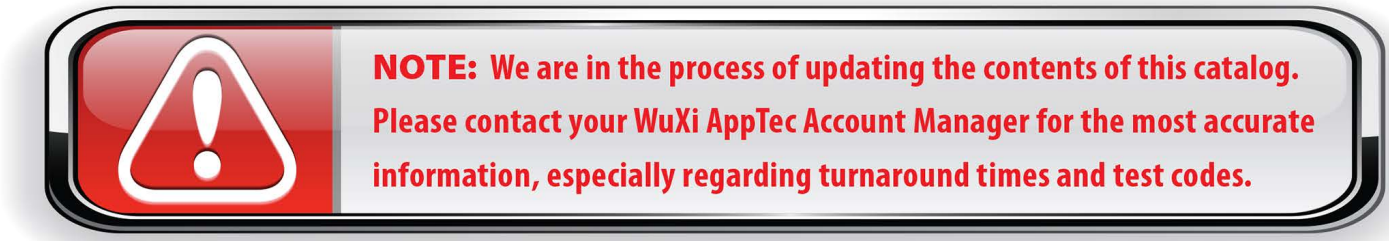


## MICROBIOLOGY TESTING

Microbial assays involve a variety of tests, from the determination of the numbers and types of organisms naturally present on a product to the assessment of controlled environments. With our comprehensive menu of microbiology services, WuXi AppTec provides testing across the product development life cycle and beyond: R&D and screening, in-process release and validations, and finished product delivery.

### IN THIS SECTION

# BACTERIAL ENDOTOXIN (LAL) TESTS

Pyrogens are fever-producing materials that most often originate from Gram-negative bacterial cell walls, but can also originate as leachates from some chemicals and materials. Pyrogens from bacterial cell walls (the most commonly encountered type of pyrogen) are referred to as bacterial endotoxin and are readily detected by Limulus Amebocyte Lysate (LAL) testing systems.

LAL (Limulus Amebocyte Lysate) gel clot testing is a semi-quantitative method for testing of most medical devices/products. This method has been replaced in most cases by the more sensitive kinetic methods. The kinetic chromogenic LAL method provides direct quantification of the detected endotoxin level and is especially useful for very low-level detection, determining the endotoxin reduction of various production processes, monitoring the quality of water systems, and providing endotoxin levels for lot release of products. The kinetic turbidimetric method is similar to the chromogenic method and is used where there may be color interferences (e.g., blood-containing product). WuXi AppTec follows the FDA, USP and AAMI guidelines when performing LAL tests.

Each time a new device/product is produced, or a significant change in material formulation is made on an existing device/product, a validation must be performed on samples from three production lots. The purpose of this is to ensure that the materials used in the construction of the device do not impart an inhibiting or enhancing effect on the LAL test system. Other changes, such as a change in the testing laboratory, may only require a single lot validation.

Sample requirements for both the validation testing and routine testing are typically determined by the size of the production lots from which the samples are selected.

**NOTE:**
Chemical pyrogens, also called materials-mediated pyrogens, can be detected only by using the USP Rabbit Pyrogen Test or Materials Mediated Test.
*[See Medical Device Catalog's Biocompatibility section.]*

**The Gel Clot Method is also available. Contact Atlanta facility for details.**

**130601 Kinetic Chromogenic**

**130802 Kinetic Turbidimetric**

## LAL Test Validation

Validation of the inhibition or enhancement properties of the materials on the test system.

**SAMPLE REQUIREMENTS** [USP and AAMI Guidelines]
Samples from three (3) production lots should be tested for inhibition and enhancement before this test is considered validated for use with the test product.

***NOTE:*** Validation testing can be performed at the same time and on the same samples as the lot release (finished product) testing.

