

# MEDICAL DEVICE STERILITY ASSURANCE



**PBL**  
Pacific BioLabs



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# INTRODUCTION

A common goal of medical device manufacturers is to produce safe products. Sterility is essential to the safety of many medical devices. Most single use devices are terminally sterilized by ethylene oxide gas or radiation. The sterilization process must be validated for each product to verify that it effectively and reliably kills any organisms that may be present on the pre-sterilized product. By means of the cGMP medical device regulations, FDA has established some of the requirements for an acceptable sterility assurance program. More specific guidelines for validation of the sterilization processes are developed and published by AAMI in conjunction with ISO.

The first part of this booklet outlines AAMI/ISO requirements for the validation of ethylene oxide sterilization cycles and radiation sterilization doses. The second part contains more detailed information about tests that are integral to effective quality assurance systems for sterile medical devices. Reusable medical device validations are covered in a separate PBL booklet titled, *Reusable Device Validations*.

We recommend that clients review current AAMI publications which pertain to the method of sterilization used for their products. Some of the more relevant publications are listed below. To obtain these documents, visit the AAMI at [www.aami.org](http://www.aami.org).

- ANSI/AAMI/ISO 11137-1, *Sterilization of health care products—Radiation—Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*
- ANSI/AAMI/ISO 11137-2, *Sterilization of medical devices —Microbiological methods - Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process.*
- ANSI/AAMI/ISO 11737-3, *Sterilization of health care products —Radiation - Part 3: Guidance on dosimetric aspects*
- AAMI TIR 33, *Sterilization of Health Care Products—Radiation-Substantiation of a selected sterilization dose-Method VD<sub>max</sub>.*
- AAMI/ISO 11135, *Sterilization of health care products – Ethylene oxide – Requirements for development, validation, and routine control of a sterilization process for medical devices.*
- AAMI TIR 16, *Microbiological aspects of ethylene oxide sterilization.*

- ANSI/AAMI/ISO 10993-7, *Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residuals.*

The FDA Center for Devices and Radiological Health (CDRH) can provide assistance to medical device manufacturers. The medical device Section of the FDA website ([www.fda.gov](http://www.fda.gov)) has extensive information on a variety of topics, including the following:

- Products and Medical Procedures
- Medical Device Safety
- Device Regulatory Assistance
- Premarket Requirements
- Postmarket Requirements
- Publications
- Medical Device News



# PART 1 – AAMI/ISO EO AND RADIATION STERILIZATION VALIDATION GUIDELINES

## STERILITY ASSURANCE PROGRAM

### PREVALIDATION CHECKLIST

- ❑ Evaluate candidate materials, components and packaging compatibility with ethylene oxide gas, gamma or electron beam radiation sterilization processes. Material manufacturers and contract sterilizers can assist with this evaluation.
- ❑ Select method of sterilization.
- ❑ Screen materials for biocompatibility by *in vitro* methods.
- ❑ Pacific BioLabs offers comprehensive biocompatibility testing services in accordance with FDA, ISO and AAMI Guidelines. For more information, please call for a complimentary copy of Pacific BioLabs' booklet *Assessing Biocompatibility*. For assistance in designing an appropriate biocompatibility testing plan for your device, please call client services at 510-964-9000 or [info@PacificBioLabs.com](mailto:info@PacificBioLabs.com).
- ❑ Manufacture finished devices. Be sure to manufacture enough for both biocompatibility testing of finished devices and your sterilization validation.
- ❑ Select your contract sterilizer and execute a contract sterilization agreement with them.
- ❑ Submit a product sample to Pacific BioLabs for evaluation.
- ❑ Select validation methodology in consultation with contract sterilizer and Pacific BioLabs.
- ❑ Generate sterilization process validation protocols for both physical and microbiological aspects of validation.
- ❑ Send samples for initial phase validation testing to Pacific BioLabs, i.e. bioburden method validation, bioburden, sample item portion preparation (if required), bacteriostasis/fungistasis test.
- ❑ Schedule physical validation (e.g. dose map study or EO chamber qualification).
- ❑ Schedule post-sterilization microbiological testing with Pacific BioLabs.
- ❑ Conduct finished device biocompatibility testing.



*Bioburden testing determines the amount of microbes on a medical device and is an essential component of radiation and ETO sterilization validations.*

### STERILITY ASSURANCE LEVEL

Sterility is defined as the state of being free from viable microorganisms. A sterility assurance level (SAL) is defined as the probability of an item being nonsterile after it has been exposed to a validated sterilization process. Most medical devices are sterilized to achieve a SAL of  $10^{-6}$ , which is the probability of one in a million items being nonsterile. Any sterile medical device that may be sold in Europe must be sterilized at a SAL of  $10^{-6}$ . In the USA, some less critical devices (e.g. specimen cups)

are sterilized at a SAL of  $10^{-3}$ . In setting up a validation program, the sterility assurance level that will be required for the medical device must be chosen.

A sterility assurance program for medical devices will require that the sterilization method chosen is validated to deliver the target SAL. As long as the bioburden of the device remains consistent, the sterilization validation ensures that routine sterilization cycles will achieve the desired SAL.

## ■ HOW CAN I COMPLETE MY VALIDATION ASAP?

- **Anticipate sample requirements.** Manufacturers should adequately plan for the sample requirements of a complete sterilization validation.
  - **Submit your evaluation sample early,** even if it is only a prototype. This is particularly helpful for large and complex products. Pacific BioLabs' staff will help you anticipate possible validation problems, determine your budget for validation costs and sample requirements, and design your validation protocol.
  - **Request your validation protocol early.** A well-prepared, detailed protocol is critical for smooth and timely completion of your validation. It is also required in order to meet FDA/ISO regulations. Early preparation of the validation protocol can save you several weeks in your validation timeline.
  - **Submit bioburden test method validation samples early.** Three to fourteen sterile samples are required for bioburden method validation testing (three to five for the Bioburden Method Validation and nine for the Screening for the Release of Adverse Substances if necessary). The bioburden recovery test occasionally fails initially causing the need for additional testing. This can delay your project by several weeks. It is wise to keep it off the critical path of your validation project.
  - **Allow extra time if SIP (sample item portion) preparation is required** in a radiation dose validation. For some large devices, it may be necessary to use a scaled-down version of the product for bioburden studies and the verification dose resistance experiment. For complex devices, disassembly and/or sectioning may be required prior to irradiation and testing.
  - **Submit bacteriostasis/fungistasis(B/F) samples early.** The B/F test requires three to six sterile samples, sterilized using the same process you will use on your final product. The B/F test sometimes fails initially causing the need for additional testing. This is a frequent source of delay in validation projects.
  - **For AAMI/ISO 11137 Method 1,** reserve 100 extra samples from each validation lot. If one lot has more than double the bioburden of either of the other two lots, the 100 samples for the dose audit test should come from the lot with the highest bioburden.
  - **For  $VD_{max}$  validations, it is preferable to include 20 samples for verification dosing.** 10 samples will be tested for sterility. If growth occurs in less than one sample, the validation is acceptable. If growth occurs in two samples, the other 10 samples can be tested for sterility. If no growth occurs in the second 10 samples, the validation is acceptable. If growth occurs in greater than three of 20 samples, additional testing is required.
  - **Let us know when your samples are coming.** The more notice you give us, the easier it is for Pacific BioLabs to meet your turnaround time requirements.
  - **Include clear and complete Laboratory Service Request forms with each sample submission.** This helps us avoid errors and speeds up your sample login.
- Respond quickly if something goes wrong. Most validations go smoothly. If a problem does arise, Pacific BioLabs will notify you promptly. Your quick response will help us keep your validation on track, on budget and on time.



# ETHYLENE OXIDE STERILIZATION

## CYCLE VALIDATION – MICROBIOLOGICAL REQUIREMENTS

### OVERKILL METHOD (AAMI/ISO 11135 METHOD C)

The overkill method is most commonly used to validate ethylene oxide cycles used in the sterilization of medical devices. The overkill method is based on demonstrating that the sterilization of a microbial challenge (biological indicator) exceeds the challenge posed by the bioburden of the product.

#### Validation Protocol

For an initial validation, a protocol should be prepared which outlines the overall validation requirements. The protocol should describe the medical device and specify the test procedures that will be used. Please see page 12 for more details.

- Please submit one sample to Pacific BioLabs for evaluation.

