

METHODS COMMITTEE REPORTS

Methods Committee on Biological Threat Agents

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Committee Actions

The Methods Committee on Biological Threat Agents was established to: (1) guide and supervise the development of performance and acceptance criteria for biological threat

agent detection technologies; (2) review standards for biological threat agents; (3) review and approve study designs as part of the AOAC validation process; and (4) serve as a conduit between AOAC and the biodefense community at large.

There were many changes to the Committee on Biological Threat Agents roster this past year. The Committee gratefully acknowledges the contributions of 6 former members and extends its sincere appreciation for their service. These former members are (1) Deborah Loveys, First Chair; (2) Helen Spencer, Member; (3) Patrick Eugene Williams, Member; (4) Seth H. Pincus, Member; (5) Daryl S. Paulson, Statistical Advisor; (6) Brad Stawick, TDRM Representative. In January 2008, Patrick J. Treado accepted the position of Chair. The Committee also welcomed members Chris Aston, Ed Brown, and Bela Matyas.

Almost all of the Committee work is conducted in support of one or more government-sponsored projects. AOAC currently has contracts with the Department of Homeland Security (DHS) and the Department of Defense (DOD) for several biological threat agent detection projects. These projects are anything but "business as usual" for AOAC; often they require entirely new approaches to study design, testing, and method validation. The projects are often impacted by rapidly shifting priorities and may be subject to abrupt scheduling changes. For these reasons, along with the small size of the Committee and the anticipated volume of work, Committee on Biological Threat Agents asked AOAC to establish a streamlined review process specific to the Committee. Most of the methods likely to come before the Committee will be nontraditional methods (i.e., an atypical procedural protocol where the scope of experimentation may be outside the range of routine AOAC collaborative studies requiring unique experimental design/analytical processes). Specific review guidance was developed by AOAC and documented in SOP# CommLRevProcess Version 1.

Stakeholders' Panel on Agent Detection Assays (SPADA)

Committee on Biological Threat Agents members are participants of the Stakeholders' Panel on Agent Detection Assays (SPADA), a panel comprising over 100 principal stakeholders in the biothreat detection community from government, industry, and academia. Supported by the DOD (including the Critical Reagents Program) and the DHS Chemical and Biological Research and Development Section, SPADA was created to develop the criteria for the development, evaluation, and validation of biological threat agent assays and detectors. The focus of SPADA's initial efforts has been to develop voluntary consensus standards and testing methods to evaluate polymerase chain reaction (PCR)

assays for the detection of the biological threat agents *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis* from aerosol samples collected on dry filters or into a liquid.

During this report period, SPADA meetings were convened December 13–14, 2007, and April 7–8, 2008. The group has established inclusivity and exclusivity testing panels for 3 biological agents and is working to establish performance requirements for PCR-based assays, develop protocols for testing and evaluation, and establish acceptance criteria for the performance of assays for these 3 biological agents.

SPADA Recommendations to Committee on Biological Threat Agents (as of January 15, 2008)

SPADA recommends:

(1) Acceptance of an inclusivity panel of 15 *B. anthracis* strains.

(2) Acceptance of an exclusivity panel of 20 *B. anthracis* near-neighbor strains.

(3) Acceptance of an inclusivity panel of 12 strains of *F. tularensis*.

(4) Acceptance of an exclusivity panel of 7 *F. tularensis* near-neighbor strains.

(5) Acceptance of the Environmental Factors zoo panel as presented with the addition of tick DNA, and the specification of *Clostridium botulinum* HALL strain and *E. coli* K-12 strain.

(6) Acceptance of the soil panel as presented, recognizing that they must define: (a) Who will test and characterize the soils and what standardized methods will be used. (b) What is meant by a large quantity? (c) Inhibition testing is for informational purposes only; to be documented in the package insert. (d) The filter quantity to be used in aerosol testing.

(7) That all strain panels be reconsidered as new scientific information becomes available.

Committee on Biological Threat Agents response to SPADA's recommendations was documented in a memorandum from AOAC to SPADA dated January 15, 2008. Committee on Biological Threat Agents accepted most of SPADA's recommendations, with the following provisions:

(1) Inclusivity and exclusivity panels referenced are suitable for PCR-based testing only; future types of tests will require reconsideration and re-evaluation of strain panels.

(2) The number of strains selected for each panel represents the minimum number to support evaluation of detection performance, and the Committee encourages developers to evaluate more than the minimum numbers of strains.

(3) Committee on Biological Threat Agents requested that the scientific rationale for strain selection be fully documented and that all supporting materials (PowerPoint presentations, assay data, literature citations, etc.) used to define/describe the scientific rationale be collected and consolidated for future reference. AOAC staff documented the scientific rationale for strain selection for the inclusivity and exclusivity panels in a memorandum from AOAC to Committee on Biological Threat Agents dated January 2, 2008.

(4) Committee on Biological Threat Agents requested that each of the 4 working group chairs review draft SPADA meeting minutes, all available PowerPoint presentations, and all cited literature references to ensure that AOAC accurately and adequately captured the scientific rationale for the selection and development of the inclusivity and exclusivity panels.