# BACTERIAL ENDOTOXIN (LAL) TESTS

| | |
|---|---|
| Quantitative determination of endotoxin level for finished devices or other materials.<br><br>**SAMPLE REQUIREMENTS**<br>For lots of less than 30 units – 2 sample devices<br>For lots of 30-100 units – 3 sample devices<br>For lots of 101 units or greater – 3% of lot, up to maximum of 10<br>*[It is recommended that samples be sterile.]* | **130501 Kinetic Chromogenic**<br><br>**130800 Kinetic Turbidimetric**<br><br>**LAL Limit Test – Finished Product Testing** |
| Endotoxin testing of water system samples or other non-biological liquids.<br><br>**SAMPLE REQUIREMENTS**<br>Minimum of 1 mL in sealed endotoxin-free polystyrene or glass container. | **130701 Kinetic Chromogenic**<br><br>**130801 Kinetic Turbidimetric**<br><br>**LAL Liquid Test** |

Contact your Account Manager for the most accurate information regarding test codes and turnaround times.

# BIOBURDEN TESTING

Bioburden testing is an assessment of the numbers and types of microorganisms present on a product, and may be used for assessment of incoming materials, as an indicator of manufacturing conditions, and to support sterilization validations. A determination of the recovery efficiency and characterization (grouping micro-organisms into categories) are both required for compliance with bioburden standards. All aspects of bioburden testing – test parameters, characterization and recovery efficiency – are performed according to specified ISO, AAMI, USP, or FDA standards.

| | |
|---|---|
| **1606000**<br>**Aerobe Bioburden** | Aerobe microflora count. (Test conditions may recover mold and yeasts as well as bacteria.) |
| **1604000**<br>**Fungi Bioburden** | Mold and yeast count. Extracts are plated using media designed to select for yeast and mold organisms (fungi). |
| **1605500**<br>**Spore Bioburden** | Aerobic spore count. Extracts are heat shocked to eliminate vegetative cells but recover spores. |
| **1605600**<br>**Anaerobe Bioburden** | Anaerobe microflora count. Extracts are incubated under anaerobic conditions. (Test conditions may recover facultative organisms as well.) |
| **1607000**<br>**Aerobe and Fungi Bioburden** | Separate aerobe and fungi microflora counts. |

| | |
|---|---|
| Full aerobe characterization for products that have demonstrated no anaerobe bioburden. | **1603010**<br>**Aerobe Bioburden Panel**<br>**Aerobe / Spore / Fungi** |
| Intended for items for which a full characterization of the bioburden is needed. | **1605000**<br>**Total Bioburden Panel**<br>**Aerobe / Anaerobe / Spore / Fungi** |
| Devices are extracted multiple times to determine overall efficiency of the first extraction. The percent efficiency and the correction factor are calculated for use in future bioburden evaluations performed on the product. This method is *not* recommended for items which typically display a very low bioburden (e.g., less than 50 CFU per device).<br>**SUGGESTED SAMPLE** 5 devices | **1601000**<br>**Bioburden Recovery Efficiency – Repetitive Recovery Method** |
| Devices are inoculated with a known quantity of bacterial spores and then subjected to the established bioburden procedure. The recovered spores are counted and a correction factor is calculated for use in future bioburden evaluations.<br>**SUGGESTED SAMPLE** 3 sterile devices | **1602000**<br>**Bioburden Recovery Efficiency – Spore Inoculation Method** |

# BIOBURDEN TESTING – MICROBIOLOGICAL EXAMINATION

USP <61> - Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests

- Suitability
- Testing of Products

USP <62> - Microbiological Examination of Nonsterile Products: Tests for Specified Organisms

- Suitability
- Testing of Products

Microbiological Examination Tests, as outlined in USP <61> and <62> are "intended to determine whether a substance or preparation complies with an established specification for microbiological quality" and are designed to "allow determination of the absence of, or limited occurrence of, specified microorganisms that may be detected under the conditions described" in the procedure. These test methods can be applied to pharmaceutical articles, both finished and raw materials, and may also be useful for evaluating the presence of organisms on select materials used in some medical devices or biologics. It is designed to provide an estimate of the number of viable aerobic microorganisms, both bacteria and fungi, and/or to screen for specific target microbial species.

