BET METHODOLOGY & SUPPORT SERVICES

BET Method Options

Methodology & Applications

Through free consultation and support, we will assist you to determine the method that best suits your needs and sample type. ACC offers all regulatory-compliant kinetic and gel-clot BET methods.

Introduction

Limulus Amebocyte Lysate (LAL) tests detect and quantify bacterial endotoxins derived from the outer cell wall membrane of gram-negative bacteria. The critical component of the LAL reagents used in endotoxin tests is derived from blood cells (amebocytes) of the horseshoe crab, *Limulus polyphemus*. Amebocytes contain the proteins of the blood-clotting mechanism, which is triggered primarily by endotoxins and also by (1→3)-β-D-glucan. LAL reagents are primarily used to test for endotoxins in injectable pharmaceuticals, biological products, medical devices, and renal dialysis centers. Endotoxin tests are described in the Bacterial Endotoxins Test chapter in the US Pharmacopeia (Chapter <85>) and in the equivalent chapters in the European Pharmacopoeia (Chapter 2.6.14) and the Japanese Pharmacopoeia (General Tests, No. 4.01). Modified LAL reagents can be used for specific detection of (1→3)-β-D-glucan.

Selecting a Method

Consider the following when deciding which bacterial endotoxin test method to use:

- What are the regulatory requirements, if any?
- What type of product or sample is to be tested?
- What test sensitivity is required? (What is the endotoxin limit specification for the sample?)
- Is quantitative analysis desired?

There are three principal bacterial endotoxin test methods: the chromogenic, turbidimetric, and gel-clot methods. The first two may be grouped together as kinetic photometric methods, as they require a timed optical reader.

Both chromogenic and turbidimetric methods offer the greatest sensitivity, allowing detection of low endotoxin concentrations and greater dilution of samples, which is important for overcoming interference. Both kinetic methods utilize software to quantify your test results. The gel-clot method is a simple, positive/negative, low start-up cost alternative that has been the reference method for years.

Kinetic Testing Methods

Chromogenic Method

The BET reagent is formulated with a synthetic substrate which produces a chromophore when cleaved by endotoxin-activated enzymes.

- Requires either the Pyros Kinetix® Flex tube reader or an incubating plate reader system, such as the BioTek ELx808 IUTM*
- Maximum sensitivity to 0.001 EU/mL, the highest chromogenic sensitivity available in the BET industry when using ACC's Pyrochrome® reagent
- Electronically stored data
- Incubation time varies depending on the standard curve range
- High sensitivity allows for greater dilution to overcome interference

Turbidimetric Method

The optical density (turbidity) increase that accompanies the clotting reaction is read in our Pyros Kinetix® Flex tube reader or in an incubating microplate reader.

- Requires either the Pyros Kinetix® Flex tube reader system or an incubating microplate reader such as the BioTek ELx808 IUTM*
- Maximum sensitivity to 0.001 EU/mL, the highest sensitivity available in the BET industry when using ACC's Pyrotell®-T reagent
- Quantitative test results and electronically stored data
- Incubation time varies depending on the standard curve range; results can be obtained in as little as 15 minutes with ACC reagents
- High sensitivity allows for greater dilution to overcome interference

Gel-Clot BET Testing Method

Gel-Clot Method

The formation of a gel clot indicates the presence of endotoxin in a sample. The method is performed in small test tubes and is read manually by inverting the test tubes.

- Requires non-circulating water bath or dry bath incubator
- Manually read test
- Reagents of differing sensitivity are available: 0.25, 0.125, 0.06 and 0.03 EU/mL
- May be less sensitive to interference than other methods
- Is the referee method as per BET chapters in the US, European and Japanese Pharmacopeia

Overview of Testing Procedures

The following section summarizes the procedures/steps to be taken to perform routine product release testing of a sample in a regulated environment. In an unregulated environment, or when testing for informational purposes only, follow the procedures described under Preliminary Testing.

Qualification of Reagent, Technician & Laboratory

The reagent must be tested to ensure that it is performing to specification. Technicians must be qualified to perform the test and the absence of significant day-to-day or inter-technician variability in the laboratory should be documented. This requires testing using endotoxin standards only, not samples.

Preliminary Testing

Preliminary Testing is not a regulatory requirement but is an important step to develop a set of conditions for the test method that can be used in the Test for Interfering Factors to demonstrate the absence of interference. During Preliminary Testing, samples should be characterized for endotoxin contamination and/or potential interference. It is typically performed by testing a series of dilutions of sample with and without a Positive Product Control (PPC). PPCs consist of sample with a known amount of endotoxin standard. The purpose is to indicate that added endotoxin is appropriately detected and that the sample does not interfere with the detection of endotoxin.

