American National Standard

ANSI/AAMI ST72:2011/(R)2016

Bacterial endotoxins — Test methods, routine monitoring, and alternatives to batch testing

AAMI
Advancing Safety in Healthcare Technology

Objectives and uses of AAMI standards and recommended practices

It is most important that the objectives and potential uses of an AAMI product standard or recommended practice are clearly understood. The objectives of AAMI's technical development program derive from AAMI's overall mission: the advancement of medical instrumentation. Essential to such advancement are (1) a continued increase in the safe and effective application of current technologies to patient care, and (2) the encouragement of new technologies. It is AAMI's view that standards and recommended practices can contribute significantly to the advancement of medical instrumentation, provided that they are drafted with attention to these objectives and provided that arbitrary and restrictive uses are avoided.

A voluntary *standard* for a *medical device* recommends to the manufacturer the information that should be provided with or on the product, basic safety and performance criteria that should be considered in qualifying the device for clinical use, and the measurement techniques that can be used to determine whether the device conforms with the safety and performance criteria and/or to compare the performance characteristics of different products. Some standards emphasize the information that should be provided with the device, including performance characteristics, instructions for use, warnings and precautions, and other data considered important in ensuring the safe and effective use of the device in the clinical environment. Recommending the disclosure of performance characteristics often necessitates the development of specialized test methods to facilitate uniformity in reporting; reaching consensus on these tests can represent a considerable part of committee work. When a drafting committee determines that clinical concerns warrant the establishment of *minimum* safety and performance criteria, referee tests must be provided and the reasons for establishing the criteria must be documented in the rationale.

A *recommended practice* provides guidelines for the use, care, and/or processing of a medical device or system. A recommended practice does not address device performance *per se*, but rather procedures and practices that will help ensure that a device is used safely and effectively and that its performance will be maintained.

Although a device standard is primarily directed to the manufacturer, it may also be of value to the potential purchaser or user of the device as a frame of reference for device evaluation. Similarly, even though a recommended practice is usually oriented towards healthcare professionals, it may be useful to the manufacturer in better understanding the environment in which a medical device will be used. Also, some recommended practices, while not addressing device performance criteria, provide guidelines to industrial personnel on such subjects as sterilization processing, methods of collecting data to establish safety and efficacy, human engineering, and other processing or evaluation techniques; such guidelines may be useful to health care professionals in understanding industrial practices.

In determining whether an AAMI standard or recommended practice is relevant to the specific needs of a potential user of the document, several important concepts must be recognized:

All AAMI standards and recommended practices are *voluntary* (unless, of course, they are adopted by government regulatory or procurement authorities). The application of a standard or recommended practice is solely within the discretion and professional judgment of the user of the document.

Each AAMI standard or recommended practice reflects the collective expertise of a committee of health care professionals and industrial representatives, whose work has been reviewed nationally (and sometimes internationally). As such, the consensus recommendations embodied in a standard or recommended practice are intended to respond to clinical needs and, ultimately, to help ensure patient safety. A standard or recommended practice is limited, however, in the sense that it responds generally to perceived risks and conditions that may not always be relevant to specific situations. A standard or recommended practice is an important *reference* in responsible decision-making, but it should never *replace* responsible decision-making.

Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific need of the user.

Particular care should be taken in applying a product standard to existing devices and equipment, and in applying a recommended practice to current procedures and practices. While observed or potential risks with existing equipment typically form the basis for the safety and performance criteria defined in a standard, professional judgment must be used in applying these criteria to existing equipment. No single source of information will serve to identify a particular product as "unsafe". A voluntary standard can be used as one resource, but the ultimate decision as to product safety and efficacy must take into account the specifics of its utilization and, of course, cost-benefit considerations. Similarly, a recommended practice should be analyzed in the context of the specific needs and resources of the individual institution or firm. Again, the rationale accompanying each AAMI standard and recommended practice is an excellent guide to the reasoning and data underlying its provision.

In summary, a standard or recommended practice is truly useful only when it is used in conjunction with other sources of information and policy guidance and in the context of professional experience and judgment.