#### Bioburden Method Validation

The method that will be used for routine determination of product bioburden levels must be validated to insure that it is effective in recovering microorganisms from the product and that it allows for adequate growth of the recovered microorganisms. Typically validating the bioburden test method involves two phases of testing: adverse substance screening and recovery testing. Please see page 12 for more details.

- Recovery Validation using the Repetitive (Exhaustive) Method (5 nonsterile samples are recommended: a minimum of 3 samples are required) -OR- Product Inoculation (Simulated) Method (5 sterile samples are recommended: a minimum of 3 samples are required). This study will determine if it is necessary to apply a recovery factor to routine bioburden test results.



*ETO Sterilization is a common sterilization method used in health care facilities but most non-reusable devices are sterilized in large industrial ETO chambers.*

#### Bioburden Enumeration

The bioburden level of the product must be determined. For most products, aerobic bacteria and fungi bioburden are appropriate. AAMI/ISO recommends the testing of 10 samples from each of three production lots. It is most appropriate to sample the production lots that will be used in the three validation cycles. The bioburden samples may be selected to represent various production or packaging times. The samples should be collected immediately prior to sterilization. Please see page 15 for more details.

- 10 nonsterile samples from each of three production lots are recommended.

#### Bacteriotasis/Fungistasis Test

The procedure used to perform product sterility testing must be validated by means of bacteriostasis/fungistasis testing. This testing will ensure that false negative results will not occur in the sterility test. A false negative result allows a nonsterile sample to appear sterile due to materials in the device that inhibit microbial growth. False negatives can be overcome by modifications to the sterility test procedure.

Sterile samples are required for B/F testing. If sterile samples are not initially available, the B/F samples can be run with the fractional or half-cycles. Please see page 15 for more details.

- 3 to 6 sterile samples are required.



### Microbial Challenge – Use of Biological Indicators

For the validation study, biological indicators (B.I.s) are used to challenge EO cycles. The indicator organism is *Bacillus atrophaeus*, a spore forming bacteria with well characterized resistance to the EO sterilization process. Most commonly, *B. atrophaeus* spore strips with a population of  $10^6$  colony forming units (cfu) per strip are used. The population label claim from each lot of biological indicators used for validation testing must be verified. Three biological indicator replicates per lot used for validation purposes should be tested.

### Product vs. B.I. Resistance to EO

One purpose of the validation process is to support the use of *B. atrophaeus* biological indicators as a release mechanism for the product following routine EO processing. This can be done by means of one or more fractional cycles. After EO processing in the fractional cycle(s), both the product and the spore strips will be tested for sterility. Sterility test results must indicate that the *B. atrophaeus* organisms on the spore strips are more resistant to the EO process than the organisms that make up the bioburden of the product. If this is verified, and if product tests sterile in the fractional cycles, it is only necessary to test the spore strips after EO processing in the half cycles. For the half cycles, the spore strips must be placed in the product in same manner that was used in the fractional cycle.

### B.I. Placement and Quantity Required

Before processing in EO cycles, the biological indicators must be placed within the product inside the product package. The product must be evaluated to determine appropriate locations to place the B.I.s. The area of the device that presents the greatest potential challenge to gas penetration should be selected.

The ISO/AAMI guideline recommends calculating the appropriate quantity of B.I. challenge locations based on usable chamber volume as follows: 20 B.I.s for the first 5 m<sup>3</sup> of chamber volume, 2 B.I.s for each additional m<sup>3</sup> between 5 m<sup>3</sup> and 10 m<sup>3</sup> and then 1 B.I. for each m<sup>3</sup> beyond 10 m<sup>3</sup>. An unexposed positive control B.I. should be sent to the lab with each group of exposed B.I.s.

- A minimum of 20 biological indicators and 1 positive control B.I. is required.

### Inclusion of Process Challenge Devices (PCDs)

PCDs are uniquely packaged biological indicators that can be placed within the sterilization chamber during sterilization cycles. The use of PCDs as a release mechanism for routine EO processed products may be included as part of the initial sterilization validation. This could eliminate the loss of product associated with allocating samples for internal B.I. placement and sterility testing. In addition to the B.I.s placed within the product, a second set of B.I.s, the PCD, is placed outside of product packages and processed in the fractional cycles. Sterility test results must indicate that the PCD has a similar or greater resistance to the EO process than the B.I. located within the product.

### Product Load Configuration

For validation cycles, the maximum quantity of product representing the densest load configuration that will be processed in full routine cycles must be loaded in the EO sterilizer chamber. The product with B.I. test samples must be widely distributed throughout the sterilizer chamber. A placement diagram must be prepared, typically by the contract sterilization facility in consultation with the device manufacturer. The diagram must indicate the location of the product with B.I. test samples plus temperature and humidity sensors in the loaded chamber.



**Biological indicators containing at least a million organisms of *Bacillus atrophaeus* are used to validate EO cycles.**

## EO CYCLE PARAMETERS AND MONITORING

All EO cycles must be run using sterilization equipment that has been calibrated and for which IQ and OQ processes have been completed. Verify equipment status with the contract sterilizer before initiating the validation cycles. The parameters for the fractional, half and full cycles must be established in consultation with the contract sterilizer. During validation cycles, the temperature of the product at several widely distributed locations should be monitored and compared with the chamber control temperature. The monitored locations should include somewhere product with B.I. test samples are located. The sterilizer chamber EO concentration and the chamber and product humidity should also be monitored.

### Microbial Validation – Sample Processing

Each cycle load is preconditioned for the designated minimum time and then processed in the sterilizer chamber using the previously defined parameters. The product (if applicable) and B.I. test samples must be removed from the load either before aeration or after the minimum routine aeration time. The test samples should be sent to Pacific BioLabs for sterility testing via same day or next day delivery service.

- 1 or more fractional cycles – products and B.I.s (internal and, if desired, external) to be tested for sterility
- 3 half cycles – B.I.s (internal and, if desired, external) to be tested for sterility
- 3 full cycles – B.I.s (internal or, if applicable, external) to be tested for sterility

For the full cycles, data from the first 3 routine processing loads will be included to support the initial EO sterilization validation. The microbial validation is acceptable if the sterility test results for the product in the fractional cycle are negative and all of the B.I.s from the half cycle are negative (B.I. positive results in the fractional cycle are expected).

### Ethylene Oxide Residuals Testing

To complete the validation of the EO sterilization process, ethylene oxide residuals testing of the sterile medical device is required. AAMI/ISO has published standards for EO residuals limits. The testing can be conducted at the final desired aeration timepoint, or if desired an EO decay curve can be established. Periodic sampling and analysis of the product can establish an EO dissipation curve. This data can be used to establish quarantine times prior to product release, or to provide more information about the changes in product EO levels resulting from manufacturing, packaging or sterilization process changes. EO analyses of samples at three to five time points are normally recommended to determine an EO dissipation curve. Please see pages 22 and 23 for more details.

- At least 2 sterile samples per testing timepoint and 1 unsterilized control sample are required

### Routine Cycle Monitoring

As a result of the validation process, the use of *B. atrophaeus* spore strips in release testing for routine sterilization loads will likely be validated. Routine sterilization loads should be monitored with 10 to 20 biological indicators. It is a common practice to calculate the appropriate quantity of B.I.s based on usable chamber volume as follows: 10 B.I.s for the first 5 m<sup>3</sup> of chamber volume, 1 B.I. for each additional m<sup>3</sup> between 5 m<sup>3</sup> and 10 m<sup>3</sup> and then 1 B.I. for each m<sup>3</sup> beyond 10 m<sup>3</sup>. An unexposed positive control B.I. should be sent to the lab with each group of exposed B.I.s. The load can be released following acceptable sterility testing of the B.I.s.

- A minimum of 10 biological indicators and 1 positive control B.I. is required.

### Routine Bioburden Monitoring

Product bioburden is an important indication of the microbiological cleanliness and control of the manufacturing process. Control of product bioburden is required to maintain a validated sterility assurance level. Many factors can affect product bioburden. Among these are changes in materials, vendors, manufacturing personnel, procedures or equipment, water systems used in manufacturing and seasonal changes. Therefore, we strongly recommend a minimum of quarterly bioburden testing for all routinely produced sterile medical devices, regardless of the means of terminal sterilization.

### Revalidation

An annual documented review of all manufacturing and sterilization processes should be performed to demonstrate that nothing has changed that will affect the performance of the validated sterilization process. In addition to the annual review, AAMI recommends revalidating the EO sterilization process at least every two years to verify the effectiveness of the sterilization process. This revalidation should consist of (at a minimum) bioburden testing, one sub-lethal cycle, one half-cycle, and ethylene oxide residual testing. If any significant changes are made in the product, packaging or manufacturing, a complete revalidation is required.