From the results of the Preliminary Testing, a product dilution and possibly product treatment is selected for the Test for Interfering Factors (see below). The endotoxin limit for the product must be detectable at the dilution selected.

Test for Interfering Factors (Validation)

The Test for Interfering Factors is performed to validate the test conditions and dilution for the particular sample type. It is accomplished by demonstrating that endotoxin added to the sample in PPCs can be readily detected within required limits.

Routine Testing

Routine testing is conducted using the sample method preparation and conditions for the Test for Interfering Factors and includes a parallel PPC to check for interference. Tests also include negative controls and appropriate standards. Multiple number of units per lot of finished product should be tested, usually sampled from the beginning, the middle, and the end of the production run. For medical devices, aqueous extracts of up to 10 units are tested, usually after pooling.

Support Services

ACC offers its customers extensive technical support. Our Global Technical Service department is staffed with experienced professionals who provide customer assistance for the full range of ACC products and services.

Technical support is available by telephone, email, and in person, through workshops, on-site training, or on-site consultation. Customers who have questions about individual products, test methods, instrumentation, and/or software are invited to call our staff.

SOFTWARE VALIDATION PROTOCOLS

ACC offers Validation Protocols that provide the end user with a comprehensive set of integrated documents to guide them through the system validation process. The protocol files allow users to edit the documents to meet their company's specific validation requirements.

REAGENT TRANSFER PROTOCOL

The Reagent Transfer Protocol document (RTP) is used to validate the change from another manufacturer to ACC BET reagents. If changing BET reagent manufacturers, ACC offers assistance with guidance and instructions for using the Validation Protocol. This can be used as verification of the validation process. The Reagent Transfer Protocol is designed to assist users in completing validation of their switch from the current BET reagent to an ACC product. During this process, if you require any assistance, you will be able to obtain help and advice through the Technical Services department of ACC.

EXPERTISE & RESOURCES

Assistance with selecting a test method or reagent sensitivity is always available from our Technical Service Department and representatives in the field. Our staff can help with Preliminary Testing, Testing for Interfering Factors or Routine Testing. The LAL Update®, our newsletter, includes useful technical articles and is available on our website. Our Contract Test Services (see page 38) team regularly performs Preliminary Testing and method development and can provide results using all test methods. Regardless of which method is selected, you can always be assured of the full support of ACC.

For details on endotoxin testing in the United States, users should consult the current revision of the US Pharmacopeia (USP), chapter <85>, "Bacterial Endotoxins Test," and chapter <86>, "Bacterial Endotoxins Test Using Recombinant Reagents." For those testing outside the US, you should consult your local regulatory requirements for the BET.

ON-SITE CONSULTING SERVICES

ACC staff is available to visit client sites to assist investigations and troubleshooting. These visits often address Bacterial Endotoxin Testing (BET) procedures, in addition to identifying sources of contamination in test laboratories and manufacturing processes.

#CSOS01	On-site Consulting Services (per day)
#SCOS01	On-site Service Call (per day)

CUSTOMIZED ON-SITE WORKSHOPS

ACC can customize a workshop for you and your staff and conduct it at your facility or ours. Instructors work with you to create a training program tailored to your specific requirements.

#WKSP01	One-Day BET Workshop (per workshop, up to 5 attendees)
#WKSP02	Two-Day BET Workshop (per workshop, up to 5 attendees)
#WKSP03	Three-Day BET Workshop (per workshop, up to 5 attendees)
OSCP-01	One-Day On-site Compounding Pharmacy Training

METHODOLOGY BACKGROUND

This course is designed to introduce BET methodologies to technicians and managers who are new to endotoxin testing. Topics include:

- Endotoxins—What they are, where they come from, and why they are important
- BET—An overview of the BET/endotoxin reaction, with emphasis on sources of interference
- Detailed instruction of the test methods, including a discussion of laboratory setup, materials, and aseptic techniques
- Sample handling and preparation
- Practical approaches to sample characterization and overcoming interference
- Technician and laboratory certification and validation of the BET

HANDS-ON LABORATORY

The laboratory courses for kinetic and gel-clot methods are designed to give the attendee hands-on experience conducting endotoxin tests. Participants perform tests and learn to read and interpret results. Familiarity with general laboratory techniques (especially pipetting) is essential.