INTERPRETATIONS OF AAMI STANDARDS AND RECOMMENDED PRACTICES

Requests for interpretations of AAMI standards and recommended practices must be made in writing, to the AAMI Vice President, Standards Policy and Programs. An official interpretation must be approved by letter ballot of the originating committee and subsequently reviewed and approved by the AAMI Standards Board. The interpretation will become official and representation of the Association only upon exhaustion of any appeals and upon publication of notice of interpretation in the "Standards Monitor" section of the *AAMI News*. The Association for the Advancement of Medical Instrumentation disclaims responsibility for any characterization or explanation of a standard or recommended practice which has not been developed and communicated in accordance with this procedure and which is not published, by appropriate notice, as an *official interpretation* in the *AAMI News*.

Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing

Developed by
Association for the Advancement of Medical Instrumentation

Approved 19 December 2011 and reaffirmed 9 June 2016 by
American National Standards Institute, Inc.

Abstract: This recommended practice specifies general criteria to be applied in the determination of bacterial endotoxins (pyrogens) on sterilized or sterilizable healthcare products, components or raw materials. Endotoxin methodologies covered include both qualitative (limit) methods and quantitative (end-point) methods. The recommended practice excludes determination of pyrogens other than bacterial endotoxins.

Keywords: Limulus amebocyte lysate, LAL, pyrogenic labeling, maximum valid dilution, MVD, RSE:CSE standardization, analyst qualification, product qualification, gel-clot technique, chromogenic technique, turbidimetric technique, medical device, batch testing, laboratory quality system, product family, set, sample frequency, kinetic assay

AAMI Recommended Practice

This Association for the Advancement of Medical Instrumentation (AAMI) recommended practice implies a consensus of those substantially concerned with its scope and provisions. The existence of an AAMI recommended practice does not in any respect preclude anyone, whether they have approved the recommended practice or not, from manufacturing, marketing, purchasing, or using products, processes, or procedures not conforming to the standard. AAMI recommended practices are subject to periodic review, and users are cautioned to obtain the latest editions.

CAUTION NOTICE: This AAMI recommended practice may be revised or withdrawn at any time. AAMI procedures require that action be taken to reaffirm, revise, or withdraw this recommended practice no later than five years from the date of publication. Interested parties may obtain current information on all AAMI documents by calling or writing AAMI.

All AAMI standards, recommended practices, technical information reports, and other types of technical documents developed by AAMI are *voluntary*, and their application is solely within the discretion and professional judgment of the user of the document. Occasionally, voluntary technical documents are adopted by governmental regulatory agencies or procurement authorities, in which case the adopting agency is responsible for enforcement of its rules and regulations.

Published by

Association for the Advancement of Medical Instrumentation
4301 N. Fairfax Drive, Suite 301
Arlington, VA 22203-1633
www.aami.org

Printed in the United States of America

ISBN 1–57020–426–8

Contents

Glossary of equivalent standards

International Standards adopted in the United States may include normative references to other International Standards. For each International Standard that has been adopted by AAMI (and ANSI), the table below gives the corresponding U.S. designation and level of equivalency to the International Standard. NOTE: Documents are sorted by international designation. The code in the US column, "(R)20xx" indicates the year the document was officially reaffirmed by AAMI. E.g., ANSI/AAMI/ISO 10993-4:2002/(R)2009 indicates that 10993-4, originally approved and published in 2002, was reaffirmed without change in 2009.

Other normatively referenced International Standards may be under consideration for U.S. adoption by AAMI; therefore, this list should not be considered exhaustive.