Boxes of medical devices enter a concrete bunker by conveyor belt where they are sterilized using an e-beam accelerator.

## RADIATION STERILIZATION

### ANSI/AAMI/ISO METHODS

#### DOSE VALIDATION REQUIREMENTS

Several ANSI/AAMI/ISO methods are available for validation of the radiation dose used to sterilize a medical device. All of the methods use product bioburden enumeration and a bioburden organism resistance sterility test which is referred to as the verification dose resistance experiment. The most appropriate methodology is selected based on the projected production schedule and size. The four most commonly used methods are summarized below.

When planning a validation, be sure to manufacture enough samples for the entire validation study. Depending on the product's availability and complexity, an initial validation study can generally be completed in 6 to 8 weeks. Cost is contingent on product size and complexity.

#### **AAMI TIR 33 $VD_{max}$** (For Frequent or Infrequent Production Batches)

AAMI TIR 33, *Sterilization of health care products – Radiation Substantiation of a selected sterilization dose – Method  $VD_{max}$* , is used to establish a minimum sterilization dose for products manufactured frequently or infrequently in large or small batches. A minimum sterilization dose of 15, 17.5, 20, 22.5, 25, 27.7, 30, 32.5, or 35 kGy is selected based on the product's average bioburden. For the validation of a single lot, 10 products are tested for bioburden and then a verification dose resistance experiment is performed on 10 products irradiated at the calculated verification dose (or 20 products if growth occurs in two of the first 10 tested). For frequently produced lots, the initial validation includes

bioburden testing of 10 products from each of three separate lots and then a verification dose resistance experiment on 10 products from one lot. Revalidation consists of quarterly bioburden testing of 10 products followed by a verification dose resistance experiment on 10 products from the same lot.

#### **ANSI/AAMI/ISO 11137-2 Method 1** (For Large and Frequent Production Batches)

Dose Setting Method 1 is outlined in ANSI/AAMI/ISO 11137-2, *Sterilization of health care products—Radiation-Part 2: Establishing the sterilization dose* and is used less frequently than the  $VD_{max}$  methods. The initial validation includes bioburden testing of three separate lots and a verification dose resistance experiment on 100 samples from one lot irradiated at the calculated verification dose. Revalidation consists of quarterly bioburden testing of 10 samples followed by a verification dose resistance experiment on 100 samples from the same production lot.

#### **ANSI/AAMI/ISO 11137-2 $VD_{max}$** (For Frequent or Infrequent Production Batches)

ANSI/AAMI/ISO 11137-2, *Sterilization of health care products—Radiation-Part 2: Establishing the sterilization dose*, is used to establish a minimum sterilization dose of 15 kGy for products with an average bioburden <1.5 CFU or 25 kGy for products with an average bioburden <1,000 CFU. The number of devices required to conduct a validation is the same as indicated under AAMI TIR 33 –  $VD_{max}$ .



## VALIDATION STAGES

### VALIDATION PROTOCOL

For the initial validation, a protocol outlining the overall validation requirements must be generated. The protocol should define the device (or product family) and specify the test procedures that will be used. Please see page 12 for additional information.

- Please submit one sample to Pacific BioLabs for evaluation.

### Sample item portion (SIP) Preparation

For some large devices, it may be necessary to use a simulated, scaled-down version of the product for bioburden testing and the verification dose resistance experiment. For complex devices, disassembly and/or sectioning may be required prior to irradiation and testing. Each product must be evaluated individually to determine an appropriate SIP preparation procedure. The number of samples required for SIP preparation is the total of that required for bioburden and sterility test method validation, bioburden enumeration, and the verification dose resistance experiment. Please see page 12 for additional information.

### Bioburden Method Recovery Validation


The method that will be used for routine determination of product bioburden levels must be validated to ensure that it is effective in recovering microorganisms from the product and that it allows for adequate growth of the recovered microorganisms. Typically validating the bioburden test method involves two phases of testing: adverse substance screening and recovery testing. Please see page 12 for more details.

- Recovery Validation using the Repetitive (Exhaustive) Method (5 nonsterile samples are recommended: a minimum of 3 samples are required) -OR- Product Inoculation (Simulated) Method (5 sterile samples are recommended: a minimum of 3 samples are required). This study will determine if it is necessary to apply a correction factor to routine bioburden test results.

### Bioburden Enumeration

The bioburden level of the product must be determined. For most products, aerobic bacteria and fungi bioburden are appropriate. The bioburden samples may be selected to represent various production or packaging times. The samples should be collected so that they are representative of the product just prior to sterilization. Please see page 15 for more details.

- For ANSI/AAMI/ISO 11137-2 Method 1, 3-10 samples from each of 3 production lots are required.
- For VDMax, 3-10 samples from the production lot are required for a single lot validation. For validation of frequently produced lots, 3-10 samples from each of 3 production lots are required.



*Because it is generally less destructive to plastics than gamma, irradiation by e-beam sterilization is becoming increasingly popular.*



*Sterility testing is a crucial aspect of the irradiation dose resistance experiment and dose auditing.*

### Bacteriostasis/Fungistasis Test

The procedure that will be used to perform sterility testing (the verification dose resistance experiment) must be validated by means of bacteriostasis/fungistasis testing. This testing will ensure that false negative results will not occur in the sterility test. A false negative result allows a nonsterile sample to appear sterile due to inhibition of the microbial growth. This type of reaction is caused by certain materials utilized in some medical devices, but can be overcome by modifications to the sterility test procedure.

- 3 sterile samples are required to validate the AAMI/ISO sterility test.



*Any sterility tests that are positive for growth are thoroughly investigated.*

### Verification Dose Resistance Experiment

The purpose of the verification dose resistance experiment is to determine if any of the organisms that are part of the product bioburden are unusually resistant to the radiation sterilization process. The resistance experiment consists of a sterility test of products which have been irradiated at a defined dose. The verification dose is calculated based on the bioburden data. Products from a single lot must be irradiated at the verification dose. For AAMI/ISO 11137 Method 1, the dose is designed to deliver a  $10^{-2}$  sterility assurance level. For V<sub>D</sub>max the verification dose is at a  $10^{-1}$  sterility assurance level. To interpret the verification dose resistance experiment results, refer to the table on page 11. For additional information about dose auditing, please see page 16.

- For AAMI/ISO 11137 Method 1, 100 product samples from a single lot are required. Please submit 3 to 5 extra samples.
- For V<sub>D</sub>max, 10 product samples from a single lot are normally required. However, if growth occurs in two of the first 10 tested, 10 additional samples (that have been dosed at the verification dose) are required. Please submit 11 to 12 extra samples in the event that a confirmatory sterility test is required.

### Dose Auditing – Revalidation

The dose release validation program must generally be audited quarterly for frequently produced products. For products produced less frequently than quarterly, each production lot is validated for release. The dose audit interval may be extended to semiannually or annually if it is demonstrated over time that the product bioburden is stable with respect to levels, microorganism types, and microorganism resistance.

- For AAMI/ISO 11137, 10 nonsterile samples from a single lot are required for bioburden testing and 100 dosed samples from the same lot are required for the verification dose resistance experiment. Please submit 3 to 5 extra samples for the dose audit.
- For V<sub>D</sub>max, 10 nonsterile samples from a single lot are required for bioburden testing and 10 dosed samples from the same lot are required for the dose audit. Please submit 11 to 12 extra samples for the dose audit, in the event that a confirmatory sterility test is required.