| | |
|---|---|
| **161402**<br><br>**Microbial Enumeration Tests – Suitability** | Test should be performed at least once (and as circumstances require subsequently) to demonstrate that test sample does not inhibit recovery or multiplication, under test conditions, of microorganisms that may be present. Aliquots of the diluted sample are inoculated with separate, diluted cultures of *Staphylococcus aureus*, *Pseduomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. Confirmed inoculum counts are compared to counts recovered in the presence of the test material to determine whether the method provides for satisfactory neutralization of any inhibitory properties from the test material and appropriate recovery of the inoculum organisms. |
| **161400**<br><br>**Microbial Enumeration Tests – Testing of Products** | Test is designed to determine total aerobic microbial count, and total yeast and mold count that can be recovered from the test material under the conditions and by the methods outlined in USP <61>. |

| | |
|---|---|
| Test should be performed at least once (and as circumstances require subsequently) to demonstrate that test sample does not inhibit recovery or multiplication, under test conditions, of microorganisms that may be present. Aliquots of the diluted sample are inoculated with separate, diluted cultures of *Staphylococcus aureus*, *Pseduomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Candida albicans* and *Clostridium sporogenes*. Confirmed inoculum counts are compared to counts recovered in the presence of the test material to determine whether the method provides for satisfactory neutralization of any inhibitory properties from the test material and appropriate recovery of the specified organisms. | **161403**<br><br>**Tests for Specified Microorganisms – Suitability** |
| Test is designed to demonstrate freedom of the test material from Bile-tolerant Gram-Negative Bacteria, *Escherichia coli, Salmonella*, *Pseduomonas aeruginosa*, *Staphylococcus aureus*, *Clostridia* and *Candida albicans*. | **161401**<br><br>**Tests for Specified Microorganisms – Testing of Products** |

# CONTROLLED ENVIRONMENT TESTING – MICROBIAL SAMPLING TESTS

**1701000**

**Environmental Air Sample**

Growth on each environmental sample unit (either plates or strips) is enumerated. Gram stain and/or identification are available.

**SAMPLE REQUIREMENTS** Client provides air sample(s) for incubation and enumeration.

**PREFERRED SHIPPING** Overnight air. Protect from temperature extremes.

---

**1702000**

**Environmental Surface Sample**

Growth on each surface sample (RODAC plate) is enumerated. Gram stain and/or identification are available.

**SAMPLE REQUIREMENTS** Client provides surface sample(s) for incubation and enumeration.

**PREFERRED SHIPPING** Overnight air. Protect from temperature extremes.

---

**170300 – 100 mL**

**170400 – 1 mL**

**Water System Microbial Counts**

Sample aliquots obtained from a water system are evaluated for number of viable microorganisms in each 100 mL or 1 mL sample.

**SAMPLE REQUIREMENTS** 100 mL or 1 mL sample in sterile sealable container. (Client provides sterile collection containers.) Keep refrigerated.

**PREFERRED SHIPPING** Overnight air. Ship with cold packs.

---

**107500**

**Water System Coliform Count**

Used as an indicator of the quality of water. Samples are screened for coliform bacteria, a major intestinal bacterial pathogen.

**SAMPLE REQUIREMENTS** 100 mL. Keep refrigerated.

**PREFERRED SHIPPING** Overnight air. Ship with cold packs.

---

**107501**

**Water System Microbial Counts and Water System Coliform Count**

In this test, both Water System Microbial Counts (Test Code: 170300/170400) and Water System Coliform Counts (Test Code: 107500) are performed.