International designation	U.S. designation	Equivalency
IEC 60601-1:2005 Technical Corrigendum 1 and 2	ANSI/AAMI ES60601-1:2005 and ANSI/AAMI ES60601-1:2005/A2:2010 ANSI/AAMI ES60601-1:2005/C1:2009 (amdt)	Major technical variations C1 Identical to Corrigendum 1 & 2
IEC 60601-1-11:2010	ANSI/AAMI HA60601-1-11:2011	Major technical variations
IEC 60601-1-2:2007	ANSI/AAMI/IEC 60601-1-2:2007	Identical
IEC 60601-2-2:2009	ANSI/AAMI/IEC 60601-2-2:2009	Identical
IEC 60601-2-4:2010	ANSI/AAMI/IEC 60601-2-4:2010	Identical
IEC 60601-2-16:2008	ANSI/AAMI/IEC 60601-2-16:2008	Identical
IEC 60601-2-19:2009	ANSI/AAMI/IEC 60601-2-19:2009	Identical
IEC 60601-2-20:2009	ANSI/AAMI/IEC 60601-2-20:2009	Identical
IEC 60601-2-21:2009	ANSI/AAMI/IEC 60601-2-21:2009	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:2004/(R)2009	Major technical variations
IEC 60601-2-27:2011	ANSI/AAMI/IEC 60601-2-27:2011	Identical
IEC 60601-2-47:2001	ANSI/AAMI EC38:2007	Major technical variations
IEC 60601-2-50:2009	ANSI/AAMI/IEC 60601-2-50:2009	Identical
IEC 80001-1:2010	ANSI/AAMI/IEC 80001-1:2010	Identical
IEC 80601-2-30:2009 and Technical Corrigendum 1	ANSI/AAMI/IEC 80601-2-30:2009 and ANSI/AAMI/IEC 80601-2-30:2009/C1:2009 (amdt) – consolidated text	Identical (with inclusion) C1 Identical to Corrigendum 1
IEC 80601-2-58:2008	ANSI/AAMI/IEC 80601-2-58:2008	Identical
IEC/TR 60878:2009	ANSI/AAMI/IEC TIR60878:2003	Identical
IEC/TR 62296:2009	ANSI/AAMI/IEC TIR62296:2009	Identical
IEC 62304:2006	ANSI/AAMI/IEC 62304:2006	Identical
IEC/TR 62348:2006	ANSI/AAMI/IEC TIR62348:2006	Identical
IEC/TR 62354:2009	ANSI/AAMI/IEC TIR62354:2009	Identical
IEC 62366:2007	ANSI/AAMI/IEC 62366:2007	Identical
IEC/TR 80002-1:2009	ANSI/IEC TIR80002-1:2009	Identical
ISO 5840:2005	ANSI/AAMI/ISO 5840:2005/(R)2010	Identical
ISO 7198:1998	ANSI/AAMI/ISO 7198:1998/2001/(R)2010	Identical
ISO 7199:2009	ANSI/AAMI/ISO 7199:2009	Identical
ISO 8637:2010	ANSI/AAMI/ISO 8637:2010	Identical
ISO 8638:2010	ANSI/AAMI/ISO 8638:2010	Identical
ISO 10993-1:2009	ANSI/AAMI/ISO 10993-1:2009	Identical
ISO 10993-2:2006	ANSI/AAMI/ISO 10993-2:2006/(R)2010	Identical
ISO 10993-3:2003	ANSI/AAMI/ISO 10993-3:2003/(R)2009	Identical
ISO 10993-4:2002 and Amendment 1:2006	ANSI/AAMI/ISO 10993-4:2002/(R)2009 and Amendment 1:2006/(R)2009	Identical
ISO 10993-5:2009	ANSI/AAMI/ISO 10993-5:2009	Identical
ISO 10993-6:2007	ANSI/AAMI/ISO 10993-6:2007/(R)2010	Identical
ISO 10993-7:2008	ANSI/AAMI/ISO 10993-7:2008	Identical
ISO 10993-9:2009	ANSI/AAMI/ISO 10993-9:2009	Identical
ISO 10993-10:2010	ANSI/AAMI/ISO 10993-10:2010	Identical
ISO 10993-11:2006	ANSI/AAMI/ISO 10993-11:2006/(R)2010	Identical
ISO 10993-12:2007	ANSI/AAMI/ISO 10993-12:2007	Identical
ISO 10993-13:2010	ANSI/AAMI/ISO 10993-13:2010	Identical
ISO 10993-14:2001	ANSI/AAMI/ISO 10993-14:2001/(R)2006	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000/(R)2006	Identical
ISO 10993-16:2010	ANSI/AAMI/ISO 10993-16:2010	Identical
ISO 10993-17:2002	ANSI/AAMI/ISO 10993-17:2002/(R)2008	Identical
ISO 10993-18:2005	ANSI/AAMI BE83:2006/(R)2011	Major technical variations
ISO/TS 10993-19:2006	ANSI/AAMI/ISO TIR10993-19:2006	Identical
ISO/TS 10993-20:2006	ANSI/AAMI/ISO TIR10993-20:2006	Identical
ISO 11135-1:2007	ANSI/AAMI/ISO 11135-1:2007	Identical
ISO/TS 11135-2:2008	ANSI/AAMI/ISO TIR11135-2:2008	Identical