## Verification Dose Test Results – Acceptance and Action Criteria

| SAMPLES TESTED | POSITIVES OBSERVED | AAMI/ISO 11137 METHOD 1<br>ACCEPTANCE AND ACTION CRITERIA  |
|----------------|--------------------|--|
| 100            | 0 – 2              | The initial validation or quarterly dose audit results are acceptable. The routine sterility assurance level dose is determined based on the bioburden test data or the dose calculated in the original study remains valid.   |
| 100            | ≥ 3                | For an initial validation, investigate product bioburden. If appropriate and possible, identify and eliminate resistant organisms. Reestablish bioburden and dose. Perform a new verification dose resistance experiment.  |
| 100            | 3 – 4              | For a quarterly audit, augment SAL dose based on new bioburden or assumed logarithmic increase in bioburden. Repeat verification dose experiment. If 2 or less positives on repeat, return to original SAL dose. If 3 to 6 positives on repeat, augment SAL dose, then reestablish SAL dose. If 7 or more positives on repeat, reestablish SAL dose. |
| 100            | 5 – 6              | For a quarterly audit, augment SAL dose based on new bioburden or assumed logarithmic increase in bioburden, then reestablish dose.  |
| 100            | ≥ 7                | Reestablish Dose   |

| SAMPLES TESTED | POSITIVES OBSERVED | VD <sub>MAX</sub> METHOD<br>ACCEPTANCE AND ACTION CRITERIA   |
|----------------|--------------------|--|
| 10             | 0 – 1              | Validation testing results are acceptable. The selected dose is validated for the production lot (for a single lot validation) or for 3 months if 3 lots were tested for bioburden.  |
|                | 2                  | Perform another confirmatory sterility test of 10 samples exposed to the same verification dose. If there are 0 positives in the second test, the selected dose is validated. If there are any positives in the second test of 10 samples, the selected dose is not validated. See action indicated below. |
|                | ≥ 3                | Investigate product bioburden. If appropriate and possible, identify and eliminate resistant organisms. Reestablish bioburden and perform a new verification dose resistance experiment or use an alternate dose setting method.   |



# PART 2 – STERILITY ASSURANCE COMPLIANCE

## TEST DESCRIPTIONS

### STERILIZATION PROCESS VALIDATION PROTOCOL

For an initial validation of the sterilization process for a medical device, cGMP regulations require a written validation protocol. The validation protocol is an outline of the requirements of a proposed validation effort. The protocol will include a complete identification of the medical device, a statement of purpose, the name of the contract sterilizer, the name of the testing laboratory, a list of the tests that are required, references for the test procedures to be used and criteria that will be used to judge if the validation effort has been successful. Specific test procedures can also be provided to clients who request them.

Pacific BioLabs will customize a sterilization validation protocol to meet individual client requirements. Once the initial validation is successfully completed, no additional validation protocols are normally required for subsequent revalidation, i.e. quarterly dose audits or subsequent single lot validations for the same product using the same method. Please call Pacific BioLabs to discuss your sterilization validation requirements.

### SAMPLE ITEM PORTION (SIP) PREPARATION

A sample item portion is a specially prepared portion of a medical device that is used in AAMI/ISO dose setting procedures. Some large or complex devices cannot practically be tested in their entirety. Using nonsterile samples, a defined portion of the device is aseptically removed and packaged. This portion (SIP) is used for the bioburden and verification dose studies.

For some complex devices, it may be necessary to disassemble or cut up the device so that it can fit into rinse fluid and media containers used for bioburden and verification dose testing. This disassembly or cutting must be done aseptically so that the natural bioburden of the product is not affected. It also must not reduce the challenge to the sterilizing process.

The adequacy of the SIP must be demonstrated by means of a sterility test of 20 non-sterile SIP samples. The SIP is considered adequate if at least 17 of the samples test positive in the sterility test.

Please submit a product sample to Pacific BioLabs for evaluation so that an appropriate SIP preparation procedure can be determined.

### BIOBURDEN METHOD VALIDATION

ANSI/AAMI/ISO Guideline 11737-1, *Sterilization of health care products – Microbiological Methods - Part 1: Determination of the Population of Microorganisms on Products* requires that the bioburden test method be validated for each medical device. The purpose of this validation is to ensure that the bioburden test method which will be used to determine the product bioburden level, is effective in a) recovering microorganisms that are present on the product and b) does not inhibit growth of the recovered microorganisms. Insufficient recovery or inhibition would result in underestimation of a product's true bioburden and could lead to an inadequate sterilization cycle or dose. The recovery data from validation testing will indicate if a recovery factor should be applied to results obtained by routine bioburden testing.

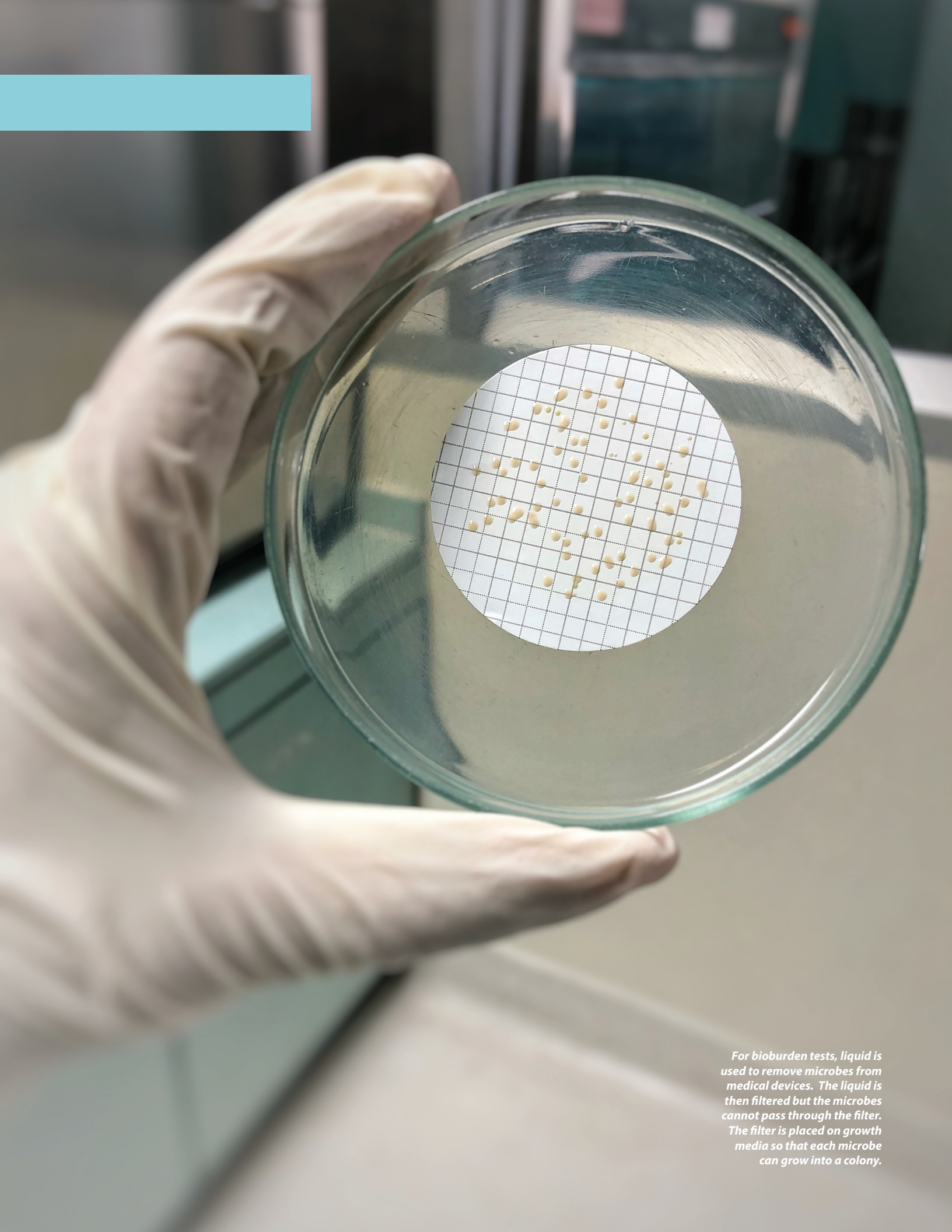
The bioburden method validation must be performed prior to proceeding with actual bioburden testing of the product. If any changes affecting materials, assembly or configuration are made to the product, the bioburden method should be revalidated.

- **Repetitive (Exhaustive) Recovery**

This method uses the naturally occurring bioburden of the product. A bioburden test is performed on the same device three or more times. The counts obtained from the replicate extractions are used to calculate a percent recovery. Pacific BioLabs recommends this method for devices which have a moderate to high bioburden level and do not contain absorbent materials.

- **Product Inoculation (Simulated) Recovery**

This method simulates a product bioburden by inoculating a known amount of spores onto a sterile device. The device is then tested for bioburden with the same method proposed for use in routine analysis. The recovered level is compared to the known inoculation level and the percent recovery is calculated. Pacific BioLabs recommends this method for devices with low bioburden levels or complex configurations. It also may be used for devices which absorb the rinsing fluid used in bioburden tests. The method does have limitations, because the spore inoculation may not reproduce the adherence properties of the natural bioburden. Please note that sterile samples are usually required for simulated recovery tests.



