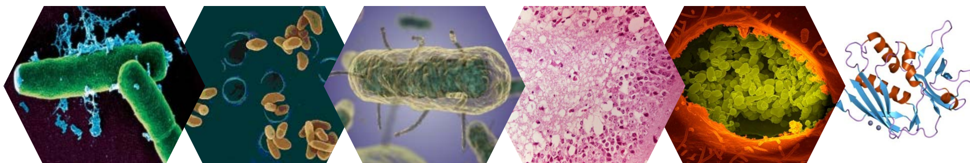
The Scientific Association Dedicated to Analytical Excellence®

Stakeholder Panel on Agent Detection Assays

SEPTEMBER 1-2, 2015

AOAC INTERNATIONAL Headquarters
Conference Room 110
2275 Research Boulevard
Rockville, Maryland, 20850



contact: spada@aoac.org

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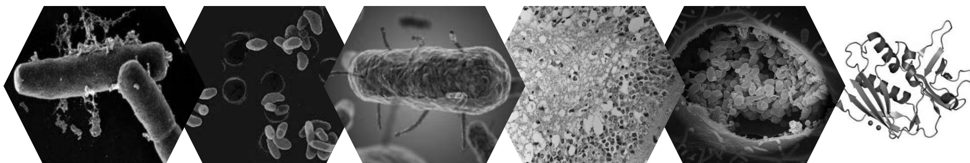


contact: spada@aoac.org

Appendix W

POLICY AND PROCEDURES ON VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

Illustrations of Conflicts of Interest

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.

2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.

3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

4. Sponsorship of a collaborative study by an interest (which may include the referee) which stands to profit from the results; such sponsorship usually involving the privilege granted by the investigator to permit the sponsor to review and comment upon the results prior to AOAC evaluation.

5. A volunteer asked to review a manuscript submitted for publication when the manuscript contains information which is critical of a proprietary or other interest of the reviewer.

The foregoing are intended as illustrative and should not be interpreted to be all-inclusive examples of conflicts of interest AOAC volunteers may find themselves involved in.

Do's and Don't's

Do avoid the appearance as well as the fact of a conflict of interest.

Do make written disclosure of any material interest which may constitute a conflict of interest or the appearance of a conflict of interest.

Do not accept payment or gifts for services rendered as a volunteer of the Association without disclosing such payment or gifts.

Do not vote on any issue before an AOAC decision-making body where you have the appearance of or an actual conflict of interest regarding the recommendation or decision before that body.

Do not participate in an AOAC decision-making body without written disclosure of actual or potential conflicts of interest in the issues before that body.

Do not accept a position of responsibility as an AOAC volunteer, without disclosure, where the discharge of the accepted responsibility will be or may appear to be influenced by proprietary or other conflicting interests.

Procedures

Each volunteer elected or appointed to an AOAC position of responsibility shall be sent, at the time of election or appointment, a copy of this policy and shall be advised of the requirement to adhere to the provisions herein as a condition for active participation in the business of the Association. Each volunteer, at the time of his or her election or appointment, shall indicate, in writing, on a form provided for this purpose by AOAC, that he or she has read and accepts this policy.

Each year, at the spring meeting of the AOAC Board of Directors, the Executive Director shall submit a report certifying the requirements of this policy have been met; including the names and positions of any elected or appointed volunteers who have not at that time indicated in writing that they have accepted the policy.

Anyone with knowledge of specific instances in which the provisions of this policy have not been complied with shall report these instances to the Board of Directors, via the Office of the Executive Director, as soon as discovered.

* * * * * *

Adopted: March 2, 1989
Revised: March 28, 1990
Revised: October 1996
Reviewed by outside counsel March 2000 (Fran Dwornik) and found to be current and relevant

Appendix U

ANTITRUST POLICY STATEMENT AND GUIDELINES

Introduction

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on-going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

Responsibility for Antitrust Compliance

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance -- which includes avoidance of even an appearance of improper activity -- belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

Antitrust Guidelines

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits. Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment. Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard
2. Don't discuss with competitors your own or the competitors' prices, or anything that might

affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.

3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.

4. Don't disclose to others at meetings or otherwise any competitively sensitive information.

5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.

6. Don't stay at a meeting where any such price or anti-competitive talk occurs.

7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.

8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.

9. Do send copies of meeting minutes and all AOAC-related correspondence to the staff member involved in the activity.

10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

Conclusion

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: March 11, 1991
Revised October 1996

Appendix V

POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

Introduction

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.

The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.

The Scientific Association Dedicated to Analytical Excellence®

Policy

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

> "The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.
>
> The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for

the purposes for which permission has been specifically granted.

The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization."

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a member of the Association.

Instructions

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.

2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.

3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.

5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.

6. Do not print one part of the logo or insignia in one color and other parts in another color.

7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, elected and appointed officers, staff, sections, or committees; except by special permission.

   Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

   Copies of all correspondence using AOAC letterhead or conducting AOAC official business,

whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

   Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.

2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.

3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

* * * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

SPADA

Stakeholder Panel on Agent Detection Assays

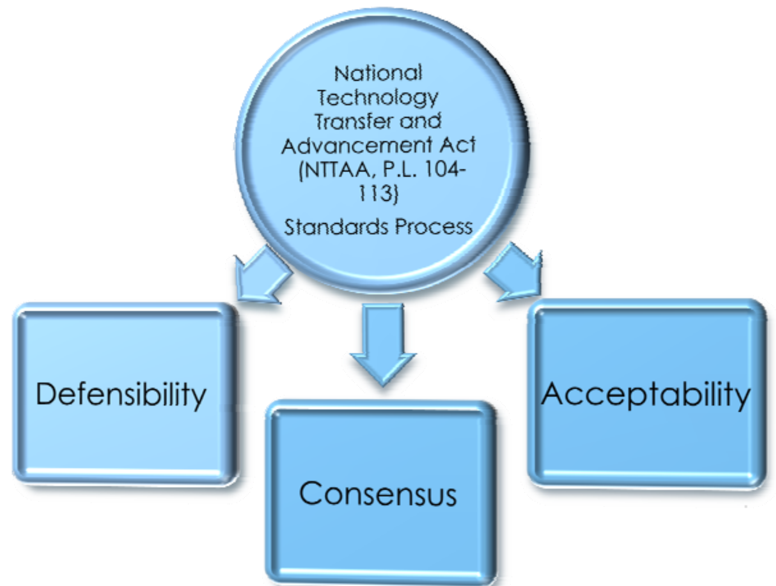
a Voluntary Consensus Body established by AOAC INTERNATIONAL with funding from US DHS S&T, US DHS OHA, and US DOD

FACT SHEET

Performance Standards for Threat Detection Equipment

SPADA, an enduring national capability established to leverage the threat community to develop national voluntary consensus standards for third-party evaluation of threat detection tools.

Standards guide procurement decisions of end-users, provide first responders with independently validated threat detection tools for incident management, can provide state and local government with evaluation criteria for regulation, can provide minimum acceptance criteria to federal agencies for acquisition decisions.



Participating Organizational Stakeholders

Public Safety	Military	Academic Institutions
Public Health	Professional Organizations	Contract Research
Method Developers	Federal Law Enforcement	Federal/State Regulatory
Reference Materials Providers	State & Local Law	Federal/State Coordinating
End Users	National Laboratories	Industry - General

Reference to SPADA: ***US DHS Framework for a Biothreat Field Response Mission Capability, April 5, 2011***

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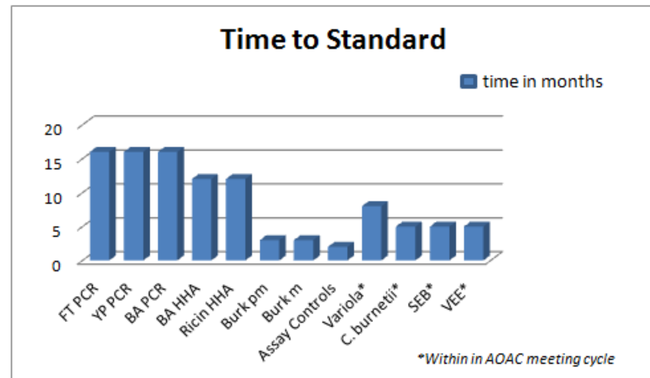
Accomplishments

15 Stakeholder Meetings

16 Specialized Working Groups

11 Consensus Based Standards

1 Town Hall Meeting



Publications & Completed Standards and Methods

Development of Standard Method Performance Requirements for Biological Threat Agent Detection Methods. **Coates, Scott G.; Burnelle, Sharon L.; Davenport, Matthew G. (2011) Vol. 94, No. 4, pp. 1328-1337(10).**

AOAC SMPR 2010.001: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Francisella tularensis* in Aerosol Collection Filters and/or Liquids (2011) Vol. 94, No. 4, pp. 1338-1341(4).

AOAC SMPR 2010.002: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Yersinia pestis* in Aerosol Collection Filters and/or Liquids (2011) Vol. 94, No. 4, pp. 1342-1346(5).

AOAC SMPR 2010.003: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* in Aerosol Collection Filters and/or Liquids (2011) Vol. 94, No. 4, pp. 1347-1351(5).

AOAC SMPR 2010.004: Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of *Bacillus anthracis* Spores in Visible Powders (2011) Vol. 94, No. 4, pp. 1352-1355(4).

AOAC SMPR 2010.005: Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Ricin in Visible Powders (2011) Vol. 94, No. 4, pp. 1356-1358(3).

AOAC SMPR 2011.xxx: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Burkholderia pseudomallei* in Aerosol Collection Filters and/or Liquids – PUBLICATION FORTHCOMING

AOAC SMPR 2011.xxx: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Burkholderia mallei* in Aerosol Collection Filters and/or Liquids – PUBLICATION FORTHCOMING

AOAC SMPR 2011.xxx: Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* in Visible Powders – PUBLICATION FORTHCOMING

AOAC SMPR 2014.006: Standard Method Performance Requirements for Detection and Identification of *Variola Virus* DNA in Aerosol Collection Filters and/or Liquids – (2015) Vol. 98, No. 4, pp. 1046-1049(4).

AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures (2011) Vol. 94, No. 4, pp. 1359-1381(23). OMA Appendix I.

AOAC Official Method of Analysis Standard Practice for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents from Nonporous Surfaces: Collaborative Study. **Locascio, Laurie E.; Harper, Bruce; Robinson, Matthew; Badar, T., (2007) Vol. 90, No. 1, pp. 299-333(28), AOAC 2006.04, ASTM E2458**

AOAC Performance Tested℠ Certification No. 101103, RAZOR® EX Anthrax Air Detection System. **Spaulding, Usha K.; Christensen, Clarissa J.; Crisp, Robert J.; Vaughn, Michael B.; Trauscht, Robert C.; Gardner, Jordan R.; Thatcher, Stephanie A.; Clemens, Kristine M.; Teng, David H.; Bird, Abigail; Ota, Irene M., (2012) Vol. 95, No. 3, pp. 860-891(32)**

AOAC Official Methods of Analysis **- AOAC 2012.06 *RAZOR® EX Anthrax Air Detection System for Detection of Bacillus anthracis Spores from Aerosol Collection Samples: Collaborative Study*. Hadfield, Ted; Ryan, Valorie; Spaulding, Usha K.; Clemens, Kristine M.; Ota, Irene M.; Brunelle, Sharon L. (2013) Vol. 96, No. 2, pp. 392-398(7)**

AOAC Performance Tested℠ **Certification No. 121201, *Individual Hand Held Assay for Ricin Toxin*. Brunelle, Sharon L; Hadfield, Ted; Ryan, Valorie – PUBLICATION FORTHCOMING**

AOAC Official Methods of Analysis **– AOAC 2013.08 *Individual Hand Held Assay for Ricin Toxin*. Brunelle, Sharon L; Hadfield, Ted; Ryan, Valorie – PUBLICATION FORTHCOMING**

Reference to SPADA: US House of Representatives Appropriations Committee, Subcommittee on Homeland Security; Testimony of the Honorable Tara O'Toole, MD, MPH - Undersecretary, Directorate of Science and Technology, Department of Homeland Security, February 25, 2010

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MATTHEW DAVENPORT, PhD
PROGRAM MANAGER, BIOSCIENCES AND INFORMATICS
THE JOHNS HOPKINS UNIVERSITY APPLIED PHYSICS LABORATORY

Chair, AOAC Stakeholder Panel on Agent Detection Assays

Matt is a Program Manager in Biosciences and Informatics at the Johns Hopkins University Applied Physics Laboratory (JHU/APL) to include projects in personalized genomics, the Microbiome, and functional biology. Matt also works in the areas of human performance and austere medicine with military communities. Prior to JHU/APL, Matt was a Program Manager in the Department of Homeland Security Science and Technology Directorate (DHS S&T) where he established the DHS Public Safety Actionable Assay (PSAA) program and the Stakeholder Panel for Agent Detection Assays (SPADA) to develop voluntary consensus standards for the validation of biothreat detection technologies used by first responders and private-sector end users. In addition to the PSAA program, Matt coordinated a number of bioinformatics efforts including: the development of new databases and software to identify signatures that can be used to specifically detect biothreat agents; sequencing strains of biothreats and their genetic near-neighbors; and application of next generation sequencing to biothreat detection. He also served on numerous interagency committees and co-chaired a working group under the National Science and Technology Council that produced *A National Strategy for CBRNE Standards*.

Matt joined DHS S&T as a Science and Technology Policy Fellow from the American Association for the Advancement of Science (AAAS) where he worked in the same areas of biological countermeasures. Prior to DHS, he was a postdoctoral fellow at both The Johns Hopkins University School of Medicine and the Memorial Sloan-Kettering Cancer Center studying the biochemical mechanisms that control replication of the human genome and the repair of genome when it becomes damaged. Matt earned his doctorate from the Department of Microbiology and Immunology at the University of North Carolina at Chapel Hill and a B.S. in microbiology from North Carolina State University.

Linda C. Beck, PhD, MT (ASCP)

Naval Surface Warfare Center

SPADA COXIELLA BURNETII WORKING GROUP CO-CHAIR

Dr. Linda Beck works for the Department of Defense at the Naval Surface Warfare Center Dahlgren Division (NSWCDD) as a Lead Scientist/Microbiologist in the CBR Concepts and Experimentation Branch. Prior to her current position, she worked for the Department of Homeland Security for three years, and served as the Deputy Program Manager and Director for Laboratory Operations for the BioWatch Program, the biosurveillance system designed to detect select aerosolized biological agents. Preceding her DHS position, Dr. Beck was working at NSWCDD and developed and implemented the BioWatch Quality Assurance Samples laboratory and served as the Program Manager for the DHS effort at Dahlgren. During that tenure, she also served as the Head of the Micro/Molecular Biology Section, supported the development of methods for testing the efficacy of decontaminants on biotoxins, and served as a Chem/Bio Subject Matter Expert on the Hazard Mitigation, Materiel and Equipment Restoration Advance Technology Demonstration program sponsored by the Defense Threat Reduction Agency, Joint Science and Technology Office (DTRA JSTO).

In addition to her Federal government work, Dr. Beck has 15 years of experience in a career in academia. She was a professor in the Biological Sciences Department at the University of Mary Washington prior to her appointment to serve on the faculty in the School of Allied Health Professions at the Medical College of Virginia/Virginia Commonwealth University.

Dr. Beck graduated from the Medical College of Virginia, Virginia Commonwealth University (MCV/VCU) with a PhD in Pathology/Clinical Microbiology followed by two years as a Postdoctoral Research Fellow in the School of Medicine at MCV/VCU.

Ted Hadfield, PhD

Owner, Hadeco LLC.

SPADA BACILLUS ANTHRACIS WORKING GROUP CO-CHAIR

Ted L. Hadfield, Ph.D., Co-chair of the Variols Working Group, graduated from University of Utah in 1976. He did a post doctoral in Clinical Immunology at the Latter Day Saints Hospital in Salt Lake City, UT. He subsequently was an assistant professor at California State University in Los Angeles. In 1980 he joined the United States Air Force as a Laboratory Officer. He was stationed at the Armed Forces Institute of Pathology as Chief of Bacteriology. In 1984 he was transferred to Wilford Hall USAF Medical

Center in San Antonio Texas as Chief, Clinical Microbiology. In 1989, he transferred back to the Armed Forces Institute of Pathology as Chief of Microbiology. Dr. Hadfield retired from the Air Force in 2000 and was appointed as a Distinguished Scientist at the American Registry of Pathology. He continued as Chief of Microbiology and as Deputy Director of Infectious and Parasitic Diseases Pathology. In 2003 he moved to MRIGlobal's Florida Division as Chief, Bioscience Advisor. In 2012 he retired from MRIGlobal and became president of HADECO, LLC, a consultation service for microbiological, immunology and molecular biology solutions. Dr Hadfield has more than 100 scientific publications and remains active in research projects at MRIGlobal, University of Florida, Gainesville and consultations with clinical laboratories.

Luther Lindler, PhD

Department of Homeland Security, Science and Technology Directorate

SPADA YERSINIA PESTIS WORKING GROUP CHAIR

Dr. Lindler joined the DHS Science and Technology Directorate in October 2003 as a Senior Science Advisor. Dr. Lindler currently serves as the DHS S&T liaison to the Department of Defense Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD). He also serves as the Chief Scientist for the DHS Chemical and Biological Defense Division providing biodefense expertise to both DOD as well as DHS in the area of infectious disease threats from a global perspective. Dr. Lindler's previous work provided strategic investments to bring forward deployed rapid molecular diagnostics to U.S. forces. Dr. Lindler provided technical leadership in the Federal Material Threat Assessment and Biological Risk Assessment programs. He helped plan the National Biodefense Analysis and Countermeasures Center forensics and threat characterization programs as well as the first DHS laboratory building on the Fort Detrick National Biodefense Campus. Before joining DHS, Dr. Lindler was a leader in the U.S. Army Biodefense program. He was a principle investigator at the Walter Reed Army Institute of Research leading a team of professionals studying the pathogenesis of the plague bacterium. He served on the Army's plague vaccine steering committee and the emerging threats steering committee within the Biodefense program. The peak of his career with the Army culminated with his senior editorship of the well-acclaimed Biodefense book entitled, "Biological Weapons Defense; Infectious Diseases and Counterbioterrorism." Dr. Lindler was a postdoctoral fellow in the laboratory of Dr. Susan Straley at the University of Kentucky in Lexington from 1987 until 1989. Dr. Lindler received his Ph.D. in Microbiology from the Medical College of Virginia in 1987, his Masters of Science in Microbiology from Clemson University in 1981 and his Bachelor's of Science in Medical Technology from Lenoir Rhyne College in North Carolina in 1978.

Paul Jackson, PhD

Los Alamos and Lawrence Livermore National Laboratories (Retired)

SPADA BACILLUS ANTHRACIS WORKING GROUP CO-CHAIR

Paul received his Bachelor's of Science degree from the University of Washington in Cellular Biology and his Ph.D. from the University of Utah in Molecular Biology. He was a visiting scholar at the Center for International Security and Cooperation (CISAC) at Stanford University from September 2011-September 2012 and is now a CISAC affiliate. He is also an adjunction professor at the Middlebury Institute of International Studies at Monterey (formerly the Monterey Institute of International Studies) where he team teaches a class entitled "Science and Technology for Non-proliferation and Terrorism Studies". For the past 24 years he has been studying bacterial pathogens, first working to develop DNA-based methods of detecting these microbes and their remnants in environmental and laboratory samples, then developing methods to differentiate among different strains of the same pathogenic species. Research interests include the study of different methods of interrogating biological samples for detection and characterization of content, and development of bioforensic tools that provide detailed information about biothreat isolates including full interrogation of samples for strain content and other genetic traits. Methods he and collaborators developed have been applied to forensic analysis of samples and aid in identifying the source of disease outbreaks. He contributed to analysis of the *Bacillus anthracis* present in the 2001 Amerithrax letters and conducted detailed analyses of human tissue samples preserved from the 1979 Sverdlovsk anthrax outbreak, providing evidence that was inconsistent with Soviet government claims of a natural anthrax outbreak. His current interests continue to focus on development of assays that rapidly detect specific signatures including antibiotic resistance in threat agents and other pathogens. More recent activities include identification and characterization of new antimicrobial compounds that are based on the pathogens' own genes and the products they encode. These include development of such materials as therapeutic antimicrobials, their application to remediate high value contaminated sites and materials, and their use to destroy large cultures and preparations of different bacterial threat agents. Efforts to address issues of antibiotic resistance and treatment of resistant organisms have recently been expanded to look at non-threat agent pathogens that cause problematic nosocomial or community-acquired infections of particular interest to the military.

Paul spent 24 years as a Technical Staff Member at Los Alamos National Laboratory where he was heavily involved in development of the biological threat reduction efforts there. He was appointed a Laboratory Fellow at Los Alamos – a lifetime appointment - in recognition of his efforts. He moved to Lawrence Livermore National Laboratory in 2005 where he was a Senior Scientist in the Global Security and Physical and Life Sciences Directorates until his retirement in 2013. In addition to his work at the National Laboratories, he has served on the FBI's Scientific Working Group for Microbial Forensics, on NIH study sections and review panels, and continues to serve on steering and oversight committees for other federal agencies.

Eileen N. Ostlund, DVM, MS, PhD

Head, Equine and Ovine Viruses Section, Diagnostic Virology Laboratory

National Veterinary Services Laboratories, STAS, VS, APHIS, USDA APHIS

SPADA STAPHYLOCOCCAL ENTEROTIXIN TYPE-B (SEB) WORKING GROUP CHAIR

Eileen N. Ostlund received her DVM (1980) and MS (1982) from the University of Illinois and then spent 5 years in private veterinary practice with an equine focus. Subsequently, she completed a PhD in Veterinary Science from the University of Kentucky (1992) and conducted postdoctoral research in infectious diseases at the Animal Health Trust, Newmarket, England and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. She then served on the faculty at the University of Missouri, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. In 1998, Dr. Ostlund joined USDA/APHIS/VS as the Head of the Equine and Ovine Viruses Section in the Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Ames, Iowa. Dr. Ostlund served as the USDA co-chair of the Intragovernmental Select Agent and Toxin Technical Advisory Committee (ISATTAC) from 2005 through 2013. She has been named a designated expert for the World Organization for Animal Health, Office International des Epizooties (OIE), for eastern, western, and Venezuelan encephalomyelitis, West Nile Fever, equine infectious anemia, and bluetongue.

James Samuel, PhD

Professor and Chair of Microbial Pathogenesis and Immunology

Texas A&M

SPADA Q-FEVER WORKING GROUP CO-CHAIR

Professor and Chair at the Department of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center College of Medicine. His research is primarily focused on the human respiratory

pathogen, *Coxiella burnetii*, the agent of Q fever, with basic studies on mechanisms of pathogenesis and applied goals of novel vaccine and diagnostic development. Dr. Samuel has taught courses in genetics, microbiology and microbial pathogenesis for both medical and graduate education.

Sandra M. Tallent, PhD

Center for Food Safety and Nutrition, United States Food and Drug Administration

SPADA VENEZUALAN EQUINE ENCEPHALITIS (VEE) WORKING GROUP CHAIR

Sandra McKenzie Tallent received her Bachelor of Science from Florida Southern College, Lakeland Florida and, upon graduation, attended Orlando Regional Medical Center's School of Medical Technology. The challenges of antimicrobial resistance prompted her to alter her career focus from clinical microbiology to public health research. She earned her Master's and Doctorate in Microbiology and Immunology from Virginia Commonwealth University in Richmond, Virginia followed by a CDC Emerging Infectious Disease Research Fellow appointment with Virginia's Division of Consolidated Laboratory Services. She has been with the U.S. FDA for seven years where her work involves assay development to detect *Staphylococcus aureus* and *Bacillus cereus* and their enterotoxins in food matrices.

David Wagner, PhD

Associate Professor, Department of Biological Sciences

Associate Director, Center for Microbial Genetics and Genomics

Northern Arizona University

SPADA F. TULARENSIS WORKING GROUP CO-CHAIR

Dave Wagner has been working with dangerous pathogens, including *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, and *Burkholderia pseudomallei*, in field and laboratory settings since 1999. He is the Associate Director of the Center for Microbial Genetics and Genomics at NAU, which employs more than 60 faculty, staff, and students. Dr. Wagner has established research collaborations around the world, including *F. tularensis* research in Europe and Asia and *Y. pestis* research in Africa, Asia, Europe, and South America, among many others. His is broadly interested in the evolutionary history, phylogeography, and ecology of infectious disease agents.

STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Tuesday – Wednesday, September 1 – 2, 2015

AOAC INTERNATIONAL Headquarters
Conference Room 110
2275 Research Blvd., Rockville, Maryland, 20850
9:00 a.m. – 5:00 p.m.

STAKEHOLDER PANEL AGENDA – SEPTEMBER 1, 2015 (Day 1)

I. Welcome, Introductions, & Call to Order – *Jim Bradford, AOAC INTERNATIONAL & Matthew Davenport, DHS & SPADA Chair* **(9:00 a.m. – 9:15 a.m.)**

II. Overview (**9:15 a.m. – 10:30 a.m.)**
   a. Meeting Objectives – *Jim Bradford, AOAC INTERNATIONAL*
   b. SPADA Standards Development Priorities Discussion– *Matthew Davenport, DHS, SPADA Chair*
      i. Historical Perspective
      ii. Current Project Summary
      iii. SPADA Long-Term Goals

III. Working Group Draft Standard Method Performance Requirements (SMPR) Presentations (**10:45 a.m. – 2:45 p.m.)**
   a. AOAC Policies and Procedures for Adopting an SMPR – *Deborah McKenzie, AOAC INTL.* **(10:45 a.m. – 10:50 a.m.)**
   b. Environmental Factors* – *Scott Coates, AOAC INTERNATIONAL* (**10:50 a.m.-11:30 a.m.)**
   c. *Coxiella brunetii* – Linda Beck, NSWC and James Samuel, Texas A&M* **(11:30 a.m. – 12:15 p.m.)**
   d. *Staphylococcus* enterotoxin A-C* – *Sandra Tallent, FDA* **(1:00 p.m. – 1:45 p.m.)**
   e. Venezuelan Equine Encephalitis* - *Eileen Ostlund, USDA* **(1:45 p.m. – 2:30 p.m.)**

IV. Launch of New Working Groups (**2:45 p.m. – 5:00 p.m.)**
   a. AOAC Policies and Procedures for WGs – *Deborah McKenzie, AOAC INTL.* **(2:45 p.m. – 3:00 p.m.)**
   b. *Bacillus anthracis – Ted Hadfield, Hadeco, LLC* and *Paul Jackson, LLNL/LANL (Ret)* **(3:00 p.m. – 3:40 p.m.)**
      i. Fitness for Purpose
   c. *Francisella tularensis – Paul Keim, NAU* and *David Wagner, NAU* **(3:40 p.m. – 4:20 p.m.)**
      i. Fitness for Purpose
   d. *Yersinia pestis – Robert Bull, FBI* **(4:20 p.m. – 5:00 p.m.)**
      i. Fitness for Purpose

V. Wrap Up – *Matthew Davenport, DHS & SPADA Chair*

VI. Adjourn

**Item requires a vote* (italic)

Lunch 12:15 – 1:15

AOAC INTERNATIONAL Stakeholder Panel on Agent Detection Assays
Working Group Sessions - SEPTEMBER 2, 2015 (Day 2)
9:00 a.m. – 4:30 p.m.