International designation	U.S. designation	Equivalency
ISO 11137-1:2006	ANSI/AAMI/ISO 11137-1:2006/(R)2010	Identical
ISO 11137-2:2006 (2006-08-01 corrected)	ANSI/AAMI/ISO 11137-2:2006	Identical
ISO 11137-3:2006	ANSI/AAMI/ISO 11137-3:2006/(R)2010	Identical
ISO 11138-1:2006	ANSI/AAMI/ISO 11138-1:2006/(R)2010	Identical
ISO 11138-2:2006	ANSI/AAMI/ISO 11138-2:2006/(R)2010	Identical
ISO 11138-3:2006	ANSI/AAMI/ISO 11138-3:2006/(R)2010	Identical
ISO 11138-4:2006	ANSI/AAMI/ISO 11138-4:2006/(R)2010	Identical
ISO 11138-5:2006	ANSI/AAMI/ISO 11138-5:2006/(R)2010	Identical
ISO/TS 11139:2006	ANSI/AAMI/ISO 11139:2006	Identical
ISO 11140-1:2005	ANSI/AAMI/ISO 11140-1:2005/(R)2010	Identical
ISO 11140-3:2007	ANSI/AAMI/ISO 11140-3:2007	Identical
ISO 11140-4:2007	ANSI/AAMI/ISO 11140-4:2007	Identical
ISO 11140-5:2007	ANSI/AAMI/ISO 11140-5:2007	Identical
ISO 11607-1:2006	ANSI/AAMI/ISO 11607-1:2006/(R)2010	Identical
ISO 11607-2:2006	ANSI/AAMI/ISO 11607-2:2006/(R)2010	Identical
ISO 11663:2009	ANSI/AAMI/ISO 11633:2009	Identical
ISO 11737-1:2006	ANSI/AAMI/ISO 11737-1:2006	Identical
ISO 11737-2:2009	ANSI/AAMI/ISO 11737-2:2009	Identical
ISO/TS 12417:2011	ANSI/AAMI/ISO TIR12417:2011	Identical
ISO 13408-1:2008	ANSI/AAMI/ISO 13408-1:2008	Identical
ISO 13408-2:2003	ANSI/AAMI/ISO 13408-2:2003	Identical
ISO 13408-3:2006	ANSI/AAMI/ISO 13408-3:2006	Identical
ISO 13408-4:2005	ANSI/AAMI/ISO 13408-4:2005	Identical
ISO 13408-5:2006	ANSI/AAMI/ISO 13408-5:2006	Identical
ISO 13408-6:2006	ANSI/AAMI/ISO 13408-6:2006	Identical
ISO 13485:2003	ANSI/AAMI/ISO 13485:2003/(R)2009	Identical
ISO 13958:2009	ANSI/AAMI/ISO 13958:2009	Identical
ISO 13959:2009	ANSI/AAMI/ISO 13959:2009	Identical
ISO 14155:2011	ANSI/AAMI/ISO 14155:2011	Identical
ISO 14160:2011	ANSI/AAMI/ISO 14160:2011	Identical
ISO 14161:2009	ANSI/AAMI/ISO 14161:2009	Identical
ISO 14708-3:2008	ANSI/AAMI/ISO 14708-3:2008	Identical
ISO 14708-4:2008	ANSI/AAMI/ISO 14708-4:2008	Identical
ISO 14708-5:2010	ANSI/AAMI /ISO 14708-5:2010	Identical
ISO 14937:2009	ANSI/AAMI/ISO 14937:2009	Identical
ISO/TR 14969:2004	ANSI/AAMI/ISO TIR14969:2004	Identical
ISO 14971:2007	ANSI/AAMI/ISO 14971:2007/(R)2010	Identical
ISO 15223-1:2007 and A1:2008	ANSI/AAMI/ISO 15223-1:2007 and A1:2008	Identical
ISO 15223-2:2010	ANSI/AAMI/ISO 15223-2:2010	Identical
ISO 15225:2010	ANSI/AAMI/ISO 15225:2010	Identical
ISO 15674:2009	ANSI/AAMI/ISO 15674:2009	Identical
ISO 15675:2009	ANSI/AAMI/ISO 15675:2009	Identical
ISO 15882:2008	ANSI/AAMI/ISO 15882:2008	Identical
ISO 15883-1:2006	ANSI/AAMI ST15883-1:2009	Major technical variations
ISO/TR 16142:2006	ANSI/AAMI/ISO TIR16142:2005	Identical
ISO 17664:2004	ANSI/AAMI ST81:2004	Major technical variations
ISO 17665-1:2006	ANSI/AAMI/ISO 17665-1:2006	Identical (with inclusions)
ISO/TS 17665-2:2009	ANSI/AAMI/ISO TIR17665-2:2009	Identical
ISO 18472:2006	ANSI/AAMI/ISO 18472:2006/(R)2010	Identical
ISO/TS 19218-1:2011	ANSI/AAMI/ISO TIR19218:2011	Identical
ISO 20857:2010	ANSI/AAMI/ISO 20857:2010	Identical
ISO 22442-1:2007	ANSI/AAMI/ISO 22442-1:2007	Identical
ISO 22442-2:2007	ANSI/AAMI/ISO 22442-2:2007	Identical
ISO 22442-3:2007	ANSI/AAMI/ISO 22442-3:2007	Identical
ISO/TR 22442-4:2010	ANSI/AAMI/ISO TIR22442-4:2010	Identical
ISO 23500:2011	ANSI/AAMI/ISO 23500:2011	Identical
ISO/TS 23810:201X[1]	ANSI/AAMI/ISO TIR23810:201X	Identical
ISO 25539-1:2003 and A1:2005	ANSI/AAMI/ISO 25539-1:2003/(R)2009 and A1:2005/(R)2009	Identical
ISO 25539-2:2008	ANSI/AAMI/ISO 25539-2:2008	Identical
ISO 26722:2009	ANSI/AAMI/ISO 26722:2009	Identical
ISO 27186:2010	ANSI/AAMI/ISO 27186:2010	Identical
ISO 80369-1:2010	ANSI/AAMI/ISO 80369-1:2010	Identical
ISO 81060-1:2007	ANSI/AAMI/ISO 81060-1:2007	Identical
ISO 81060-2:2009	ANSI/AAMI/ISO 81060-2:2009	Identical