*For bioburden tests, liquid is used to remove microbes from medical devices. The liquid is then filtered but the microbes cannot pass through the filter. The filter is placed on growth media so that each microbe can grow into a colony.*



*Isolates are streaked and submitted to an approved vendor for identification.*





## BIOBURDEN ENUMERATION

Bioburden is the population of microorganisms on a raw material, product component or finished medical device just prior to sterilization. For finished medical devices, the bioburden test data is used to establish parameters for an effective sterilization process. To insure the ongoing safety of the sterilization process, it is necessary to verify that the bioburden level remains consistent over time. There are two important aspects of product bioburden control – maintaining consistency from lot to lot and avoiding spikes within a single lot. A bioburden spike occurs when the bioburden for an individual product is 2 or more times greater than the group average. A significant increase in the device bioburden would reduce the sterility assurance level of the sterilization process.

For new medical devices, ten randomly selected samples from three separate newly manufactured lots should be tested for bioburden. It is useful to track samples by date and time of assembly or packaging to determine when and where any inconsistencies may be occurring. After initial data is generated, bioburden tests should be conducted monthly to quarterly, depending on frequency and volume of production, as part of an ongoing environmental monitoring program. It is also important to check bioburden levels whenever any changes are made in packaging locations, manufacturing processes, raw material vendors, or personnel involved with production. If bioburden data increases significantly or shows extreme variability, the manufacturing process should be investigated so that corrective measures can be implemented.

Bioburden studies are also used to monitor microorganism levels on materials that could affect the bioburden of the finished device, such as product components, manufacturing fluids and product packaging. The bioburden test data may provide useful information in the investigation of bioburden spikes and dose audit failures. Bioburden testing can also be used as a material qualifications tool. For additional information refer to ANSI/AAMI/ISO 11737-3:2004, *Sterilization of medical devices—Microbiological methods - Part 3: Guidance on evaluation and interpretation of bioburden data*.

To perform a bioburden test, a sample is aseptically transferred to an appropriate volume of extraction fluid and then mechanically agitated to remove microorganisms. Membrane filtration is the preferred method for the culturing and microbial enumeration of the extraction fluid. Pacific BioLabs uses this method for products with filterable extraction fluid. When the extraction fluid cannot be filtered, the plate count method is used. Bioburden results are reported on individual samples showing total aerobic count with a breakdown of bacteria and fungi. Anaerobic bioburden and heatshocking methods for enumeration of spores are also available upon request.

### Bioburden Analyses

- Bioburden Test Method Validation
  - Recovery Study – Repetitive Treatment (3 to 5 extractions/sample)
  - Recovery Study – Simulated (spore inoculation of products)
- Bioburden – Total Aerobic Bacteria and Fungi by Filtration Method:
  - Small Devices (low level bioburden)
  - Medium Devices (moderate level bioburden)
  - Large Devices, Small Kits, Papers, Fabrics (higher level bioburden)
- Total Anaerobic Bacteria
- Total Aerobic Spores
- Process Fluids (Aerobic Bacteria by Filtration Method)

## MICROBIAL IDENTIFICATION

Microbial identification of bioburden isolates or for sterility test positives can help determine where microbial contamination may be coming from. If there is a sharp rise in bioburden levels, it is important for manufactures to bring levels back down to so that the sterilization dose does remains effective. The identification of the species can provide hints into how the device was contaminated. For example, Staphylococci are commonly found on skin and therefore the gowning procedure may need to be reviewed. Heterotrophic bacteria are found in water which may point to needed improvements with the rinsing water supply.

## BACTERIOSTASIS/ FUNGISTASIS TEST

The bacteriostasis/fungistasis test is designed to validate the procedure used to test a product for sterility by demonstrating that microorganisms present on the product will be detected in the course of the sterility test. Regulatory bodies require this test because some products contain substances that inhibit the growth of microorganisms. Although a product may harbor microorganisms and be nonsterile, the presence of growth inhibition substances can cause a false negative sterility test.

The test is conducted by performing a simulated sterility test, then adding low levels of selected bacteria and fungi as challenge microorganisms to the culture media. The organisms will remain viable, grow and be detectable in the culture media if the product does not exert a bacteriostatic or fungistatic effect. If the product is found to be bacteriostatic or fungistatic, the sterility test procedure must be modified and another bacteriostasis/fungistasis test must be performed. This testing should be performed on all new products and when any significant changes are made in the manufacturing or materials of an existing product. Pacific BioLabs strongly recommends repeating the B/F test biannually to account for any possible changes to the product or manufacturing process. For medical devices, three to six sterile samples are required for the B/F test.

- Bacteriostasis/Fungistasis Test – Direct Transfer Method
  - 3 organisms in SCDM (Radiation Dose Audits)
  - 6 organisms – 3 in FTM and 3 in SCDM (USP; EO sterilized products)

## AAMI/ISO DOSE AUDIT

A dose audit is a sterility test of samples which have been irradiated at a defined kGy level determined as part of an ANSI/AAMI/ISO dose validation study. The ANSI/AAMI/ISO standard requires that dose audits be performed quarterly (or with each lot if production is less frequent than quarterly) for all devices that been validated according to ANSI/AAMI/ISO 11137 Method 1 or AAMI TIR 33 VD<sub>max</sub>. The dose audit interval may be extended to semiannually or annually if it is demonstrated over time that the product bioburden is stable with respect to levels, microorganism types, and microorganism resistance. All samples are tested using Soybean Casein Digest Medium and incubated for 14 days at 30°±2°C.

The cost of a dose audit is usually based on the amount of medium required to test the sample. Samples which are difficult to aseptically handle will incur an additional charge. Samples which can be easily cut with scissors can be aseptically divided and possibly tested in smaller containers. To minimize the chance of a sample being compromised in sterility testing, it is highly desirable to minimize sample manipulation. We prefer to use the smallest container and volume which will allow the test sample to be submerged in media (although it is not always possible to completely submerge all samples). An appropriate media volume must be validated for each product by bacteriostasis-fungistasis testing. If the product cannot be cut to fit into any of the following containers, SIP preparation will be required (see page 12).

## STERILITY TESTING

Sterility testing of products and/or biological indicators (i.e. spore strips) exposed to a sterilization process is an important part of all sterility assurance programs. Most manufacturers of EO sterilized medical devices monitor their validated EO sterilization loads with *B. atrophaeus* spore strips and release their products for distribution based on negative sterility test results of the spore strips. The spore strips may be placed inside or outside the product depending on the type of spore strip testing performed during the validation. The spore strips should be distributed in locations throughout the

sterilization chamber. A positive control (i.e. unprocessed spore strip) should be included with all spore strip sterility tests. These routine sterility tests must be supplemented periodically with more extensive cycle validations.

Routine lot release of terminally sterilized medical devices by means of end product sterility testing is not recommended for several reasons. The bioburden of most medical devices generally is a lesser challenge to the sterilization process than biological indicators, and overkill cannot be demonstrated. Statistically, the probability that a sterility test of 20 or 40 product samples will detect nonsterile samples among a much larger number of products is very limited. Also, it is generally recognized that the process of sterility testing has a significantly lower sterility assurance level than most validated terminal sterilization processes. However, end product sterility testing of medical devices is occasionally performed as part of investigations or to support other information in making a lot release decision.

### Spore Strips Only

This test is appropriate for devices sterilized by steam, EO, and some types of chemical sterilization such as hydrogen peroxide techniques. Generally 10 to 20

spore strips are used to monitor a cycle. Spore strips are cultured in SCDM and then usually incubated for 7 days. Shorter incubation times can be used depending on the manufacturer's instructions.

### Product with Spore Strips

This test is generally used for fractional and half cycles in ethylene oxide. Spore strips are normally cultured in SCDM and incubated for at least 7 days. Products are usually tested in SCDM and FTM and incubated for 14 days.