**SAMPLE REQUIREMENTS** 110 mL or 200 mL. Keep refrigerated.

**PREFERRED SHIPPING** Overnight air. Ship with cold packs.

## CONTROLLED ENVIRONMENT TESTING – SAMPLING PRODUCTS

| | |
|---|---|
| The RCS impact sampler is used in the detection of microbes in air samples from manufacturing zones. | **1705000**<br>**Rental of RCS Impact Sampler** |
| These strips have Tryptic Soy Agar (TSA) for isolation and cultivating a variety of aerobic microorganisms. | **1705010**<br>**RCS Impact Sampler Strips – Tryptic Soy Agar** |
| These plates, with Tryptic Soy Agar (TSA), contain neutralizing agents to inactivate residual disinfectants when the sample is being collected and are used for isolating and cultivating a variety of aerobic microorganisms from a surface or air (fallout) sample. Surface sampling is used to determine the quality of routine cleaning and sanitization procedures. The RODAC method will semiquantify the amount of microbial content on the surface. | **1705030**<br>**RODAC Sample Plates – Tryptic Soy Agar with Lecithin & Polysorbate 80** |

# GROWTH PROMOTION

Prepared media must be tested prior to use to ensure it will support the growth of low levels of microorganisms. WuXi AppTec offers this testing per USP requirements as well as customized per client request.

| | |
|---|---|
| **190411**<br><br>**Growth Promotion for Liquid Media (3 orgs per USP <71>)** | Sterility Test Medium is tested for growth promotion using the current USP <71> organisms for Soybean-Casein Digest Medium (SCDM) or Fluid Thioglycollate Medium (FTM). (Additional organisms available upon request.)<br><br>**SAMPLE REQUIREMENTS** 3 samples |
| **190412**<br><br>**Growth Promotion for Solid / Liquid Media (5 orgs per USP <61>)** | Growth Medium is tested for growth promotion using the current USP <61> organisms in agar or broth for Soybean-Casein Digest Medium (SCDM) or Sabouraud Dextrose Medium . (Additional organisms available upon request.)<br><br>**SAMPLE REQUIREMENTS** 5 samples |
| **190410**<br><br>**Growth Promotion per Organism** | Growth Medium is tested for growth promotion using selected organisms in specified media.<br><br>**SAMPLE REQUIREMENTS** 1 sample per organism |

# INOCULATED PRODUCT TESTS

Inoculated product consists of actual devices or materials that have been inoculated with a specified level of a liquid biological indicator (BI) suspension. Inoculated products are used to validate and / or monitor certain sterilization processes. Testing is performed by product immersion using either the BI manufacturer's parameters or those found in USP, ISO or AAMI standards.

Biological indicators (BIs) are carriers, such as a paper strip, that are inoculated with a specified level of a particular organism (typically *Bacillus* species). BIs are used to validate and/or monitor certain sterilization processes.

---

Devices are inoculated (usually in a location determined as most difficult to sterilize) with an indicator organism appropriate to the sterilization system in use.

**SAMPLE REQUIREMENTS** No minimum.

*Indicate required population and sterilization method.*

**1902000** *B. atrophaeus*
**1902100** *G. stearothermophilus*

**Product Inoculation**

---

After devices are cleaned per manufacturer's instruction, a bioburden test is performed to evaluate the effectiveness of the cleaning. Results are reported as a percent reduction.

**SAMPLE REQUIREMENTS** 4 product samples (1 to be used as positive control)

**160210**

**Spore Count for Inoculated Product**

---

Individual spore strips are transferred from their primary package to Soybean-Casein Digest Medium (SCDM) and incubated for recovery of the indicator organism.

**SAMPLE REQUIREMENTS** Spore strips
(Client-provided positive control recommended.)

**SHIPPING** Overnight air. Protect from temperature extremes.

**1204100**

**Biological Indicator Direct Transfer**

---

Spore strips that have been placed within a product or its package are retrieved from the product or package and transferred to Soybean-Casein Digest Medium (SCDM) for recovery of the indicator organism.

**SAMPLE REQUIREMENTS** Spore strips
(Client-provided positive control recommended.)

**SHIPPING** Overnight air. Protect from temperature extremes.

**1205100**

**Biological Indicator Within Product**

# INOCULATED PRODUCT TESTS

## Inoculated Product Sterility

**1221010**
Extra Small [≤ 100 mL of media]

**1221000**
Small [100 and 200 mL of media]

**1221210**
Medium [300 and 400 mL of media]

**1221410**
Large [500 and 600 mL of media]

**1221610**
Extra Large [800 and 1000 mL of media]

**1221620**
Jumbo [1200 and 1500 mL of media]

**1221630**
Extra Jumbo [2000 mL of media]

Product that has been inoculated with a liquid spore solution and exposed to a sterilization process is tested in Soybean-Casein Digest Medium (SCDM) to detect surviving organisms.