[1] In production

Committee representation

Association for the Advancement of Medical Instrumentation

Sterilization Standards Committee

This AAMI Recommended Practice was developed by the AAMI Microbiological Methods Working Group, under the auspices of the AAMI Sterilization Standards Committee.

At the time this document was published, the **AAMI Sterilization Standards Committee** had the following members:

Cochairs:
Victoria M. Hitchins, PhD, FDA/CDRH
Michael H. Scholla, Dupont Protection Technologies

Members:
Trabue D. Bryans, WuXi AppTec Inc.
Peter A. Burke, PhD, Steris Corporation
Nancy Chobin, RN CSPDM, St Barnabas Healthcare System
Charles Cogdill, Boston Scientific Corporation
Ramona Conner, RN MSN CNOR, Association of Perioperative Registered Nurses
Jacqueline Daley, Association for Professionals in Infection Control and Epidemiology
Kimbrell Darnell, CR Bard
Dave Dion, Cardinal Health (MP&S)
Lisa Foster, Sterigenics International
Joel R. Gorski, PhD, NAMSA
Deborah A. Havlik, Hospira Worldwide Inc.
Victoria M. Hitchins, PhD, FDA/CDRH
Lois Atkinson Jones, MS, (Independent Expert)
Susan G. Klacik, CCSMC FCS ACE, IAHCSMM
Byron J. Lambert, PhD, Abbott Laboratories
Colleen Patricia Landers, RN, Canadian Standards Association
Lisa N. Macdonald, Becton Dickinson & Company
Jeff Martin, Alcon Laboratories Inc.
Patrick J. McCormick, PhD, Bausch & Lomb Inc.
Rainer Newman, Johnson & Johnson
Janet M. Prust, 3M Healthcare
Nancy Rakiewicz, Moog Medical Devices
Michael H. Scholla, Dupont Protection Technologies
Mark Seybold, Baxter Healthcare Corporation
Andrew Sharavara, PhD, Propper Manufacturing Co Inc.
Mark N. Smith, Getinge USA
William N. Thompson, Covidien
Martell Kress Winters, BS SM, Nelson Laboratories Inc.
William E. Young, (Independent Expert)

