

METHODS COMMITTEE REPORTS

Biological Threat Agents

DEBORAH A. LOVEYS, CHAIR

Georgia Public Health Laboratory, 1749 Clairmont Rd,
Decatur, GA 30033

MARY MCBRIDE, SECRETARY

Agilent Technologies, Inc., 5301 Stevens Creek Blvd,
Santa Clara, CA 95051

CHERYL GAUTHIER

Massachusetts Department of Public Health, 305 South St,
Jamaica Plain, MA 02130

HELEN SPENCER

CBRN Research and Technology Initiative (CRTI) Biology,
Rm 5-38, 344 Wellington St, Ottawa, ON K1A 0K2 Canada

PATRICK J. TREADO

ChemImage Corp., 7301 Penn Ave, Pittsburgh, PA 15208

CHRISTINA EGAN

New York State Department of Health, 120 New Scotland
Ave, Albany, NY 12208

PATRICK EUGENE WILLIAMS

Anzen Biosciences, 12595 Palisade Dr, Nevada City, CA
95959

SETH H. PINCUS

Children's Hospital, 200 Henry Clay Ave, New Orleans,
LA 70118

ROBERT A. LABUDDE, STATISTICAL ADVISOR

Least Cost Formulations, Ltd., 824 Timberlake Dr, Virginia
Beach, VA 23464

DARYL S. PAULSON, STATISTICAL ADVISOR

BioScience Laboratories Inc., 300 N. Wilson Ave, Suite 1,
Bozeman, MT 59715

CHRISTINA Z. THOMPSON, SAFETY ADVISOR

Eli Lilly and Co., 2410 Wayne Dr, Greenfield, IN 46140

MARK R. COLEMAN, OFFICIAL METHODS BOARD ADVISOR

Eli Lilly and Co., Elanco Animal Health (GL10), 2001 W
Main St, Greenfield, IN 46140

ANTHONY HITCHINS, GENERAL REFEREE (*Bacillus anthracis*)

U.S. Food and Drug Administration, Center for Food
Safety and Applied Nutrition, 5100 Paint Branch Pkwy,
College Park, MD 20740

MARK POLI, GENERAL REFEREE (Biological Toxins)

U.S. Army Medical Research Institute for Infectious
Diseases, Integrated Toxins Division, Fort Detrick, MD
21702

BRAD STAWICK, TDRM REPRESENTATIVE

Stawick Laboratory Management, LLC, 3446 Patricia Ellen
Dr, Bartlett, TN 38133

Background

Committee L was formally approved by AOAC's Board of Directors on December 12, 2006. The Committee was constituted and met for the first time for training and orientation on January 29, 2007. The mission of Committee L is to (1) review and approve methods for biological threat agents as AOAC Official MethodsSM and approve modifications to existing Official MethodsSM; (2) guide and supervise the development of performance and acceptance criteria for biological threat agent technologies; and (3) ensure that method protocol development meets AOAC requirements for a successful collaborative study.

Committee L members also serve on the Stakeholders Panel on Agents for Detection Assays (SPADA). SPADA was born of the need for the Science and Technology Directorate of the Department of Homeland Security (DHS-S&T) to develop voluntary consensus standards for methods for the detection and identification of biological threat agents. SPADA includes stakeholders from the federal government, state governments, academia, and the private sector. SPADA will reach consensus on the standards required for polymerase chain reaction (PCR) testing methods from samples of air particulates obtained by aerosol collection and on elements of what constitutes a Public Health Actionable Assay. SPADA will establish inclusivity, exclusivity, and environmental factors panels for several select agents. SPADA will develop experimental designs, and performance and acceptance criteria of the relevant method studies. SPADA will recommend those to AOAC approving bodies such as Committee L, the General Referee (GR), and the Official Methods Board.

Four independent Working Groups (WGs) were also established to enable internationally recognized biological threat agent-specific subject matter experts (SMEs) to focus on the development of agent-specific panels, to establish scientific criteria for strain selection from candidate lists of available strains, and to recommend experimental designs and methods. The 4 WGs are (1) *Bacillus anthracis* (Ba) WG; (2) *Francisella tularensis* (Ft) WG; (3) *Yersinia pestis* (Yp) WG; and (4) Environmental Factors WG. At least 1 Committee L voting member is embedded into each of these WGs, and the GR for Ba is very active in all of the WGs.

