



## BIOBURDEN / MICROBIAL ASSAYS

Microbial assays involve a variety of tests, from the determination of the numbers and types of organisms naturally present on a product or material, to the assessment of a product or ingredient to have an antibacterial or preservative action against microorganisms. WuXi AppTec can perform the types of microbial assays listed below as well as customized studies.

Other tests are available.  
Contact your WuXi AppTec  
Account Manager.

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## 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.

<b>1606000</b> <b>Aerobe Bioburden</b>	Aerobe microflora count. (Test conditions may also recover mold and yeasts as well as bacteria.) <b>TURNAROUND TIME</b> 7-10 days
<b>1604000</b> <b>Fungi Bioburden</b>	Mold and yeast count. Extracts are plated using media designed to select for yeast and mold organisms (fungi). <b>TURNAROUND TIME</b> 7-10 days
<b>1605500</b> <b>Spore Bioburden</b>	Aerobic spore count. Extracts are heat shocked to eliminate vegetative cells but recover spores. <b>TURNAROUND TIME</b> 5-10 days
<b>1605600</b> <b>Anaerobe Bioburden</b>	Anaerobe microflora count. Extracts are incubated under anaerobic conditions. (Some facultative organisms may also grow.) <b>TURNAROUND TIME</b> 5-10 days
<b>1607000</b> <b>Aerobe and Fungi Bioburden</b>	Separate aerobe and fungi microflora counts. <b>TURNAROUND TIME</b> 7-10 days
<b>1603010</b> <b>Aerobe Bioburden Panel</b> <b>Aerobe / Spore / Fungi</b>	Full aerobe characterization for products that have demonstrated no anaerobe bioburden. <b>TURNAROUND TIME</b> 7-10 days

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Intended for items for which a full characterization of the bioburden is needed.

**TURNAROUND TIME** 7-10 days

**1605000**

**Total Bioburden Panel**

**Aerobe / Anaerobe / Spore / Fungi**

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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).

**SUGGESTED SAMPLE** 5 devices  
Contact laboratory for sample details.

**TURNAROUND TIME** 7-10 days

**1601000**

**Bioburden Recovery Efficiency –  
Repetitive Recovery Method**

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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.

**SUGGESTED SAMPLE** 3 sterile devices  
Contact laboratory for sample details.

**TURNAROUND TIME** 3-5 days

**1602000**

**Bioburden Recovery Efficiency –  
Spore Inoculation Method**

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## MICROBIAL IDENTIFICATION

190601

**Gram Stain**

Differential staining technique used to categorize microorganisms.

**TURNAROUND TIME** 1-2 days

190640

**Colony Morphology**

Description of an organism's macroscopic (colony) appearance, including shape, color and texture.

**TURNAROUND TIME** 1-2 days

190630

**Gram Stain and  
Colony Morphology**

Description of an organism's macroscopic (colony) appearance, including shape, color and texture, plus differential staining to determine organism category.

**TURNAROUND TIME** 1-2 days

190401

**Bacterial Identification**

Determination of the genus *or* genus and species of a bacterial isolate.

**TURNAROUND TIME** 3-14 days

190650

**Fungi/Yeast/Actinomyces  
Identification**

Determination of the genus *or* genus and species of a fungi, yeast or *Actinomyces* isolate.

**TURNAROUND TIME** 3-14 days

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.

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*, *Pseudomonas 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.

**TURNAROUND TIME** 10-14 days

**161402**

**Microbial Enumeration Tests – Suitability**

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>.

**TURNAROUND TIME** 7-10 days

**161400**

**Microbial Enumeration Tests – Testing of Products**

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*, *Pseudomonas 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.

**TURNAROUND TIME** 3-10 days

**161403**

**Tests for Specified Microorganisms – Suitability**

Test is designed to demonstrate freedom of the test material from Bile-Tolerant Gram-Negative Bacteria, *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridia* and *Candida albicans*.