Alternates:
Lloyd Brown, Covidien
Glenn W. Calvert, Becton Dickinson & Company
Steven J. Elliott, WuXi AppTec Inc.
Thomas J. Frazar, Johnson & Johnson
Kathy Hoffman, Sterigenics International
Jim Kaiser, Bausch & Lomb Inc.
Natalie Lind, IAHCSMM
Reynaldo Lopez, Cardinal Health (MP&S)
Ralph Makinen, Boston Scientific Corporation
Mary S. Mayo, CR Bard
David Ford McGoldrick, BS, Abbott Laboratories
Jerry R. Nelson, PhD, Nelson Laboratories Inc.
Karen Polkinghorne, Dupont Protection Technologies
Wallace E. Puckett, PhD, Steris Corporation
Mike Sadowski, Baxter Healthcare Corporation
Jason Voisinet, Moog Medical Devices

Craig A. Wallace, 3M Healthcare
Valerie Welter, Hospira Worldwide Inc.

NOTE--Participation by federal agency representatives in the development of this document does not constitute endorsement by the federal government or any of its agencies.

At the time this document was published, the **AAMI Microbiological Methods Working Group** had the following members:

Cochairs:
Kimbrell Darnell, CR Bard
Martell Kress Winters, BS SM, Nelson Laboratories Inc.

Members:
Anne F. Booth, MS, Conmed Corp
Carolyn Braithwaite, CaridianBCT Sterilization Services Inc.
Trabue D. Bryans, WuXi AppTec Inc.
Gary J. Chilson, Moog Medical Devices
Charles Cogdill, Boston Scientific Corporation
Gary N. Cranston, Consulting & Technical Services/PCS
Kimbrell Darnell, CR Bard
Douglas D. Davie, Sterilization Validation Services
David Dominguez, CareFusion
Sylvie Dufresne, PhD, TSO3 Inc.
Niki Fidopiastis, Sterigenics International
Naomi Gamm, St Jude Medical Inc.
Steve N. Goldstine, PhD, Steve Goldstine Consultants
Thomas L. Hansen, Terumo Medical Corporation
Deborah A. Havlik, Hospira Worldwide Inc.
Katherine E. Hill, 3M Healthcare
Victoria M. Hitchins, PhD, FDA/CDRH
Robert Dennis Houlsby, Abbott Laboratories
Carolyn L. Kinsley, LexaMed
Roland C. Kippenhan, Minntech Corporation
John Lohr, PhD, Alcon Laboratories Inc.
Reynaldo Lopez, Cardinal Health (MP&S)
Gerald E. McDonnell, PhD, Steris Corporation
Joseph M. Mello, Ethide Laboratories Inc.
Russell D. Mills, Zimmer Inc.
Gerry A. O'Dell, MS, Gerry O'Dell Consulting
David Opie, Noxilizer Inc.
Dave Parente, NAMSA
Miriam Rozo, Johnson & Johnson
Matthew Russell, Cook Inc.
Terri Rymer, Baxter Healthcare Corporation
Manuel Saavedra Jr., Kimberly-Clark Corporation
Michael J. Schoenly, Bausch & Lomb Inc.
Zenius V. Seskas, Stericon Inc.
Harry L. Shaffer, JM Hansen & Associates
Barb Smith, Getinge USA
Somheak Sohn, MPH, Quality Tech Services Inc.
Radhakrishna S. Tirumalai, US Pharmacopeia Convention Inc.
Hong Van Trinh, Covidien
Donald Tumminelli, SPS Medical Supply Corp
Richard L. Weisman, Fresenius Medical Care Renal Therapies Group
Martell Kress Winters, BS SM, Nelson Laboratories Inc.
Curtrice Zeigler, Becton Dickinson & Company