Embedded members update Committee L at each Committee L meeting. Due to the security-sensitive nature of these studies, the collaborative studies in their entirety shall remain confidential, with only the method details dissemination being permissible.

Committee Actions

(1) *Ba HHA*.—Committee L and the GR for Ba were engaged immediately to review, comment, and approve (if acceptable) the study design for a Ba hand-held laboratory evaluation. Committee L spent several months (February–June) on this effort. This project was terminated in June due to lack of funding.

(2) *Ricin HHA*.—Committee L and the GR for Biological Toxins were engaged immediately to review, comment, and approve (if acceptable) the study design for a ricin hand-held laboratory evaluation. Committee L spent several months (February–June) on this effort. This project was terminated in June due to lack of funding.

(3) *Department of Defense agent detection method design and validation*.—A novel study design was proposed to allow for evaluation of discrete modules. This nontraditional approach to study development was introduced and discussed for several months (February–June) but later abandoned due to lack of consensus on application of an appropriate statistical model for interpretation.

(4) *Inclusivity, exclusivity, and rational for strain selection panels*.—SPADA and the WGs are working to reach consensus on recommendations for both the identity and number of strains to be included in study design proposals. The SPADA group recommended that genetic diversity and antigenic diversity should be considered the primary criteria for which multitarget PCR methods should be evaluated, with genetic diversity being the principal factor determining strain selection.

(5) *Development of guidelines for establishing panels of select agent strains*.—While the WGs were working hard to develop the panels, it became clear that each of the WGs could substantively benefit if strain selection guidance was available, at least for the inclusivity panel development. Committee L has been asked to provide such guidance; this document is currently in draft format, and Committee L members recently voted to accept or modify the document. The outcome of this vote will be discussed at the next Committee L meeting (August 30, 2007) and if approved, the guidance will be forwarded to the AOAC Official Methods Board for adoption as consensus guidance for the selection of representative analytes for use in nontraditional method validation studies.

(6) *Discussion of review of NGB environmental swab collection study design protocol*.—Committee L has reviewed and commented on this study design. Review and consolidation of reviewer comments are currently in progress, with further discussion of statistical design anticipated at the upcoming AOAC Annual Meeting. The protocol will require some revisions before it can be approved. Comments and

recommendations will be provided to the study director for action.

Summary of General Referee Reports

Biological threat agents.—Anthony Hitchins and Mark Poli. In 2003, DHS partnered with AOAC to develop and validate voluntary consensus standards for hand-held assays (HHAs) against biological threat agents. Since then, AOAC has facilitated the development of a standard protocol for the collection of “white powders” from hard surfaces as well as a comparison of several HHAs for *Bacillus anthracis* spores. In 2006, AOAC formed a WG on Standards for Hand-Held Assays (WGS). This working group was tasked by DHS with overseeing further development and validation of *B. anthracis* HHAs, field-testing the protocol for collecting white powders, and moving forward with developing assay specifications and study designs for HHAs for ricin.

Bacterial select agents.—As reported last year, a series of validation studies on *Bacillus anthracis* detection methods (1–5), organized by AOAC INTERNATIONAL, was conducted for DHS. This year, in broadening of the scope of such studies to encompass other select agents, DHS through AOAC set up SPADA. The mission of SPADA was to establish inclusivity, exclusivity and environmental factors panels for potential select agent methods with current focus on PCR methods and on samples of air particulates obtained by filtration. In addition of course, SPADA will design the experimental designs and acceptance criteria of the relevant method studies. In order to facilitate its mission SPADA set up several WGs for the various select agents and one for environmental factors. The WGs were composed of internationally recognized experts on the detection of various agents and environmental substances. Each WG had a few representatives from AOAC including Committee L. The role of the experts was to establish scientific criteria for choosing the strains for select agent panels from candidate lists of available strains, and for choosing the environmental factors.