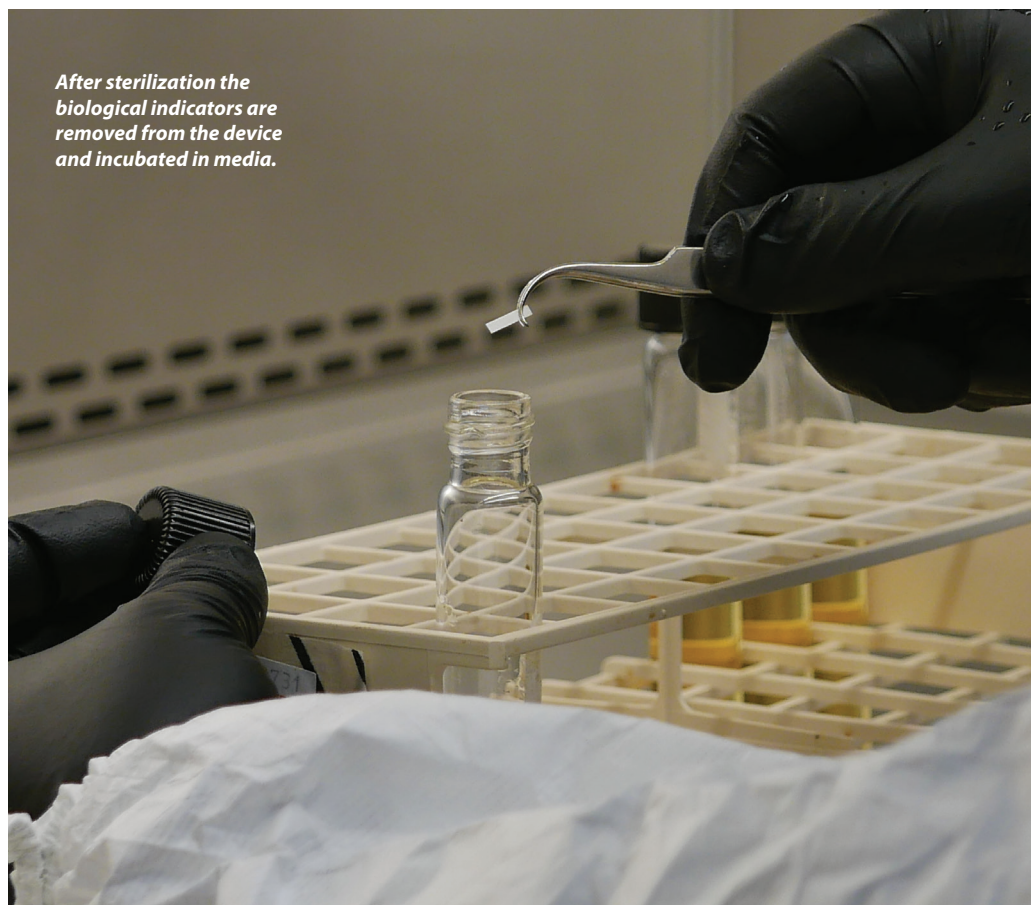
### Product Only – Direct Transfer or Fluid Path

Entire devices or portions of devices are rinsed with or submerged in Soybean Casein Digest Medium. Usually 10 product samples are required. Incubation time is 14 days.

### Inoculated Product

For this test, product samples which have been inoculated with a microorganism that is resistant to the sterilization process are used as biological indicators. This test is used most frequently to verify steam or EO penetration into an area of a medical device that is too small to be monitored with a spore strip. It is also used as part of the steam sterilization validation for reusable devices. Incubation time is 7 days.

*After sterilization the biological indicators are removed from the device and incubated in media.*







*Sterility testing at Pacific BioLabs is conducted in an ISO Class 5 environment.*

## WHY PACIFIC BIOLABS SHOULD CONDUCT YOUR DOSE AUDITS AND STERILITY TESTS

- Over 35 years of experience providing sterility testing services for medical device and pharmaceutical manufacturers.
- Pacific BioLabs' proximity to radiation contract sterilization facilities minimizes sample shipment time and expense. Pick-up service is available at some sterilization facilities.
- Pacific BioLabs works closely with contract sterilizers to expedite the processing of your samples.
- Pacific BioLabs has extensive standard operating procedures covering all aspects of sterility testing operations.
- Product sterility testing is aseptically performed in a certified cleanroom by experienced technicians.
- Samples for lot release are put on test within 2 days of sample receipt.
- Environmental controls are performed for all sterility tests.
- Samples are monitored daily. Clients are immediately advised by phone should growth occur.
- Growth occurring in sterility tests is thoroughly investigated.
- MyPBL, PBL's client portal, provides constant access to published reports and current study status.

Pacific BioLabs has a dedicated cleanroom sterility testing suite. The suite is constantly maintained under positive pressure that cascades from core cleanroom to the gowning room to our sample disinfection room to the ambient pressure entrance area. Sterility testing is performed in HEPA filtered hoods by fully gowned test technicians. All media used in sterility testing is steam sterilized in validated autoclave cycles. Each batch of media is tested for growth promotion. The temperature in all chambers used to incubate samples is continuously monitored by a Rees Scientific Facilities Monitoring System which provides audible, phone, and email alerts in the event of temperature excursions beyond defined parameters.



## MICROBIAL ENVIRONMENTAL MONITORING

Medical device manufacturers use microbial environmental monitoring programs to evaluate the effectiveness of cleaning and disinfection procedures and to assess the overall microbial cleanliness of their manufacturing environment. An effective program to control microorganism levels in the manufacturing environment is essential to minimize the bioburden on the medical device being manufactured and reduce potential for bioburden spikes. Spikes in the bioburden of finished medical devices can cause a reduction in the sterility assurance level for the product.

Air, surface and compressed gas samples are taken during routine production operations to obtain a microbiological profile of the manufacturing environment. Observation of work practices are made during the survey. Test data and other information are evaluated to determine what actions can be taken to reduce or stabilize the bioburden of the medical device. Once an intensive survey has been conducted and strategic sampling locations are determined, samples can be taken by the manufacturer's personnel on a regular schedule. Pacific BioLabs can provide the necessary supplies for microbiological sampling. Exposed materials are returned to Pacific BioLabs for enumeration and reporting. If the facility is within the SF Bay Area, PBL analysts can travel to the facility to take the samples as well.

Monitoring the environment for nonviable particulates and microorganisms is an important control function because they both are essential in achieving product compendial requirements for particulate matter and sterility, especially for parenteral products. The number of nonviable particulates is also critical in the electronic industry. It is generally accepted that if fewer particulates are present in an operational clean room or other controlled environment, the microbial count under operational conditions will be less, provided that there are no changes in airflow, temperature and humidity.

Pacific BioLabs trained personnel can collect samples of nonviable particulates at the same time the microbial environmental monitoring samples are taken. Non-viable particulate air samples aid in demonstrating compliance with ISO classification guidelines.

If any major changes are made at the facility or in the manufacturing process, or if product bioburden levels increase significantly, a re-

evaluation of environmental conditions should be conducted. For additional information about environmental monitoring, refer to USP general chapter <1116> *Microbiological Control and Monitoring of Aseptic Processing Environments* or PDA TR 13 (revised 2014) *Fundamentals of a Microbiological Environmental Monitoring Program*.

Pacific BioLabs works with many of its clients to establish and maintain cost-effective programs to meet FDA requirements for monitoring the microbial

cleanliness of their manufacturing environments and assessing the efficacy of production area disinfection procedures. Our microbiologists can train quality assurance and manufacturing personnel to collect microbial environmental samples or PBL analysts can perform the sampling. Following is an overview of aspects of a microbial environmental monitoring program. Please contact business development at [Info@PacificBioLabs.com](mailto:Info@PacificBioLabs.com) to schedule a sampling.



### MICROBIAL ENVIRONMENTAL MONITORING PLAN FOR PRODUCTION AREAS

- **Review manufacturing procedures**
  - movement of materials
  - personnel practices
  - cleaning and disinfection procedures
- **Visit production and packaging areas. Observe manufacturing process.**
- **Evaluate possible sources of microorganisms and potential to impact product bioburden.**
  - water and ancillary fluid used in production
  - HVAC systems
  - manufacturing equipment
  - manufacturing personnel
  - Formulate sampling plan.
  - prepare a production area schematic
  - identify sites for air and surface samples
  - determine if water or other process fluid sampling is required
  - determine if compressed gas sampling is needed
- **Collect environmental samples.**
  - identify each with a site code and the date and time collected
- **Ship samples for next day delivery to Pacific BioLabs if needed.**
- **Pacific BioLabs incubates samples at temperatures appropriate for the various environmental samples.**
  - Samples are incubated 3-5 days at 30-35°C for bacteria and then transferred to another incubator at 20-25°C for 4-7 days to grow yeast and mold.
- **Following incubation, bacteria and mold colonies are enumerated.**
  - microorganisms will be characterized or identified if desired
- **Results are reported to client.**

## MATERIALS USED FOR MICROBIAL ENVIRONMENTAL MONITORING

**RCS Air Sampler:** A mechanical instrument which pulls in a preset volume of air, impacting microorganisms onto a strip filled with a nutrient agar.