I. Introduction and Overview of Assays and Validation (9:00 a.m. – 9:15 a.m.)

Matt Davenport, DHS, SPADA Chair

II. Review of Environmental Factors Panels (9:15 a.m. – 10:00 a.m.)

Ted Hadfield, Hadeco, LLC

III. *Bacillus anthracis* (10:15 a.m. – 12:00 p.m.)

Chair: Ted Hadfield, Hadeco, LLC and *Paul Jackson, LLNL/LANL (Retired)*

a. Review Fitness for Purpose
SMPR Development

IV. *Francisella tularensis* (12:45 p.m. – 2:30 p.m.)

Co-Chairs: Paul Keim, NAU and *David Wagner, NAU*

a. Review Fitness for Purpose
b. SMPR Development

V. *Yersinia pestis* (2:45 p.m. – 4:30 p.m.)

Chair: Luther Lindler, DHS

a. Review Fitness for Purpose
b. SMPR Development

**Item requires a vote*

Lunch 12:00 pm – 1:45 pm

AOAC INTERNATIONAL STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

E. James Bradford
Executive Director &CEO, AOAC INTERNATIONAL
September 1, 2015

Rockville, MD

Meeting Objectives

- SPADA Executive Steering Panel Session
  - Under current DHS Contract
    - Convene stakeholders from different government agencies
    - Purpose is to establish priorities for standards development for the detection of threat agents

Meeting Objectives

- SPADA to vote on three standards
  - Under JHU/DoD/AOAC contract
  - Developed by Working Groups of SMEs

Meeting Objectives

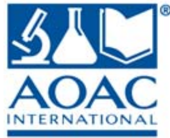
- Presentation and Approval of SMPRs
  - Working Group Draft SMPRs
    - *Coxiella burnetti* – Chairs, James Samuel, Texas A&M and Linda Beck, Naval Warfare Surface Center
    - *Staphylococcus* Enterotoxin *A-C* – Chair, Sandra Tallent, FDA, CFSAN
    - Venezuelan Equine Encephalitis – Chair, Eileen Ostlund, USDA, APHIS
- SMPRs approved by SPADA
  - become AOAC voluntary consensus standards – SMPR®
  - Published in *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis of AOAC INTERNATIONAL*

Meeting Objectives

- SPADA launch of three new Working Groups
  - JHU/DoD/AOAC contract 2015 – 2016
    - Develop standards for seven additional standards
    - Begin with:
      - *Bacillus anthracis*
      - *Francisella tularensis*
      - *Yersinia pestis*
    - SPADA to form Working Groups and suggest additional SMEs
    - WG launch development of SMPRs
      - Fitness-for-Purpose statement
      - Inclusivity/exclusivity panels and environmental factors

SPADA Meetings 2016

- March 2016
  - Present BA, FT, and YP Draft SMPRs for SPADA approval
  - Launch WGs and SMPR development for four additional agents
    - *Variola major*
    - *Burkholderia mallei*
    - *Brucella*
    - *Botulinum neurotoxin A*
- September 2016
  - Present Draft SMPRs for the four agents for SPADA approval



AOAC Stakeholder Panel on Agent Detection Assays (SPADA)

AOAC STANDARDS DEVELOPMENT PROCESS

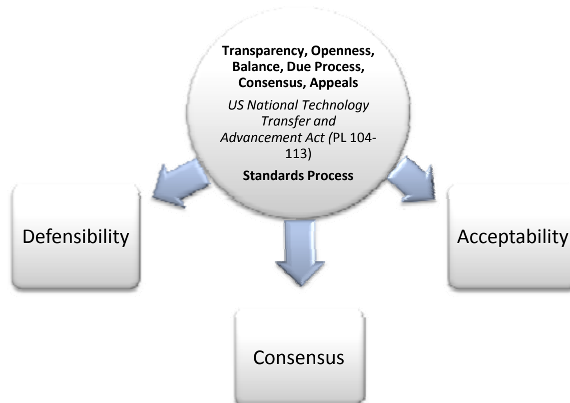
APPROVAL OF AN AOAC SMPR®

Deborah McKenzie
Sr. Director,
AOAC Standards Development
AOAC INTERNATIONAL
September 1, 2015

AOAC INTERNATIONAL HEADQUARTERS
2275 Research Blvd, Ste 300
Rockville, Maryland 20850

The Scientific Association Dedicated to Analytical Excellence®

AOAC Standards Development Processes

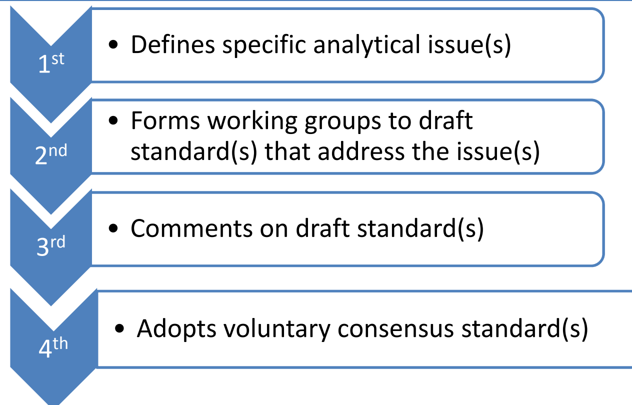


AOAC INTERNATIONAL

As an international standards development organization, AOAC must maintain the following principles throughout all its standard setting activities:

- Transparency
- Openness
- Balance of Interests
- Due Process
- Consensus
- Appeals

Stakeholder Panel Role and Output



AOAC Voluntary Consensus Standards

- **Standard Method Performance Requirements (SMPRs)**
  - Published in *Official Methods of Analysis of AOAC INTERNATIONAL*
  - Manuscript published in *Journal of AOAC INTERNATIONAL*

Stakeholder Panel Composition

- Product Manufacturers
- Analyte/Method Subject Matter Experts
- Technology Providers
- Method Developers
- Government and Regulatory Agencies
- Contract Research Organizations
- Reference Materials Developers
- Ingredient Manufacturers
- Method End Users
- Academia & Research
- Non-Governmental Organizations (ISO, IDF, etc…)
- Other…. as identified

Anyone with a material interest can participate
Balanced group of representative voting stakeholders
Chair and voting members vetted

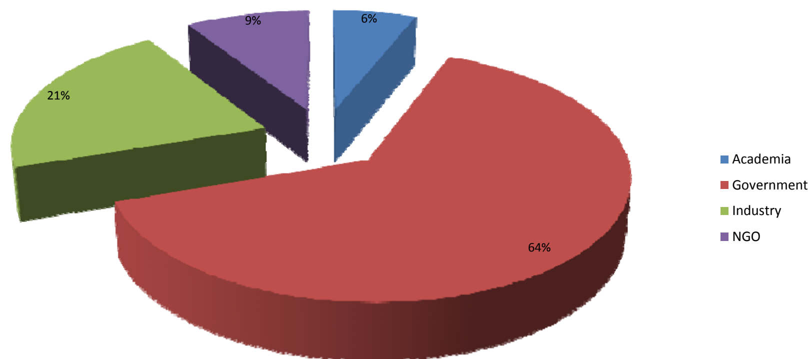
Organizational Meeting Registrants

- Association of Public Health Laboratories
- ATCC
- bioMérieux
- Censeo Insight
- US Defense Threat Reduction Agency
- FBI
- Hadeco
- Ibis Biosciences
- InSilixa
- Interagency Board
- Gerstel
- JPEO
- Lawrence Livermore National Lab
- Maryland Department of Agriculture
- Minnesota Department of Health
- NIH/NIAID
- Naval Medical Research Center
- New Horizons Diagnostics Corporation
- NIST
- North Carolina DHHS
- Northern Arizona University
- Northrup Grumman
- R-Biopharm
- Texas A&M
- USAMRIID
- US Army Edgewood Chemical Biological Center
- US CDC
- USDA APHIS
- US DHS S&T
- US DHS OHA
- US DOD Critical Reagents Program
- US DoD Navy
- US DoD Dugway Proving Ground
- US FDA-CFSAN
- US FDA ORS

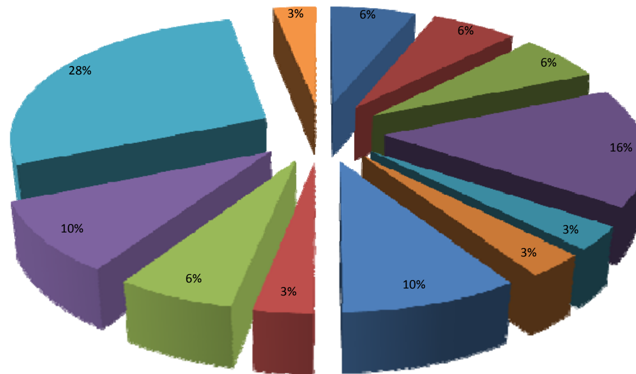
Registered Organizations by Broad Perspectives

- Academia/Research
  - Northern Arizona University
  - Texas A&M
- Industry
  - New Horizons Diagnostics Corporation
  - Northrup Grumman
  - R-Biopharm
  - ATCC
  - bioMérieux
  - Censeo Insight
  - Hadeco
  - Ibis Biosciences
  - InSilixa
  - Gerstel
- Non Governmental Organization
  - APHL
- Government
  - FBI
  - IAB
  - Lawrence Livermore National Lab
  - Maryland Department of Agriculture
  - Minnesota Department of Health
  - NIH/NIAID
  - NIST
  - North Carolina DHHS
  - US CDC
  - USDA APHIS
  - US DHS S&T
  - US DHS OHA
  - US FDA-CFSAN
  - US FDA ORS
- Government, Military
  - JPEO
  - Naval Medical Research Center
  - USAMRIID
  - US Army Edgewood Chemical Biological Center
  - US DOD Critical Reagents Program
  - US DOD Defense Threat Reduction Agency
  - US DoD Navy
  - US DoD Dugway Proving Ground

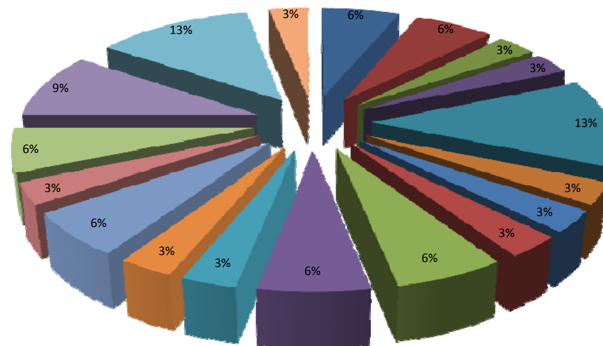
Registrants by Broad Perspectives



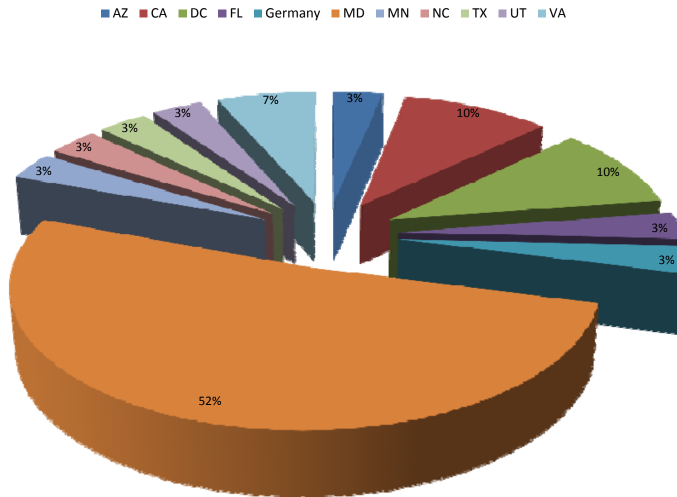
Registrants by Specific Perspectives



Registrants by Broad and Specific Perspectives

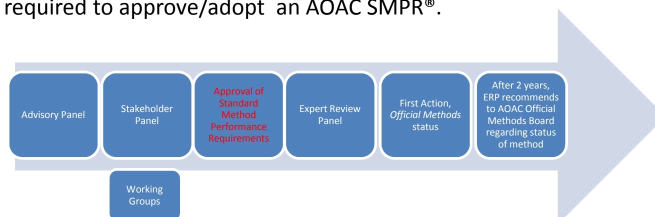


Registrants by Region - In/Out of USA



Approving AOAC Standards

- Working Group Chair or designee will present on the draft standard method performance requirements including reconciled comments received on behalf of the working group and moves for SPADA to adopt the SMPR® as presented
- SPADA chair will entertain deliberation on the draft standard
- After due deliberation, SPADA chair will call for a vote
- Voting members will be able to vote in favor of the motion, against the motion, or abstain from voting
- 2/3 vote in favor required to approve/adopt an AOAC SMPR®.



Documentation and Communication

- AOAC carefully documents the actions of Stakeholder Panel and the Working Groups
- AOAC will prepare summaries of the meetings
  - Communicate summaries to the stakeholders
  - Publish summaries in the *Referee* section of AOAC's *Inside Laboratory Management*
- AOAC publishes its voluntary consensus standards and *Official Methods*
  - *Official Methods of Analysis of AOAC INTERNATIONAL*
  - *Journal of AOAC INTERNATIONAL*
- AOAC publishes the status of standards in the Referee section of AOAC's *Inside Laboratory Management*

Roles and Responsibilities

- Stakeholder Panel
  - Establish working groups to develop standards
  - Comment, deliberate, and establish voluntary consensus standards
- Stakeholder Panel Working Groups
  - Develop draft standard method performance requirements
  - Reconcile comments
  - Present draft standard to stakeholders
- Official Methods Board
  - Vet and approve stakeholder panel chair and representative voting members
  - Assign representative to serve as a resource to stakeholder panel
- AOAC Staff
  - Coordinate stakeholder panel, working groups, and facilitate their meetings.
  - Document actions/decisions of working groups and stakeholder panel
  - Post SMPRs and collect comments for draft SMPRs

Contact Information

Contact AOAC Staff:

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Web: www.aoac.org

- **E. James Bradford**, Executive Director & CEO, jbradford@aoac.org, ext. 102
- **Krystyna McIver**, Executive for Scientific Engagement and Communication, kmciver@aoac.org, ext. 111
- **Scott Coates**, Chief Scientific Officer, scoates@aoac.org, ext. 137
- **Deborah McKenzie**, Sr. Director – Standards Development and AOAC Research Institute, dmckenzie@aoac.org, ext. 157

Environmental Factors For Validating Biological Threat Agent Detection Assays

Scott Coates, AOAC CSO

September 1, 2015

AOAC INTERNATIONAL, 2275 Research Blvd., Rockville, Maryland, 20850

Background

- First version appeared in 2004
  - for hand held immunoassays.
- Second version first appeared in 2007
  - for PCR assays.
  - to demonstrate robustness in the face of external contaminants that might inhibit the PCR chain reaction.
  - to demonstrate specificity in the field in which a practically endless variety of DNA is found.

Background

- Created a Working Group on Environmental Factors led by Dr. Steven Morse with CDC assigned to consider what DNAs should be tested
- *Environmental Factors For Validating Biological Threat Agent Detection Assays*
  - Part 1: Environmental Matrix Samples - Aerosol Environmental Matrices.
  - Part 2: Environmental Panel Organisms

Part 1: Environmental Matrix Samples

Method developers shall obtain environmental matrix samples that are representative and consistent with the collection method that is anticipated to ultimately be used in the field.

Part 2: Environmental Panel Organisms

Environmental Panel with:

- 46 bacteria & viruses
- 14 fungi
- 32 eukaryotic DNA
- 6 biological insecticides
- 26 powders and chemicals

DUSA Project

- Apparent that *Environmental Factors For Validating Biological Threat Agent Detection Assays* needed to be updated and DoD applications needed to be considered.
- *Ad hoc* Joint Working Group on Environmental factors consisting of all three current working groups formed.
  - Met by conference call twice in June 2015 to discuss and refine the environmental background panels for all three SMPRs.

Ad hoc Joint Working Group Results

- Part 1 was revised to clarify, but remained essentially the same.
- Part 2 essentially the same but updated to:
  - remove near neighbors.
  - remove unavailable DNA.
  - make adjustment specific to specific targets.
- Part 3 for Potential Interferents added.

Part 3 : Potential Interferants Study

- Supplements the Environmental Factors Study.
- Applicable to all biological threat agent detection assays for Department of Defense applications.
- Interferants shall be spiked at a final test concentration of 1 µg/ml directly into the sample collection buffer. Interferants may be pooled.

Part 3 : Potential Interferants Study

- List of potential interferants that are likely to be encountered in various Department of Defense applications:
  - working group discussion .
  - Joint Biological Point Detection System Test and Evaluation Master Plan (TEMP)
  - Joint Concept Test Plan for Whole System Live Agent Testing.
  - Joint Biological Tactical Detection System TEMP.
  - Generation Diagnostic System TEMP.
- One additional Potential Intereferent was identified after the *Ad hoc* working group concluded its work: “sand”.

Organization of Potential Interferents Table

- Grouped.
- Additional information provided.
- Potential Theater of Operations.

Organization of Potential Interferents Table

COMPOUNDS		POTENTIAL THEATERS OF OPERATION
GROUP 1: PETROLEUM-BASED	JP-8	AIRFIELD
	JP-5	NAVAL
	DIESEL/GASOLINE MIXTURE	GROUND
	FOG OIL (STANDARD GRADE FUEL NUMBER 2)	NAVAL, GROUND
	BURNING RUBBER	GROUND, AIRFIELD

Today's Objective

- Review and confirm revisions to **Environmental Factors For Validating Biological Threat Agent Detection Assays.**
  - Add "sand" to group 4: environmental?

AOAC INTERNATIONAL STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Co-Chairs: Linda Beck, NSWC and James Samuel, Texas A&M
Chair, SPADA Q-Fever Working Group
September 1, 2015

AOAC INTERNATIONAL, 2275 Research Blvd., Rockville, Maryland, 20850

Fitness for Purpose

"Detection of C. burnetii by PCR in liquid samples. Field deployable PCR assay would be desirable."

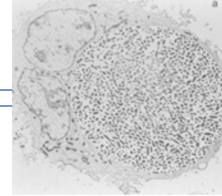
Q-Fever
Working Group Members

Linda Beck, Naval Surface Warfare Center (Co-Chair)
James Samuel, Texas A&M (Co-Chair)
Jessica Appler, HHS BARDA
Jeff Ballin, ECBC
Ron Busher, JRO-CBRND
Ryan Cahall, Censeo Insight
Matthew Davenport, DHS
Christina Egan, NYSDOH
Joan Gebhardt, NMRC
Ted Hadfield, Hadeco, LLC.
Martha Hale, USAMRIID
Kia Hopkins, ECBC
Cecilia Kato, CDC
Alexander Kayatani, PFPA
Karen Kesterson, PFPA
Katalin Kiss, ATCC
John Lednicky, University of Florida
Pejman Naraghi-Arani, InSilixa
Sean O'Brien, DoD DUSA T&E
Kate Ong, JEPO
Roberto Rebeil, ECBC
Kristian Roth, FDA
Mark Scheckelhoff, DHS/OHA
Emily Yost, ATEC

Q-Fever Working Group
Work to Date

- 2 In Person Meetings
- 3 teleconferences (March 2015 – May 2015)
- 1 SMPR Drafted
- Public comment period (June 30, 2015 – July 31, 2015)
- SMPRs made ready for SPADA review and approval

Background



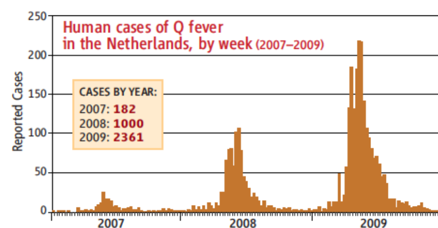
Baca *et al.* 1984

- Legionellales, obligate intracellular parasite
- Gram negative with LPS: Phase I and Phase II
- Metabolically active axenically, esp. at lower pH
- Life cycle: LCV and SCV
- Acute (>50% seroconvert-asymptomatic) and chronic infectious disease
- Broad zoonotic reservoir; high seropositivity rate
- Replicates in a "remodeled" phagolysosome-like vacoule
- Genomic predictions * : ~2150 ORFs
  - Complete TCA, various aa auxotrophs
  - Large group of transporters
  - Proteomic skew to high pI
  - Complete Type 4 secretion element
  - >200 genes with single/point mutation "pseudogene"

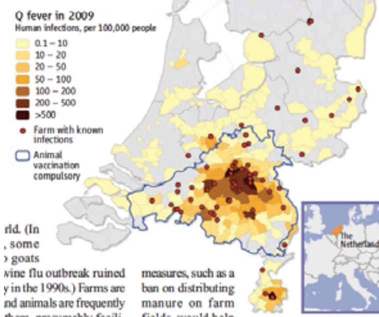
MEDICINE
TEXAS A&M HEALTH SCIENCE CENTER

*Seshadri *et al.* PNAS 2003

Background



Rising tide. The number of human Q-fever cases exploded in the past 3 years, and the disease, originally concentrated in the south, spread north and east.



Hundreds of Q fever cases reported during Operation Iraqi Freedom and Afghanistan war

MEDICINE
TEXAS A&M HEALTH SCIENCE CENTER

*15 JANUARY 2010 VOL 327 SCIENCE

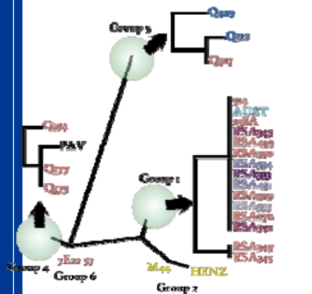
Background

Comparative genomics: Pathotype model?

Group(*)	Plasmid type(&)	Isolate(+)	Phase($)	Original source	Disease	Passage([])
I	QpH1	Nine Mile RSA493	I	Montana tick, 1935	-	307GP/1TC/1EP
		Nine Mile RSA439(>)	II	Montana tick, 1935	-	90EP/1TC/1EP
		Nine Mile RSA514(>)	I/II#	Montana tick, 1935	-	4 EP/306GP/3EP
		Nine Mile RSA285-A(>)	I/II#	Montana tick, 1935	-	4 EP/306GP/3EP
		Dyer RSA 345	II	USA, human blood, 1938	Acute	81EP
		American Q Dyer(>)	I	USA, human blood, 1938	Acute	75EP/1GP
		Australia QD RSA425	II	Australia, human blood, ~1939	Acute	177EP
		Turkey RSA333	II	Turkey, human blood, 1948	Acute	31EP
		African RSA334(>)	I	Central Africa, human blood, 1949	Acute, Congolese Red Fever	3HP/4EP
		Giround RSA431(>)	I	Central Africa, human blood, 1949	Acute, Congolese Red Fever	2 GP/2EP
		El Tayeb RSA342	I	Egypt, tick, 1967	-	4GP/2EP
		Panama RSA335	I	Panama, chiggers, 1961	-	4EP
		California 33 RSA329	I	California, cow's milk, 1947	Persistent	6EP
		California 16 RSA350	II	California, cow's milk, 1947	Persistent	38EP
		Ohio 314 RSA270	I	Ohio, cow's milk,1956	Persistent	4EP
		Ohio 314 RSA338	II	Ohio, cow's milk,1956	Persistent	42EP
II	QpH1	M44 RSA459	II	Italo-Greek, 'Grita', ~1945	Acute	?/2EP
		M44 Q141(>)	II	Italo-Greek, 'Grita', ~1945	Acute	?/1GP/2EP
		Henzerling RSA331	II	Italy, human blood,1945	Acute	36EP
III	QpH1	Idaho goat Q195	I	Idaho goat, 1981	Abortion	2EP
		Idaho goat	I	Idaho goat placenta, 1975	Abortion	5EP
		Koka	I	Ethiopia, tick, 1963	-	1GP/6EP
IV	QpRS	MSU Goat Q177	I	Montana, goat cotyledon, 1980	Abortion	GP/2EP
		Canada Goat Q218	I	Ontario, Canada, goat spleen,1981	Abortion	1GP/1EP
		Idaho Sheep 80-1	I	Idaho sheep liver,1980	Abortion	1GP/4EP
		K Q154	I	Oregon, human heart valve,1976	Endocarditis	HV/2EP
		P Q173	I	California, human heart valve, 1979	Endocarditis	HV/2EP
		F Q228	I	Washington, human heart valve, 1982	Endocarditis	HV/3EP
		H WSU101	I	California, human heart valve, 1986	Endocarditis	HV/2EP
V	NP	L Q216	I	Nova Scotia, human heart valve, 1981	Endocarditis	HV/2EP
		G Q212	I	Nova Scotia, human heart valve, 1981	Endocarditis	HV/2EP
		S Q217	I	Montana, human liver biopsy, 1981	Hepatitis	BX/2EP
		Ko Q229	I	Nova Scotia, human heart valve, 1982	Endocarditis	HV/2EP
VI	QpDG	Dugway 7E22-57	I	Utah, rodents, 1958	-	3EP
		Dugway 7E9-12	I	Utah, rodents, 1958	-	3EP

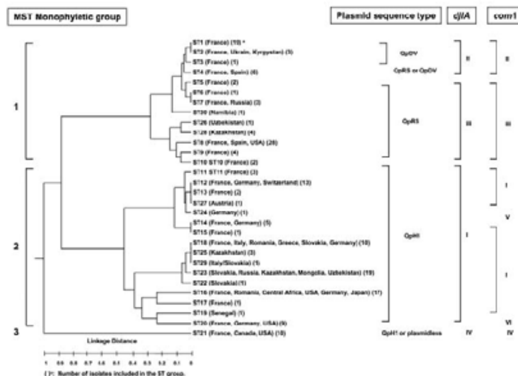
Samuel et al. 1985

C. burnetii phylogenetic organization



SNP and VNTR based trees for 25 worldwide isolates of *Coxiella burnetii.* Geographical distribution shown below.

Pearson, Keim et al. ASM2005



Glazunova *et al.* EID 2005

SMPR Key Points

Intended Use:

Laboratory or field use by Department of Defense trained operators.