Alternates:
Nancy Blaszko, Sterigenics International
Marjean Boyter, Fresenius Medical Care Renal Therapies Group
Sandra Budden, Alcon Laboratories Inc.
April J. Doering, St Jude Medical Inc.
Steven J. Elliott, WuXi AppTec Inc.
J.C Fulghum, Hospira Worldwide Inc.
Marilyn J. Gould, PhD, Consulting & Technical Services/PCS
Joyce M. Hansen, JM Hansen & Associates
Annetta Herrington, Moog Medical Devices

Renate Johnson, Boston Scientific Corporation
Amy Karren, Nelson Laboratories Inc.
Bert Kingsbury, Terumo Medical Corporation
Maggie Ladd, BA BS, Kimberly-Clark Corporation
Sharon K. Lappalainen, FDA/CDRH
Helene Leblond, TSO3 Inc.
Antonio F Lopez, CareFusion
Ronald G. Lulich, 3M Healthcare
Ruby Martinez, Cardinal Health (MP&S)
Mary S. Mayo, CR Bard
David Ford McGoldrick, BS, Abbott Laboratories
Laurie Nawrocki, NAMSA
Michael Nolan, SPS Medical Supply Corp
Susan E Norton, Bausch & Lomb Inc.
Richard T. O'Donnell, Steris Corporation
Jonathan Karl Olson, MA, Minntech Corporation
Ken Paddock, Baxter Healthcare Corporation
Lorra Parliament, Becton Dickinson & Company
Antonio Prado, Covidien
Claudia Romero, Johnson & Johnson
David Silor, Zimmer Inc.
Michael G. Sprague, Ethide Laboratories Inc

NOTE--Participation by federal agency representatives in the development of this document does not constitute endorsement by the federal government or any of its agencies.

Foreword

This recommended practice was developed by the AAMI Microbiological Methods Working Group under the auspices of the AAMI Sterilization Standards Committee. The objective of this document is to provide criteria to be applied in the determination of bacterial endotoxins on or in medical devices, components, or raw materials using bacterial endotoxin test methods.

This is the second edition of *Bacterial endotoxins—Test methods, routine monitoring and alternatives to batch testing*, which was first published as an American National Standard in 2002 as ANSI/AAMI ST72:2002. In comparison to the first edition, this new edition includes additional guidance on out-of-specification test results and investigation.

Compliance with this recommended practice is voluntary. The existence of the recommended practice does not preclude anyone from manufacturing, marketing, purchasing, or using products, processes, or procedures not conforming to the recommended practice.

As used within the context of this document, “shall” indicates requirements to be strictly followed in order to conform to the recommended practice; “should” indicates that among several possibilities one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action should be avoided but is not prohibited; “may” is used to indicate that a course of action is permissible within the limits of the standard; and “can” is used as a statement of possibility and capability. “Must” is used only to describe “unavoidable” situations, including those mandated by government regulation.

This recommended practice should be considered flexible and dynamic. AAMI and ANSI procedures require that recommended practice be reviewed every five years and, if necessary, revised to reflect technological advances that may have occurred since publication.

Suggestions for improving this recommended practice are invited. Comments and suggested revisions should be sent to Technical Programs, AAMI, 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633.

NOTE—This foreword does not contain provisions of the American National Standard, *Bacterial endotoxins—Test methods, routine monitoring and alternatives to batch testing* (ANSI/AAMI ST72:2011), but it does provide important information about the development and intended use of the document.

Introduction

A pyrogen is any substance that can induce fever. Testing for pyrogens is required for release of many health care products. Pyrogens can be classified into two groups: microbial (e.g., bacteria, fungi, viruses) and non-microbial (e.g., drugs, device materials, steroids, plasma fractions). The most significant pyrogens have been found to be endotoxins from Gram-negative bacteria. Although Gram-positive bacteria, fungi, and viruses may be pyrogenic, they do so through a different mechanism (systemic effects) and to a lesser degree than Gram-negative bacteria. Only Gram-negative bacterial endotoxin testing will be covered in this document.

