

Methods Committee on Biological Threat Agents

Bacillus anthracis and Other Select Agents

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Previously the U.S. Department of Homeland Security (DHS), through AOAC INTERNATIONAL, established a Stakeholders Panel on Agent Detection Assays (SPADA). The panel has continued its work this year. It has established inclusivity, inclusivity, and environmental factors panels for potential polymerase chain reaction (PCR) methods for select agents in samples of air particulates obtained by filtration. There was a separate working group (WG) for each select agent, as well as for environmental factors.

Bacillus anthracis Studies

The *Bacillus anthracis* WG recommended to SPADA, with the approval of the Methods Committee, an intrinsic panel of 15 strains and an extrinsic panel of 20 near-neighbor strains (mainly *B. cereus* and *B. thuringiensis*). In addition, a WG on study design and acceptance criteria, along with the Methods Committee on Biological Threat Agents, proposed parameters and criteria for precollaborative and collaborative studies of *B. anthracis* (and other select organisms) PCR methods which were approved by SPADA. Four PCR methods for detection of virulent and avirulent *B. anthracis* spores in air particulates (collected by dry filtration) and in liquid suspension (representing wet aerosol collection) were studied precollaboratively and collaboratively by 11 laboratories after approval of the protocols by the Methods Committee on Biological Threat Agents. The Study Director was Diane Dutt. The methods were those of the Department of Defense (DOD) Critical Reagents Program and the National Guard Bureau (NGB):

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.

Based on the current study results available from the Study Director's draft manuscripts, only NGB Methods 1 and 4 detected the chromosomal target (BA3) at the 2×10^4 spore level set by SPADA for *B. anthracis* PCR methods. Extraction efficacy appeared to be a contributing limiting factor for DOD methods 2 and 3. The detection of the 2 plasmid targets (BA1 and BA2) in spores by the 2 NGB methods (and in the 2 DOD methods) was unexpectedly nonspecific, apparently due to an unidentified systematic error.

Other Select Organisms and Environmental Interfering Factors

Francisella tularensis WG.—The group has tentatively proposed to SPADA an intrinsic panel of 9 strains and extrinsic panel of 10 near-neighbor strains.

Yersinia pestis WG.—The group has proposed to SPADA that there be intrinsic and extrinsic panel sizes of 13 and 19 strains, respectively.

Environmental Factors WG.—The candidate panel is large. 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 that may potentially crossreact in a method. The panel has not been finalized yet.

Recommendations

(1) Based on the draft precollaborative and collaborative study reports, it is recommended that PCR Methods 1 and 4 for *B. anthracis* (target BA3 only) be considered by the Methods Committee on Biological Threat Agents for First Action Official Method status.

(2) It is strongly recommended that the Study Director for the NGB *B. anthracis* methods continue retesting and/or conduct further study to resolve the unexpectedly aberrant specificity results obtained with the BA1 and BA2 plasmid primer-probe sets for the NGB methods.

(3) It is recommended that SPADA and the WGs continue working toward submission of the study protocol packages for *Francisella tularensis*, *Yersinia pestis*, and Environmental Factors for consideration by the Methods Committee on Biological Threat Agents.

Biological Weapons

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Background

A precollaborative study protocol for the evaluation of the Botulinum Toxin ELISA for the Detection of Toxins A, B, E, and F in select foods was submitted in October 2006, for *Official Methods* review by Richard Whiting of the U.S. Food and Drug Administration (FDA), Shashi Sharma of FDA/Center for Food Safety and Applied Nutrition (CFSAN), and Susan Maslanka of the Centers for Disease Control and Prevention (CDC). The protocol was reviewed by members of the Methods Committee on Microbiology and the Methods Committee on Biological Threat Agents, as the applicability of the assay is intended for the detection of *Clostridium botulinum* toxins in both general food and bioterror arenas. The protocol was approved in February, 2008.

The purpose of the precollaborative study is to establish key method parameters prior to beginning a collaborative validation study for the assessment of the Botulinum Toxin ELISA to qualitatively detect toxin in food matrixes. The study is designed to determine the probability that the method will give a positive response in a particular matrix for each toxin at a known concentration. The method will also be evaluated for inclusivity and exclusivity, the ability of the method to specifically detect a range of targets to the exclusion of nontargets. The goal is to determine if the Botulinum Toxin ELISA can be used as a prescreen test for the detection of botulinum toxins A, B, E, and F. The intended use of the method would be to have a presumptive identification of botulinum toxins A, B, E, or F in potentially contaminated food products. Advantages of the method include reduced

time-to-detection, increased sample throughput, and improved testing consistency. Implementation of the Botulinum Toxin ELISA method would expand the national testing capacity for the detection of botulinum toxins in foods, as only a limited number of laboratories are capable of running the mouse bioassay. The presumptive identification of toxin in foods by the ELISA may result in a more rapid response from public health agencies in identifying contaminated foods and removing suspect products from the food supply.

Ten foods were selected for evaluation. Whole milk, identified as being of the greatest interest, will be tested with all 4 toxins. Additional foods include broccoli and yogurt (toxin A); tomato juice, salami, and green bean liquid (toxin B); smoked salmon and liquid egg (toxin E); and orange juice and liquid infant formula (toxin F). Spiking levels and recoveries were evaluated, and sample processing issues were addressed during the second quarter. Based upon the results of these preliminary studies, spiking levels and procedures have been modified to better fit the study objectives.

Meanwhile, a schedule was approved for a pilot study to address the issue of stability during shipping, which will be critical for the full collaborative study. In addition, regulations for shipping samples spiked with botulinum toxins will be evaluated for possible restrictions that may affect the full collaborative study. Other portions of the collaborative study design are awaiting results of the precollaborative study.

FDA and CDC are updating AOAC regularly by conference call to address problems and keep the project on schedule. According to the current schedule for the precollaborative study, the final set of samples will be tested in September, 2008.

Recommendations

It is recommend that the FDA/CDC continue working towards generation of the precollaborative data set which will support a full collaborative study.