Applicability:

Specific detection of *Coxiella burnetii* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

SMPR Key Points

AMDL

2,000 genomic equivalents / mL of *Coxiella burnetii* target DNA in the candidate method sample collection buffer.

Probability of Detection at AMDL within sample collection buffer ≥ 0.95

Time-to-results: Four hours.

SMPR Key Points

INCLUSIVITY PANEL

PHYLOGENETIC GROUP	ISOLATE (EXAMPLE)
GROUP 1	NINE MILE RSA493 NINE MILE RSA439
GROUP 2	HENZERLING
GROUP 3	IDAHO GOAT
GROUP 4	K
GROUP 5	G
GROUP 6	DUGWAY

SMPR Key Points

EXCLUSIVITY PANEL

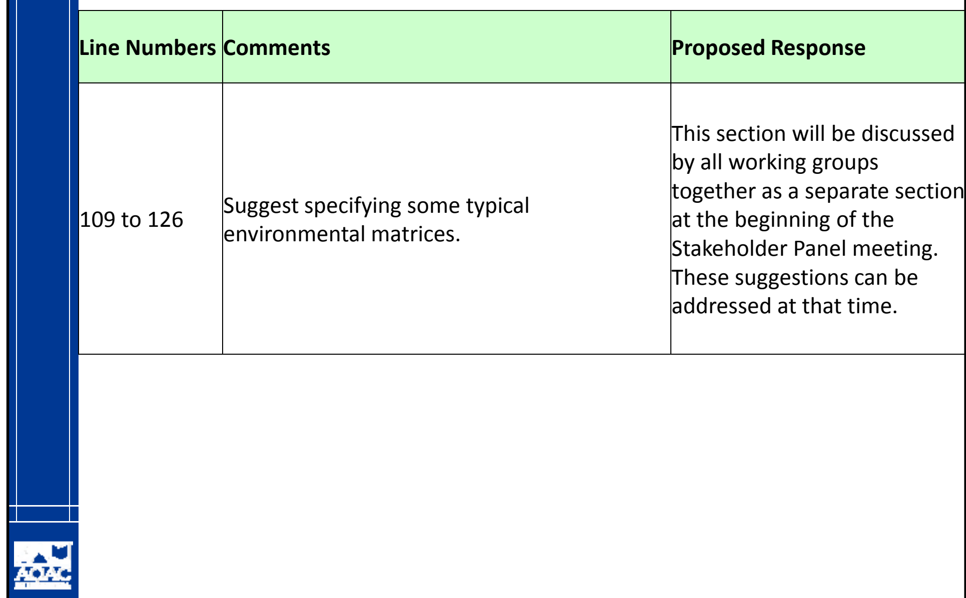
Species	Strain
Legionella pneumophila	Philadelphia 1
Legionella pneumophila	Wadsworth 1
Legionella pneumophilia	Sg6
Legionella longbeachae	
Rickettsiella spp.	

Comments

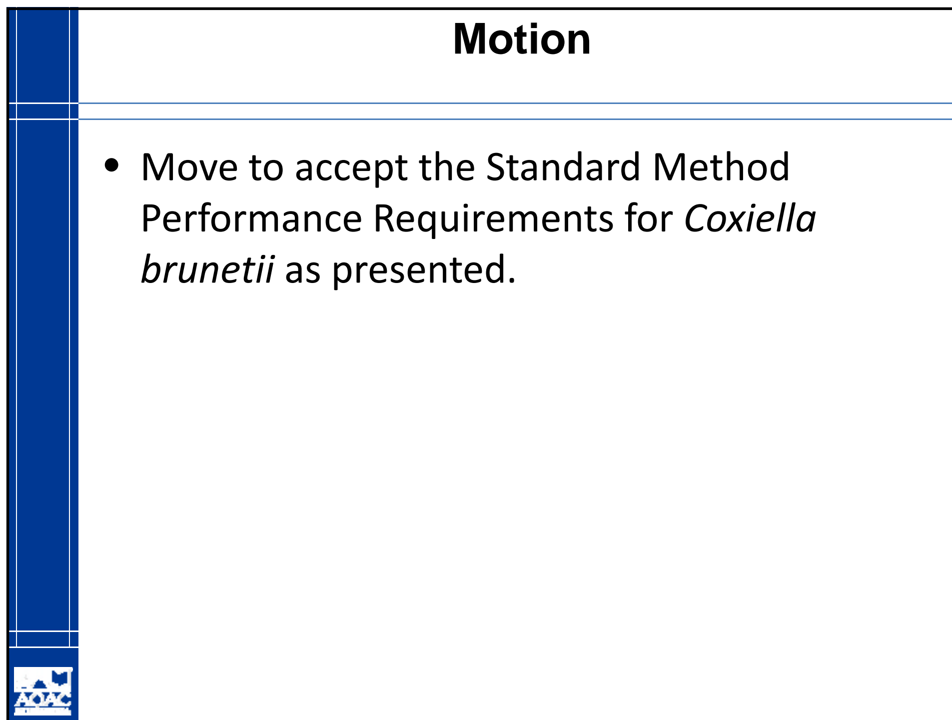
Line Numbers	Comments	Proposed Response
40-46	Are these System False Negative and Positive Rates the same as those to be determined in the presence of environmental matrix materials as indicated in the Method Performance Requirements Table (unnumbered)?	That is correct.
Section 7	Method Performance Table (unnumbered) How can a System False-Positive Rate be determined using spiked environmental matrix materials if the spike is the target organism? Seems like it should be False-Negative and the other rate without spike should be False-Positive.	The intent is that spiked and unspiked environmental matrix samples will be used to evaluate false negative and false positive rates respectively. Propose to revise Tavle IV Part 1 to clarify this requirement.

Comments

Line Numbers	Comments	Proposed Response
Table II.	Additional species suggested.	For discussion.
Table III	In the empty cells suggest putting "Investigator to choose strain(s)" if that is the intention.	Agree to insert "Investigator may choose strain(s)"



Line Numbers	Comments	Proposed Response
109 to 126	Suggest specifying some typical environmental matrices.	This section will be discussed by all working groups together as a separate section at the beginning of the Stakeholder Panel meeting. These suggestions can be addressed at that time.



Discussion?

AOAC SMPR 2015.XXX; Version 7; June 19, 2015.

Method Name: **Detection of *Coxiella burnetii***

Approved Body: *AOAC Stakeholder Panel on Agent Detection Assays*

1. Intended Use: Laboratory or field use by Department of Defense trained operators.

2. Applicability: Specific detection of *Coxiella burnetii* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

3. Analytical Technique: Molecular detection of nucleic acid.

4. Definitions:

Acceptable Minimum Detection Level (AMDL)
The predetermined minimum level of an analyte, as specified by an expert committee which must be detected by the candidate method at a specified probability of detection (POD).

Coxiella burnetii
Naturally obligate intracellular bacterial pathogen of the *Legionellales* family.

Exclusivity
Study involving pure non-target strains, which are potentially cross-reactive, that shall not be detected or enumerated by the test~~ed~~ method.

Inclusivity
Study involving pure target strains that shall be detected or enumerated by the alternative method.

Maximum Time-To- Result
Maximum time to complete an analysis starting from the test portion preparation to assay result.

Probability of Detection (POD)
The proportion of positive analytical outcomes for a qualitative method for a given matrix at a specified analyte level or concentration with a ≥ 0.95 confidence interval.

System false-negative rate
Proportion of test results that are negative contained within a population of known positives

System false-positive rate
Proportion of test results that are positive contained within a population of known negatives.

50
51 **5. System suitability tests and/or analytical quality control:**
52 The controls listed in Table I shall be embedded in assays as appropriate. Manufacturer
53 must provide written justification if controls are not embedded in the assay.
54
55 **6. Validation Guidance:** AOAC INTERNATIONAL Methods Committee Guidelines for Validation
56 of Biological Threat Agent Methods and/or Procedures (AOAC INTERNATIONAL Official
57 Methods of Analysis, 2012, Appendix I).
58
59 Inclusivity and exclusivity panel members must be characterized and documented to truly
60 be the species and strains they are purported to be.
61
62 **7. Method Performance Requirements**:

Parameter	Minimum Performance Requirement
AMDL	**2,000 genomic equivalents / mL of *Coxiella burnetii* target DNA in the candidate method sample collection buffer.**
Probability of Detection at AMDL within sample collection buffer.	≥ 0.95
Probability of Detection at AMDL in environmental matrix materials.	≥ 0.95
System False-Positive Rate using spiked environmental matrix materials.	< 0.10 %
System False-Negative Rate using environmental matrix materials.	< 0.10 %
Inclusivity	All inclusivity strains (Table II) must test positive at 2x the AMDL †
Exclusivity	All exclusivity strains (Table III and Table IV; part 2) must test negative at 10x the AMDL †

Notes:
† 100% correct analyses are expected. All aberrations are to be re-tested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures[1]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

63
64 **8. Time-to-results**: Four hours.
65
66

[1] Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, APPENDIX I; also on-line at http://www.eoma.aoac.org/app_i.pdf.

67 **TABLE I: Controls**

68

Control	Description	Implementation
Positive Control	This control is designed to demonstrate an appropriate test response. The positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to demonstrate that the assay sensitivity is performing at a previously determined level of sensitivity.	Single use per sample (or sample set) run
Negative Control	This control is designed to demonstrate that the assay itself does not produce a detection in the absence of the target organism. The purpose of this control is to rule-out causes of false positives, such as contamination in the assay or test.	Single use per sample (or sample set) run
Inhibition Control	This control is designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect the target organism.	Single use per sample run

69
70 **Table II: Inclusivity Panel**
71

Phylogenetic Group	Isolate (Example)
Group 1	Nine Mile RSA493 Nine Mile RSA439
Group 2	Henzerling
Group 3	Idaho Goat
Group 4	K
Group 5	G
Group 6	Dugway

72 73 74 75 76 77 78 79 80 81 82 83 84 85
86
87
88
89 **Table III: Exclusivity Panel (near-neighbor)**
90

Species	Strain
Legionella pneumophila	Philadelphia 1
Legionella pneumophila	Wadsworth 1
Legionella pneumophilia	Sg6
Legionella longbeachae	
Rickettsiella spp.	

91 92 93 94

95 **Table IV: Environmental Factors For Validating Biological Threat Agent Detection**
96 **Assays**
97
98 [Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]
99
100 The Environmental Factors Studies supplement the biological threat agent near-neighbor
101 exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 -
102 environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the
103 potential interferants applicable to Department of Defense applications.[2] Part 2 is not
104 applicable to techniques that do not detect nucleic acid.
105
106
107 **Part 1:**
108
109 **Environmental Matrix Samples - Aerosol Environmental Matrices**
110
111
112 Method developers shall obtain environmental matrix samples that are representative and
113 consistent with the collection method that is anticipated to ultimately be used in the field. This
114 includes considerations that may be encountered when the collection system is deployed
115 operationally such as collection medium, duration of collection, diversity of geographical areas
116 that will be sampled, climatic/environmental conditions that may be encountered and seasonal
117 changes in the regions of deployment.
118
119 Justifications for the selected conditions that were used to generate the environmental matrix
120 and limitations of the validation based on those criteria must be documented.
121
122 - Method developers shall test the environmental matrix samples for interference using
123 samples inoculated with a target biological threat agent sufficient to achieve 95%
124 probability of detection.
125 - Cross-reactivity testing will include sufficient samples and replicates to ensure each
126 environmental condition is adequately represented.
127
128

[2] Added in June 2015 for the Deprtment of Defense project.

Part 2: Environmental Panel Organisms - This list is comprised of identified organisms from the environment.

Inclusion of all environmental panel organisms is not a requirement if a method developer provides appropriate justification that the intended use of the assay permits the exclusion of specific panel organisms. Justification for exclusion of any environmental panel organism(s) must be documented and submitted.

Organisms and cell lines may be tested as isolated DNA, or as pools of isolated DNA. Isolated DNA may be combined into pools of up to 10 panel organisms, with each panel organism represented at 10 times the AMDL, where possible. The combined DNA pools are tested in the presence (at 2 times the AMDL) and absence of the target viral gene or gene fragment. If an unexpected result occurs, each of the individual environmental organisms from a failed pool must be individually re-tested at 10 times the AMDL with and without the target viral gene or gene fragment at 4,000 genome equivalents/mL in the candidate method DNA elution buffer.

- **Potential bacterial biothreat agents**
  Bacillus anthracis Ames
  Yersinia pestis Colorado-92
  Francisella tularensis subsp. *tularensis* Schu-S4
  Burkholderia pseudomallei
  Burkholderia mallei
  Brucella melitensis

- **Cultivatable bacteria identified as being present in air and soil**
  Acinetobacter lwoffii
  Agrobacterium tumefaciens
  Bacillus amyloliquefaciens
  Bacillus cohnii
  Bacillus psychrosaccharolyticus
  Bacillus benzoevorans
  Bacillus megaterium
  Bacillus horikoshii
  Bacillus macroides
  Bacteroides fragilis
  Burkholderia cepacia
  Burkholderia gladoli
  Burkholderia stabilis
  Burkholderia plantarii
  Chryseobacterium indologenes
  Clostridium sardiniense
  Clostridium perfringens
  Deinococcus radiodurans
  Delftia acidovorans
  Escherichia coli K12
  Fusobacterium nucleatum
  Lactobacillus plantarum

177 ~~*Legionella pneumophilas*~~[3]
178 *Listeria monocytogenes*
179 *Moraxella nonliquefaciens*
180 *Mycobacterium smegmatis*
181 *Neisseria lactamica*
182 *Pseudomonas aeruginosa*
183 *Rhodobacter sphaeroides*
184 *Riemerella anatipestifer*
185 *Shewanella oneidensis*
186 *Staphylococcus aureus*
187 *Stenotophomonas maltophilia*
188 *Streptococcus pneumoniae*
189 *Streptomyces coelicolor*
190 *Synechocystis*
191 *Vibrio cholerae*
192

193 • **DNA Viruses**
194 *Adenovirus* vaccine
195 *Herpes simplex virus* or *Cytomegalovirus* – whichever is available
196 *Orthopoxvirade* Vaccinia
197

198 • **Microbial eukaryotes**
199

200 Freshwater amoebae
201 *Acanthamoeba castellanii*
202 *Naegleria fowleri*
203

204 Fungi
205 *Alternaria alternata*
206 *Aspergillus fumagatis*
207 *Aureobasidium pullulans*
208 *Cladosporium cladosporioides*
209 *Cladosporium sphaerospermum*
210 *Epicoccum nigrum*
211 *Eurotium amstelodami*
212 *Mucor racemosus*
213 *Paecilomyces variotii*
214 *Penicillum chrysogenum*
215 *Wallemia sebi*
216
217

[3] Already included on the exclusivity table.

- **DNA from higher eukaryotes**

  <u>Plants</u>
  Zea mays (corn)
  Pollen from *Pinus* spp. (pine)
  ~~Cotton~~[4]

  <u>Arthropods</u>
  Aedes aegypti (ATCC /CCL-125 mosquito cell line)
  Aedes albopictus (Mosquito C6/36 cell line)
  Dermatophagoides pteronyssinus (Dust mite -commercial source)
  Xenopsylla cheopis Flea (Rocky Mountain labs)
  Drosophilia cell line
  Musca domestica (housefly) ARS, USDA, Fargo, ND
  Gypsy moth cell lines LED652Y cell line (baculovirus)– Invitrogen
  Cockroach (commercial source)
  Tick (Amblyomma)

  <u>Vertebrates</u>
  Mus musculus (ATCC/HB-123) mouse
  Rattus norvegicus (ATCC/CRL-1896) rat
  Canis familiaris(ATCC/CCL-183) dog
  Felis catus (ATCC/CRL-8727) cat
  Homo sapiens (HeLa cell line ATCC/CCL-2) human
  Gallus gallus domesticus (Chicken)
  Goat[5]

- **Biological insecticides** – Strains of *B. thuringiensis* present in commercially available insecticides have been extensively used in hoaxes and are likely to be harvested in air collectors. For these reasons, it should be used to assess the specificity of these threat assays.

  B. thuringiensis subsp*. israelensis*
  B. thuringiensis subsp*. kurstaki*
  B. thuringiensis subsp*. morrisoni*
  Serenade (Fungicide) *B. subtilis* (QST713)

  Viral agents have also been used for insect control. Two representative products are:

  Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)

  Cyd-X for coddling moths (Coddling moth granulosis virus)

[4] Removed by C. burnetii WG on 4/23/2015 due to unavailability.
[5] Specific to C. burnetii; added by CB WG on 4/23/2015.

264 **<u>Part 3: Potential Interferants Study</u>**

265

266 The Potential Interferants Study supplements the Environmental Factors Study, and is applicable
267 to all biological threat agent detection assays for Department of Defense applications. Table V
268 provides a list of potential interferants that are likely to be encountered in various Department
269 of Defense applications.

270

271 Method developers and evaluators shall determine the most appropriate potential interferants
272 for their application. Interferants shall be spiked at a final test concentration of 1 µg/ml directly
273 into the sample collection buffer. Interferants may be pooled. Sample collection buffers spiked
274 with potential interferants shall by inoculated at 2 times the AMDL (or AMIL) with one of the
275 target biological threat agents.

276

277 Spiked / inoculated sample collection buffers shall be tested using the procedure specified by
278 the candidate method.

279

280 It is expected that all samples are correctly identified as positive. If using pooled samples of
281 potential interferants, and a negative result occurs, then the pooled potential interferants shall
282 be tested separately at the 2 times the AMDL (or AMIL) with one of the target biological threat
283 agents.

284

285

286 Table V: Potential Interferants

Compounds		Potential Theaters of Operation
group 1: petroleum-based	JP-8[1]	airfield
	JP-5[2]	naval
	diesel/gasoline mixture	ground
	fog oil (standard grade fuel number 2)	naval, ground
	burning rubber[3]	ground, airfield
group 2: exhaust	gasoline exhaust	ground
	jet exhaust	naval, airfield
	diesel exhaust	ground
group 3: obscurants	terephthalic acid[4]	ground
	zinc chloride smoke[5]	ground
	solvent yellow 33[6]	ground
group 4: environmental	burning vegetation	ground, airfield
	road dust	ground
	sea water (sea spray)	naval
group 5: chemicals	brake fluid[7]	all
	brake dust[8]	ground
	cleaning solvent, *MIL-L-63460*[9]	all
	explosive residues a) high explosives[10] b) artillery propellant[11]	all

287
288 Table 4 is offered for guidance and there are no mandatory minimum requirements for the
289 number of potential interferants to be tested.

290
291
292
293

294

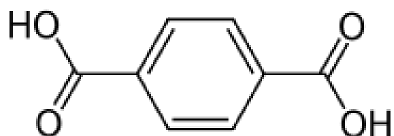
---

1 **JP-8**. Airforce formulation jet fuel.

2 **JP-5**. A yellow kerosene-based jet fuel with a lower flash point developed for use in aircraft stationed aboard aircraft carriers, where the risk from fire is particularly great. JP-5 is a complex mixture of hydrocarbons, containing alkanes, naphthenes, and aromatic hydrocarbons.

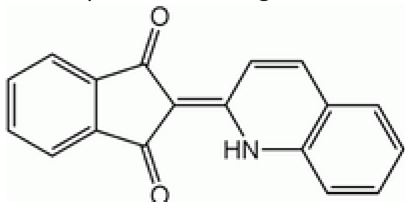
3 **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead; cadmium.

4 **Terephthalic acid.** Used in the AN/M83 hand grenade currently used by US military.



5 **Zinc chloride smoke**. Also known as “zinc chloride smoke” and “HC smoke”. Was used in the M8 grenade and still used in 155mm artillery shells. HC smoke is composed of 45% hexachloroethane, 45% zinc oxide, and 10% aluminum.

6 **Solvent yellow 33** [IUPAC name: 2-(2-quinolyl)-1,3-indandione] is a new formulation being develop for the M18 grenade.



7 **Brake fluid**. DOT 4 is the most common brake fluid, primarily composed of glycol and borate esters. DOT 5 is silicone-based brake fluid. The main difference is that DOT 4 is hydroscopic whereas DOT 5 is hydrophobic. DOT 5 is often used in military vehicles because it is more stable over time requires less maintenance

8 **Brake dust**. Fe particles caused by abrasion of the cast iron brake rotor by the pad and secondly fibers from the semi metallic elements of the brake pad. The remainder of the dust residue is carbon content within the brake pad.

9 **MIL-L-63460**, "Military Specification, Lubricant, Cleaner and Preservative for Weapons and Weapons Systems”; trade name **“**Break-Free CLP**”.** Hyperlink: Midway USA.

[10] **High explosives**. The M795 155mm projectile is the US Army / Marine Corp's current standard projectile containing 10.8 kg of TNT. The M795 projectile replaced the M107 projectile that contained Composition B which is a 60/40 mixture of RDX/TNT. RDX is cyclotrimethylene trinitramine. Suggestion: test RDX/TNT together.

[11] **Artillery propellant**. Modern gun propellants are divided into three classes: single-base propellants which are mainly or entirely nitrocellulose based, double-base propellants composed of a combination of nitrocellulose and nitroglycerin, and triple base composed of a combination of nitrocellulose and nitroglycerin and nitroguanidine. Suggestion: test total nitrocellulose/ nitroglycerin nitroguanidine together.

The near neighbors for Coxiella burnetii are shown below. They come from the tree of life, a phylogenetic representation of bacterial isolates ranked on 16S ribosome sequences.

Legionella jordanis (ATCC 33623) [590]

Legionella jamestowniensis (ATCC 35298) [591]
<----- Legionella jamestowniensis
Legionella jordanis (NCTC 11533) [589]

Fluoribacter gormanii str. LS-13 (ATCC 33342 (T)) [617]
<----- Legionella dumoffii
Fluoribacter dumoffii str. NY-23 (ATCC 33279) <Flu.dumof2> [621]jm m
<----- Legionella cherrii
Legionella cherrii (ATCC 35252) [623]
<----- Legionella gormanii
Legionella wadsworthii (ATCC 33877) [622]
<----- Legionella wadsworthii
<----- Legionella parisiensis
Legionella anisa (ATCC 35292) [616]
<----- Legionella anisa
Fluoribacter bozemanae str. WIGA <Flu.bozema> [624]
Fluoribacter bozemanae str. MI-15 [625]
<----- Legionella bozemanii (NCTC 11369)
<----- Legionella bozemanii (NCTC 11975)
<----- Legionella gratiana
Legionella longbeachae str. Long-Beach-4 [628]
Legionella sainthelensi (ATCC 35248) [626]
<----- Legionella sainthelensi
<----- Legionella cincinnatiensis
Legionella cincinnatiensis (ATCC 43753) [627]
<----- Legionella santicrucis
<----- Legionella steigerwaltii
Legionella steigerwaltii (ATCC 35302) [613]
<----- Legionella tucsonensis
Legionella sp. str. LLAP-3 [615]
Legionella lytica (PCM 2298) [614]
<----- Legionella worsleiensis
<----- Legionella quateirensis
<----- Legionella shakespearei
<----- Legionella moravica
Legionella pneumophila subsp. pneumophila <Leg.pneumo> [606]
Legionella pneumophila str. Chicago 2 sgp6 [605]
Legionella pneumophila subsp. fraseri str. Dallas-1E' [607]
Legionella pneumophila subsp. pneumophila <Leg.pneP12> [608]
Legionella pneumophila subsp. pneumophila str. Chicago-2 [604]
Legionella pneumophila subsp. pneumophila str. Knoxville-1 [609]
Legionella pneumophila subsp. pneumophila <Leg.pneuP1> [612]
Legionella pneumophila subsp. fraseri str. Dallas-1E [610]
Legionella pneumophila subsp. fraseri str. Los Angeles 1 [611]
<----- Legionella londiniensis
<----- Legionella lansingensis
<----- Legionella fairfieldensis
<----- Legionella geestiana
<----- Legionella adelaidensis (NCTC 12735)
<----- Legionella birminghamensis (NCTC 12437)
<----- Legionella quinlivanii
Legionella oakridgensis (ATCC 33761) [603]
<----- Legionella nautarum
Legionella israelensis (ATCC 43119) [602]
<----- Legionella israelensis
Legionella feeleii str. 691-WI-H [595]

Legionella feeleii str. WO-44C [594]
<----- Legionella feeleii
<----- Legionella maceachernii
Tatlockia maceachernii str. PX-1-G2-E2 <Tat.maceac> [597]
Tatlockia maceachernii str. PX-1-G2-E2 <Tat.maceE2> [596]
Tatlockia micdadei str. TATLOCK <Tat.micdad> [601]
Tatlockia micdadei str. VAMC-Pgh-12 (ATCC 33346) [598]
Tatlockia micdadei str. TATLOCK <Tat.micdTA> [600]
Tatlockia micdadei str. PPA-JC [599]
Legionella spiritensis str. Mt St Helens-9 [629]
Legionella erythra str. SE-32A-C8 [631]
<----- Legionella rubrilucens
Legionella rubrilucens (ATCC 35304) [630]
Legionella brunensis [593]
<----- Legionella brunensis
Legionella hackeliae str. Lansing-2 [592]

Fluoribacter bozemanae str. WIGA <Flu.bozeGA> [618]
Fluoribacter dumoffii str. NY-23 <Flu.dumoff> [620]
Fluoribacter dumoffii str. Tex-KL (ATCC 33343) [619]

AOAC INTERNATIONAL STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Sandra Tallent, US FDA
Chair, SPADA SEB Working Group
September 1, 2015

AOAC INTERNATIONAL, 2275 Research Blvd., Rockville, Maryland, 20850

Fitness for Purpose

"Detection of sub-nanogram levels of SEA-SEC in liquid samples, preferably using field deployable assay in less than four hours."

SEB
Working Group Members

Sandra Tallent, FDA (Chair)
Jessica Appler, HHS BARDA
Patrice Arbault, BioAdvantage Consulting
Jeff Ballin, ECBC
Linda Beck, Naval Surface Warfare Center
Don Bushner, JRO-CBRND
Ryan Cahall, Censeo Insight
Matthew Davenport, DHS
Martha Hale, USAMRIID
Kia Hopkins, ECBC
Malcolm Johns, DHS
Liz Kerrigan, ATCC
Karen Kesterson, PFPA
Katalin Kiss, ATCC
Markus Lacorn, R-Biopharm
Metthew Lesho, Luminex
Stephen Morse, CDC
Sean O'Brien, DoD DUSA T-E
Kate Ong, JPEO-CBD
Tom Phillips, MD Department of Agriculture
Roberto Rebeil, ECBC
Reinhardt Witzenberger, R-Biopharm
Emily Yost, ATEC

SEB Working Group
Work to Date

- 2 In Person Meetings
- 3 teleconferences (March 2015 – May 2015)
- 1 SMPR Drafted
- Public comment period (June 30, 2015 – July 31, 2015)
- SMPRs made ready for SPADA review and approval

Background

- Staphylococcal enterotoxins are pyrogenic proteins that lead to toxic shock and respiratory distress due to inhalation.
- Symptoms 90 minutes-24 hours post aerosol exposure fever, headache, muscle aches, pulmonary and GI symptoms.
- Biothreat agent due to universal availability and lack of discernment between other syndromes or diseases.
- Initial aerosol exposure studies performed with SEB only, SEA & SEC shown later to have similar effects.

