



Homeland Security Partners with AOAC to Evaluate Methods for the Detection and Identification of *Bacillus anthracis*

Background

The events of September 11, 2001, and the anthrax outbreak that followed, greatly increased the awareness of the vulnerability of the United States and its citizens to terrorist and bioterrorist threats. As a result of these disasters, the U.S. Department of Homeland Security (DHS) was created in 2002. One of the strategic goals of the DHS is to “lead, manage, and coordinate the national response to acts of terrorism, natural disasters, or other emergencies.” President Bush has requested \$274 million in the FY2005 budget for the Bio-Surveillance Initiative, to include funding for DHS, the Department of Health and Human Services’ Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Department of Agriculture’s Food Safety Inspection Service (FSIS), and the Animal and Plant Health Inspection Service (APHIS) for environmental and human monitoring and to help protect the safety of the food supply. In order to accomplish these goals, federal, state, and local agencies must possess the tools necessary to accurately and quickly detect and identify biological threat agents.

AOAC INTERNATIONAL is a 120-year-old nonprofit organization with 3500 members and is “committed to be a proactive, worldwide provider and facilitator in the development, use, and harmonization of validated analytical methods.” With its expertise and experience in the validation of analytical methods, it was a natural fit for the DHS to partner with AOAC in 2003 to independently evaluate methods for the detection and identification of *Bacillus anthracis* (BA), the causative agent of anthrax.

Anthrax is a treatable disease when diagnosed in the early stages of infection in humans. All three forms, cutaneous, gastrointestinal, and pulmonary (inhalational), can be fatal if untreated or if treatment is delayed. The gastrointestinal and pulmonary forms are more often fatal mainly because they go unrecognized until it is too late for effective treatment. Accurate and reliable methods, therefore, are needed for detection and identification of BA.

For either routine monitoring or disaster response, first responders must have screening methods appropriate for field use for rapid detection of BA in potentially contaminated environmental samples. In October 2001, the CDC issued a Health Advisory warning that the utility and validity of hand-held assays (HHAs) sold commercially for the rapid detection of BA is unknown. Then, in July 2002, the White House Office of Science and Technology Policy (OSTP) advised that federal agencies cease the procurement of any new equipment or services that may detect BA as the method for assaying suspicious mail or for routine environmental sampling of mail rooms. It was recognized that these HHA methods did not possess independent validation of performance claims.

Task Force for *Bacillus anthracis*

Under the direction of Scott Coates and Diana Hopkins at AOAC, a Task Force for *Bacillus anthracis* (TFBA) was created to serve as the backbone of the program. The Task Force is co-chaired by DHS’ Director of Standards, Bert Coursey, and the Assistant Director of Homeland Security at the White House OSTP, Lawrence Kerr. The TFBA consists of

approximately 50 experts on BA, assay development, validation study design, and statistics from 36 federal and 9 military agencies (including DHS, DOD, FDA, HHS/CDC, USDA, USPS, FBI), and representatives of state and municipal agencies, academia, and first responder units.

The TFBA formed five subtask groups (STGs) focused on method selection, study design, laboratory selection, selection of experts, and labeling/packaging. Together, the TFBA members recommended the criteria for method inclusion, the study design, and developed criteria for laboratory selection. Eight HHAs were identified from which six were selected for evaluation. All recommendations were provided to expert reviewers in the AOAC *Performance Tested Methods*SM (PTM) and *Official Methods*SM Programs for consideration in this project. Formation of the TFBA was key to the success of the project because it brought the stakeholders together to agree on a common path forward and provided technical expertise.

Evaluating Methods

The timeline for the entire program, from creating the Task Force to completing the studies and final review of the study reports, was tight—just 1 year. If accomplished, this would mean designing the systems, policies and processes as well as completing the evaluations for multiple test kits in about half the time normally required, obviously requiring great organizational skills and cooperation among all parties involved.

As stated above, the program included the evaluation of HHAs for screening purposes and laboratory methods for confirmatory identification of BA. The inclusion criteria for the HHAs were portability, the ability to obtain results in less than 30 minutes, the test must be easy to perform and easy to interpret, and the test must have completed its development phase, ready for validation.

The test format that best fits the inclusion criteria is the lateral flow format. The best-known examples of this format are home pregnancy tests that require application of the test sample to one end of the device, followed by a waiting period, then interpretation of results. Typically, these tests are based on the specificity of an antibody for its intended target molecule. When a sample is applied to the device, the target molecules within the sample bind to labeled antibodies contained within the device, forming target-antibody-label complexes. These antibodies are typically labeled with colloidal gold. The sample liquid causes the target-antibody-label complexes to flow through a thin membrane. The membrane contains two capture zones, one for capture of the target molecule (test zone) and one for capture of the labeled antibody (control zone). As the sample liquid flows past the test zone, the target-antibody-label complexes are captured, causing a colored line to form on the membrane due to the color of the colloidal gold or other label. As the flow continues, the liquid encounters the control zone in which the labeled antibody is captured, regardless of the presence of the target molecule, causing a colored line to form on the membrane. The test interpretation is based on the number of lines visible on the membrane. A line will always be present in the control zone, ensuring that the test has functioned properly, but a line will be present in the test zone only when the target molecule is present at a level equal to or greater than the detection limit of the test. There were five lateral flow devices identified for inclusion in the evaluation of HHAs for field use.