**Contact Plate:** A petri dish with an elevated convex surface of nutrient agar. It is used for taking samples of flat surfaces. The lid of the dish is removed. The agar surface is pressed lightly against the surface to be sampled. The lid is then immediately replaced on the dish.

**Fallout Plate:** A petri dish containing a nutrient agar. It is used for semi-quantitative air sampling. The dish is placed in the desired location, the lid is removed and the agar is exposed for a defined amount of time, usually for 30 minutes to 2 hours. Organisms falling from the air settle on the surface of the agar.

**DE Neutralizing Agar:** A neutralizing agar formulated with Tween 80 (a surfactant), lecithin (a general purpose neutralizer), sodium thiosulfate and sodium thioglycollate. This combination is used to neutralize chlorine, glutaraldehyde, iodophor, phenolic and quat based disinfectants. This agar is used to culture many types of bacteria and some fungi.

**Lethen Agar:** A neutralizing agar formulated with Tween 80 and lecithin. This combination is used to neutralize alcohol, phenolic and quaternary ammonium chloride based disinfectants.

**Sabouraud Dextrose Agar:** A high sugar, low pH agar used to select for yeast and molds.

**Sodium Thiosulfate:** A chemical added to agar to neutralize halogen based disinfectants, such as bleach.

**Sterile Buffer Solution with Swab:** The buffer solution is supplied in a 10 mL screw cap test tube. The sterile swab is provided in a paper pouch. Swabs are used for taking samples of non-flat surfaces. The swab is moistened by dipping it in the sterile buffer solution. The surface to be sampled is swabbed. The tip of the swab is then cut or broken into the test tube of solution and the cap is replaced on the test tube.

**Tryptic Soy Agar with Lecithin:** A general purpose nutrient agar used to culture many bacteria and some fungi. The additions of lecithin neutralizes disinfectants.

*Environmental monitoring samples can be sent to PBL for incubation and analysis.*

### Microbial Environmental Supplies and Services

- Microbial Environmental Monitoring Supplies:
  - Fallout Plate, 100 mm, Tryptic Soy Agar (TSA)
  - Fallout Plate, 100 mm, Sabouraud Dextrose Agar (SDA)
  - Contact Plate, DE Neutralizing Agar
  - Contact Plate, (TSA)
  - Contact Plate, (SDA)
  - RCS Air Sampler Strip, TSA
  - RCS Air Sampler Strip, SDA or Rose Bengal Agar
  - Sterile Buffer Solution with sterile swab
  - Sterile Specimen Cups, Screw Cap, 120 mL
- Microbiological Environmental Sampling (Performed by Pacific BioLabs Personnel)
- Microbial Samples Enumeration and Report:
  - Fallout Plate Count
  - Contact Plate Count
  - RCS Air Sampler Strip Count
  - Buffer Solution – Aerobic Count
- Compressed Air Sampling
- Nonviable Particulate Sampling





*Package integrity testing is a common component of a shelf life study.*

## ACCELERATED AGING – EXPIRATION DATING STUDY

An accelerated aging study is used to support the assignment of an expiration date to a sterile medical device. Accelerated aging is a process in which products and packaging are stored in an environmental chamber at an elevated temperature to simulate a longer period of real time aging.

Accelerated aging is based on the assumption that the chemical reactions involved in the deterioration of materials follow the Arrhenius reaction rate function. This function states that rises in temperature of 10°C will double the rate of a chemical reaction.

The elevated temperature condition typically used at Pacific BioLabs for accelerated aging of medical devices is 55°C with ambient humidity. Following the Arrhenius equation, 46 days at 55°C would equal 1 year of aging at 25°C. Alternative aging conditions are also available.

For packaging, recommended testing options include seal strength and package integrity by dye penetration and/or microbial challenge. For products, testing should include applicable physical performance testing and visual comparison to products that have not been aged.

Accelerated aging studies are normally conducted in accordance with a written protocol. Products that have been exposed to a validated sterilization process should be used in these studies. A typical study includes testing of samples after several aging intervals (eg. 1, 3 and 5 year equivalency). The protocol specifies the aging intervals and the tests to be performed after each interval. The sample requirements are based on the number of

aging intervals and the tests required after each interval.

Pacific BioLabs can provide accelerated aging storage services when other testing is not required. Please call for a quote for a complete accelerated aging/expiration dating/shelf life study.

## REAL TIME AGING – EXPIRATION DATING STUDY

A real time aging study must be used to support the assignment of an expiration dating claim that is based on an accelerated aging study. Products are usually stored at controlled room temperature and tested annually for as many years as desired. The tests should be the same as those used in the accelerated aging study.

## PACKAGE INTEGRITY TESTING

Package integrity tests are used to detect packaging problems that could adversely affect the sterility of a medical device. Sterile products are subjected to an environmental stress intended to simulate extreme conditions that a product might encounter in shipping or storage. The product packaging is then subjected to microbial challenge or dye penetration testing to determine if it has retained its properties as a microbial barrier. Thirteen samples are recommended for this test.



## SHELF-LIFE SERVICES

- Accelerated Aging and Packaging tests
- Accelerated Aging – Expiration Dating Protocol
- Accelerated Aging in Environmental Chambers
- Seal Peel Test (10 products/time point)
- Package Integrity by Microbial Challenge (13 products/time point)
- Package Integrity by Dye Penetration (13 products/time point)

## BACTERIAL ENDOTOXINS (LAL) TEST

A pyrogen is the product of the action of heat on an organic substance and, in medical terms, is frequently described as a fever producing substance. The most potent pyrogens originate from gram negative bacteria, which are common water-borne organisms. Although not entirely accurate, the terms pyrogen and endotoxin are often used interchangeably. Detection of bacterial endotoxin contamination is essential to ensure the safety of certain medical devices. The bacterial endotoxins test using Limulus Amebocyte Lysate (LAL) is recommended for the detection of endotoxins in medical devices. Any product that is labeled as nonpyrogenic must be tested to verify that claim. Medical devices with bloodstream, intraocular or cerebrospinal fluid contact must also be tested for the presence of bacterial endotoxins.

Whether or not a device is considered pyrogenic is based on the amount of endotoxin the device contains in correlation to the accepted human tolerance of 5 endotoxin units (EU) per kilogram of body weight. Nonpyrogenic water is used to extract medical devices.

Endotoxin not only cause a fever, they also activate other immune and cardiovascular molecules and cells such as neutrophils, platelets, and complement to induce mast cell degranulation causing hypertension. Endotoxin can also activate Factor XII causing intravascular coagulation and organ damage. Large amounts of endotoxin could lead to septic shock. Endotoxin has been linked to auto-immune diseases, breathing problems, tightness in the chest and even obesity. Because of the high number of biological processes effected by endotoxin, the FDA has taken a very proactive stance on endotoxin testing.

The LAL test is usually performed on a composite of the extracts. It is possible that one device of the composite could contain > 20 EU, when others in the composite would contain < 20 EU, and the composite test would pass. The most endotoxin one device could contain, if all others in a 10 sample composite contained zero endotoxin, would be 200 EU. This is still below the human tolerance of 350 EU, based on an average human body weight of 70 kg ( $70 \text{ kg} \times 5 \text{ EU/kg} = 350 \text{ EU}$ ). In a composite test, 20 EU is the average endotoxin limit for most medical devices. However, the limit for the devices which contact cerebrospinal fluid is 2.15 EU per device. The maximum allowable extraction volume is calculated to ensure that the average endotoxin burden per device in the LAL test does not exceed these limits.

The current LAL reference document is ANSI/AAMI ST72/Ed. 2, *Bacterial endotoxins – Test methodologies, routine monitoring, and alternatives to batch testing* which specifies methods and criteria the determination of bacterial endotoxins on healthcare products, components or raw materials. The three common LAL testing methods are the gel-clot method, kinetic chromogenic method and turbidimetric method. All three methods measure endotoxin, but differ in their sensitivities and procedure.

For medical devices, the following sampling strategy is recommended for the LAL test: 2 samples for lot sizes less than 30, 3 samples for lot sizes 30-100, and 3% of the lot not to exceed 10 samples for lot sizes equal to or greater than 101. One test is performed on a composite of the test samples. Because water is a source of pyrogens, it is important to routinely monitor water systems using the bacterial endotoxins test. For process water samples, the amount of sample required is 10 mL. Please contact Pacific BioLabs if you need sample collection containers.