**TURNAROUND TIME** 7-10 days

**161401**

**Tests for Specified Microorganisms – Testing of Products**

## ANTIMICROBIAL EFFICACY TESTING – IN VITRO ASSAYS FOR DEVICES / COMBINATION PRODUCTS

WuXi AppTec offers the following assays to determine the antimicrobial activity of medical devices, components or other materials treated with antimicrobial agents. Any of the standard test methods listed here can be modified to include different/additional organisms as well as different/additional exposure times based on product application, claims and characteristics.

Contact our Atlanta laboratory for more information or to discuss specific modifications.

**MINIMUM SAMPLE REQUIREMENTS:** Contact lab. *Untreated controls are recommended for all tests.*

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**190660**

**USP <51>  
Antimicrobial Effectiveness**

Determines the effectiveness of antimicrobial (preservative) substances for the following products: injections and other parenterals including emulsions, otic products, sterile nasal products and ophthalmic products made with aqueous bases or vehicles; topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes; oral products other than antacids, made with aqueous bases or vehicles; antacids made with an aqueous base. Typically conducted prior to conducting microbial recovery assays involving products with potential inhibitory or microbicidal activity.

Samples are inoculated with known levels of micro-organisms and are evaluated for degree of inhibition over a 28-day period.

**TURNAROUND TIME** 6-7 weeks

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**190661**

**USP <1227>  
Validation of Microbial Recovery  
(Neutralization Validation)**

Evaluates the method chosen to neutralize the anti-microbial properties of any product with inhibitory or microbiocidal activity. The purpose of the assay is to ensure the validity of test results achieved during the USP <51> Antimicrobial Effectiveness test and other microbial recovery tests. It is conducted prior to estimating the number of viable micro-organisms.

**TURNAROUND TIME** 7-10 days

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**190665**

**JIS Z 2801 / ISO 22196  
Antimicrobial Activity,  
Quantitative**

Specifies the testing methods to evaluate antimicrobial activity and antimicrobial efficacy of bacteria on the surface of antimicrobial products. Recommended test organisms are *Staphylococcus aureus* and *Escherichia coli*. Triplicate samples are inoculated and evaluated for antimicrobial activity over selected contact periods between 1 to 24 hours.

**TURNAROUND TIME** 5-8 days

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**110790**

**Zone of Inhibition /  
Kirby-Bauer Susceptibility**

Demonstrates activity/potency of antimicrobials or antibiotics, based on measuring the zone of inhibition observed for specified microorganisms. Areas of particular application include materials treated or infused with an antimicrobial agent that leaches out of the material.

**TURNAROUND TIME** 3-5 days

**ANTIMICROBIAL EFFICACY TESTING –  
IN VITRO ASSAYS FOR  
DEVICES / COMBINATION PRODUCTS**

Quantitatively evaluates the effectiveness of a sample treated with a non-leaching antimicrobial by shaking in an organism suspension. The typical challenge organism is *Klebsiella pneumoniae*. Samples are exposed to the challenge organism in a liquid suspension for one (1) hour under continuous agitation. The percent reduction of the challenge organism is then calculated.

**TURNAROUND TIME** 5-8 days

**110780**

**ASTM E-2149 – Dynamic Contact  
[Antibacterial, Quantitative]**

Quantitatively evaluates the effectiveness of an antimicrobial agent incorporated into hydrophobic polymeric material. Recommended challenge organisms are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (or *Klebsiella pneumoniae*). An aqueous-based bacterial inoculum remains in close contact with the treated material as a “pseudo-biofilm.” Treated and non-treated (control) samples are compared for determination of percent reduction over a defined time period.

**TURNAROUND TIME** 7-10 days

**110770**

**ASTM E-2180 –  
Bound Antibacterial Activity**

Duplicate samples are inoculated with the selected challenge microorganisms and changes in that inoculum population are evaluated at time points selected based on intended use of the material or over a longer period of time to develop a kill model for the material.

**TURNAROUND TIME** 5-8 days

**110795**

**ASTM E-2315 –  
Time-Kill Procedure**

Qualitatively evaluates ability of synthetic polymeric test material to support the growth of a mixture of five (5) fungi. Synthetic polymeric test material is usually provided in the form of molded and fabricated articles, tubes, rods, sheets and film materials. Samples are inoculated with the fungi mixture and evaluated for the degree of growth over a 21-day period.