**SAMPLE REQUIREMENTS** Dependent on method and sterilizer volume.

---

**190501**

## Liquid Sample Population Confirmation

Liquid samples, including spore suspensions and inoculated liquids, are enumerated to confirm spore population.

**SAMPLE REQUIREMENTS** Dependent upon expected population.

---

**190300** 3 Sample Composite
**120200** Single Sample

## Biological Indicator – Total Viable Spore Count

Before using a new lot of BIs for sterilization load monitoring, the average population per unit should be independently confirmed per USP regulations.

**SAMPLE REQUIREMENTS** Dependent on selected test.

**SHIPPING** Overnight air. Protect from temperature extremes.

## MICROBIAL IDENTIFICATION

| | |
|---|---|
| Differential staining technique used to categorize microorganisms. | **190601**<br>**Gram Stain** |
| Description of an organism's macroscopic (colony) appearance, including shape, color and texture. | **190640**<br>**Colony Morphology** |
| Description of an organism's macroscopic (colony) appearance, including shape, color and texture, plus differential staining to determine organism category. | **190630**<br>**Gram Stain and Colony Morphology** |
| Identification of a microbial isolate to at least the *genus* level. | **190401**<br>**Bacterial / Microbial Identification** |

# STERILITY TESTING – BIOLOGICAL INDICATORS

Biological indicators (BIs) are carriers, such as a paper strip, that are inoculated with a specified level of a particular organism (typically *Bacillus* species). BIs are used to validate and/or monitor certain sterilization processes. Testing is performed according to either the BI manufacturer's recommendations or USP, ISO, or AAMI requirements.

**1201000**

## Biological Indicator Direct Transfer

Individual spore strips are transferred from their primary package to Soybean-Casein Digest Medium (SCDM) and incubated for recovery of the indicator organism.

**SAMPLE REQUIREMENTS** Spore strips
(Client-provided positive control recommended.)

**SHIPPING** Overnight air. Protect from temperature extremes.

**1203000**

## Biological Indicator Within Product

Spore strips that have been placed within a product or its package are retrieved from the product or package and transferred to Soybean-Casein Digest Medium (SCDM) for recovery of the indicator organism.

**SAMPLE REQUIREMENTS** Spore strips
(Client-provided positive control recommended.)

**SHIPPING** Overnight air. Protect from temperature extremes.

**120080** Self-Contained
**120081** Within Product

## Biological Indicator – Self-Contained

Self-contained BIs that have been placed within a product or its package are removed, activated and incubated for the recovery of the indicator organism.

**SAMPLE REQUIREMENTS** Spore strips
(Client-provided positive control recommended.)

**SHIPPING** Overnight air. Protect from temperature extremes.

**190300** 3 Sample Composite
**120200** Single Sample

## Biological Indicator – Total Viable Spore Count

Before using a new lot of BIs for sterilization load monitoring, the average population per unit should be independently confirmed per USP regulations.

**SAMPLE REQUIREMENTS** Dependent on selected test.

**SHIPPING** Overnight air. Protect from temperature extremes.

# STERILITY TESTING – STERILITY METHOD SUITABILITY TEST (B/F)

The Sterility Method Suitability Test (B/F) is necessary to demonstrate that there are no substances produced by the test materials (in the specified volume of test medium) that would cause inhibition of bacterial or fungal growth in a sterility test (i.e., a false negative interpretation). Testing is performed by inoculating sterility test samples in media with low levels of selected organisms to ensure growth. The parameters for the Sterility Method Suitability Test (B/F) are based on USP, ISO, CFR or AAMI requirements.