### Bacterial Endotoxins Tests

Kinetic Chromogenic tests are available:

- LAL Validation – Inhibition and Enhancement
- Immersion
- Fluid Path



## ETHYLENE OXIDE RESIDUALS ANALYSES

About half of the sterile disposable medical devices used in the United States are sterilized with gaseous ethylene oxide (EO). This method is widely used because it avoids the heat and radiolytic stress often associated with steam or radiation sterilization. It is also commonly used for kits which contain a drug or component that cannot be sterilized with radiation.

EO gas penetrates the intricate design configurations and cavities which are often found in complex medical devices. Despite the volatility of this gas, certain plastics and polymers readily adsorb EO and retain it for a period of time following sterilization. If chloride ions or moisture are present, EO will react with them to form ethylene chlorohydrin (ECH) and ethylene glycol as by-products. Both EO and ECH are acknowledged as physiological toxins.

Acceptable limits for ethylene oxide residuals are published in ANSI/AAMI/ISO 10993-7, Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residuals. This standard specifies the maximum allowable dose (mass) of EO and ECH residues which can

be delivered to a patient. Limits are based on the use classification for the device: limited exposure (24 hours), prolonged exposure (up to one month) or permanent exposure (lifetime use).

ANSI/AAMI/ISO 10993-7 provides test methods for analyzing ethylene oxide residuals in sterilized products. To simulate patient exposure, product samples are generally subjected to an aqueous extraction. The extract is analyzed for EO and ECH (and EG in certain circumstances) by gas chromatography. To determine if residue levels are acceptable, the weight of the device or device component being tested is required. Results are calculated in relation to the daily dose (mg/day) for daily, monthly and lifetime exposure, and are also reported in ppm (parts per million).

An alternate method for extracting EO from a sample is the headspace method. This method is also referred to as the exhaustive method because the entire amount of EO present in the sample is determined. A defined portion of the device is weighed and transferred

to a sampling vial. EO is thermally extracted from the sample and driven into the headspace of the vial. The gas in the headspace is analyzed by gas chromatography. Results are reported in ppm (parts per million), and calculated to ANSI/AAMI/ISO limits.

For EO residuals analyses, we generally recommend that two sterile samples be submitted. Additionally, ANSI/AAMI/ISO recommends that one non-sterilized control sample be tested to insure that the product does not cause chromatographic interference during testing. AAMI recommends that samples be shipped under dry ice.

### Ethylene Oxide Residues Tests

- Ethylene Oxide Residual Analysis (EO only)
  - Aqueous Extraction
  - Headspace Sampling
  - Acetone or DMF Extraction
- Ethylene Chlorohydrin Residuals Analyses



## ETHYLENE OXIDE DISSIPATION STUDIES

Ethylene oxide will slowly dissipate from sterilized devices. It is useful to determine how long it takes for the residual EO to dissipate to a target level. By periodic sampling and analysis of the product, an EO dissipation curve can be established. This data can be used to establish quarantine times prior to product release, or to provide more information about the changes in product EO levels resulting from manufacturing, packaging or sterilization process changes. Either aqueous or headspace extraction methods mentioned on the previous page may be used in establishing dissipation curves.

To determine an ethylene oxide dissipation curve, EO analyses of samples at three to five time points are normally recommended (e.g. 1, 3, 5, 7 and 10 days after sterilization). One sample per time point and one non-sterilized control sample are required.

## ISO GUIDELINES FOR STERILANT RESIDUES

### DEVICE CLASSIFICATION

**Limited Exposure:** devices that have single or multiple use or contact is likely to be less than 24 hours

**Prolonged Exposure:** devices whose single, multiple, or long-term use or contact is likely to exceed 24 hours but not 30 days

**Permanent Contact:** devices whose single, multiple, or long-term use or contact is greater than 30 days.

### ALLOWABLE LIMITS FOR ETHYLENE OXIDE AND ETHYLENE CHLOROHYDRIN

#### Permanent Contact Devices

The average daily dose of EO to patient shall not exceed 0.4 mg/day. In addition, the maximum EO dose shall not exceed: 4 mg in the first 24 hours, 60 mg in the first 30 days, and 2.5 grams in a lifetime.

The average daily dose of ECH to patient shall not exceed 0.4 mg/day. In addition, the maximum ECH dose shall not exceed: 9 mg in the first 24 hours, 60 mg in the first 30 days, and 10 grams in a lifetime.

#### Prolonged Exposure Devices

The average daily dose of EO to patient shall not exceed 2 mg/day. In addition, the maximum EO dose shall not exceed: 4 mg in the first 24 hours, and 60 mg in the first 30 days.

The average daily dose of ECH to patient shall not exceed 9 mg in the first 24 hours, and 60 mg in the first 30 days.

#### Limited Exposure Devices

The average daily dose of EO to patient shall not exceed 4 mg.

The average daily dose of ECH to patient shall not exceed 9 mg.

## REUSABLE MEDICAL DEVICE TESTING

To address regulatory and liability concerns, manufacturers of medical devices that are intended for reuse and sterilization in health care facilities must provide specific cleaning, disinfection and/or sterilization instructions to their customers. To ensure that reliable cleaning, disinfection and/or sterilization will result, the instructions must be validated. For more information, refer to AAMI TIR 12 Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities – A guide for medical device manufacturers and AAMI TIR 30– A compendium of processes, materials, test methods, and acceptance criteria for cleaning reusable medical device.

Pacific BioLabs can simulate most conventional hospital cleaning and sterilization procedures. Fees for the cleaning and sterilization validation studies will vary depending on the validation protocol. Please contact [Info@PacificBioLabs.com](mailto:Info@PacificBioLabs.com) to discuss your requirements. To learn more about reusable device validations please download our reusable device validation booklet at [www.PacificBioLabs.com](http://www.PacificBioLabs.com)

- Cleaning, Disinfection and Sterilization Validation Protocols
- Cleaning Validation Studies
- Disinfection Validation Studies
- Sterilization Validation Studies
  - Ethylene Oxide
  - Steam Sterilization at 121°C to 135°C
  - Flash, Gravity and High Vacuum Cycles
  - Liquid Chemical Sterilization
  - Dry Heat Sterilization and Depyrogenation
  - Hydrogen Peroxide Sterilization System



## THE PACIFIC BIOLABS ADVANTAGE

### THE SERVICE LEADER IN BIOSCIENCE TESTING

Pacific BioLabs (PBL) is an independent laboratory offering GLP/GMP testing services to the medical device and pharm/biopharm industries. PBL specializes in biocompatibility, sterility assurance, microbiology and preclinical toxicology/pharmacology services.

SERVING THE BIOSCIENCE INDUSTRY SINCE 1982

Pacific BioLabs clients range from small start-ups to Fortune 500 giants. Our staff is widely recognized for their experience, technical competence and commitment to client service. Over the years, PBL has gained a national reputation for quality in service and excellence in science.

### STATE OF THE ART VIVARIUM AND LABS

Pacific BioLabs conducts its operations in a stunning 32,000 square foot facility in Hercules, CA, overlooking the San Francisco Bay. The building houses a 12,000 square foot vivarium with a surgery suite, necropsy lab, radiation lab, procedure rooms, and ample support areas. The semi-barrier SPF rodent suite has a HEPA-filtered air supply and dedicated procedure space. Animal facilities and critical equipment are monitored 24/7. Emergency power is supplied by an on-site generator. The site can accommodate a planned 18,000 square foot facility expansion.

### RIGOROUS REGULATORY COMPLIANCE

In the regulatory science arena, quality means compliance. PBL has an outstanding track record in audits by FDA, EPA, MHRA, and other agencies, not to mention hundreds of client auditors.

At Pacific BioLabs we conduct all testing in accordance with applicable Good Manufacturing Practice (cGMP) and Good Laboratory Practice (GLP) regulations. To insure data integrity, our Quality Assurance Unit staff routinely audits all aspects of lab operations and administer our world class CAPA system. PBL's extensive body of Standard Operating Procedures is at the core of a thorough, documented training system which ensures that all technical staff can capably perform their assigned procedures.

For most biocompatibility submissions, the FDA and EPA require that testing be performed in accordance with GLP regulations. It is the client's responsibility to determine when GLP treatment is required for their product and to inform PBL in writing of this requirement at the time of sample submission. (An additional fee for GLP treatment will be incurred, typically 10-20% of total test costs.)

Pacific BioLabs is FDA-registered and certified by ANAB to ISO 17025:2017. Our animal science program is AAALAC accredited.

## NOTES

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**PBL**  
Pacific BioLabs

*The Service Leader in Bioscience Testing*

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