**TURNAROUND TIME** 4-5 weeks

**110760**

**ASTM G-21 –  
Antifungal, Semi-Quantitative**

Determines the efficacy of sanitizing agents on various inanimate surfaces, such as counters, floors and other areas/materials that are routinely cleaned. Typically conducted to validate sanitizing procedures for cleanrooms or aseptic processing areas.

**TURNAROUND TIME** 4-5 weeks

**Custom**

**ASTM E-1153 –  
Sanitizer Efficacy**

**See next page for: Antimicrobial Efficacy Testing – In Vivo Studies**

# ANTIMICROBIAL EFFICACY TESTING – IN VIVO STUDIES FOR DEVICES / COMBINATION PRODUCTS

## Custom

### Antimicrobial Efficacy (AME) – In Vivo Studies

FDA-accepted animal models (developed by WuXi AppTec) test the efficacy of antimicrobial components incorporated into medical devices, providing an infectious agent-challenge study to evaluate antimicrobial-device combinations. Each antimicrobial efficacy (AME) study is custom designed, using a number of clinically relevant bacterial strains, and several implant methodologies to produce a consistent and non-lethal *in vivo* device infection to assess and compare antimicrobial device components. Analyses of infection at device explant include quantitative assessment of remaining bacteria from device and surrounding tissue, imaging capabilities, and specific strain identification to confirm the bacterial strain of the resulting infection.

#### Bacterial Strains

WuXi AppTec's library of bacterial strains continues to expand. The bacterial strains most commonly used in developing *in vivo* device infections include:

- *Staphylococcus aureus*
- *Staphylococcus epidermis*
- *Staphylococcus capitis*
- *Escherichia coli*
- *Acinetobacter baumannii*
- Methicillin-resistant *Staph A* (MRSA)
- Clinical isolates provided by Sponsor

#### Additional Analyses

Additional available analyses include:

- *In vitro* antimicrobial tests, including Zone of Inhibition
- Histopathology to assess local response and microscopic evidence of bacterial infection
- Biochemical and DNA identification of recovered bacterial strains
- Hematology and clinical chemistry analyses to assess signs of infection and disease progression
- Drug analysis of serum and tissue samples
- Imaging analysis of explanted device surfaces, including scanning electron microscopy and confocal laser scanning microscopy

**Samples for Antimicrobial Efficacy *In Vivo* Studies  
should be shipped to our St. Paul facility.**

**Contact your WuXi AppTec Account Manager for  
further information regarding these studies.**

## ANTIMICROBIAL ASSAYS FOR INDUSTRIAL PRODUCTS

Antimicrobial assays are used to determine or confirm the effectiveness of treatments applied to commercial textiles and other industrial products. Test methods for measuring the antimicrobial activity include ASTM, AATCC, USP and other standard or modified methods. Testing includes antibacterial and antifungal activity, with both qualitative and quantitative assays available.

Note: Test parameters in the following descriptions are those found in the standard method referenced. Many test methods allow modifications to these parameters based on product application, claims and characteristics. Contact the lab for more information or to discuss specific modifications.