Rapid screening tests must be backed up by accurate laboratory methods for confirmation of presumptive results. Thus, the DHS-AOAC contract, which initially encompassed the validation of rapid test kits (also known as hand-held assays or “HHAs”) for environmental samples, was later expanded to include confirmatory methods for positive identification of BA in environmental samples. Two confirmatory methods were identified to be included in the program: The Laboratory Response Network (LRN), Gamma Phage and Direct Fluorescence Assay (DFA); and the MIDI Inc. SherlockTM fatty acid profile method.

AOAC Validation Programs

AOAC has two widely known programs for validation of analytical methods, namely, the *Official Methods*SM and the PTM Programs. The *Official Methods*SM Program offers a two-step validation process in which the test sponsor first submits “precollaborative” data for review by the appropriate AOAC *Official Methods*SM committee. The precollaborative data consist of pure cultures for inclusivity/exclusivity evaluation and testing of adulterated and nonadulterated “field” samples. This testing is typically done in the sponsor’s laboratory. The second phase of the validation is a collaborative study, which evaluates the performance of the test in 10-12 laboratories to test for lab-to-lab reproducibility. This testing typically includes several sets of adulterated and nonadulterated “field” samples, and the study report is again sent to the Methods Committee for review.

The PTM Program is a one-step validation program that requires ruggedness testing, lot-to-lot reproducibility testing, and stability data (for the determination of the shelf-life of the test kit), in addition to the *Official Method*SM precollaborative data requirements. The PTM program also includes test kit performance evaluation by a single independent laboratory. A single study report, encompassing both the internal and independent evaluations, is sent to one AOAC-appointed General Referee and two independent expert reviewers.

Recently, the PTM and *Official Methods*SM Programs were harmonized so that the PTM study can be used to fulfill the OMA precollaborative requirements. It offers test kit companies the option of obtaining a PTM certificate upon successful completion of the first (precollaborative) phase, followed by Official First Action status upon successful completion of the second (collaborative) phase. The review processes for the harmonized program include both independent expert reviewers and the AOAC Methods Committee. This harmonized program was used as the model for the study designs created by the TFBA for evaluation of BA detection and identification methods.

Using the harmonized program, Phase 1 of the HHA evaluation included determination of inclusivity (the ability to detect a variety of *B. anthracis* strains), exclusivity (the ability to distinguish *B. anthracis* strains from non-*B. anthracis* strains), sensitivity (the reliability of assays when known numbers of *B. anthracis* spores are present), and specificity (the rate of correct results when assays are challenged with known numbers of non-*B. anthracis* spores) in the independent laboratory. Also determined were matrix effects (cross-reactions with common hoax materials) and preparation effects (reliability of assay results when spore samples are prepared using different preparation procedures) in the independent laboratory, and ruggedness (how variation of procedural factors affect the analytical performance of assays) in the test kit sponsors’ laboratories.

Phase 2 of the HHA evaluation was a collaborative study in which 12 laboratories analyzed common samples to determine the reproducibility of the HHA methods.

To facilitate the execution of the Phase 1 and Phase 2 studies, a single laboratory (U.S. Army Dugway Proving Grounds) was chosen to serve as the Phase 1 independent laboratory for the five simultaneous HHA evaluations and the lead laboratory for the Phase 2 collaborative study, preparing spore samples and shipping these to each of the collaborating laboratories. In this way, test sample preparation was consistent between studies and was expedited. Similarly, the same set of 12 collaborators performed the collaborative studies for all of the HHAs and all five HHAs were evaluated simultaneously. AOAC performed extensive on-site evaluations to ensure that each collaborating laboratory possessed the proper facilities, training, and quality assurance practices to conduct such a study as determined by the TFBA. A multi-day training session for the collaborating laboratories was organized by AOAC to ensure that all laboratories received the same level of training prior to conducting the study. This model was also followed for the evaluation of BA identification methods.

Upon completion of the studies, each test kit company, together with an independent consultant, prepared a PTM/Official MethodSM study report, which was submitted to two expert reviewers, a General Referee, and the Official Methods Committee. To expedite the

review process, a multi-day review session was organized by AOAC, at which the reviewers as a group were able to ask questions of the test kit company representatives and then discuss their opinions privately. Following review, all Study Directors met, including the Method Committee chair and Associate Referee, to respond to reviewers' comments and to submit additional data before final review.

Conclusion

As of the time of this writing, the CDC identification method has been granted First Action status, the five HHA detection methods are in the final stages of review, and the second identification method is in collaborative study. A final decision on the HHAs and confirmatory test method are expected sometime this fall.

Considering that the 1-year timeframe of the DHS contract was initially intended only for the evaluation of HHAs for screening and did not include efforts for the evaluation of confirmatory identification methods, much has been accomplished by AOAC and the TFBA in 1 year.

The model developed for the AOAC-DHS Bacillus anthracis project is a science-based decision-making process adapted for evaluation of threat agent methodologies. Key to the success of the project was the ability of AOAC to generate consensus among federal agencies and to promote the sharing of resources among federal agencies (see sidebar for more keys to success). As a result, AOAC has created a one-of-a-kind infrastructure for the evaluation of threat agent detection methods and has created a trained, experienced, nationwide network of evaluation laboratories. The value of the project for the citizens of the United States lies in the fact that it provided independent third-party assessments of detection technologies that were previously unvalidated. Upon completion of the project, AOAC will have provided the federal government with a thorough knowledge of the performance capabilities and limitations of five HHAs and two confirmatory identification methods. With this knowledge, informed science-based decisions can be made on matters of importance to national security.



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