SMPR Key Points

- Field use by DOD trained operators
- Analytical protein detection method in liquid samples of SEA, SEB, SEC_1, SEC_2,& SEC_3.
- Acceptable minimum detection level (AMDL) of 0.25ng/mL with a maximum time to result of four hours.
- Probability of detection (POD) of the analyte shall be > 0.95.

Comments Submitted (if any)

SMPR	Line Numbers	Comments	Proposed Response
SEA-C	52 - 53	Change 'Samples..' to 'Sample containers..' and 'randomly mixed together..' to 'randomly associated..' because as is it stands it reads like samples are to be mixed.	Agreed.
	58	If non-target related SE toxin(s) were tested positive below 10 x the AMDL this does not strictly exclude a method to meet the requirements of this SMPR. The method developer may communicate this result from the selectivity study to the method user. Iinsert second footnote for AMDL of non-target compounds	For discussion. The current footnote does stae that "Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users."
		There is no table III. change table IV to table III and table V to table IV; change text accordingly	Agreed.
	113	State reason for non-applicability even though it may be obvious or would be if the types of detection were indicated at the documents start near l. 3, e.g. immunochemical, mass spectroscopy?	Agreed.
	138	Table V is referred to as Table 4. Correct if necessary.	Agreed.
	Table II.	State current commercially available sources, recognizing that sources and availabilities may change in the future. Otherwise the impression is given that none are available currently.	Propose to remove the "Commercial availability" column.

Motion

- Move to accept the Standard Method Performance Requirements for *Staphylococcal Enterotoxin* A-C as presented.

Discussion?

AOAC SMPR 2015.XXX; Version 9; June 19, 2015

Method Name: **Detection of Staphylococcal Enterotoxin A-C**

Approval Body: *AOAC Stakeholder Panel on Agent Detection Assays*

1. **Intended Use**: Laboratory or field use by trained operators within the Department of Defense.

2. **Applicability**: Detection of SEA, SEB and SEC1, SEC2, SEC3 in liquid samples. The preferential method would be a field-deployable assay or assays.

3. **Analytical Technique**: Any analytical method that can detect the protein and meets the requirements of this SMPR.

4. **Definitions**:

Acceptable Minimum Detection Level (AMDL)
The predetermined minimum level of an analyte, as specified by an expert committee which must be detected by the candidate method at a specified probability of detection (POD).

Maximum Time-To-Assay Result
Maximum time to complete an analysis starting with recovery of toxins from the collection matrix s and ending with the assay result.

Probability of Detection (POD)
The proportion of positive analytical outcomes for a qualitative method for a given matrix at a specified analyte level or concentration with a ≥ 0.95 confidence interval.

SEA-C
Staphylococcus enterotoxin is a pyrogenic protein implicated in toxic shock and respiratory disorders and superantigenic response due to inhalation Staphylococcal enterotoxin A (SEA), Staphylococcal enterotoxin B (SEB), and Staphylococcal enterotoxin C (SEC) are a part of a set of exotoxins produced by *S. aureus* which comprise about 23 serologically distinct proteins that include: SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, SEH, SEG, SEI, SEJ, SEK and SEU.

Selectivity Study
A study designed to demonstrate a candidate method's ability to detect SEA, SEB, and SEC; and at the same time, demonstrate that a candidate method does not detect nontarget compounds and nontarget related toxins

5. **System suitability tests and/or analytical quality control:**
The controls listed in Table I shall be made available in assays as appropriate. Manufacturer or method developer must provide written justification if controls are not available in the assay.

6. **Validation Guidance:** AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures (AOAC INTERNATIONAL Official Methods of Analysis, 2012, Appendix I).

50
51 Equal numbers SEA, SEB and SEC 1, SEC 2, SEC 3 samples must be represented in the
52 selectivity study. Use pristine buffer solution. Samples with target and nontarget
53 compounds must be: 1) blind coded; 2) randomly mixed together; 3) evaluated at the same
54 time, and 4) masked, so that the sample identity remains unknown to the analysts. Batches
55 are permissible provided 6.1, 6.2, 6.3, and 6.4 are followed.
56
57 **7. Method Performance Requirements**
58

Parameter	**Minimum Performance Requirement**
AMDL	0.25 ng /mL recovered toxin in liquid
Selectivity Study	POD ≥ 0.95 at AMDL for SEA, SEB, & SEC 1, SEC 2, SEC 3.
	All nontarget compounds (Table II and Table III) must test negative at 10x the AMDL†
System False-Negative Rate using spiked aerosol environmental matrix at the AMDL	≤ 5% (Table III Part 1)
System False-Positive Rate using aerosol environmental matrix at the AMDL	≤ 5% (Table III; Part 1)
Notes: † 100% correct analyses are expected. All aberrations are to be re-tested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures[1]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.	

59
60 **8. Maximum Time for Assay Results:** Four hours
61
62
63
64
65
66

[1] Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, APPENDIX I; also on-line at http://www.eoma.aoac.org/app_i.pdf.

67 **Table I: Controls**
68
69
70
71

Control	Description	Implementation
Positive Control	This control is designed to demonstrate an appropriate test response. The positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to demonstrate that the assay sensitivity is performing at a previously determined level of sensitivity.	Single use per sample (or sample set) run
Negative Control	This control is designed to demonstrate that the assay itself does not produce detection in the absence of the target organism. The purpose of this control is to rule-out causes of false positives, such as contamination in the assay or test.	Single use per sample (or sample set) run

72 **Table II: Nontarget Toxins**

73

Toxin	Commercial availability
SED	
SEE	
SEH	
SEI	
SEJ	
SEK	

74

75 **Table IV: Environmental Factors For Validating Biological Threat Agent Detection**
76 **Assays**
77
78 [Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]
79
80 The Environmental Factors Studies supplement the biological threat agent near-neighbor
81 exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 -
82 environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the
83 potential interferants applicable to Department of Defense applications.[2] Part 2 is not
84 applicable to techniques that do not detect nucleic acid; and therefore not included in this
85 SMPR.
86
87
88 **Part 1:**
89
90 **Environmental Matrix Samples - Aerosol Environmental Matrices**
91
92
93 Method developers shall obtain environmental matrix samples that are representative and
94 consistent with the collection method that is anticipated to ultimately be used in the field. This
95 includes considerations that may be encountered when the collection system is deployed
96 operationally such as collection medium, duration of collection, diversity of geographical areas
97 that will be sampled, climatic/environmental conditions that may be encountered and seasonal
98 changes in the regions of deployment.
99
100 Justifications for the selected conditions that were used to generate the environmental matrix
101 and limitations of the validation based on those criteria must be documented.
102
103 - Method developers shall test the environmental matrix samples for interference using
104 samples inoculated with a target biological threat agent sufficient to achieve 95%
105 probability of detection.
106 - Cross-reactivity testing will include sufficient samples and replicates to ensure each
107 environmental condition is adequately represented.
108
109

[2] Added in June 2015 for the Deprtment of Defense project.

110
111 **Part 2: Environmental Panel Organisms -**
112
113 **Not applicable to this SMPR and therefore removed.**

114 **Part 3: Potential Interferants Study**
115
116 The Potential Interferants Study supplements the Environmental Factors Study, and is applicable
117 to all biological threat agent detection assays for Department of Defense applications. Table V
118 provides a list of potential interferants that are likely to be encountered in various Department
119 of Defense applications.
120
121 Method developers and evaluators shall determine the most appropriate potential interferants
122 for their application. Interferants shall be spiked at a final test concentration of 1 µg/ml directly
123 into the sample collection buffer. Interferants may be pooled. Sample collection buffers spiked
124 with potential interferants shall by inoculated at 2 times the AMDL (or AMIL) with one of the
125 target biological threat agents.
126
127 Spiked / inoculated sample collection buffers shall be tested using the procedure specified by
128 the candidate method.
129
130 It is expected that all samples are correctly identified as positive. If using pooled samples of
131 potential interferants, and a negative result occurs, then the pooled potential interferants shall
132 be tested <u>separately</u> at the 2 times the AMDL (or AMIL) with one of the target biological threat
133 agents.
134
135

136 Table V: Potential Interferants

Compounds		Potential Theaters of Operation
group 1: petroleum-based	JP-8[1]	airfield
	JP-5[2]	naval
	diesel/gasoline mixture	ground
	fog oil (standard grade fuel number 2)	naval, ground
	burning rubber[3]	ground, airfield
group 2: exhaust	gasoline exhaust	ground
	jet exhaust	naval, airfield
	diesel exhaust	ground
group 3: obscurants	terephthalic acid[4]	ground
	zinc chloride smoke[5]	ground
	solvent yellow 33[6]	ground
group 4: environmental	burning vegetation	ground, airfield
	road dust	ground
	sea water (sea spray)	naval
group 5: chemicals	brake fluid[7]	all
	brake dust[8]	ground
	cleaning solvent, *MIL-L-63460*[9]	all
	explosive residues a) high explosives[10] b) artillery propellant[11]	all

137

138 Table 4 is offered for guidance and there are no mandatory minimum requirements for the
139 number of potential interferants to be tested.

140

141

142

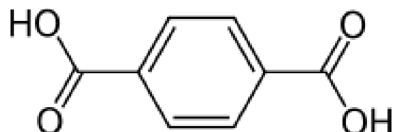
143

[1] **JP-8**. Airforce formulation jet fuel.

[2] **JP-5**. A yellow kerosene-based jet fuel with a lower flash point developed for use in aircraft stationed aboard aircraft carriers, where the risk from fire is particularly great. JP-5 is a complex mixture of hydrocarbons, containing alkanes, naphthenes, and aromatic hydrocarbons.

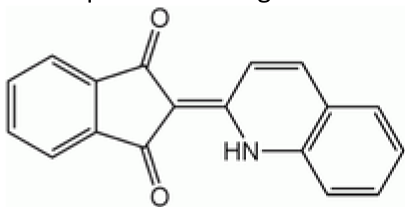
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead; cadmium.

[4] **Terephthalic acid.** Used in the AN/M83 hand grenade currently used by US military.



[5] **Zinc chloride smoke**. Also known as “zinc chloride smoke” and “HC smoke”. Was used in the M8 grenade and still used in 155mm artillery shells. HC smoke is composed of 45% hexachloroethane, 45% zinc oxide, and 10% aluminum.

[6] **Solvent yellow 33** [IUPAC name: 2-(2-quinolyl)-1,3-indandione] is a new formulation being develop for the M18 grenade.



[7] **Brake fluid**. DOT 4 is the most common brake fluid, primarily composed of glycol and borate esters. DOT 5 is silicone-based brake fluid. The main difference is that DOT 4 is hydroscopic whereas DOT 5 is hydrophobic. DOT 5 is often used in military vehicles because it is more stable over time requires less maintenance

[8] **Brake dust**. Fe particles caused by abrasion of the cast iron brake rotor by the pad and secondly fibers from the semi metallic elements of the brake pad. The remainder of the dust residue is carbon content within the brake pad.

[9] **MIL-L-63460**, "Military Specification, Lubricant, Cleaner and Preservative for Weapons and Weapons Systems”; trade name **“**Break-Free CLP**”.** Hyperlink: Midway USA.

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[10] **High explosives**. The M795 155mm projectile is the US Army / Marine Corp's current standard projectile containing 10.8 kg of TNT. The M795 projectile replaced the M107 projectile that contained Composition B which is a 60/40 mixture of RDX/TNT. RDX is cyclotrimethylene trinitramine. Suggestion: test RDX/TNT together.

[11] **Artillery propellant**. Modern gun propellants are divided into three classes: single-base propellants which are mainly or entirely nitrocellulose based, double-base propellants composed of a combination of nitrocellulose and nitroglycerin, and triple base composed of a combination of nitrocellulose and nitroglycerin and nitroguanidine. Suggestion: test total nitrocellulose/ nitroglycerin nitroguanidine together.

AOAC INTERNATIONAL STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Eileen Ostlund, USDA
Chair, SPADA VEE Working Group
September 1, 2015

AOAC INTERNATIONAL, 2275 Research Blvd., Rockville, Maryland, 20850

VEE
Working Group Members

Eileen Ostlund, USDA (Chair)
Jessica Appler, HHS BARDA
Jeff Ballin, ECBC
Linda Beck, Naval Surface Warfare Center
Don Bushner, JRO-CBRND
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Tom Phillips, MD Dept. of Agriculture
Ann Powers, CDC
Jon Rayner, SRI
Mark Scheckelhoff, DHS/OHA
Darci Smith, SRI
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Emily Yost, ATEC

Fitness for Purpose

"Identification of VEEV, and possible EEEV and WEEV RNA by assays in liquid samples. The limit of detection must be less than 100 genome copies per reaction. The preferential method would be a field-deployable assay."

[AOAC SMPR launch meeting, February, 2015]

VEE Working Group
Work to Date

- 2 In Person Meetings
- 4 teleconferences (March 2015 – May 2015)
- 1 SMPR Drafted
- Public comment period (June 30, 2015 – July 31, 2015)
- SMPRs made ready for SPADA review and approval

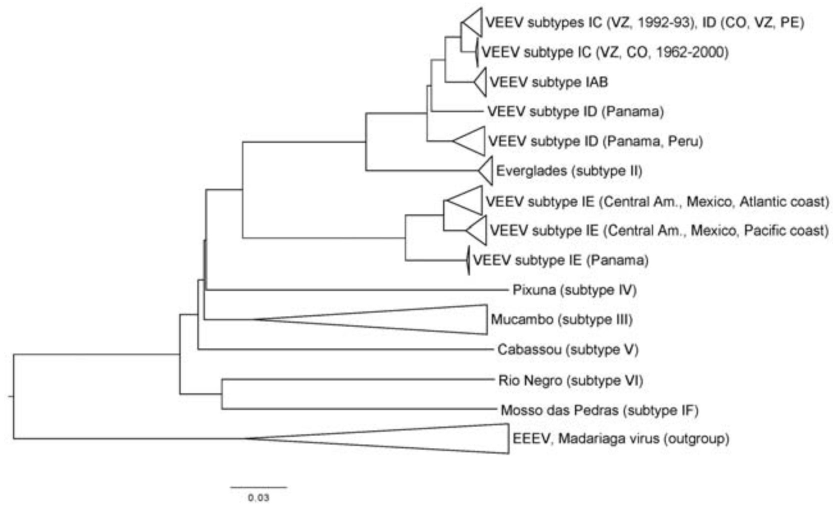
Background

- Original request from DoD emphasized VEE.
- Inclusion of EEE and WEE in same SMPR not practical.
- Limited to VEEV that are known human pathogens.
- Primary need to know which VEEV involved, therefore required identification to assist in appropriate decision-making by DoD personnel.

Fitness for Purpose

Identification of VEEV by assays in liquid samples from aerosol collectors. The acceptable minimum identification level must be less than 5,000 genome copies per ml. The assay is intended for laboratory or field use by Department of Defense trained operators. The preferential method would be a field-deployable assay.

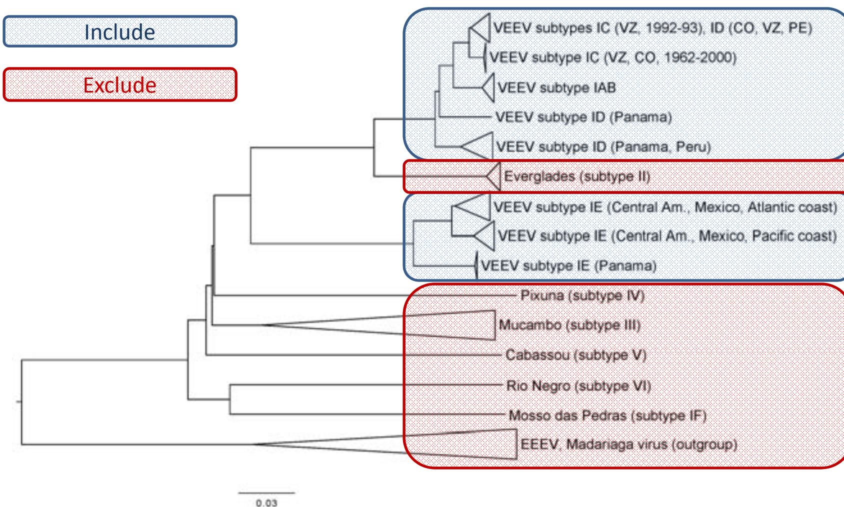
Background – VEE complex



Background – VEE complex

Include

Exclude



Phylogenetic tree provided by Scott Weaver

SMPR Key Points

- A separate SMPR is needed if identification of EEE and/or WEE is desired.
- Method defined as “molecular”; not limited to PCR.
- Minimum genome copies/ml for the AMIL have been standardized by AOAC. Reaction volumes are at the discretion of the method developer.
- Environmental matrix, environmental panel organisms and potential interferants have been standardized for SMPRS with similar intended uses.

Comments Submitted

Line Numbers	Comments	Proposed Response
17 & 53	Briefly explain why AMIL rather than the usual AMDL. The implication seems to be that any VEEV detection method may not be specific as to 1AB, 1C and 1D variants (serovars).	Propose to add additional language to the POI definition specifically stating that acceptable methods must be able to identify the individual serovars in table II.
53	State the mandatory number of matrices for system false +/- rates testing.	This was purposefully left open and is covered by the statement in table 4 part 1 that: "Justifications for the selected conditions that were used to generate the environmental matrix and limitations of the validation based on those criteria must be documented."
64	State if all the 9 inclusive strains are mandatory or not.	Propose to add language to Table III: Inclusivity Panel that: "One representative strain from each serotype is sufficient."
	The VEEV subtype ID strains listed do not represent the entire genetic diversity known. I can recommend additional strains or substitutes	For discussion.

Comments Submitted (continued)

Line Numbers	Comments	Proposed Response
70	Designing primers that include all strains of VEEV subtypes IAB, IC, ID and IE but exclude Everglades virus will be very challenging if not impossible. Consider removing Everglades virus from exclusion list.	For discussion.
71	State if all 10 near neighbors are mandatory or not.	Propose to add additional language stating that all nine viruses must be evaluated but that method developer/evaluator may choose one representative strain of Mucambo virus.
84	Environmental matrices - Aerosols: State what types to be tested? Its too vague as it stands.	This section will be discussed by all working groups together as a separate section at the beginning of the Stakeholder Panel meeting. These suggestions can be addressed at that time.
202	To avoid potential confusion with recent proposals for revisions of mosquito genera I recommend adding subgenus: Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus.	

Motion

- Move to accept the Standard Method Performance Requirements for Venezuelan Equine Encephalitis as presented.

Discussion?

AOAC SMPR 2015.XXX; Version 5, June 19, 2015

Method Name: Identification of Venezuelan Equine Encephalitis Virus (VEEV)

Approved Body: *AOAC Stakeholder Panel on Agent Detection Assays*

1. Intended Use: Laboratory or field use by Department of Defense trained operators.

2. Applicability: Identification of VEEV in liquid samples from aerosol collectors. The preferential method would be a field-deployable assay.

3. Analytical Technique: Molecular methods of detecting target-specific viral component(s).

4. Definitions:

Acceptable Minimum Identification Level (AMIL)
The predetermined minimum level of an analyte, as specified by an expert committee which must be detected and identified by the candidate method with a specified probability of identification (POI).

Exclusivity
Study involving pure non-target strains and species, which are potentially cross-reactive, that shall not be detected or identified by the test method.

Inclusivity
Study involving pure target strains or species that shall be detected and identified by the alternative method.

Maximum Time-To-Assay Result
Maximum time to complete an analysis starting from the test portion preparation to assay result.

Probability of Identification (POI)
The proportion of positive analytical outcomes for an identification method for a given matrix at a given analyte level or concentration.

***Venezuelan Equine Encephalitis(VEE) Virus* (VEEV)** VEEV encompasses several viruses all of which are within the Alphavirus genus of the *Togaviridae* family. For the purpose of this SMPR VEEV includes the human pathogenic virus variants VEEV-1AB, VEEV-1C, VEEV-1D, and VEE-1D.

5. System suitability tests and/or analytical quality control:
The controls listed in Table I shall be made available in assays as appropriate. Manufacturer must provide written justification if controls are not available with the assay.

48 **6. Validation Guidance:** AOAC INTERNATIONAL Methods Committee Guidelines for Validation
49 of Biological Threat Agent Methods and/or Procedures (AOAC INTERNATIONAL Official
50 Methods of Analysis, 2012, Appendix I).
51
52 **7. Method Performance Requirements**:
53

Parameter	Minimum Performance Requirement
AMIL	**5000 genome copies / mL**
POI at AMIL within sample collection buffer	≥ 0.95
POI at AMIL in an aerosol environmental matrix	≥ 0.95 (Table IV; part 1)
System False-Negative Rate using spiked aerosol environmental matrix	≤ 5% (Table IV; Part 1)
System False-Positive Rate using aerosol environmental matrix	≤ 5% (Table IV; Part 1)
Inclusivity panel purified DNA	All inclusivity strains (Table II) must be correctly identified at 2x the AMIL†
Exclusivity panel purified DNA	All exclusivity strains (Table III and Annex IV; part 2) must test negative at 10x the AMIL†

Notes:
† 100% correct analyses are expected. All aberrations are to be re-tested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures[1]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

54
55 **8. Maximum Time-to-Results**: Four hours.
56
57
58

- [1] Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, APPENDIX I; also on-line at http://www.eoma.aoac.org/app_i.pdf.

59 **Table I: Controls**
60

Control	Description	Implementation
Positive Control	This control is designed to demonstrate an appropriate test response. The positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to demonstrate that the assay sensitivity is performing at a previously determined level of sensitivity.	Single use per sample (or sample set) run
Negative Control	This control is designed to demonstrate that the assay itself does not produce a detection in the absence of the target organism. The purpose of this control is to rule-out causes of false positives, such as contamination in the assay or test.	Single use per sample (or sample set) run
Inhibition Control	This control is designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect the target organism.	Single use per sample run

Table II: Inclusivity Panel

VIRUS	Serotype / Variant	Representative Strain (s)	Human Illness?	
VEEV	VEE-IAB	Trinidad Donkey	Yes	
		MF-8	Yes	
	VEE-IC	ICVE93, ICVE95	Yes	
	VEE-IE	IEMX63, IEPA62	Yes	
	VEE-ID	1DPA61, 1DPE98, IDPE06	Yes	

67 **Table III: Exclusivity Panel (near-neighbor)**

68

69

70

VIRUS	Representative Strain (s)
Mosso das Pedras	78V 3531
Everglades	Fe-3-7c
Mucambo	A
	C (strain 71D-1252)
	D
Tonate	Tonate
Pixuna	Pixuna
Cabassou	Cabassou
Rio Negro	AG 80-663
EEE	PE6
WEE	CBA87

71 **These viruses are related to VEE and are in the same antigenic complex.**

72 **Table IV: Environmental Factors For Validating Biological Threat Agent Detection**
73 **Assays**
74
75 [Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]
76
77 The Environmental Factors Studies supplement the biological threat agent near-neighbor
78 exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 -
79 environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the
80 potential interferants applicable to Department of Defense applications.[2] Part 2 is not
81 applicable to techniques that do not detect nucleic acid.
82
83
84 **Part 1:**
85
86 **Environmental Matrix Samples - Aerosol Environmental Matrices**
87
88
89 Method developers shall obtain environmental matrix samples that are representative and
90 consistent with the collection method that is anticipated to ultimately be used in the field. This
91 includes considerations that may be encountered when the collection system is deployed
92 operationally such as collection medium, duration of collection, diversity of geographical areas
93 that will be sampled, climatic/environmental conditions that may be encountered and seasonal
94 changes in the regions of deployment.
95
96 Justifications for the selected conditions that were used to generate the environmental matrix
97 and limitations of the validation based on those criteria must be documented.
98

99 • Method developers shall test the environmental matrix samples for interference using
100 samples inoculated with a target biological threat agent sufficient to achieve 95%
101 probability of detection.
102 • Cross-reactivity testing will include sufficient samples and replicates to ensure each
103 environmental condition is adequately represented.
104
105

[2] Added in June 2015 for the Deprtment of Defense project.

106
107 **Part 2: Environmental Panel Organisms -** This list is comprised of identified organisms from the
108 environment.
109
110 Inclusion of all environmental panel organisms is not a requirement if a method developer provides
111 appropriate justification that the intended use of the assay permits the exclusion of specific panel
112 organisms. Justification for exclusion of any environmental panel organism(s) must be documented
113 and submitted.
114
115 Organisms and cell lines may be tested as isolated DNA, or as pools of isolated DNA. Isolated DNA
116 may be combined into pools of up to 10 panel organisms, with each panel organism represented at
117 10 times the AMDL, where possible. The combined DNA pools are tested in the presence (at 2 times
118 the AMDL) and absence of the target viral gene or gene fragment. If an unexpected result occurs,
119 each of the individual environmental organisms from a failed pool must be individually re-tested at
120 10 times the AMDL with and without the target viral gene or gene fragment at 4,000 genome
121 equivalents/mL in the candidate method DNA elution buffer.
122
123 - **Potential bacterial biothreat agents**
124 *Bacillus anthracis* Ames
125 *Yersinia pestis* Colorado-92
126 *Francisella tularensis* subsp. *tularensis* Schu-S4
127 *Burkholderia pseudomallei*
128 *Burkholderia mallei*
129 *Brucella melitensis*
130
131 - **Cultivatable bacteria identified as being present in air and soil**
132 *Acinetobacter lwoffii*
133 *Agrobacterium tumefaciens*
134 *Bacillus amyloliquefaciens*
135 *Bacillus cohnii*
136 *Bacillus psychrosaccharolyticus*
137 *Bacillus benzoevorans*
138 *Bacillus megaterium*
139 *Bacillus horikoshii*
140 *Bacillus macroides*
141 *Bacteroides fragilis*
142 *Burkholderia cepacia*
143 *Burkholderia gladoli*
144 *Burkholderia stabilis*
145 *Burkholderia plantarii*
146 *Chryseobacterium indologenes*
147 *Clostridium sardiniense*
148 *Clostridium perfringens*
149 *Deinococcus radiodurans*
150 *Delftia acidovorans*
151 *Escherichia coli* K12
152 *Fusobacterium nucleatum*
153 *Lactobacillus plantarum*

154 ~~*Legionella pneumophilas*~~[3]
155 *Listeria monocytogenes*
156 *Moraxella nonliquefaciens*
157 *Mycobacterium smegmatis*
158 *Neisseria lactamica*
159 *Pseudomonas aeruginosa*
160 *Rhodobacter sphaeroides*
161 *Riemerella anatipestifer*
162 *Shewanella oneidensis*
163 *Staphylococcus aureus*
164 *Stenotophomonas maltophilia*
165 *Streptococcus pneumoniae*
166 *Streptomyces coelicolor*
167 *Synechocystis*
168 *Vibrio cholerae*
169

170 - **DNA Viruses**

171 *Adenovirus* vaccine
172 *Herpes simplex virus* or *Cytomegalovirus* – whichever is available
173 *Orthopoxvirade* Vaccinia
174

175 - **Microbial eukaryotes**

176
177 Freshwater amoebae
178 *Acanthamoeba castellanii*
179 *Naegleria fowleri*
180
181 Fungi
182 *Alternaria alternata*
183 *Aspergillus fumagatis*
184 *Aureobasidium pullulans*
185 *Cladosporium cladosporioides*
186 *Cladosporium sphaerospermum*
187 *Epicoccum nigrum*
188 *Eurotium amstelodami*
189 *Mucor racemosus*
190 *Paecilomyces variotii*
191 *Penicillum chrysogenum*
192 *Wallemia sebi*
193
194

[3] Already included on the exclusivity table.