Bacillus anthracis WG.—The group has agreed to recommend to SPADA an intrinsic panel of 15 strains and extrinsic panel of 15 near-neighbor strains (*Bacillus cereus* and *Bacillus thuringiensis*). The group has now started the process of recommending study parameter values for method acceptance.

Francisella tularensis WG.—The group has tentatively agreed to recommend to SPADA an intrinsic panel of at least 15 strains and extrinsic panel of at least 15 near-neighbor strains.

Yersinia pestis WG.—The group has agreed on intrinsic and extrinsic panel sizes of 25 strains each.

Environmental factors WG.—The WG is finalizing its potentially very large candidate list before beginning the culling process to a more manageable sized panel. The candidate panel is large because the factors to be considered include (1) organic or inorganic compounds that comprise white powders and soil varieties and (2) lower and higher eukaryotic organisms including pollen, insect and mammalian components that may potentially cross react in a candidate

method. In addition some further strains of the microbes considered by the select agent WGs will also be included.

Recommendations

That SPADA and the WGs continue working towards submission of the combined study protocol package for consideration by Committee L.

Toxic Select Agents

The WGSB assigned specific task groups to address different aspects of ricin HHAs. A Task Group on Product Specification and Study Design was chaired by Tetsuhisa Goto and Mark Poli and consisted of 14 individuals representing government, assay developers, law enforcement, and first responders. This Task Group worked closely with AOAC staff to address the following specific tasks: (1) Identify HHA users; (2) develop and implement a user survey to determine performance expectations of HHAs; (3) document user product performance expectations; (4) draft acceptance criteria for HHAs; (5) submit recommendations for acceptance criteria and supporting documentation to WGSB; and (6) draft study designs for internal, independent, and collaborative studies.

At a WGSB meeting on December 8, 2006, the Task Group submitted the results of the user survey as well as draft acceptance criteria for ricin HHAs addressing the following parameters: inclusivity testing, exclusivity testing, sensitivity, specificity, threshold concentration, reference method, lot-to-lot variability, interferences, and ruggedness. After some discussion and modification by the WGSB, these criteria were accepted.

Study designs for in-house, independent laboratory, and collaborative studies were also drafted and sent to the newly formed Methods Committee on Biological Threat Agents (Committee L) for review.

Other Task Groups were assembled for ricin HHAs as well, including the Task Group on Experts, the Task Group on Laboratory Selection, and the Task Group on Labeling and Package Inserts. However, work among these task groups was suspended when DHS suspended the project.

A study design for a precollaborative study for a botulinum toxin test kit entitled "Evaluation of the Botulinum Toxin ELISA for the Detection of Toxins A, B, E, and F in Select Foods" is under development at AOAC on behalf of Richard Whiting and Sasha Sharma of U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition and Joanne Andreadis and Susan Maslanka from the Botulism Public Health Research and Preparedness Laboratory, Centers for Disease Control and Prevention. This study design is currently awaiting further input from the Study Directors and will be submitted to Committee L (newly-formed Committee on Methods for Biological Threat Agents) in the near future.

Recommendations

(1) *Ricin HHA acceptance criteria*: Recommend acceptance by WGSB.

(2) *Ricin study design(s)*: Recommend acceptance. DHS has suspended the project to support studies and requests publication of study designs in *J. AOAC Int.* as guidelines for interested companies. Recommend moving forward with this.

(3) *Botulinum toxin ELISA study design*: Recommend further review after input from Study Directors.

References

- (1) Stephenson, J. (2005) *J. AOAC Int.* **88**, 202–203
- (2) *Official Methods of Analysis* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **2004.04**
- (3) *Official Methods of Analysis* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **2004.11**
- (4) ASTM, Work Item Number 8273, Version 10.2, *Standard Practice for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents from Nonporous Surfaces: Collaborative Study*
- (5) Locascio, L.E., Harper, B., & Robinson, M. (2007) *J. AOAC Int.* **90**, 299–333
- (6) *Official Methods of Analysis* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **2004.08**