IN-DEPTH TOPICS

This course provides the experienced technician with a more detailed understanding of how a BET program can be applied to quality control. Topics include:

- Techniques for testing non-aqueous or highly interfering substances
- (1→3)-β-D-glucan—Contamination, recognition and investigation
- Medical device extraction and validation of extraction protocols
- Regulatory considerations

Contact Global Technical Services

For course dates and fees, please contact your local ACC representative or check our website at acciusa.com. The Bacterial Endotoxin Testing Workshop schedule can be accessed from the BET Products section or from the Calendar section of the ACC website. To receive additional information or to register for a course, contact the appropriate office below.

UNITED STATES

t 800.848.3248
e techservice@acciusa.com

UNITED KINGDOM

t (44) 151.547.7444
e TechnicalServices@acciuk.co.uk

BEST QC Microbiology Training

Bioburden, Endotoxin, Sterility Testing — Innovative educational programs designed specifically for you.

BEST is a three-day innovative educational program designed specifically for you and your laboratory staff.

This program, brought to you by ACC and MilliporeSigma, will focus on both in-process and product release quality control and will provide an overview of relevant methods in each area. Basic technical laboratory skills, however, are assumed as a prerequisite for participation. The course will consist of class presentations and demonstrations of laboratory applications. This program is only available in the US.

DAY ONE

Bioburden Testing

Dependable Tests Based on Membrane Filtration

Bioburden testing is critical for monitoring water quality and raw materials and for ensuring that manufacturing processes remain in microbiological control. During the first day of the training, you will learn about:

- Advantages and limitations of membrane filtration
- How to choose the right membrane for your application
- The regulations governing bioburden testing
- How to develop a sampling plan for bioburden testing
- How to qualify and validate a method
- How to set alert and action limits
- How to interpret bioburden test results
- How to troubleshoot membrane filtration issues
- Rapid methods for bioburden testing
- Hands-on session using a manifold and Milliflex Plus Pump

DAY TWO

Endotoxin Testing

BET Methodology & Background

The Bacterial Endotoxin Test (BET) is used for the detection and quantitation of endotoxins from gram-negative bacteria. Reagents are primarily used to test for endotoxins in injectable pharmaceuticals, biological products, and medical devices. They are also used in renal dialysis centers and a wide range of other applications. During the second day of training, you will learn:

- What are endotoxin and BET reagents
- The regulations governing bacterial endotoxin testing
- The methodology for bacterial endotoxin testing
- How to qualify a chosen BET method
- How to validate samples and how to test them routinely
- How to analyze and interpret data
- How to address sample interference
- Hands-on session
- Learn about sustainable recombinant reagents

DAY THREE

Sterility Testing

A Complete Solution for Reliable Results

Sterility testing is considered the most essential QC Microbiological test for releasing sterile final product. This test is heavily regulated and harmonized across most of the globe. During the third day of training, you will learn about:

- The history of sterility testing
- The global harmonized regulations overview
- Environmental monitoring requirements for sterility testing
- Deep dive into USP <71>
- Advantages and limitations of direct inoculation sterility testing
- Advantages and limitations of open funnel sterility testing
- Advantages and limitations of closed system sterility testing
- Overview of sterility testing media and rinse fluids
- Most common sterility questions
- Hands-on session — Steritest Equinox

Program Schedule & Fees

This program is only available in the US. For program dates and fees, please contact your local ACC representative or check our website at acciusa.com. To receive additional information or to register for a program, contact the US office below.

UNITED STATES

t 800.848.3248 | **e** techservice@acciusa.com

PROTECTION THROUGH DETECTION

CORPORATE HEADQUARTERS

Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive
Falmouth, MA 02536-4445
USA

t 888.395.2221
t 508.540.3444
f 508.540.8680

acciusa.com

CUSTOMER SERVICE
custservice@acciusa.com

TECHNICAL SERVICE
techservice@acciusa.com

CONTRACT TEST SERVICES
testservice@acciusa.com

UNITED KINGDOM

Associates of Cape Cod Int'l., Inc.
Unit 1 F/G/H Academy Business Park
Lees Road, Knowsley, Liverpool L33 7SA
United Kingdom

t (44) 151.547.7444
f (44) 151.547.7400
e info@acciuk.co.uk

acciuk.co.uk

UK CUSTOMER SERVICE
customerservices@acciuk.co.uk

COMPANY REGISTRATION NUMBER
BR002906