195 • **DNA from higher eukaryotes**
196 Plants
197 *Zea mays* (corn)
198 Pollen from *Pinus* spp. (pine)
199 ~~Cotton~~[4]
200
201 Arthropods
202 *Aedes aegypti* (ATCC /CCL-125 mosquito cell line)
203 *Aedes albopictus* (Mosquito C6/36 cell line)
204 *Dermatophagoides pteronyssinus* (Dust mite -commercial source)
205 *Xenopsylla cheopis* Flea (Rocky Mountain labs)
206 *Drosophilia* cell line
207 *Musca domestica* (housefly) ARS, USDA, Fargo, ND
208 Gypsy moth cell lines LED652Y cell line (baculovirus)– Invitrogen
209 Cockroach (commercial source)
210 Tick (Amblyomma)
211
212 Vertebrates
213 *Mus musculus* (ATCC/HB-123) mouse
214 *Rattus norvegicus* (ATCC/CRL-1896) rat
215 *Canis familiaris*(ATCC/CCL-183) dog
216 *Felis catus* (ATCC/CRL-8727) cat
217 *Homo sapiens* (HeLa cell line ATCC/CCL-2) human
218 *Gallus gallus domesticus* (Chicken)
219 Goat[5]
220
221 • **Biological insecticides** – Strains of *B. thuringiensis* present in commercially available
222 insecticides have been extensively used in hoaxes and are likely to be harvested in
223 air collectors. For these reasons, it should be used to assess the specificity of these
224 threat assays.
225
226 *B. thuringiensis* subsp*. israelensis*
227 *B. thuringiensis* subsp. *kurstaki*
228 *B. thuringiensis* subsp. *morrisoni*
229 Serenade (Fungicide) *B. subtilis* (QST713)
230
231 Viral agents have also been used for insect control. Two representative products
232 are:
233
234 Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)
235
236 Cyd-X for coddling moths (Coddling moth granulosis virus)
237
238
239

[4] Removed by C. burnetii WG on 4/23/2015 due to unavailability.
[5] Specific to C. burnetii; added by CB WG on 4/23/2015.

240
241 **Part 3: Potential Interferants Study**
242
243 The Potential Interferants Study supplements the Environmental Factors Study, and is applicable
244 to all biological threat agent detection assays for Department of Defense applications. Table V
245 provides a list of potential interferants that are likely to be encountered in various Department
246 of Defense applications.
247
248 Method developers and evaluators shall determine the most appropriate potential interferants
249 for their application. Interferants shall be spiked at a final test concentration of 1 µg/ml directly
250 into the sample collection buffer. Interferants may be pooled. Sample collection buffers spiked
251 with potential interferants shall by inoculated at 2 times the AMDL (or AMIL) with one of the
252 target biological threat agents.
253
254 Spiked / inoculated sample collection buffers shall be tested using the procedure specified by
255 the candidate method.
256
257 It is expected that all samples are correctly identified as positive. If using pooled samples of
258 potential interferants, and a negative result occurs, then the pooled potential interferants shall
259 be tested separately at the 2 times the AMDL (or AMIL) with one of the target biological threat
260 agents.
261
262

263 Table V: Potential Interferants

Compounds		Potential Theaters of Operation
group 1: petroleum-based	JP-8[1]	airfield
	JP-5[2]	naval
	diesel/gasoline mixture	ground
	fog oil (standard grade fuel number 2)	naval, ground
	burning rubber[3]	ground, airfield
group 2: exhaust	gasoline exhaust	ground
	jet exhaust	naval, airfield
	diesel exhaust	ground
group 3: obscurants	terephthalic acid[4]	ground
	zinc chloride smoke[5]	ground
	solvent yellow 33[6]	ground
group 4: environmental	burning vegetation	ground, airfield
	road dust	ground
	sea water (sea spray)	naval
group 5: chemicals	brake fluid[7]	all
	brake dust[8]	ground
	cleaning solvent, *MIL-L-63460*[9]	all
	explosive residues a) high explosives[10] b) artillery propellant[11]	all

264
265 Table 4 is offered for guidance and there are no mandatory minimum requirements for the
266 number of potential interferants to be tested.

267
268
269
270

271
272

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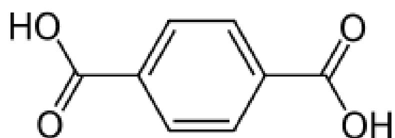
[1] **JP-8**. Airforce formulation jet fuel.

[2] **JP-5**. A yellow kerosene-based jet fuel with a lower flash point developed for use in aircraft stationed aboard aircraft carriers, where the risk from fire is particularly great. JP-5 is a complex mixture of hydrocarbons, containing alkanes, naphthenes, and aromatic hydrocarbons.

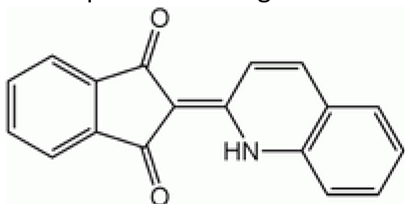
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead; cadmium.

[4] **Terephthalic acid.** Used in the AN/M83 hand grenade currently used by US military.



[5] **Zinc chloride smoke**. Also known as “zinc chloride smoke” and “HC smoke”. Was used in the M8 grenade and still used in 155mm artillery shells. HC smoke is composed of 45% hexachloroethane, 45% zinc oxide, and 10% aluminum.

[6] **Solvent yellow 33** [IUPAC name: 2-(2-quinolyl)-1,3-indandione] is a new formulation being develop for the M18 grenade.



[7] **Brake fluid**. DOT 4 is the most common brake fluid, primarily composed of glycol and borate esters. DOT 5 is silicone-based brake fluid. The main difference is that DOT 4 is hydroscopic whereas DOT 5 is hydrophobic. DOT 5 is often used in military vehicles because it is more stable over time requires less maintenance

[8] **Brake dust**. Fe particles caused by abrasion of the cast iron brake rotor by the pad and secondly fibers from the semi metallic elements of the brake pad. The remainder of the dust residue is carbon content within the brake pad.

---

[9] **MIL-L-63460**, "Military Specification, Lubricant, Cleaner and Preservative for Weapons and Weapons Systems"; trade name **"**Break-Free CLP**".** Hyperlink: Midway USA.

[10] **High explosives**. The M795 155mm projectile is the US Army / Marine Corp's current standard projectile containing 10.8 kg of TNT. The M795 projectile replaced the M107 projectile that contained Composition B which is a 60/40 mixture of RDX/TNT. RDX is cyclotrimethylene trinitramine. Suggestion: test RDX/TNT together.

[11] **Artillery propellant**. Modern gun propellants are divided into three classes: single-base propellants which are mainly or entirely nitrocellulose based, double-base propellants composed of a combination of nitrocellulose and nitroglycerin, and triple base composed of a combination of nitrocellulose and nitroglycerin and nitroguanidine. Suggestion: test total nitrocellulose/ nitroglycerin nitroguanidine together.

AOAC Stakeholder Panel on Agent Detection Assays (SPADA)

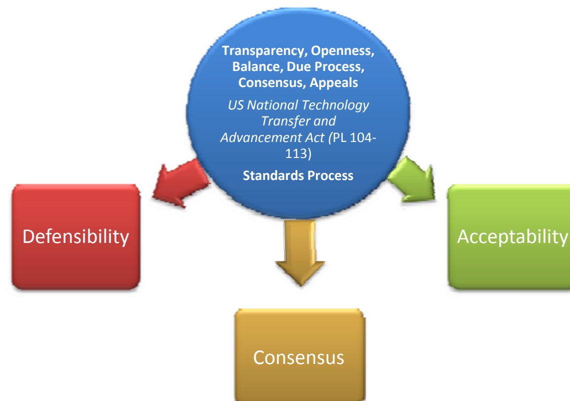
AOAC STANDARDS DEVELOPMENT PROCESS

Stakeholder Panel Working Groups

Deborah McKenzie
Sr. Director,
AOAC Standards Development
AOAC INTERNATIONAL
September 1, 2015

AOAC INTERNATIONAL HEADQUARTERS
2275 Research Blvd, Ste 300
Rockville, Maryland 20850

AOAC Standards Development Processes

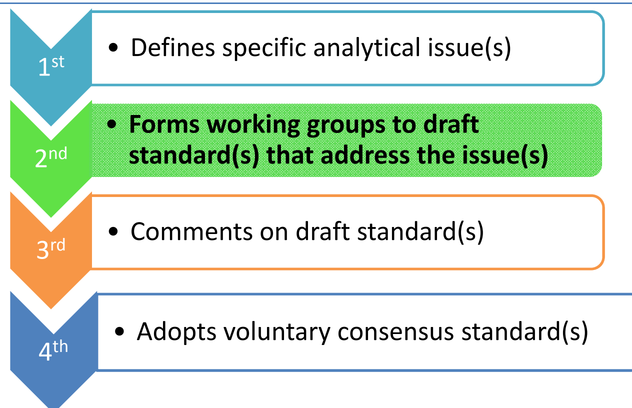


AOAC INTERNATIONAL

As an international standards development organization, AOAC must maintain the following principles throughout all its standard setting activities:

- Transparency
- Openness
- Balance of Interests
- Due Process
- Consensus
- Appeals

Stakeholder Panel Role and Output



AOAC Voluntary Consensus Standards

- **Standard Method Performance Requirements (SMPRs)**
  - Published in *Official Methods of Analysis of AOAC INTERNATIONAL*
  - Manuscript published in *Journal of AOAC INTERNATIONAL*

Stakeholder Panel Working Groups

- Engage in the detailed discussions and work of the stakeholders
- Develop draft fitness for purpose and standard method performance requirements (SMPRs)or other draft standard as proposed by stakeholder panel
- Recommend draft standards to the stakeholder panel
- Can review and recommend methods for consideration
- Formed based on efficiency for work
- Managed by staff
- Conducted in accordance with national and international standards development criteria

Working Group Membership

- Working group membership is not vetted
- Membership is a microcosm of stakeholder panel
  - Experts from government, industry, research, states, military, public health sector, public safety sector, method developers, method end users, etc…
- Moderated by working group chair(s)
- Facilitated by AOAC staff
- Working groups are often formed during the stakeholder meetings and joint working group meetings

Working Group Chair(s)

- Make a launch presentation to the stakeholder panel introducing the topic of the standard
- Moderate the working group discussions
- Work with AOAC CSO and staff
  - On presentations
  - Draft standard
  - Reconcile comments on standard
- Make a SMPR® presentation proposing the draft SMPR to the stakeholder panel.

Working Group Meetings

- In person meetings
  - Wednesday, September 2, 2015
- Teleconference meetings
  - To be scheduled after SPADA meetings
- Facilitated by AOAC staff
- Moderated by Working Group chair(s)
- Discuss the contents of SMPR®
- Goal of Working Group deliberations
  - To reach a general agreement on draft SMPR®

Draft Standards

- AOAC will post the draft SMPR® for a 30 day public comment period
- AOAC CSO and Working Group Chair will reconcile comments
  - Revise SMPR® if needed
  - Reconvene working group if needed

Standard Methods Performance Requirements (SMPRs)

- Documents a community's analytical method needs.
- Very detailed description of the analytical requirements.
- Includes method acceptance requirements.
- Used to adopt AOAC Official Methods by Expert Review Panels.
- Published as a standard.

Documentation and Communication

- AOAC carefully documents the actions of Stakeholder Panel and the Working Groups
- AOAC will prepare summaries of the meetings
  - Communicate summaries to the stakeholders
  - Publish summaries in the *Referee* section of AOAC's *Inside Laboratory Management*
- AOAC publishes its voluntary consensus standards and *Official Methods*
  - *Official Methods of Analysis of AOAC INTERNATIONAL*
  - *Journal of AOAC INTERNATIONAL*
- AOAC publishes the status of standards in the Referee section of AOAC's *Inside Laboratory Management*

Working Groups - Timelines

- **September 1, 2015:**
  - **Launching of new working group topic of new standards**
- **September 2, 2015:**
  - **First meeting of the working groups**
- **September through December 2015:**
  - **Teleconferences of working groups**
- **January – February 2016:**
  - **Public Comment Period**
- **February – March 2016:**
  - **Reconciliation of Comments**
- **March 2016:**
  - **Presentation of draft SMPR during next SPADA meeting**

SPADA Chair & Working Group Chairs

B. anthracis **Working Group (BAWG)**
Paul Jackson (LLNL /LANL) & Ted Hadfield (Hadeco)

Y. pestis **Working Group (YPWG)**
Luther Lindler (DHS)

F. tularensis **Working Group (FTWG)**
Peter Emanuel (DoD) & Mark Wolcott (DoD);
Paul Keim (NAU) & David Wagner (NAU)

Environmental Factors Working Group (EFWG)
Stephen Morse (CDC)

Public Health Actionable Assay Working Group (PHAAWG)
Peter Estacio (LLNL)

B. anthracis **Handheld Assay Working Group (BaHHAWG)**
Marian McKee (BioReliance Corp.)

Ricin Handheld Assay Working Group (RicinHHAWG)
Mark Poli (DoD)

Burkholderia **Working Group (BurkWG)**
Paul Keim (NAU) & Alex Hoffmaster (CDC)

Assay Controls Working Group (ACWG)
Christina Egan (NYSDH) & Larry Blynn (Ibis)

Variola Working Group (VWG)
Victoria Olson (CDC) & Ted Hadfield (Hadeco)

Coxiella burnetii **(CWG)**
Linda Beck (NSWC) & James Samuels (Texas A&M)

Staphylococcus **Enterotoxins (SEWG)**
Sandra Tallent (US FDA)

Venezuelan Equine Encephalitis (VEEWG)
Eileen Ostlund (USDA)

Contact Information

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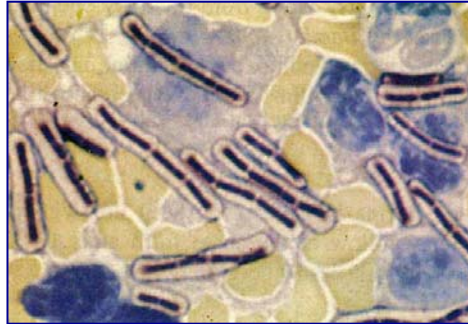
- **Deborah McKenzie**, Sr. Director – Standards Development and AOAC Research Institute, dmckenzie@aoac.org, ext. 157

Selection of the original *Bacillus anthracis* Inclusivity & Exclusivity Panels



Toluidine blue stain. Bacillus anthracis cells in a bovine spleen. Anthrax bacilli in tissue are seen in short chains surrounded by a common capsule

Strains picked and rationale for selection

Presented by
Paul J. Jackson
For the SPADA BA Working Group

Previous SPADA Ba Subgroup Members

Ted Hadfield, MRI (co-chair)

Paul Jackson, LLNL (co-chair)

Alex Hoffmaster, CDC

Rich Meyer, CDC

Matthew Van Ert, MRI

Mark Wolcott, USAMRIID

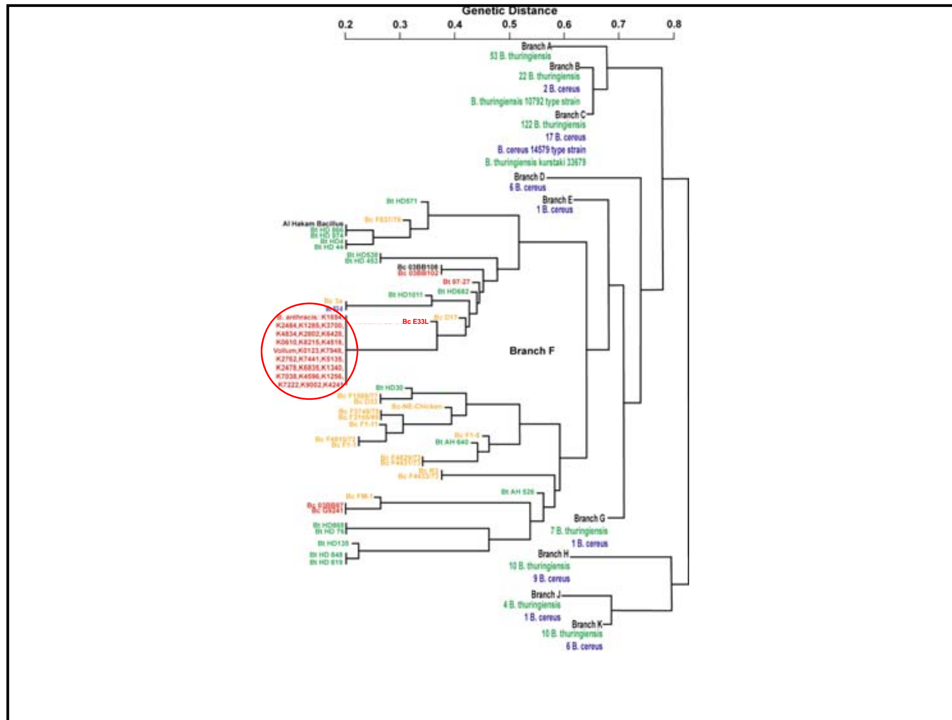
The Charge

- Select *B. anthracis* strains representative of the current available *B. anthracis* collection that represent the diversity across this species
- These strains will be used to validate detection assays
  - A successful assay should detect all of these isolates*

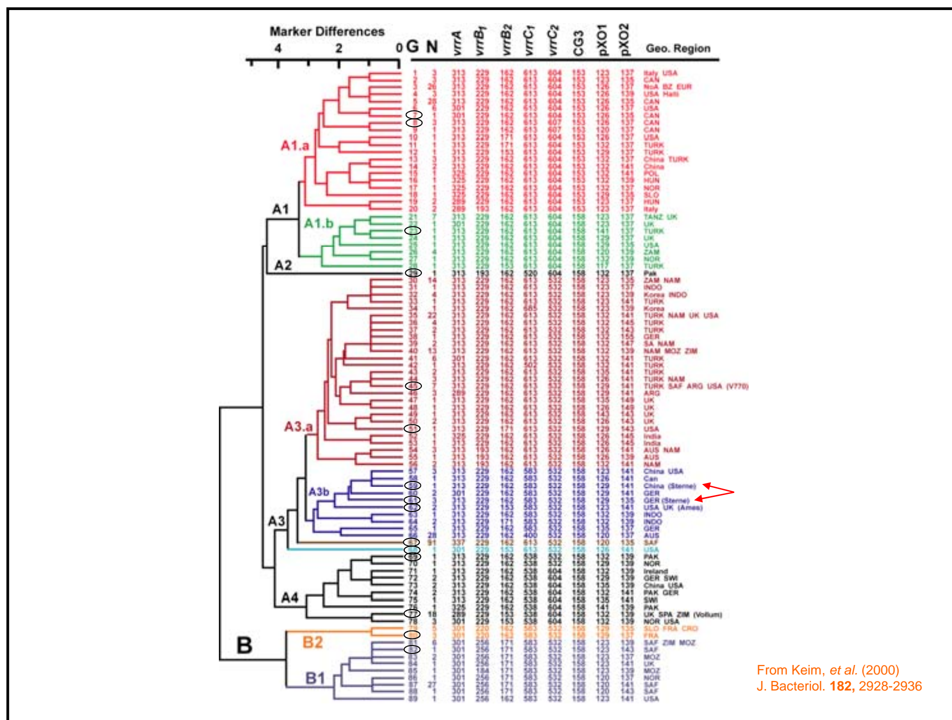
**Some assays may detect sequences or materials not present in all isolates*

What is a *B. anthracis* isolate?

- The causative agent of anthrax
- A bacterium that meets clinical criteria for being *B. anthracis*
  - Colony morphology, Gram stain, presence of capsule, motility, hemolytic activity, γ phage sensitivity, pen^s, etc.
- An isolate containing pXO1 and pXO2 unless otherwise identified as missing one of these plasmids
- An isolate that maps, by multiple phylogenetic methods, to a tight cluster of microbes sharing these characteristics



Genetic Distance
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Branch A
53 B. thuringiensis
Branch B
22 B. thuringiensis
2 B. cereus
B. thuringiensis 10792 type strain
Branch C
122 B. thuringiensis
17 B. cereus
B. cereus 14579 type strain
B. thuringiensis kurstaki 33679
Branch D
6 B. cereus
Branch E
1 B. cereus
Al Hakam Bacillus
Bc E33L
Branch F
Branch G
7 B. thuringiensis
1 B. cereus
Branch H
10 B. thuringiensis
9 B. cereus
Branch J
4 B. thuringiensis
1 B. cereus
Branch K
10 B. thuringiensis
6 B. cereus



Marker Differences
4
2
0
G
N
vrrA
vrrB1
vrrB2
vrrC1
vrrC2
CG3
pXO1
pXO2
Geo. Region
A1.a
A1
A1.b
A2
A3.a
A3b
A3
A4
B
B2
B1
From Keim, et al. (2000)
J. Bacteriol. 182, 2928-2936

***Bacillus anthracis* PCR Inclusivity panel**

No.	Cluster	Genotype	Strain	Origin	Characteristics
BA1	A1a	7	Canadian bison	Wood bison	pXO1^{+}. pXO2^{+}, VNTR genotype group A1a
BA2	A3a	45[b]	V770-NP-1R	Vaccine (USA)	pXO1^{+}. pXO2^{-}, VNTR genotype group A3a
BA3	A2	29	PAK-1	Sheep (Pakistan)	pXO1^{+}. pXO2^{+}, VNTR genotype group A2
BA4	A3a	51	BA1015	Bovine (MD)	pXO1^{+}. pXO2^{+}, VNTR genotype group A3a
BA5	A3b	62	Ames	Bovine (TX)	pXO1^{+}. pXO2^{+}, VNTR genotype group A3b
BA6	A3c	67	K3	South Africa	pXO1^{+}. pXO2^{+}, VNTR genotype group A3c
BA7	A3d	68	Ohio ACB	Pig	pXO1^{+}. pXO2^{+}, VNTR genotype group A3d
BA8	A4	69	SK-102 (Pakistan)	Imported wool (Pakistan)	pXO1^{+}. pXO2^{+}, VNTR genotype group A4
BA9	A4	77	Vollum 1B	USAMRIID[a]	pXO1^{+}. pXO2^{+}, VNTR genotype group A4
BA10	B1	82	BA1035	Human (South Africa)	pXO1^{+}. pXO2^{+}, VNTR genotype group B1
BA11	B2	80	RA3	Bovine (France)	pXO1^{+}. pXO2^{+}, VNTR genotype group B2
BA12	C	Unk[c]	2002013094 (240)	Louisiana	pXO1^{+}. pXO2^{+}, VNTR genotype group C
BA13	A1a	8	Pasteur	USAMRIID	pXO1^{-}. pXO2^{+}, VNTR genotype group A1a
BA14	A3b	59, 61[b]	Sterne	USAMRIID	pXO1^{+}. pXO2^{-}, VNTR genotype group A3b
BA15	A1b	23	Turkey No. 32	Human (Turkey)	pXO1^{+}. pXO2^{+}, VNTR genotype group A1b

[a]USAMRIID = The United States Army Medical Research Institute for Infectious Diseases

[b]Organism contains only seven of eight MLVA markers due to the lack of pXO2. Genotypes listed are consistent with seven of the eight markers used.

[c]Unk = Unknown

***Bacillus anthracis* PCR Exclusivity panel**

No.	Species	Strain	Plasmid status
BANN1	*B. cereus*	S2-8	pXO1^{-}, pXO2^{-}
BANN2	*B. cereus*	3A	pXO1^{-}, pXO2^{-}
BANN3	*B. thuringiensis*	HD1011	pXO1^{-}, pXO2^{-}
BANN4	*B. thuringiensis*	97-27*	pXO1^{-}, pXO2^{-}
BANN5	*B. thuringiensis*	HD682	pXO1^{-}, pXO2^{-}
BANN6	*B. cereus*	E33L*	pXO1^{-}, pXO2^{-}
BANN7	*B. cereus*	D17	pXO1^{-}, pXO2^{-}
BANN8	*B. thuringiensis*	HD571	pXO1^{-}, pXO2^{-}
BANN9	*B. thuringiensis*[a]	Al Hakam	pXO1^{-}, pXO2^{-}
BANN10	*B. cereus*	ATCC 4342	pXO1^{-}, pXO2^{-}
BANN11	*B. cereus*	FM1	pXO1^{-}, pXO2^{-}
BANN12	*B. cereus*	G9241*	pBCXO1^{+}[b], pXO2^{-}
BANN13	*B. cereus*	03BB102*	pXO1^{+}, capA^{+}, CapB^{+}, CapC^{+}[c]
BANN14	*B. cereus*	03BB108	pXO1^{+}, capA^{+}, CapB^{+}, CapC^{+}[c]
BANN15	*B. thuringiensis*	subsp. *israelensis* HD1002	pXO1^{-}, pXO2^{-}
BANN16	*B. thuringiensis*	subsp. *Kurstaki* HD1	pXO1^{-}, pXO2^{-}
BANN17	*B. thuringiensis*	Subsp. *Morrisoni* HD600	pXO1^{-}, pXO2^{-}
BANN18	*B. coagulans*	ATCC 7050	pXO1^{-}, pXO2^{-}
BANN19	*B. mycoides*	ATCC 6462	pXO1^{-}, pXO2^{-}
BANN20	*B. megaterium*	ATCC 14581	pXO1^{-}, pXO2^{-}

* Known pathogen in animals or humans

[a]Referred to as a *B. thuringiensis* because the Iraqis referred to it as such. Has no characteristics of a normal *B. thuringiensis* and may more accurately be referred to as a *B. cereus.*

[b]pBCXO1 is pXO1-like, but with some differences

[c]CapA, B, and C are contained within the *Bacillus anthracis* pXO2 plasmid; however only the capA, B and C sequences are found in 03BB102 and 03BB108.