**MINIMUM SAMPLE REQUIREMENTS:** Contact lab. *Untreated controls are recommended for all tests.*

<p>Samples are inoculated with <i>Aspergillus brasiliensis</i> and evaluated for the degree of growth over a 7-day period.</p> <p><b>TURNAROUND TIME</b> 8-10 days</p>	<p><b>110700</b></p> <p><b>AATCC Method 30, Part III</b> <b>Antifungal Evaluation, Qualitative</b></p>
<p>Samples are inoculated with <i>Staphylococcus aureus</i> and <i>Klebsiella pneumoniae</i> and evaluated for percent reduction of the bacteria over selected contact periods between 1 to 24 hours.</p> <p><b>TURNAROUND TIME</b> 5-8 days</p>	<p><b>110710</b></p> <p><b>AATCC Method 100</b> <b>Antibacterial Evaluation,</b> <b>Quantitative</b></p>
<p>Samples placed in direct contact with <i>Staphylococcus aureus</i> and <i>Klebsiella pneumoniae</i> inoculum streaks are evaluated for inhibition of growth and zones of inhibition within 24 hours.</p> <p><b>TURNAROUND TIME</b> 3-5 days</p>	<p><b>110720</b></p> <p><b>AATCC Method 147</b> <b>Antibacterial Evaluation,</b> <b>Qualitative</b></p>
<p>The same principle as AATCC Method 147 (<i>above</i>), but specific for carpets.</p> <p><b>TURNAROUND TIME</b> 3-5 days</p>	<p><b>110730</b></p> <p><b>AATCC Method 174, Part 1</b> <b>Antibacterial, Qualitative</b> <b>(Carpets)</b></p>
<p>The same principle as AATCC Method 100 (<i>above</i>), but specific for carpets.</p> <p><b>TURNAROUND TIME</b> 5-8 days</p>	<p><b>110740</b></p> <p><b>AATCC Method 174, Part 2</b> <b>Antibacterial, Quantitative</b> <b>(Carpets)</b></p>
<p>The same principle as AATCC Method 30, Part III (<i>above</i>), but specific for carpets.</p> <p><b>TURNAROUND TIME</b> 8-10 days</p>	<p><b>110750</b></p> <p><b>AATCC Method 174, Part 3</b> <b>Antifungal, Qualitative (Carpets)</b></p>

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<p><b>Custom</b></p> <p><b>ASTM E-1153 – Sanitizer Efficacy</b></p>	<p>Determines the efficacy of sanitizing agents on various inanimate surfaces, such as counters, floors other areas/materials that are routinely cleaned. It is typically conducted with variety of microorganisms, most often those of concern in the environment or in product application.</p> <p><b>TURNAROUND TIME</b> 4-5 weeks</p>
<p><b>110780</b></p> <p><b>ASTM E-2149 – Dynamic Contact [Antibacterial, Quantitative]</b></p>	<p>Quantitatively evaluates the effectiveness of a sample treated with a non-leaching antimicrobial by shaking in an organism suspension. The typical challenge organism is <i>Klebsiella pneumoniae</i>.</p> <p><b>TURNAROUND TIME</b> 5-8 days</p>
<p><b>110770</b></p> <p><b>ASTM E-2180 – Bound Antibacterial Activity</b></p>	<p>Quantitatively evaluates the effectiveness of an antimicrobial agent incorporated into hydrophobic polymeric material. Recommended challenge organisms are <i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i> or <i>Klebsiella pneumoniae</i>.</p> <p><b>TURNAROUND TIME</b> 7-10 days</p>
<p><b>110795</b></p> <p><b>ASTM E-2315 – Time-Kill Procedure</b></p>	<p>Quantitatively evaluates the antimicrobial activity of a test material within a specified time period using a time-kill procedure. The procedure utilizes test organisms that may be representative of the microbial flora encountered under conditions of use, or may represent standardized strains.</p> <p><b>TURNAROUND TIME</b> 5-8 days</p>
<p><b>110785</b></p> <p><b>ASTM E-2471 – Antimicrobial Activity in Carpet</b></p>	<p>Qualitatively evaluates (both stereo-microscopically and visually) antibacterial and antifungal activity at the fiber layer and at the primary backing layer of carpet when challenged with <i>Aspergillus niger</i>, <i>Serratia marcescens</i> and <i>Staphylococcus aureus</i>.</p> <p><b>TURNAROUND TIME</b> 5-8 days</p>
<p><b>110760</b></p> <p><b>ASTM G-21 – Antifungal, Semi-Quantitative</b></p>	<p>Samples are inoculated with a mixture of five (5) fungi and evaluated for the degree of growth over a 21-day period.</p> <p><b>TURNAROUND TIME</b> 4-5 weeks</p>
<p><b>110790</b></p> <p><b>Zone of Inhibition / Kirby-Bauer</b></p>	<p>This is a modified susceptibility test where the sample is in direct contact with an inoculum of a specified organism. The sample is evaluated for zone of inhibition.</p> <p><b>TURNAROUND TIME</b> 3-5 days</p>