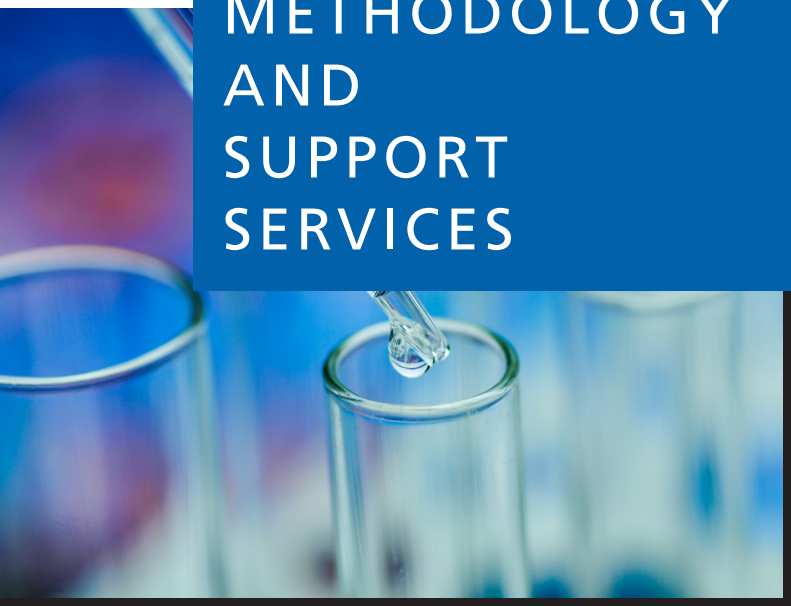
Associates of Cape Cod, Inc.
Your Endotoxin & Glucan Experts

CELEBRATING OUR 45 YEAR ANNIVERSARY

BET METHODOLOGY AND SUPPORT SERVICES



Your Endotoxin Experts!

With the best training and technical support in the field, we'll partner with you every step of the way.

MEDICAL DEVICES
ISO 13485:2016
NSAI Certified

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 United States 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-Glucans.

Selecting a Method

Consider the following when deciding which Bacterial Endotoxins 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 Endotoxins 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 IU™*
- Maximum sensitivity to 0.001 EU/mL, 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 IU™*
- Maximum sensitivity to 0.001 EU/mL, 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

**Trademark of BioTek Instruments, Inc.*

- 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 United States, 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 and 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 ten units are tested, usually after pooling.

SUPPORT SERVICES

ACC offers its customers extensive technical support. Our 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 the user requires any assistance you will be able to obtain help and advice through the Technical Services department of ACC.

Expertise and 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 Service (see page 33) 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 Associates of Cape Cod, Inc.

For details on endotoxin testing in the United States, users should consult the current revision of the United States Pharmacopia (USP), chapter <85>, "Bacterial Endotoxins Test". 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)

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Bacterial Endotoxin Testing (BET) Workshop

Associates of Cape Cod, Inc. offers training courses on all aspects of bacterial endotoxin testing. Courses an be conveniently conducted on-site or at our facilities in East Falmouth, MA; Liverpool, UK; or Mörfelden-Walldorf, Germany.

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	1 Day BET Workshop (per workshop, up to 5 attendees)
#WKSP02	2 Day BET Workshop (per workshop, up to 5 attendees)
#WKSP03	3 Day BET Workshop (per workshop, up to 5 attendees)
OSCP-01	1 Day On-site Compounding Pharmacy Training

We offer a comprehensive workshop that covers methodology, background and in-depth courses, as well as hands-on laboratory experience. A complete schedule can be found on our website, www.acciusa.com.

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 set-up, 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

Course Schedule and Fees

For course dates and fees, please contact your local ACC representative or check our website at www.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: Tel: (800) 848–3248
techservice@acciusa.com

United Kingdom: Tel: (44) 151–547–7444
info@acciuk.co.uk

Europe: Tel: (49) 61 05–96 10 0
service@acciusa.de

ORDERING INFO AND SALES OFFICES

Ordering Information
Customer service representatives are available to assist you with orders, pricing requests and Certificates of Analysis.

Method of Payment For United States

- Check (in US dollars) made payable to Associates of Cape Cod, Inc.
- Wire Transfer (contact Accounts Receivable for routing information)
- Credit Card (AMEX®, VISA®, MasterCard®)
  If payment is to be made by credit card, the following information is required: Type of Credit Card, Card Number, Credit Card Security Code, Expiration Date of Card, and Name (as it appears on the card).

Additional Information
ACC reserves the right to institute, modify or discontinue credit limits provided to customers at any time for any or no reason.

The use of credit cards for payment may incur a fee, please see our website for ACC's policy on credit card usage at www.acciusa.com/pdfs/acctc.pdf

Outside the U.S.
Please contact your local office for information regarding method of payment. For a list of your country specific distributors please visit www.acciusa.com.

Product listings, information and fill sizes are subject to change at any time without prior notice.

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536-4445 USA
Tel: (800) LAL–TEST (525–8378) or (508) 540–3444
Fax: (508) 540–8680
www.acciusa.com
Customer Service: custservice@acciusa.com
Technical Service: techservice@acciusa.com
Contract Test Service: testservice@acciusa.com

United Kingdom
Associates of Cape Cod Int'l., Inc.
Deacon Park, Moorgate Road
Knowsley, Merseyside, L33 7RX, United Kingdom
Tel: (44) 151–547–7444
Fax: (44) 151–547–7400
E-mail: info@acciuk.co.uk
www.acciuk.co.uk
UK Customer Service: customerservices@acciuk.co.uk
Company Registration Number: BR002906

Europe
Associates of Cape Cod Europe GmbH
Opelstrasse 14
D-64546 Mörfelden-Walldorf, Germany
Tel: (49) 61 05–96 10 0
Fax: (49) 61 05–96 10 15
E-mail: service@acciusa.de
www.acciusa.de

ASSOCIATES OF
CAPE COD
INCORPORATED

Specialists in Endotoxin and Glucan Detection

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536-4445 USA
Tel: (800) LAL–TEST (525–8378) or
(508) 540–3444
Fax: (508) 540–8680
www.acciusa.com
Customer Service:
custservice@acciusa.com
Technical Service:
techservice@acciusa.com
Contract Test Service:
testservice@acciusa.com

United Kingdom
Associates of Cape Cod Int'l., Inc.
Deacon Park, Moorgate Road
Knowsley, Liverpool, L33 7RX
United Kingdom
Tel: (44) 151–547–7444
Fax: (44) 151–547–7400
E-mail: info@acciuk.co.uk
www.acciuk.co.uk
UK Customer Support:
customerservices@acciuk.co.uk
Company Registration Number:
BR002906

Europe
Associates of Cape Cod Europe GmbH
Opelstrasse 14
D-64546 Mörfelden-Walldorf
Germany
Tel: (49) 61 05–96 10 0
Fax: (49) 61 05–96 10 15
E-mail: service@acciusa.de
www.acciusa.de