Genetic Distance

0.2 0.3 0.4 0.5 0.6 0.7 0.8

Branch A
53 B. thuringiensis
Branch B
22 B. thuringiensis
2 B. cereus
B. thuringiensis 10792 type strain
Branch C
122 B. thuringiensis
17 B. cereus
B. cereus 14579 type strain
B. thuringiensis kurstaki 33679
Branch D
6 B. cereus
Branch E
1 B. cereus

Bt HD571
Bc F837/76
Al Hakam Bacillus
Bt HD 866
Bt HD 974
Bt HD4
Bt HD 44
Bt HD538
Bt HD 453
Bc 03BB108
Bc 03BB102
Bt 97-27
Bt HD682
Bt HD1011
Bc 3a
Bc 524
B. anthracis: K1694, K2484,K1285,K3700, K4834,K2802,K6428, K0610,K8215,K4516, Vollum,K0123,K7948, K2762,K7441,K5135, K2478,K6835,K1340, K7038,K4596,K1256, K7222,K9002,K4241
Bc E33L
Bc D17

Branch F

Bt HD30
Bc F1589/77
Bc D33
Bc NE-Chicken
Bc F1-11
Bc F4810/72
Bc F1-1
Bc F1-6
Bt AH 640
Bc R3
Bc F4433/73
Bt AH 526
Bc FM-1
Bc 03BB87
Bc G9241
Bt HD868
Bt HD 76
Bt HD135
Bt HD 848
Bt HD 819

Branch G
7 B. thuringiensis
1 B. cereus
Branch H
10 B. thuringiensis
9 B. cereus
Branch J
4 B. thuringiensis
1 B. cereus
Branch K
10 B. thuringiensis
6 B. cereus

Green: *B. thuringiensis* – no known pathogenic properties in vertebrates
Blue: *B. cereus* soil isolate
Gold: *B. cereus* toxigenic isolate
Red: *Bacillus* isolate pathogenic in humans and/or other mammals

Genetic Distance

0.2 0.4 0.6 0.8

Branch A-C
Bc F2-19
Bc D21
Branch D
Bc D14
Bc AH 527 (37)
Bc AH 540
Bc D23
Branch E
Bc ATCC 4342
Bt HD 866 (H17)
Bt HD 974 (H8a8d)
Bt HD 4 (H3a)
Bt HD 44 (H3a)
Bt HD 571 (H11a11c)
Bc F837/76
Bt HD 453 (H4a4c)
Bt HD 538 (H9)
Bt 97-27 (H34)
Bc D17
B.anthracis: K2484, K3700,K4516,K8215, K4834,K0610,K2802, K6428,K7441,K2762, K7948,K5135,K2478, K6835,K1340,K7038, K1256,K4241,K4596, K0404,K0123,K7222, K9002, K1694,K1285
Bt HD 1011 (H20)
Bt HD 682 (H2)
Branch F
Bc 3A
Bc S2-8
Bt HD 76 (H3a)
Bt HD 868 (H19)
Bc FM-1
Bt AH 526 (H19)
Bt HD 135 (H7)
Bt HD 819 (not identified) (39)
Bt HD 848 (H7) (39)
Bc F4433/73
Bc R3
Bc F1589/77
Bt HD 30 (H5a)
Bc D33
Bc NE-Chicken
Bc F1-11 (36)
Bc F1-1
Bc F4810/72
Bc F2105/89
Bc F3748/75
Bc F4431/73
Bc F1-6
Bt AH 640 (H6)
Bc F4429/73
Bc D5
Branch G
Bt HD 99 (H2)
Bt HD 527 (H2)
Bt HD 529 (H2)
Bt HD 528 (H2)
Bt HD 3 (H2) (38)
Bt HD 19 (H2)
Branch H-K

B. thuringiensis isolates used in U.S. EPA-approved pesticides

- *Bacillus thuringiensis* (Berliner) (no subsp. given)
- *Bacillus thuringiensis* subsp. *aizawai*
- *Bacillus thuringiensis* subsp. *israelensis*
- *Bacillus thuringiensis* subsp. *kurstaki*
- *Bacillus thuringiensis* subsp. *tenebrionis**

**aka* subsp. *morrisoni*

B. thuringiensis isolates used in U.S. EPA-approved pesticides

- *Bacillus thuringiensis* (Berliner) (no subsp. given)
- *Bacillus thuringiensis* subsp. *aizawai*
- *Bacillus thuringiensis* subsp. *israelensis*
- *Bacillus thuringiensis* subsp. *kurstaki*
- *Bacillus thuringiensis* subsp. *tenebrionis**

**aka* subsp. *morrisoni*

Genetic Distance

0.5 0.6 0.7 0.8

Cluster 1

Branch A
53 *B. thuringiensis*

Branch B
22 *B. thuringiensis*
2 *B. cereus*
B. thuringiensis 10792 type strain

Branch C
122 *B. thuringiensis*
17 *B. cereus*
B. cereus 14579 type strain
B. thuringiensis kurstaki 33679

Cluster 2

Branch D
6 *B. cereus*

Branch E
1 *B. cereus*

Branch F
18 *B. thuringiensis*
18 *B. cereus*
25 *B. anthracis*

Branch G
7 *B. thuringiensis*
1 *B. cereus*

Cluster 3

Branch H
10 *B. thuringiensis*
9 *B. cereus*

Branch J
4 *B. thuringiensis*
1 *B. cereus*

Branch K
10 *B. thuringiensis*
6 *B. cereus*

From Hill, K.K., *et al.* (2004) Fluorescent amplified fragment length polymorphism analysis of *Bacillus anthracis, Bacillus cereus* and *B. thuringiensis* isolates. Appl. Environ. Microbiol. **70,** 1068-1080

Genetic Distance

0.2 0.4 0.6 0.8

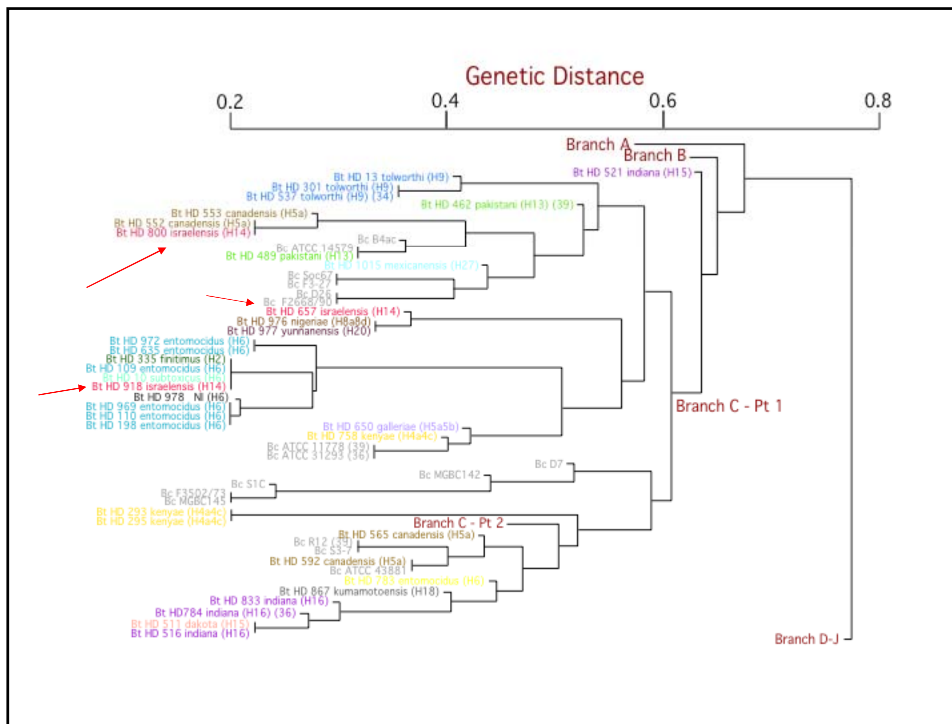
Bt HD 201 toumanoffi (H11a11b)
Bt HD 540 toumanoffi (H11a11b)
Bt HD 771 sotto (H4a4b)
Bt HD 500 israeliensis (H14)
Bt HD 659 israeliensis (H14)
Bt HD 658 israeliensis (H14)
Bt HD 789 israeliensis (H14)
Bt HD 795 israeliensis (H14)
Bt HD 661 israeliensis (H14)
Bt HD 921 israeliensis (H14)
Bt HD 799 israeliensis (H14)
Bt HD 1002 israeliensis (H14)
Bt HD 1008 israeliensis (H14)
Bt HD 603 darmsadiensis (H10)
Bt HD 612 darmsadiensis (H10)
Bt HD 602 darmsadiensis (H10)
Bt HD 625 darmsadiensis (H10)
Bt HD 604 darmsadiensis (H10)
Bt HD 626 darmsadiensis (H10) (36)
Bt HD 498 darmsadiensis (H10)
Bt HD 628 darmsadiensis (H10)
Bt HD 487 alesti (H3a)
Bt HD 83 alesti (H3a)
Bt HD 82 alesti (H3a)
Bt HD 84 alesti (H3a)
Bt HD 754 darmstadiensis (H10)
Bt HD 226 sotto (H4a4b)
Bt HD 633 NI (H4a4b)
Bt HD 772 sotto (H4a4b)
Bt HD 681 fititimus (H2)
Bt HD 127 aizawai (H7)
Bt HD 34 dendrolimus (H4a4b)
Bt HD 7 dendrolimus (H4a4b)
Bt HD 930 sotto (H4a4b)
Bt HD 615 morrisoni (H8a8b)
Bt HD 47 dendrolimus (H4a4b)
Bt HD 33 dendrolimus (H4a4b)
Bt HD 963 sotto (H4a4b)
Bt HD 568 dendrolimus (H4a4b)
Bt HD 774 sotto (H4a4b)
Bt HD 773 sotto (H4a4b)
Bt HD 557 dendrolimus (H4a4b)
Bt HD 584 dendrolimus (H4a4b)
Bt HD 586 dendrolimus (H4a4b)
Bt HD 621 morrisoni (H8a8b)
Bt HD 597 morrisoni (H8a8b) (39)
Bt HD 600 morrisoni (H8a8b)
Bt HD 530 morrisoni (H8a8b)
Bt HD 534 morrisoni (H8a8b)
Bt HD 324 morrisoni (H8a8b)
Bt HD 518 morrisoni (H8a8b)
Bt HD 711 galleriae (H5a5b)

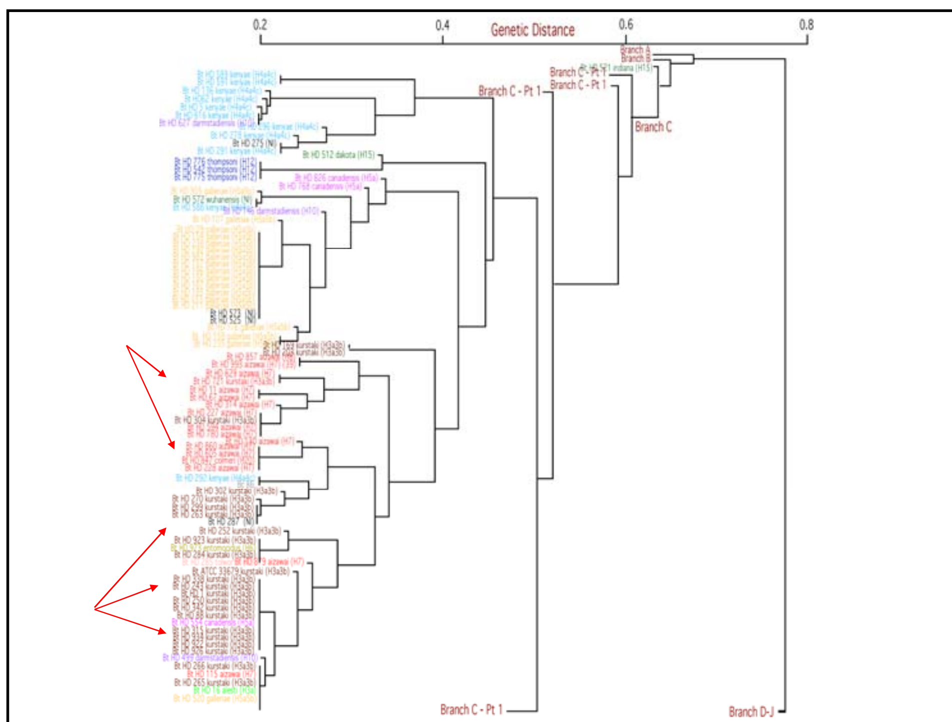
Branch A

Branch B

Branch C

Branches D-K


Genetic Distance
0.2
0.4
0.6
0.8
Branch A
Branch B
Bt HD 521 indiana (H15)
Bt HD 13 tolworthi (H9)
Bt HD 301 tolworthi (H9)
Bt HD 537 tolworthi (H9) (34)
Bt HD 462 pakistani (H13) (39)
Bt HD 553 canadensis (H5a)
Bt HD 552 canadensis (H5a)
Bt HD 800 israelensis (H14)
Bc B4ac
Bc ATCC 14579
Bt HD 489 pakistani (H13)
Bt HD 1015 mexicanensis (H27)
Bc Soc67
Bc F3-27
Bc D26
Bc F2668/90
Bt HD 657 israelensis (H14)
Bt HD 976 nigeriae (H8a8d)
Bt HD 977 yunnanensis (H20)
Bt HD 972 entomocidus (H6)
Bt HD 635 entomocidus (H6)
Bt HD 335 finitimus (H2)
Bt HD 109 entomocidus (H6)
Bt HD 918 israelensis (H14)
Bt HD 978 NI (H6)
Bt HD 969 entomocidus (H6)
Bt HD 110 entomocidus (H6)
Bt HD 198 entomocidus (H6)
Bt HD 650 galleriae (H5a5b)
Bt HD 758 kenyae (H4a4c)
Bc ATCC 11778 (39)
Bc ATCC 31293 (36)
Branch C - Pt 1
Bc D7
Bc MGBC142
Bc S1C
Bc F3502/73
Bc MGBC145
Bt HD 293 kenyae (H4a4c)
Bt HD 295 kenyae (H4a4c)
Branch C - Pt 2
Bt HD 565 canadensis (H5a)
Bc R12 (39)
Bc S3-7
Bt HD 592 canadensis (H5a)
Bc ATCC 43881
Bt HD 783 entomocidus (H6)
Bt HD 867 kumamotoensis (H18)
Bt HD 833 indiana (H16)
Bt HD784 indiana (H16) (36)
Bt HD 511 dakota (H15)
Bt HD 516 indiana (H16)
Branch D-J


0.2
0.4
Genetic Distance
0.6
0.8
Branch A
Branch B
Branch C - Pt 1
Branch C - Pt 1
Branch C - Pt 1
Branch C
Branch C - Pt 1
Branch D-J

The last three isolates in the Exclusivity Panel do not map closely to *B. anthracis*

Environmental Factors Panel

Other biothreat agents

Yersinia pestis Colorado-92
Francisella tularensis subsp. *tularensis* Schu-S4
Burkholderia pseudomallei (strain?)
Coxiella burnetii Nine Mile Phase 1
Brucella melitensis
Ricinus communis (use castor bean plant leaves as source of DNA)
Clostridium botulinum Type A

Cultivatable bacteria known to be present in air and soil

Acinetobacter lwoffii
Agrobacterium tumefaciens
Bacillus cohnii
Bacilllus psychrosaccharolyticus
Bacillus benzoevorans
Bacillus megaterium
Bacillus horikoshii
Bacillus macrolides
Bacteroides fragilis
Burkholderia cepacia
Burkholderia gladioli
Burkholderia stabilis
Burkholderia plantarii
Chryseobacterium indologenes
Clostridium sardiniense
Clostridium perfringens
Deinococcus radiodurans
Delftia acidovorans
Escherichia coli K12
Fusobacterium nucleatum
Lactobacillus plantarum
Moxaxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphlococcus aureus
Stenotrophomonas maltophilia
Steptococcus pneumonia
Streptomyces coelicolor
Synechocystis
Vibrio cholera
Legionella pneumophila
Listeria monocytogenes

Environmental Factors Panel

DNA viruses	
Vaccinia virus (pox)	Herpes simplex or CMV
Adenovirus vaccine	
Microbial eukaryotes	
Freshwater amoebae:	
Acanthamoeba castellanii	*Naegleria fowleri*
Fungi:	
Alternaria alternate	*Eurotium amstelodami*
Aspergillus fumigates	*Mucor racemosus*
Aureobasidium pullulans	*Paecilomyces variotii*
Cladosporium cladosporioides	*Penicillum chrysogenum*
Cladosporium sphaerospermum	*Saccharomyces cerevisiae*
Epicoccum nigrum	*Wallemia sebi*

Environmental Factors Panel

DNA from higher eukaryotes	
Plants:	
Zea mays (corn)	Cotton (use leaves of cotton plant as source of DNA
Pollen from *Pinus* spp. (pine)	
Arthropods:	
Aedes aegypti (ATCC/CCL-125) mosquito cell line	*Musca domestica* (housefly; ARS, USDA, Fargo, ND)
Aedes albopictus (C6/36) mosquito	Gypsy mother LED652Y cell line (baculovirus; Invitrogen)
Dust mite (commercial source)	Cockroach (commercial source)
Flea (Rocky Mountain Labs)	Tick (*Amblyomma*)
Drosophila cell line	
Mammals:	
Mus musculus (ATCC/HB-123) mouse	*Felis catus* (ATCC/CRL-8727) cat
Rattus norvegicus (ATCC/CRL-1896) rat	*Hom sapiens* HeLa) human
Canis familiarus (ATCC/CCL-183) dog	
Avian:	
Chicken	
Biological insecticides	
B. thuringiensis subsp. *israelensis**	Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)
B. thuringiensis subsp. *kurstaki**	Cyd-X for coddling moths (Coddling moth granulosis virus)
B. thuringiensis subsp. *morrisoni**	

Already in Exclusivity panel

Environmental Factors Panel

Substances	
Soils	
Sandy	Subsoil
Loam	Silt
Clay	
Dust	
Powders and chemicals	
Bacillus thuringiensis powders (e.g., Dipel)*	Baking powder
Powdered milk	GABA (GAMA aminobutyric acid)
Powdered infant formula (Fe fortified)	L-Glutamic acid
Powdered infant formula (low Fe formulation	Kaolin
Powdered coffee creamer (which one?)	Chitin
Powdered sugar[1]	Chitosan
Talcum powder	$MgSO_4$
Wheat flower	Boric acid
Baking soda	Powdered toothpaste
Chalk dust	Popcorn salt
Brewer's yeast	EDTA
Dry wall dust	ZEP
Cornstarch[1]	Rid-X

These contain insect toxins and debris from the different *B. thuringiensis* isolates listed in the Exclusivity panel

Powdered sugar contains corn starch – that's what differentiates it from granulated sugar

What have we learned from previous testing against the environmental panel?

- Can some testing be eliminated based on previous testing results?
- Have deployed assays shown vulnerability to environmental factors not considered when the first panel was assembled?

What we've learned – new information

Johnson, S.L., Daligault, H.E., Davenport, K.W., Jaissle, J., Grey, K.G., Ladner, J.T., broomall, S.M., Bishop-Lilly, K.A., Bruce, D.C., Gibbons, H.S., Coyne, S.R., Lo, C.-C., Meincke, LI, Munk, A.C., Koroleva, G.I., Rosenzweig, C.N., Palacios, f., Redden, C.L., Minogue, T.D. and Chain, P.S. (2015)

Complete Genome Sequences of 35 Biothreat Assay-Relevant *Bacillus* Species.

Genome Announc. **3,** e00151-15.

ABSTRACT: In 2011, the Association of Analytical Communities (AOAC) International released a list of *Bacillus* strains relevant to biothreat molecular detection assays. We present the complete and annotated genome assemblies of the 15 strains listed on the inclusivity panel, as well as the 20 strains listed on the exclusivity panel.

Something to Consider

- Given full genome sequences for all members of the Inclusivity and Exclusivity panels, anyone designing assays using DNA sequences can use *in silico* methods to select sequences to target with their assays that are specific to *B. anthracis*, based on signatures found only in genomes of isolates in the Inclusivity panel but absent from genomes of organisms in the Exclusivity panel.
- It will be straightforward to demonstrate that the assays were designed in such a manner and, *in silico* "testing" will quickly demonstrate whether the targeted sequences – or sets of sequences – are specific to the target.

QUESTION: Given the new sequencing information and demonstrations that the modeling provides accurate results, should we still require extensive testing against the Inclusivity and Exclusivity panels or would more limited testing against these panels suffice?

What was learned from testing?

Information from previous testing:

- Have all isolates in the inclusivity panel given identical results – with the exception of plasmids?
    - If so, do we need as many isolates in the inclusivity panel?
    - If not, which ones performed differently?
- Do some isolates appear to give more sensitive results with the same amount of assay DNA than others?
- Are some inclusivity panel isolates difficult to grow or maintain; do they lose genetic material (for example, plasmids) upon passage?
    - If so, is it likely that such isolates would ever be used to produce "weaponized" material?

What was learned from testing?

Information from previous testing:

- Are some exclusivity panel members always negative with previously tested assays – or are they positive only when other exclusivity panel members provide a positive result?
  - If so, do they need to be included in the panel?
- Which soils are problematic for testing and why?
  - Do some soils inhibit assay reactions?
  - Do some soils routinely give positive results with multiple assays?
  - Are the soil samples used representative of areas where assays might be deployed?
  - Do we need to use a more diverse collection of soil samples in testing?
- There is redundancy in the panels -
  - *B. thuringiensis* isolates and their derivatives are included in the Exclusivity panel, in the Environmental panel as organisms and as products under "powders and chemicals"
  - Powdered sugar is simply sugar grounded finally and supplemented with corn starch – can we take corn starch off the environmental panel?

Fitness for Purpose (proposal)

Detection of *Bacillus anthracis* spores in field-deployable, Department of Defense aerosol collection devices.

Motion

To develop standards for development of assays for detection of *Bacillus anthracis* spores in field-deployable, Department of Defense aerosol collection devices.

Questions?
Discussion?

STANDARD METHOD PERFORMANCE REQUIREMENTS

AOAC SMPR 2010.003

Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* in Aerosol Collection Filters and/or Liquids

Intended Use: Laboratory use for analysis of aerosol collection filters and/or liquids

Method Developer and Independent Validation

Probability of Detection at the Acceptable Minimum Detection Level

1 **Definitions**: Probability of detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given agent level or concentration. POD is concentration-dependent. The acceptable minimum detection level (AMDL) is the predetermined minimum level of a biological threat agent, which must be detected by the candidate method with an estimated 5% lower confidence limit on the POD of 0.95 or higher. The AMDL is dependent on the intended use.

2 **Test conditions**: AMDL is 20,000 standardized *Bacillus anthracis* Ames spores per filter; 2000 standardized spores per mL; 2000 genome equivalents per mL.

3 **Acceptance criteria**: No more than one failure in 96 replicates.

Inclusivity

1 **Definition**: Strains or isolates or variants of the target agent(s) that the method can detect (Table 1).

2 **Test conditions**: Test inclusivity panel at AMDL.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a negative result, retest that strain 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher.*

Exclusivity

1 **Definition**: Nontarget agents, which are potentially cross-reactive, that are not detected by the method (Table 2).

2 **Test conditions**: Test exclusivity near neighbor panel at 10 times AMDL.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a positive result, retest that strain 96 times with no failures allowed to demonstrate a 95% upper confidence limit on the POD of 0.05 or lower.*

Environmental Interference

1 **Definition**: Ability of the assay to detect target organism in the presence of nontarget organisms or environmental substances and to be free of cross-reaction from environmental organisms and substances (Appendix A).

2 **Test conditions**: Test pooled environmental panel organisms at 10 times AMDL in the presence or absence of *Bacillus anthracis* Ames at the AMDL. Test environmental substances as suspensions in the presence or absence of *Bacillus anthracis* Ames at the AMDL.

3 **Acceptance criteria**: 100% expected results for environmental organisms (i.e., no false negatives in the presence of *Bacillus anthracis* Ames, and no false positives in the absence of *Bacillus anthracis* Ames).

***Note**: In the case of an unexpected result, retest individual strains 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher. Data from environmental substances are for informational purposes only.*

Collaborative Validation Study

Reproducibility

1 **Definition**: Precision under conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

2 **Test conditions**: Test *Bacillus anthracis* Ames spores at AMDL and near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per material per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Must produce at least 10 valid data sets. Report standard deviation of reproducibility (s_R).

POD at the AMDL Under Reproducibility Conditions (formerly termed System False-Negative Rate)

1 **Definition**: Rate of positive system results in a population of known positive test portions.

2 **Test conditions**: Test *Bacillus anthracis* Ames spores at AMDL on dust-loaded filters or in dust-loaded aerosol collection

Received February 18, 2011.

Table 1. *Bacillus anthracis* PCR method: Inclusivity panel

No.	Cluster	Genotype	Strain	MRI No.[a]	Origin	Characteristics
BA1	A1a	7	Canadian bison	107448	Wood bison	pX01+, pX02+, VNTR genotype group A1a
BA2	A3a	45[b]	V770-NP-1R	107240	Vaccine (USA)	pX01+, pX02-, VNTR genotype group A3a
BA3	A2	29	PAK-1	107518	Sheep (Pakistan)	pX01+, pX02+, VNTR genotype group A2
BA4	A3a	51	BA1015	107446	Bovine (MD)	pX01+, pX02+, VNTR genotype group A3a
BA5	A3b	62	Ames	107517	Bovine (Texas)	pX01+, pX02+, VNTR genotype group A3b
BA6	A3c	67	K3	107497	South Africa	pX01+, pX02+, VNTR genotype group A3c
BA7	A3d	68	Ohio ACB	107339	Pig	pX01+, pX02+, VNTR genotype group A3d
BA8	A4	69	SK-102 (Pakistan)	107449	Imported wool (Pakistan)	pX01+, pX02+, VNTR genotype group A4
BA9	A4	77	Vollum 1B	107539	USAMRIID[a]	pX01+, pX02+, VNTR genotype group A4
BA10	B1	82	BA1035	107451	Human (South Africa)	pX01+, pX02+, VNTR genotype group B1
BA11	B2	80	RA3	107520	Bovine (France)	pX01+, pX02+, VNTR genotype group B2
BA12	C	Unk[c]	2002013094 (240)	124030	Louisiana	pX01+, pX02+, VNTR genotype group C
BA13	A1a	8	Pasteur	107171	USAMRIID	pX01-, pX02+, VNTR genotype group A1a
BA14	A3b	59, 61[b]	Sterne	107453	USAMRIID	pX01+, pX02-, VNTR genotype group A3b
BA15	A1b	23	Turkey No. 32	107255	Human (Turkey)	pX01+, pX02+, VNTR genotype group A1b

[a] MRI = MRI Global; USAMRIID = The United States Army Medical Research Institute For Infectious Diseases.