(5) Committee on Biological Threat Agents elected not to vote to approve the matrix panel (soils, powders, etc.) because additional information was needed.

At the SPADA meeting on April 7–8, 2008:

(1) SPADA voted unanimously to accept an inclusivity panel of 13 strains of *Y. pestis*, with several provisions: (a) source information on each strain in the panel would be documented; (b) the availability of each of the strains would be confirmed; and (c) 2 U.S. strains to be selected by the *Y. pestis* Working Group would be added to the panel, bringing the total to 15 strains.

(2) SPADA voted unanimously to accept an exclusivity panel of 19 *Y. pestis* near-neighbor strains with several provisions: (a) source information would be provided; (b) the availability of each of the strains would be confirmed.

(3) SPADA voted to recommend the minimum detection level (MDL) be defined for filters as 20 000 standardized BA Ames spores and for liquid samples as 2000 standardized BA Ames spores per total liquid sample.

(4) SPADA edited/revised protocols (Sponsor Validation Laboratory protocol, Independent Laboratory Validation protocol, Collaborative Study Validation protocol).

These SPADA recommendations have not yet been sent to Committee on Biological Threat Agents.

The next planned SPADA activity was a town hall-style meeting of the biothreat detection community stakeholders on September 12, 2008, in Rockville, MD. At this time, AOAC brought together 200 key decision makers from emergency responder and public health groups, government, and private industry technology providers in an effort to shape the future of SPADA. The town hall-style meeting provided a forum for discussion from many interested parties in a moderated venue.

Department of Defense Validation Plans

AOAC is working with the DOD, the Critical Reagents Program, and National Guard Bureau (NGB) to develop a series of validation test plans for 3 assays systems. The study consists of 4 different methods. Three methods are intended for the detection of virulent and avirulent *B. anthracis* spores from dry filter unit filters using PCR, and the fourth method is intended for detection of *B. anthracis* spores in suspension. The Study Director is Diane Dutt, 5183 Blackhawk Rd, Aberdeen Proving Ground, Aberdeen, MD 21010, USA, Tel: 410-436-7831, E-mail: diane.dutt1@us.army.mil. The 4 methods are:

Method 1.—Evaluation of the NGB Triple Signature QFlow-JBAIDS (ID Tech) TaqMan PCR Method for *Bacillus anthracis* Spores on Filters: Precollaborative and Collaborative Studies

Method 2.—Evaluation of the DOD Triple Signature QIAamp-JBAIDS (ID Tech) TaqMan PCR Method for *Bacillus anthracis* Spores on Filters: Precollaborative and Collaborative Studies

Method 3.—Evaluation of the DOD Critical Reagents Program Triple Signature QIAamp-7900HT (ABI) TaqMan PCR Method for *Bacillus anthracis* Spores on Filters: Precollaborative and Collaborative Studies

Method 4.—Evaluation of the NGB Triple Signature QFlow-JBAIDS (ID Tech) TaqMan PCR Method for *Bacillus anthracis* Spores in Suspension: Precollaborative and Collaborative Studies

Significant effort has been made to leverage SPADA recommendations for these studies. For example, SPADA recommended and Committee on Biological Threat Agents approved inclusivity and exclusivity panels for *B. anthracis* PCR-based test methods. The approved strain panels were incorporated into these study designs. However, SPADA has not yet completed its work to establish performance requirements for PCR-based assays, develop protocols for testing and evaluation, and establish acceptance criteria for the performance of assays for these 3 biological agents. Therefore, Committee on Biological Threat Agents and a Working Group (that included some members from SPADA)

proposed parameters and criteria for these studies. Ideally, the precollaborative study would have been performed first, with the results provided far enough in advance to inform and impact the study designs used in the collaborative studies. Unfortunately, the precollaborative and collaborative studies were conducted in parallel, between May and June 2008. Results are being compiled into 3 draft manuscripts. These manuscripts will be reviewed by Committee on Biological Threat Agents when finalized.

Collaborative Field Study of NGB Swab Environmental Sampling Method for Recovery of Bacillus anthracis from Seven Nonporous Surfaces.—Study Director Diane Dutt, 5183 Blackhawk Rd, Aberdeen Proving Ground, Aberdeen, MD 21010, Tel: 410-436-7831, E-mail: diane.dutt1@us.army.mil. Protocol approved October, 2007.

Evaluation of the Botulinum Toxin ELISA for the Detection of Toxins A, B, E, and F in Select Foods.—Study Directors Richard Whiting, FDA, Tel: 301-436-1925, Fax: 301-436-2632, E-mail: Richard.whiting@fda.hhs.gov and Susan Maslanka, CDC, E-mail: sht5@cdc.gov. This precollaborative study design, intended to determine if the Botulinum Toxin ELISA can be used as a prescreen test for the detection of botulinum toxic A, B, E, and F, was approved in January 2008.