Endotoxin is the high molecular weight lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria that causes fever, meningitis, and a rapid fall in blood pressure if introduced into blood or tissues of the body. The outer cell wall components, which are composed primarily of proteins, phospholipids, and LPS, are constantly released into the environment when Gram-negative bacteria divide or lyse. Endotoxin contamination is difficult to prevent, because it is ubiquitous in nature, stable, and small enough to pass through conventional sterilizing filters.

The non-pyrogenicity of a health care product can be achieved through the following:

1) manufacturing techniques that prevent or control the accumulation of endotoxin,

2) depyrogenation by endotoxin inactivation (e.g., dry heat) or physical removal (e.g., rinsing, distillation, ultrafiltration).

This document will focus primarily on products manufactured under conditions that do not require a depyrogenation step as part of the manufacturing process.

The purpose of this document is to consolidate the requirements and guidance for testing for bacterial endotoxins. This includes the selection of product units for testing, selection and validation of testing technique, use of technique for routine testing, and interpretation of test results. This document also addresses the requirements for manufacturing operation validation that would support alternatives to batch testing.

Information on the following is provided in the annexes:

— the background/history of endotoxin testing,

— guidance on endotoxin test methods,

— guidance on alternatives to batch testing and validating manufacturing operations, and

— guidance on out of specification test results and investigation.

The annexes to this Recommended Practice/American National Standard are for information only.

Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing

1 Scope

1.1 This document specifies general criteria to be applied in the determination of bacterial endotoxins on or in medical devices, components, or raw materials using bacterial endotoxin test methods.

NOTE Although the scope of this standard is limited to medical devices, it also includes requirements and provides guidance that may be applicable to other health care products.

1.2 This document is not applicable to the evaluation of pyrogens other than bacterial endotoxin.

2 Normative references

The following documents contain provisions that, through reference in this text, constitute provisions of this guideline. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this guideline are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

ISO 13485:2003, *Quality Management Systems—Requirements for regulatory purposes.*

The United States Pharmacopoeia (USP) <85>, current edition, United States Pharmacopeial Convention (USP), Rockville MD.

The United States Pharmacopoeia (USP) <161>, current edition, United States Pharmacopeial Convention (USP), Rockville MD.

U.S. Food and Drug Administration:1998*, Quality System Regulation*, 21 CFR, Part 820.

3 Definitions

For the purpose of this document, the following definitions apply.

3.1 bacterial endotoxins test (BET): Assay for measuring active bacterial endotoxin by combining a liquid test sample with *Limulus* amebocyte lysate (LAL) reagent and measuring the resulting proportional reaction via visual, turbidimetric, chromogenic, or other validated means of detection.

3.2 batch: Defined quantity of bulk, intermediate, or finished product, intended or purported to be uniform in character and quality, which has been produced during a defined cycle of manufacture.

[ANSI/AAMI/ISO 11139:2006]

3.3 chromogenic technique: BET methodology that quantifies or detects endotoxin on the basis of a measured color-producing reaction proportional to the interaction of LAL and endotoxin.

3.4 control standard endotoxin (CSE): Endotoxin standard preparation whose potency has been standardized against the Reference Standard Endotoxin (RSE) for a specific batch of LAL.

3.5 depyrogenation: Validated process designed to remove or inactivate endotoxin.

3.6 endotoxin or bacterial endotoxin: High molecular weight complex associated with the cell wall of Gram-negative bacteria that is pyrogenic in humans and specifically interacts with LAL.

3.7 endotoxin unit (EU): Standard unit of measure for endotoxin activity initially established relative to the activity contained in 0.2 ng of the U.S. Reference Standard Endotoxin Lot EC-2 (USP standard reference material).

NOTE FDA's reference endotoxin EC-6, USP Lot G, and the World Health Organization's primary international endotoxin standard (IS) are sub-lots of the same endotoxin preparation, making the EU and IU equal (Poole et al., 1997).

