an "unidentifiable" result will be provided. The databases for genetic method (e.g, NCBI) are significantly more extensive than those available for MALDI-ToF, therefore the potential for an "unidentifiable" result is significantly lower with the genetic analysis. However, genetic testing can be slower and more expensive than MALDI-ToF analysis due to the additional processing steps that are required to get sufficient samples for testing and the specialist processing environment to prevent cross contamination. MALDI-ToF does not require the same level of processing or specialist environment that genetic testing does, which may offer faster identifications with a lower cost per organism.

For more information on microbial identification testing, click here.

FOR MORE INFORMATION

STERIS Applied Sterilization Technologies
www.steris-ast.com
Email: ast_info@steris.com
(EMEA-APAC) is +44 330 236 8344
(Americas) +1 877.783.7479

STERIS

TECHTIP

Microbiology Testing FAQ

Q11: Should bioburden testing be performed pre- or post-sterilization?

Answer: The timing for performance of bioburden testing will be dependent on the intended use of the bioburden data. However, bioburden testing is typically performed on pre-sterilized/unprocessed products to obtain information on number of organisms present after manufacture and prior to undergoing a sterilization/bioburden reduction process.

Examples where testing on pre-sterilized/unprocessed products can provide useful information:

1. To assess the level of control of the manufacturing process itself and data to assist with control of this process, i.e. trending and prevention/corrective actions
2. To indicate the levels of organism to be killed by the sterilization process being used, i.e. radiation verification dose setting or routine radiation dose audits
3. To indicate the potential risks to the sterilization process being used by determining the quantity and/or type of microorganism present on the product
4. To conduct bioburden testing on unprocessed samples as part of investigation work during a non-conformance/out of specification/failure event
5. To examine the impact of a proposed change(s) in the manufacture of the product

Examples where testing on sterilized/processed products can provide useful information:

1. To provide additional evidence for product release, for example a log reduction study (note: this would be required to be completed both pre- and post-sterilization)
2. To examine the impact of a proposed change(s) in the manufacture of the product

Therefore, the timing for performance of bioburden testing should be based on the intended use of the data and how best to obtain this information.

For more information on bioburden testing, click here.

FOR MORE INFORMATION

STERIS Applied Sterilization Technologies
www.steris-ast.com
Email: ast_info@steris.com
(EMEA-APAC) is +44 330 236 8344
(Americas) +1 877.783.7479

STERIS