Approved by AOAC SPADA on April 24, 2007.

[b] Organism contains only seven of eight MLVA markers due to the lack of pX02. Genotypes listed are consistent with seven of the eight markers. (*Note*: Footnote applies to BA2 and BA14 genotype designations.)

[c] Unk = Unknown.

Table 2. *Bacillus anthracis* PCR method: Exclusivity panel

No.	Species	Strain	Plasmid status
BANN1	*B. cereus*	S2-8	pXO1-, pXO2-
BANN2	*B. cereus*	3A	pXO1-, pXO2-
BANN3	*B. thuringiensis*	HD1011	pXO1-, pXO2-
BANN4	*B. thuringiensis*	97-27	pXO1-, pXO2-
BANN5	*B. thuringiensis*	HD682	pXO1-, pXO2-
BANN6	*B. cereus*	E33L	pXO1-, pXO2-
BANN7	*B. cereus*	D17	pXO1-, pXO2-
BANN8	*B. thuringiensis*	HD571	pXO1-, pXO2-
BANN9	*B. cereus*	Al Hakam	pXO1-, pXO2-
BANN10	*B. cereus*	ATCC 4342	pXO1-, pXO2-
BANN11	*B. cereus*	FM1	pXO1-, pXO2-
BANN12	*B. cereus*	G9241	pBCXO1+[a], pXO2-
BANN13	*B. cereus*	03BB102	pXO1+, capA+, capB+, capC+[b]
BANN14	*B. cereus*	03BB108	pXO1+, capA+, capB+, capC+[b]
BANN15	*B. thuringiensis*	subsp. *israelensis* HD 1002	pXO1-, pXO2-
BANN16	*B. thuringiensis*	subsp. *kurstaki* HD 1	pXO1-, pXO2-
BANN17	*B. thuringiensis*	subsp. *morrisoni* HD 600	pXO1-, pXO2-
BANN18	*B. coagulans*	ATCC 7050	pXO1-, pXO2-
BANN19	*B. mycoides*	ATCC 6462	pXO1-, pXO2-
BANN20	*B. megaterium*	ATCC 14581	pXO1-, pXO2-

[a] pBCXO1 is pXO1-like, but not identical.

[b] capA, B, and C are contained within the pXO2 plasmid of *Bacillus anthracis*; however, only the capA, B, and C sequences are found in 03BB102 and 03BB108.

Approved by AOAC SPADA on December 12, 2007.

liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for target agent must demonstrate an estimated 5% lower confidence limit on the CPOD of 0.95 or higher, where CPOD is the probability of detection calculated from pooled valid collaborative data.

POD in the Absence of Analyte Under Reproducibility Conditions (formerly termed System False-Positive Rate)

1 **Definition**: Rate of positive system results in a population of known negative test portions.

2 **Test conditions**: Test near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for near neighbor must demonstrate a 95% upper confidence limit on the CPOD of 0.05 or lower, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Acknowledgments

All or part of this work was funded by the Department of Homeland Security Science and Technology Directorate, award HSHQDC-08-C-00012.

AOAC SPADA approved PCR SMPRs as amended on January 22, 2009. PCR SMPRs (version 4) were revised on May 12, 2009 to reflect OMB proposal and to correct retest statistics. The final version as shown here was approved by SPADA on June 2, 2010 and contained revision to OMB requirement of 10 valid data sets for qualitative methods in the collaborative study.

Appendix A: Environmental Factors Panel

Organisms

1 Other biothreat agents

Yersinia pestis Colorado-92

Francisella tularensis subsp. *tularensis* Schu-S4

Burkholderia pseudomallei

Coxiella burnetii Nine Mile Phase I

Brucella melitensis

Ricinus communis (use ricin plant leaves as source of DNA)

Clostridium botulinum Type A

2 Cultivatable bacteria identified as being present in air and soil

Acinetobacter lwoffii

Agrobacterium tumefaciens

Bacillus cohnii

Bacillus psychrosaccharolyticus

Bacillus benzoevorans

Bacillus megaterium

Bacillus horikoshii

Bacillus macroides

Bacteroides fragilis

Burkholderia cepacia

Burkholderia gladoli

Burkholderia stabilis

Burkholderia plantarii

Chryseobacterium indologenes

Clostridium sardiniense

Clostridium perfringens

Deinococcus radiodurans

Delftia acidovorans

Escherichia coli K12

Fusobacterium nucleatum

Lactobacillus plantarum

Moraxella nonliquefaciens

Mycobacterium smegmatis

Neisseria lactamica

Pseudomonas aeruginosa

Rhodobacter sphaeroides

Riemerella anatipestifer

Shewanella oneidensis

Staphylococcus aureus

Stenotrophomonas maltophilia

Streptococcus pneumoniae

Streptomyces coelicolor

Synechocystis

Vibrio cholerae

Legionella pneumophila

Listeria monocytogenes

3 DNA viruses

Vaccinia virus (pox)

Adenovirus vaccine

Herpes simplex or CMV (whichever is available)

4 Microbial eukaryotes

Freshwater amoebae:

Acanthamoeba castellanii

Naegleria fowleri

Fungi:

Alternaria alternata

Aspergillus fumigatis

Aureobasidium pullulans

Cladosporium cladosporioides

Cladosporium sphaerospermum

Epicoccum nigrum

Eurotium amstelodami

Mucor racemosus

Paecilomyces variotii

Penicillum chrysogenum

Saccharomyces cerevisiae

Wallemia sebi

5 DNA from higher eukaryotes

Plants:

Zea mays (corn)

Pollen from *Pinus* spp. (pine)

Cotton (use leaves from cotton plant as source of DNA)

Arthropods:

Aedes aegypti (ATCC/CCL-125) mosquito cell line

Aedes albopictus (C6/36) mosquito

Dust mite (commercial source)

Flea (Rocky Mountain labs)

Drosophila cell line

Musca domestica (housefly; ARS, USDA, Fargo, ND)

Gypsy moth cell lines LED652Y cell line (baculovirus; Invitrogen)

Cockroach (commercial source)

Tick (*Amblyomma*)

Mammals:

Mus musculus (ATCC/HB-123) mouse

Rattus norvegicus (ATCC/CRL-1896) rat

Canis familiarus (ATCC/CCL-183) dog

Felis catus (ATCC/CRL-8727) cat

Homo sapiens (HeLa) human

Avian:

Chicken

6 Biological insecticides

B. thuringiensis subsp. *israelensis*

B. thuringiensis subsp. *kurstaki*

B. thuringiensis subsp. *morrisoni*

Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)

Cyd-X for coddling moths (Coddling moth granulosis virus)

Substances

1 Soils

Sandy

Loam

Clay

Subsoil

Silt

2 Dust

3 Powders and chemicals

Bacillus thuringiensis powders (e.g., Dipel)

Powdered milk

Powdered infant formula (Fe fortified)

Powdered infant formula (low Fe formulation)

Powdered coffee creamer

Powdered sugar

Talcum powder

Wheat flour

Baking soda

Chalk dust

Brewer's yeast

Dry wall dust

Cornstarch

Baking powder

GABA (Gama aminobutyric acid)

L-Glutamic acid

Kaolin

Chitin

Chitosan

$MgSO_4$

Boric acid

Powdered toothpaste

Popcorn salt

EDTA

ZEP

Rid-X

The Environmental Factors Panel was originally approved in parts. SPADA approved the environmental organisms panel on December 13, 2007, and revised it on September 17, 2008. The soils were approved on January 22, 2009. The powders and chemicals were originally approved by SPADA on December 13, 2007, and revised on January 22, 2009. The entire Environmental Factors Panel was approved in final form as presented here on June 2, 2010.

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STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS (SPADA)

Background & Fitness for Purpose

Francisella tularensis

Paul Keim, Northern Arizona University
Dave Wagner, Northern Arizona University
Scott Coates, AOAC INTERNATIONAL

Rockville, MD, USA.
September 1, 2015

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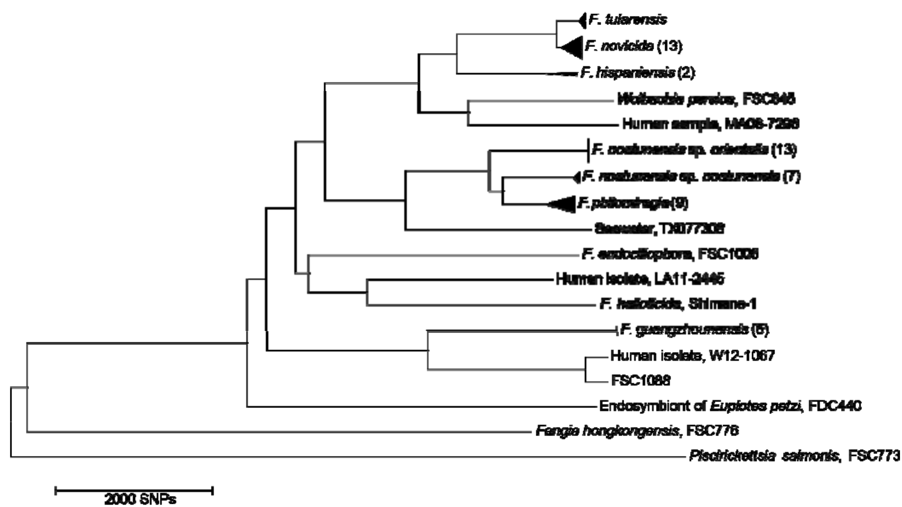

Whole genome SNP phylogeny of *Francisella* generated by the Swedish Defense Research Agency (FOI)

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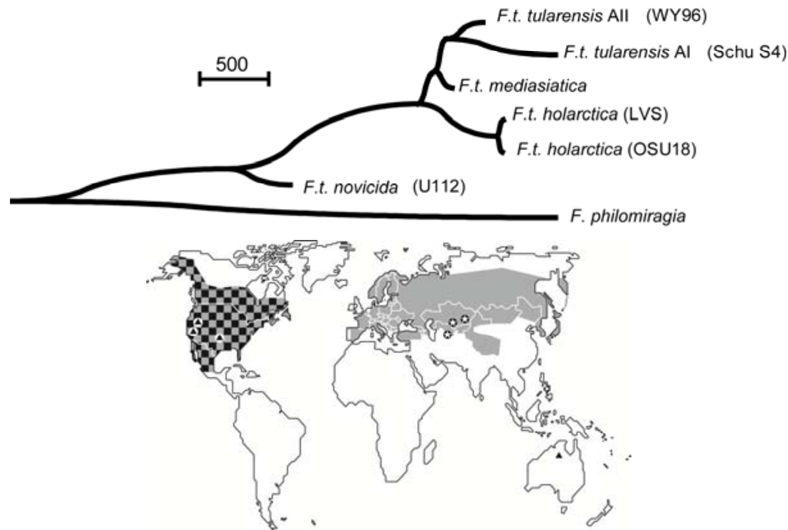

From Keim et al. 2007

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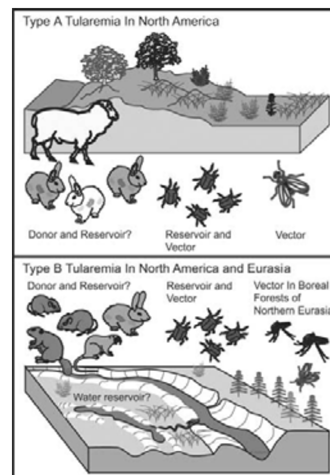
- *F. tularensis* subsp. *tularensis* (Type A): Common with high diversity – found naturally only in North America
- *F. tularensis* subsp. *holarctica* (Type B): Common with low diversity – found naturally throughout the northern hemisphere
- *F. tularensis* subsp. *mediasiatica*: Rare and reported only occasionally from Central Asia



From Keim et al. 2007

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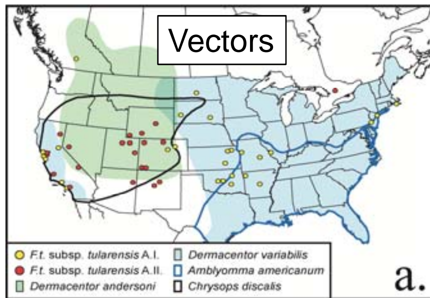

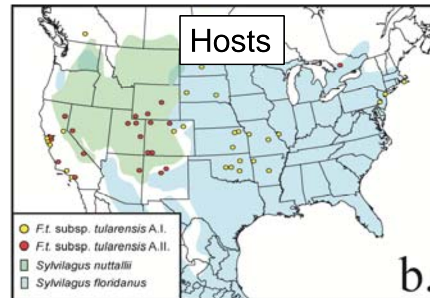

- Type A.I. found in eastern North America and west coast
- Type A.II. found in western North America
- These distributions are correlated with the distributions of specific mammal hosts and tick/fly vectors

From Farlow et al. 2005

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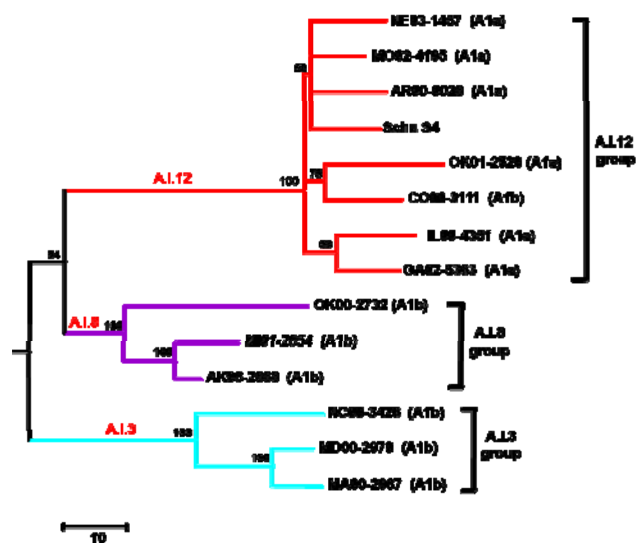

From Birdsell et al. 2014

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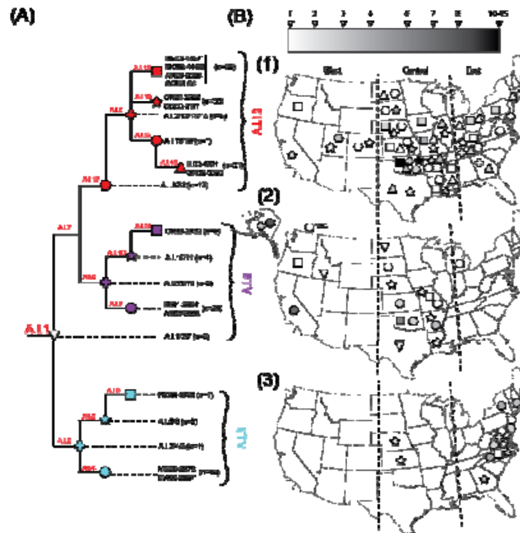

From Birdsell et al. 2014

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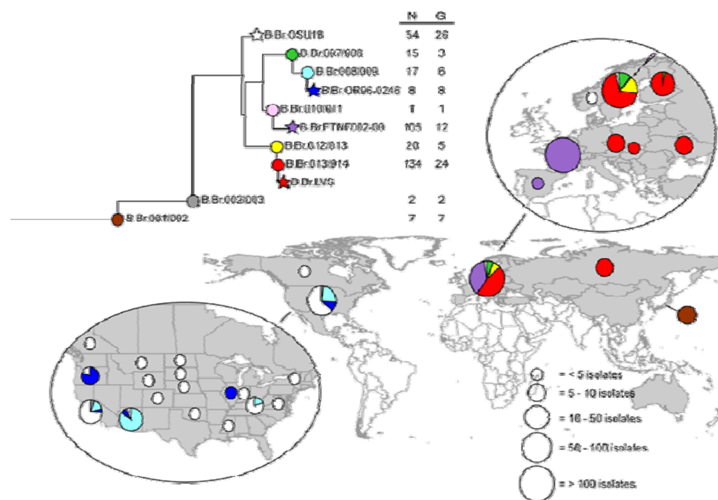

From Vogler et al. 2009

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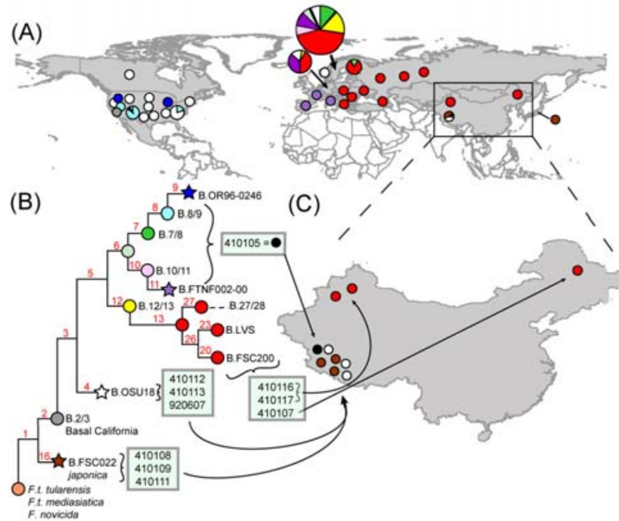

From Wang et al. 2014

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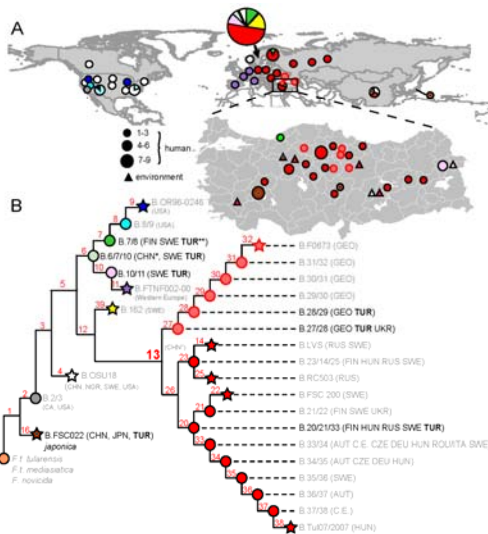

From Kilic et al. in press

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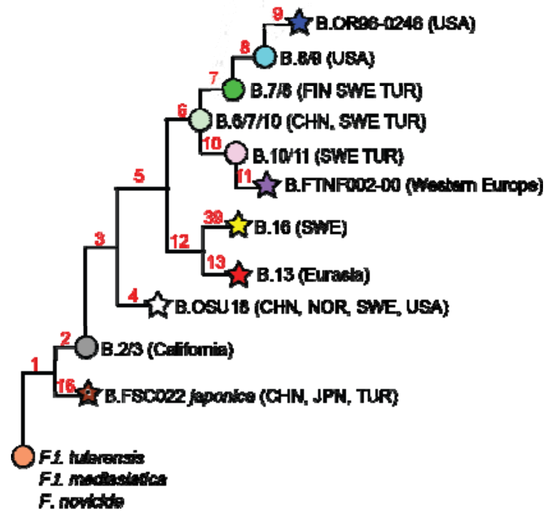

Current known phylogeographic patterns within *F. tularensis* subsp. *holarctica*

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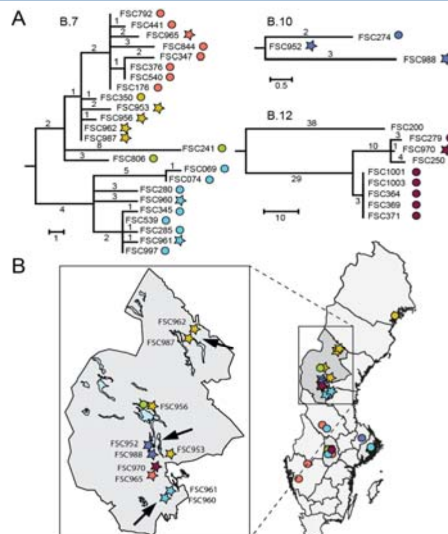

From Johansson et al. 2014

SUMMARY OF PREVIOUS SMPRS

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SMPR 2010.001

Polymerase Chain Reaction (PCR) Methods for Detection of *Francisella tularensis* in Aerosol Collection Filters and/or Liquids

- 2007-2009.
- Laboratory use for analysis of aerosol collection filters and/or liquids.
- AMDL 20,000 cells per filter; 2,000 cells per filter; 2,000 genome equivalents per ml.
- 9 FT strains.
- 10 near neighbors.
- Environmental factors panel:
  - 46 bacteria & viruses; 14 fungi; 32 eukaryotic DNA; & 6 biological insecticides.
  - 26 powders and chemicals.

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Fitness for Purpose (proposal)

Detection of *Francisella tularensis* in field-deployable, Department of Defense aerosol collection devices.

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Motion

Move to adopt the following Fitness-for-Purpose:

To develop standards for development of assays for detection of *Francisella tularensis* in field-deployable, Department of Defense aerosol collection devices.

STANDARD METHOD PERFORMANCE REQUIREMENTS

AOAC SMPR 2010.001

Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Francisella tularensis* in Aerosol Collection Filters and/or Liquids

Intended Use: Laboratory use for analysis of aerosol collection filters and/or liquids

Method Developer and Independent Validation

Probability of Detection at the Acceptable Minimum Detection Level

1 **Definitions**: Probability of detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given agent level or concentration. POD is concentration-dependent. The acceptable minimum detection level (AMDL) is the predetermined minimum level of a biological threat agent, which must be detected by the candidate method with an estimated 5% lower confidence limit on the POD of 0.95 or higher. The AMDL is dependent on the intended use.

2 **Test conditions**: AMDL is 20,000 standardized *Francisella tularensis* subsp. *tularensis* Schu-S4 cells per filter; 2000 standardized cells per mL; 2000 genome equivalents per mL.

3 **Acceptance criteria**: No more than one failure in 96 replicates.

Inclusivity

1 **Definition**: Strains or isolates or variants of the target agent(s) that the method can detect (Table 1).

2 **Test conditions**: Test inclusivity panel at AMDL.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a negative result, retest that strain 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher.*

Exclusivity

1 **Definition**: Nontarget agents, which are potentially cross-reactive, that are not detected by the method (Table 2).

2 **Test conditions**: Test exclusivity near neighbor panel at 10 times AMDL.

Received February 18, 2011.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a positive result, retest that strain 96 times with no failures allowed to demonstrate a 95% upper confidence limit on the POD of 0.05 or lower.*

Environmental Interference

1 **Definition**: Ability of the assay to detect target organism in the presence of nontarget organisms or environmental substances and to be free of cross-reaction from environmental organisms and substances (Appendix A).

2 **Test conditions**: Test pooled environmental panel organisms at 10 times AMDL in the presence or absence of *Francisella tularensis* subsp. *tularensis* Schu-S4 at the AMDL. Test environmental substances as suspensions in the presence or absence of *Francisella tularensis* subsp. *tularensis* Schu-S4 at the AMDL.

3 **Acceptance criteria**: 100% expected results for environmental organisms (i.e., no false negatives in the presence of *Francisella tularensis* subsp. *tularensis* Schu-S4, and no false positives in the absence of *Francisella tularensis* subsp. *tularensis* Schu-S4).

***Note**: In the case of an unexpected result, retest individual strains 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher. Data from environmental substances are for informational purposes only.*

Collaborative Validation Study

Reproducibility

1 **Definition**: Precision under conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

2 **Test conditions**: Test *Francisella tularensis* subsp. *tularensis* Schu-S4 at AMDL and near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per material per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Must produce at least 10 valid data sets. Report standard deviation of reproducibility (s_R).

POD at the AMDL Under Reproducibility Conditions (formerly termed System False-Negative Rate)

1 **Definition**: Rate of positive system results in a population of known positive test portions.

Table 1. *Francisella tularensis* PCR method: Inclusivity panel

No.	UCC[a] ID	Genus and species	Strain	Characteristics
FT1	FRAN001	*Francisella tularensis*	subsp. *tularensis*	Type A2 (Type strain)
FT2	FRAN004	*Francisella tularensis*	subsp. *holartica* (LVS)	Type B (Russian)
FT3	FRAN012	*Francisella tularensis*	subsp. *holartica*	Type B (United States)
FT4	FRAN016	*Francisella tularensis*	subsp. *tularensis* (SCHU S4)	Type A1 (United States)
FT5	FRAN024	*Francisella tularensis*	subsp. *holartica* JAP (Cincinnati)	Type B (Japanese)
FT6	FRAN025	*Francisella holartica*	subsp. *tularensis* (VT68)	Type B (United States)
FT7	FRAN029	*Francisella tularensis*	subsp. *holartica* (425)	Type B (United States)
FT8	FRAN031	*Francisella tularensis*	subsp. *tularensis* (Scherm)	Type A1 (United States)
FT9	FRAN072	*Francisella tularensis*	subsp. *tularensis* (WY96)	Type A2 (United States)

[a] UCC = Department of Defense Unified Culture Collection; components available through Biodefense and Emerging Infections Research Resources Repository.

Approved by AOAC SPADA on January 22, 2009.

2 **Test conditions**: Test *Francisella tularensis* subsp. *tularensis* Schu-S4 at AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for target agent must demonstrate an estimated 5% lower confidence limit on the CPOD of 0.95 or higher, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Table 2. *Francisella tularensis* PCR method: Exclusivity panel

No.	Species	Strain
FTNN1	*Francisella philomiragia*	Jensen O#319L ATCC 25015
FTNN2	*Francisella philomiragia*	Jensen O#319-029 ATCC 25016
FTNN3	*Francisella philomiragia*	Jensen O#319-036 ATCC 25017
FTNN4	*Francisella philomiragia*	Jensen O#319-067 ATCC 25018
FTNN5	*Francisella philomiragia*	D7533, GA012794
FTNN6	*Francisella philomiragia*	E9923, GA012801
FTNN7	*Francisella novicida*	D9876, GA993548
FTNN8	*Francisella novicida*	F6168, GA993549
FTNN9	*Francisella novicida*	U112, GA993550
FTNN10	*Wolbachia persica*	(Johns Hopkins)

Approved by AOAC SPADA on January 22, 2009.