Sample device or material in the sterility test medium is tested for growth inhibition using the current USP organisms for Soybean-Casein Digest Medium (SCDM) and Fluid Thioglycollate Medium (FTM). (Additional organisms available upon request.)

**SAMPLE REQUIREMENTS** 6 sterile product samples

**190105** Immersion
**190104** Membrane Filtration

**Sterility Method Suitability Test (B/F) – Two Media [USP]**

---

Sample device or material in the sterility test medium is tested for growth inhibition using the current USP organisms for Soybean-Casein Digest Medium (SCDM). This method is used when only SCDM (TSB) is used for sterility testing products. (Additional organisms available upon request.)

**SAMPLE REQUIREMENTS** 3 sterile product samples

**190106** Immersion
**190107** Membrane Filtration

**Sterility Method Suitability Test (B/F) – One Medium**

---

Sample device or material in the sterility test medium is tested for growth inhibition using selected organisms in specified media.

**SAMPLE REQUIREMENTS** 1 sterile product sample per organism per medium

**190111** Immersion
**190112** Membrane Filtration

**Sterility Method Suitability Test (B/F) – Per Organism, Per Medium**

# STERILITY TESTING – PRODUCT STERILITY TESTS

Product sterility testing is typically performed in the validation of sterilization processes, and, in some cases, for monitoring sterilization cycles. Sterility tests involve total immersion, membrane filtration, or a rinse method. The number of samples tested, the growth medium used, and the incubation conditions are based on the particular standard involved – USP, AAMI/ISO or FDA/CFR.

## AAMI/ANSI/ISO Sterility Immersion

**110100.1**
≤ 500 mL of media

**110100.3**
600 - 1000 mL of media

**110100.5**
1200 - 2500 mL of media

This test is used in sterilization validations (e.g., radiation, EO). Products are tested in Soybean-Casein Digest Medium (SCDM) at 30° ± 2°C for 14 days.

**SAMPLE REQUIREMENTS** Dependent on method used (e.g., AAMI 11137 Method 1 requires 100 samples, VDmax requires 10 samples)

## USP Sterility Immersion

**110100.2**
≤ 500 mL of media

**110100.4**
600 - 1000 mL of media

**110100.6**
**1200 - 2500 mL of media**

This testing is used to monitor sterilization loads. Products are tested in both Soybean-Casein Digest Medium (SCDM) and Fluid Thioglycollate Medium (FTM) per USP guidelines.

**SAMPLE REQUIREMENTS** Up to 40 product samples

# STERILITY TESTING – LIQUID STERILITY TESTS

This test is typically designed for aliquots ≤100 mL. Aliquot is transferred directly into the sterility test medium.

**SAMPLE REQUIREMENTS** Sample requirement is dependent upon lot size.

***For Test Codes, refer to Product Sterility Tests on opposite page.***

**Liquid Sterility Test – Direct Transfer**

---

Sterility testing is performed using sterile filtration. Liquid sample is filtered and filter is placed in a single medium [typically Soybean-Casein Digest Medium (SCDM)].

**SAMPLE REQUIREMENTS** Sample requirement is dependent upon lot size.

**1223000** < 100 mL
**1225000** 100-800 mL
**1231000** > 800 mL

**Liquid Sterility Test – Membrane Filtration**

---

Sterility testing is performed using sterile filtration. Liquid sample is filtered and filter is halved – or, alternately, liquid is halved and two filters are used – and placed in two media [typically Soybean-Casein Digest Medium (SCDM) and Fluid Thioglycollate Medium (FTM)].

**SAMPLE REQUIREMENTS** Sample requirement is dependent upon lot size.

**122310** < 100 mL
**122510** 100-800 mL
**123110** >800 mL

**USP Liquid Sterility Test – Membrane Filtration**

---

Sterility testing is performed by filling a device with liquid media and incubating the filled device.

**SAMPLE REQUIREMENTS** Sample requirement is dependent upon lot size.

**1229500** ≤ 200 mL
**1229600** 300-700 mL
**1229650** ≥ 800 mL

**Fluid Path Fill**