POD in the Absence of Analyte Under Reproducibility Conditions (formerly termed System False-Positive Rate)

1 **Definition**: Rate of positive system results in a population of known negative test portions.

2 **Test conditions**: Test near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for near neighbor must demonstrate a 95% upper confidence limit on the CPOD of 0.05 or lower, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Acknowledgments

All or part of this work was funded by the Department of Homeland Security Science and Technology Directorate, award HSHQDC-08-C-00012.

AOAC SPADA approved PCR SMPRs as amended on January 22, 2009. PCR SMPRs (version 4) were revised on May 12, 2009 to reflect OMB proposal and to correct retest statistics. The final version as shown here was approved by SPADA on June 2, 2010 and contained revision to OMB requirement of 10 valid data sets for qualitative methods in the collaborative study.

Appendix A: Environmental Factors Panel

Organisms

1 Other biothreat agents

Bacillus anthracis Ames

Yersinia pestis Colorado-92

Burkholderia pseudomallei

Coxiella burnetii Nine Mile Phase I

Brucella melitensis

Ricinus communis (use ricin plant leaves as source of DNA)

Clostridium botulinum Type A

2 Cultivatable bacteria identified as being present in air and soil

Acinetobacter lwoffii

Agrobacterium tumefaciens

Bacillus cohnii

Bacillus psychrosaccharolyticus

Bacillus benzoevorans

Bacillus megaterium

Bacillus horikoshii

Bacillus macroides

Bacteroides fragilis

Burkholderia cepacia

Burkholderia gladoli

Burkholderia stabilis

Burkholderia plantarii

Chryseobacterium indologenes

Clostridium sardiniense

Clostridium perfringens

Deinococcus radiodurans

Delftia acidovorans

Escherichia coli K12

Fusobacterium nucleatum

Lactobacillus plantarum

Moraxella nonliquefaciens

Mycobacterium smegmatis

Neisseria lactamica

Pseudomonas aeruginosa

Rhodobacter sphaeroides

Riemerella anatipestifer

Shewanella oneidensis

Staphylococcus aureus

Stenotrophomonas maltophilia

Streptococcus pneumoniae

Streptomyces coelicolor

Synechocystis

Vibrio cholerae

Legionella pneumophila

Listeria monocytogenes

3 DNA viruses

Vaccinia virus (pox)

Adenovirus vaccine

Herpes simplex or CMV (whichever is available)

4 Microbial eukaryotes

Freshwater amoebae:

Acanthamoeba castellanii

Naegleria fowleri

Fungi:

Alternaria alternata

Aspergillus fumigatis

Aureobasidium pullulans

Cladosporium cladosporioides

Cladosporium sphaerospermum

Epicoccum nigrum

Eurotium amstelodami

Mucor racemosus

Paecilomyces variotii

Penicillum chrysogenum

Saccharomyces cerevisiae

Wallemia sebi

5 DNA from higher eukaryotes

Plants:

Zea mays (corn)

Pollen from *Pinus* spp. (pine)

Cotton (use leaves from cotton plant as source of DNA)

Arthropods:

Aedes aegypti (ATCC/CCL-125) mosquito cell line

Aedes albopictus (C6/36) mosquito

Dust mite (commercial source)

Flea (Rocky Mountain labs)

Drosophila cell line

Musca domestica (housefly; ARS, USDA, Fargo, ND)

Gypsy moth cell lines LED652Y cell line (baculovirus; Invitrogen)

Cockroach (commercial source)

Tick (*Ambylomma*)

Mammals:

Mus musculus (ATCC/HB-123) mouse

Rattus norvegicus (ATCC/CRL-1896) rat

Canis familiarus (ATCC/CCL-183) dog

Felis catus (ATCC/CRL-8727) cat

Homo sapiens (HeLa) human

Avian:

Chicken

6 Biological insecticides

B. thuringiensis subsp. *israelensis*

B. thuringiensis subsp. *kurstaki*

B. thuringiensis subsp. *morrisoni*

Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)

Cyd-X for coddling moths (Coddling moth granulosis virus)

Substances

1 Soils

Sandy

Loam

Clay

Subsoil

Silt

2 Dust

3 Powders and chemicals

Bacillus thuringiensis powders (e.g., Dipel)

Powdered milk

Powdered infant formula (Fe fortified)

Powdered infant formula (low Fe formulation)

Powdered coffee creamer

Powdered sugar

Talcum powder

Wheat flour

Baking soda

Chalk dust

Brewer's yeast

Dry wall dust

Cornstarch

Baking powder

GABA (Gama aminobutyric acid)

L-Glutamic acid

Kaolin

Chitin

Chitosan

$MgSO_4$

Boric acid

Powdered toothpaste

Popcorn salt

EDTA

Rid-X

ZEP

The Environmental Factors Panel was originally approved in parts. SPADA approved the environmental organisms panel on December 13, 2007, and revised it on September 17, 2008. The soils were approved on January 22, 2009. The powders and chemicals were originally approved by SPADA on December 13, 2007, and revised on January 22, 2009. The entire Environmental Factors Panel was approved in final form as presented here on June 2, 2010.

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STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS (SPADA)

Background & Fitness for Purpose

Yersina pestis

Dr. Robert Bull, Federal Bureau of Investigation
Scott Coates, AOAC INTERNATIONAL

Rockville, MD. USA.
September 1, 2015

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Other Pathogenic Yersinia

- *Yersinia enterocolitica*- gastroenteritis
  - 54 serogroups
  - Most human pathogenic O:3, 8, 9, 5, 27
  - ~20% Identical with pestis
- *Yersinia pseudotuberculosis*- gastroenteritis
  - Serogroups I-VI with subgroups in I-IV
  - Serogroup Ib most similar to plague
  - ~90% Identical with pestis

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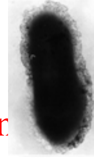
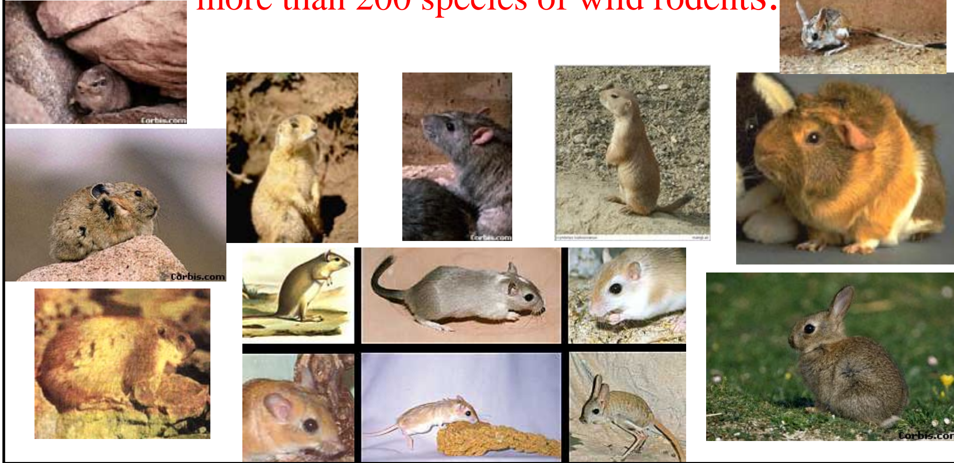


At present circulation of the plague pathogen *Yersinia pestis*, is endemic in populations of more than 200 species of wild rodents.



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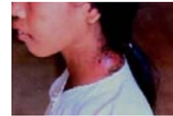
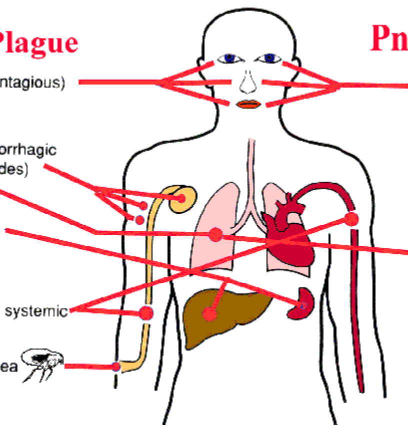
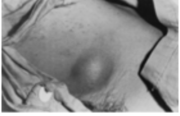


Yersinia pestis Infections (Plague)





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Devignat Classification

Biovar	Glycerol	Nitrate	Arabinose	Comment
Antiqua	+	+	+	Justinian plague AD 541-544 began in Egypt; 1st pandemic
Mediaevalis	+	-	+	Black Death 1347-1351 from central Asia to Europe; 2nd pandemic
Orientalis	-	+	+	Yunnan China 1855 global spread including Americas; 3rd pandemic
Microtus	+	-	-	New biovar described in China; virulent for rodents but avirulent for humans

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Russian Classification

Y. pestis subspecies	Rhamnose	Mellobiose	Arabinose	Devignat Classification[a]	pPla[b]	Virulence in guinea pigs[c]
pestis (epidemic)	-	-	+	A, M, O	+	+
altaica	+	+	-	M	+	-
caucasica	+	+	+	A	-	-
hissarica	+	+	-	M	+	-
ulegeica	+	+	+	M	+	-
talassica	+	+	-	M	+	-

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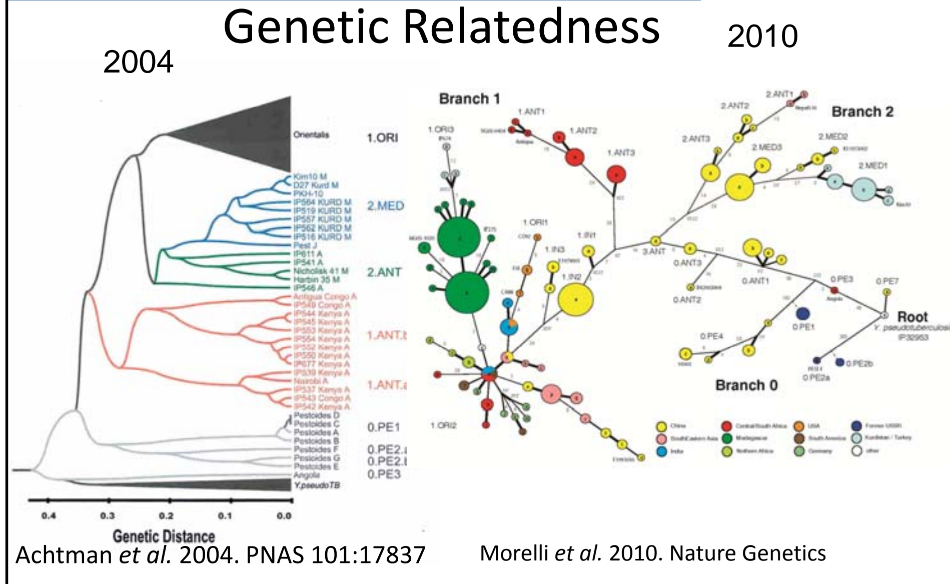
Genetic Relatedness

2004 2010



Achtman *et al.* 2004. PNAS 101:17837 Morelli *et al.* 2010. Nature Genetics

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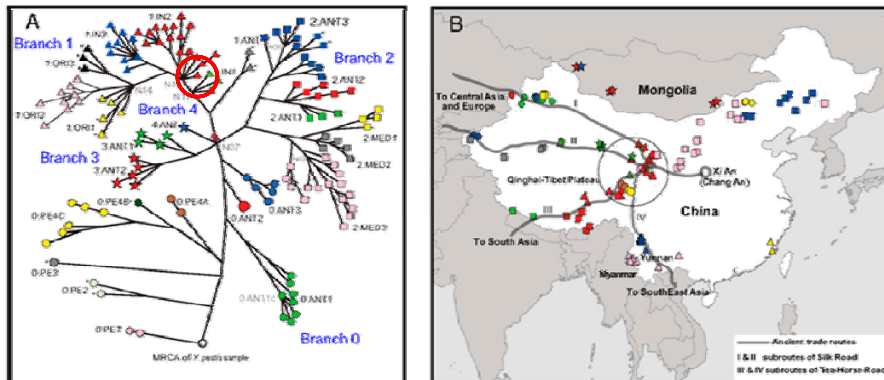
"Big Bang" Polytomy and the Black Death



Cui *et al.* 2013. PNAS 110:577-82

SUMMARY OF PREVIOUS SMPRS

SMPR 2010.002

Polymerase Chain Reaction (PCR) Methods for Detection of Y*ersina pestis* in Aerosol Collection Filters and/or Liquids

- 2007-2009.
- Laboratory use for analysis of aerosol collection filters and/or liquids.
- AMDL 20,000 cells per filter; 2,000 cells per filter; 2,000 genome equivalents per ml.
- 16 YP strains.
- 17 near neighbors.
- Environmental factors panel:
  - 46 bacteria & viruses; 14 fungi; 32 eukaryotic DNA; & 6 biological insecticides.
  - 26 powders and chemicals.

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Fitness for Purpose (proposal)

Detection of *Yersnia perstis* in field-deployable, Department of Defense aerosol collection devices.

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Motion

Move to adopt the following Fitness-for-Purpose:

Motion to develop standards for the detection of *Yersnia pestis* in field-deployable, Department of Defense aerosol collection devices.

STANDARD METHOD PERFORMANCE REQUIREMENTS

AOAC SMPR 2010.002

Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Yersinia pestis* in Aerosol Collection Filters and/or Liquids

Intended Use: Laboratory use for analysis of aerosol collection filters and/or liquids

Method Developer and Independent Validation

Probability of Detection at the Acceptable Minimum Detection Level

1 **Definitions**: Probability of detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given agent level or concentration. POD is concentration-dependent. The acceptable minimum detection level (AMDL) is the predetermined minimum level of a biological threat agent, which must be detected by the candidate method with an estimated 5% lower confidence limit on the POD of 0.95 or higher. The AMDL is dependent on the intended use.

2 **Test conditions**: AMDL is 20,000 standardized *Yersinia pestis* CO-92 cells per filter; 2000 standardized cells per mL; 2000 genome equivalents per mL.

3 **Acceptance criteria**: No more than one failure in 96 replicates.

Inclusivity

1 **Definition**: Strains or isolates or variants of the target agent(s) that the method can detect (Table 1).

2 **Test conditions**: Test inclusivity panel at AMDL.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a negative result, retest that strain 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher.*

Exclusivity

1 **Definition**: Nontarget agents, which are potentially cross-reactive, that are not detected by the method (Table 2).

2 **Test conditions**: Test exclusivity near neighbor panel at 10 times AMDL.

3 **Acceptance criteria**: 100% expected results as defined for each strain on the panel.

***Note**: In the case of a positive result, retest that strain 96 times with no failures allowed to demonstrate a 95% upper confidence limit on the POD of 0.05 or lower.*

Environmental Interference

1 **Definition**: Ability of the assay to detect target organism in the presence of nontarget organisms or environmental substances and to be free of cross-reaction from environmental organisms and substances (Appendix A).

2 **Test conditions**: Test pooled environmental panel organisms at 10 times AMDL in the presence or absence of *Yersinia pestis* CO-92 at the AMDL. Test environmental substances as suspensions in the presence or absence of *Yersinia pestis* CO-92 at the AMDL.

3 **Acceptance criteria**: 100% expected results for environmental organisms (i.e., no false negatives in the presence of *Yersinia pestis* CO-92, and no false positives in the absence of *Yersinia pestis* CO-92).

***Note**: In the case of an unexpected result, retest individual strains 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher. Data from environmental substances are for informational purposes only.*

Collaborative Validation Study

Reproducibility

1 **Definition**: Precision under conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

2 **Test conditions**: Test *Yersinia pestis* CO-92 at AMDL and near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per material per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Must produce at least 10 valid data sets. Report standard deviation of reproducibililty (s_R).

POD at the AMDL Under Reproducibility Conditions (formerly termed System False-Negative Rate)

1 **Definition**: Rate of positive system results in a population of known positive test portions.

2 **Test conditions**: Test *Yersinia pestis* CO-92 at AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At

Received February 18, 2011.

Table 1. *Yersinia pestis* PCR method: Inclusivity panel

No.	Strain	Biovar	Achtman genotype	Comment	Availability[a]
YP1	CO92	O	1.ORI.c	Well-studied example of epidemic strain of pestis, recent isolate	CDC, WRAIR, USAMRIID
YP2	KIM	M	2.Med	Well-studied strain in academic circles, virulence data extensive	CDC, WRAIR, USAMRIID
YP3	Antiqua	A	1.Ant b	Ancient strain near root of tree	CDC, WRAIR, USAMRIID
YP4	Pestoides B	M	0.PE1		CDC, WRAIR, USAMRIID
YP5	Pestoides F	A	0.PE2.a	pPst negative, old strain in terms of phylogeny	CDC, WRAIR, USAMRIID
YP6	Pestoides G	A	0.PE2.b	pPst negative	CDC, WRAIR, USAMRIID
YP7	Angola	A	0.PE3	A "pestoides" in everything except name	CDC, WRAIR, USAMRIID
YP8	Nairobi	A	1.Ant a		CDC, WRAIR, USAMRIID
YP9	Harbin35	?	2 Ant	Rumored to be used or resulted from infection during experiments by Japanese BW Unit 731	CDC, WRAIR, USAMRIID
YP10	PBM19	O	1.ORI.a		CDC, WRAIR, USAMRIID
YP11	Java9	O	1.ORI	pFra negative	CDC, WRAIR, USAMRIID
YP12	A1122	O	1.ORI.a	Well-characterized U.S. isolate that is pgm- and pCD-; also has 2X large pPst plasmid	CDC, WRAIR, USAMRIID
YP13	Nicholisk 41	M	2.ANT		CDC, WRAIR, USAMRIID
YP14	Shasta		1.ORI	YE0387; SHASTA (20 OCT 54); SHASTA; HUMAN CASE; USA: CA; 1960 6LY; UCC YERS074	CDC, USAMRIID
YP15	Dodson		1.ORI	DODSON (AUG 70); HUMAN CASE: Male age 4.5 years; USA: Arizona (Tuba City); 27 JUN 67; UCC YERS073	CDC, USAMRIID
YP16	El Dorado				

[a] CDC = Centers for Disease Control and Prevention; WRAIR = Walter Reed Army Institute of Research; USAMRIID = The United States Army Medical Research Institute for Infectious Diseases.

Version 6 approved by AOAC SPADA on January 22, 2009.

Table 2. *Yersinia pestis* PCR method: Exclusivity panel

No.	Species	Strain	Serotype	Comment	Availability[a]
YPNN1	*Yersinia ruckeri*	YERS063			USAMRIID
YPNN2	*Yersinia rohdei*	YERS062			USAMRIID
YPNN3	*Yersinia pseudotuberculosis*	PB1/+	1	Sequenced	WRAIR
YPNN4	*Yersinia pseudotuberculosis*	IP32953	1	Sequenced	WRAIR
YPNN5	*Yersinia pseudotuberculosis*	YPIII	3	Sequenced	WRAIR
YPNN6	*Yersinia pseudotuberculosis*	Pa3606	1b		WRAIR
YPNN7	*Yersinia pseudotuberculosis*	IB	1b		WRAIR
YPNN8	*Yersinia pseudotuberculosis*	EP2/+	1		WRAIR
YPNN9	*Yersinia pseudotuberculosis*	MD67	1		WRAIR
YPNN10	*Yersinia pseudotuberculosis*	1	1a		WRAIR
YPNN11	*Yersinia enterocolitica*	WA	O:8		WRAIR
YPNN12	*Yersinia enterocolitica*	8081	O:8	Sequenced	WRAIR
YPNN13	*Yersinia enterocolitica*	2516-87	O:9		WRAIR
YPNN14	*Yersinia kirstensenii*	Y231		Nonpathogenic	WRAIR
YPNN15	*Yersinia frederiksenii*	Y225		Nonpathogenic	WRAIR
YPNN16	*Yersinia intermedia*	Y228		Nonpathogenic	WRAIR
YPNN17	*Yersinia aldovae*	670-83		Nonpathogenic	WRAIR

[a] WRAIR = Walter Reed Army Institute of Research; USAMRIID = The United States Army Medical Research Institute for Infectious Diseases.

Version 6 approved by AOAC SPADA on January 22, 2009.

least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for target agent must demonstrate an estimated 5% lower confidence limit on the CPOD of 0.95 or higher, where CPOD is the probability of detection calculated from pooled valid collaborative data.

POD in the Absence of Analyte Under Reproducibility Conditions (formerly termed System False-Positive Rate)

1 **Definition**: Rate of positive system results in a population of known negative test portions.

2 **Test conditions**: Test near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 **Acceptance criteria**: Data for near neighbor must demonstrate a 95% upper confidence limit on the CPOD of 0.05 or lower, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Acknowledgments

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AOAC SPADA approved PCR SMPRs as amended on January 22, 2009. PCR SMPRs (version 4) were revised on May 12, 2009 to reflect OMB proposal and to correct retest statistics. The final version as shown here was approved by SPADA on June 2, 2010 and contained revision to OMB requirement of 10 valid data sets for qualitative methods in the collaborative study.

Appendix A: Environmental Factors Panel

Organisms

1 Other biothreat agents

Bacillus anthracis Ames

Francisella tularensis subsp. *tularensis* Schu-S4

Burkholderia pseudomallei

Coxiella burnetii Nine Mile Phase I

Brucella melitensis

Ricinus communis (use ricin plant leaves as source of DNA)

Clostridium botulinum Type A

2 Cultivatable bacteria identified as being present in air and soil

Acinetobacter lwoffii

Agrobacterium tumefaciens

Bacillus cohnii

Bacillus psychrosaccharolyticus

Bacillus benzoevorans

Bacillus megaterium

Bacillus horikoshii

Bacillus macroides

Bacteroides fragilis

Burkholderia cepacia

Burkholderia gladoli

Burkholderia stabilis

Burkholderia plantarii

Chryseobacterium indologenes

Clostridium sardiniense

Clostridium perfringens

Deinococcus radiodurans

Delftia acidovorans

Escherichia coli K12

Fusobacterium nucleatum

Lactobacillus plantarum

Moraxella nonliquefaciens

Mycobacterium smegmatis

Neisseria lactamica

Pseudomonas aeruginosa

Rhodobacter sphaeroides

Riemerella anatipestifer

Shewanella oneidensis

Staphylococcus aureus

Stenotrophomonas maltophilia

Streptococcus pneumoniae

Streptomyces coelicolor

Synechocystis

Vibrio cholerae

Legionella pneumophila

Listeria monocytogenes

3 DNA viruses

Vaccinia virus (pox)

Adenovirus vaccine

Herpes simplex or CMV (whichever is available)

4 Microbial eukaryotes

Freshwater amoebae:

Acanthamoeba castellanii

Naegleria fowleri

Fungi:

Alternaria alternata

Aspergillus fumigatis

Aureobasidium pullulans

Cladosporium cladosporioides

Cladosporium sphaerospermum

Epicoccum nigrum

Eurotium amstelodami

Mucor racemosus

Paecilomyces variotii

Penicillum chrysogenum

Saccharomyces cerevisiae

Wallemia sebi

5 DNA from higher eukaryotes

Plants:

Zea mays (corn)

Pollen from *Pinus* spp. (pine)

Cotton (use leaves from cotton plant as source of DNA)

Arthropods:

Aedes aegypti (ATCC/CCL-125) mosquito cell line

Aedes albopictus (C6/36) mosquito

Dust mite (commercial source)

Flea (Rocky Mountain labs)

Drosophila cell line

Musca domestica (housefly; ARS, USDA, Fargo, ND)

Gypsy moth cell lines LED652Y cell line (baculovirus; Invitrogen)

Cockroach (commercial source)

Tick (*Amblyomma*)

Mammals:

Mus musculus (ATCC/HB-123) mouse

Rattus norvegicus (ATCC/CRL-1896) rat

Canis familiarus (ATCC/CCL-183) dog

Felis catus (ATCC/CRL-8727) cat

Homo sapiens (HeLa) human

Avian:

Chicken

6 Biological insecticides

B. thuringiensis subsp. *israelensis*

B. thuringiensis subsp. *kurstaki*

B. thuringiensis subsp. *morrisoni*

Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)

Cyd-X for coddling moths (Coddling moth granulosis virus)

Substances

1 Soils

Sandy

Loam

Clay

Subsoil

Silt

2 Dust

3 Powders and chemicals

Bacillus thuringiensis powders (e.g., Dipel)

Powdered milk

Powdered infant formula (Fe fortified)

Powdered infant formula (low Fe formulation)

Powdered coffee creamer

Powdered sugar

Talcum powder

Wheat flour

Baking soda

Chalk dust

Brewer's yeast

Dry wall dust

Cornstarch

Baking powder

GABA (Gama aminobutyric acid)

L-Glutamic acid

Kaolin

Chitin

Chitosan

$MgSO_4$

Boric acid

Powdered toothpaste

Popcorn salt

EDTA

ZEP

Rid-X

The Environmental Factors Panel was originally approved in parts. SPADA approved the environmental organisms panel on December 13, 2007, and revised it on September 17, 2008. The soils were approved on January 22, 2009. The powders and chemicals were originally approved by SPADA on December 13, 2007, and revised on January 22, 2009. The entire Environmental Factors Panel was approved in final form as presented here on June 2, 2010.

Bacillus anthracis SMPR History

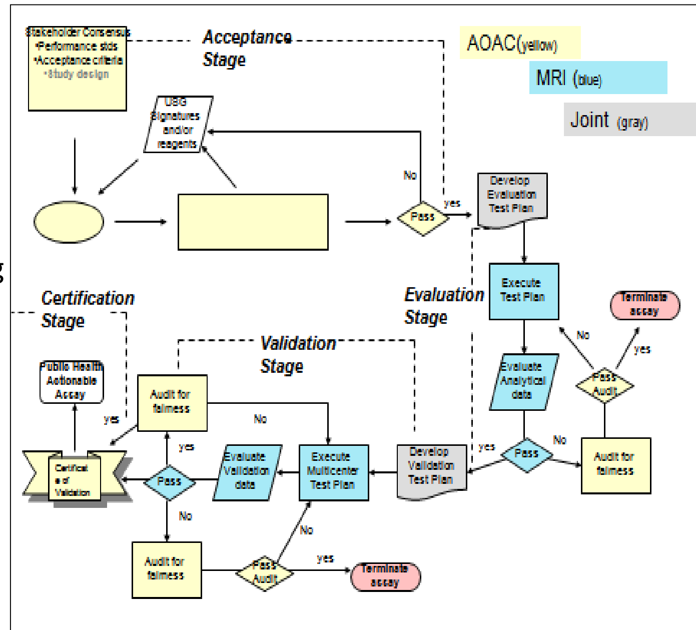
Matt Davenport, DHS
Scott Coats, AOAC
Paul Jackson, LLNL
Ted Hadfield, HADECO, LLC

In the Beginning (Matt/Scott)

- Need for BA SMPR
- Directives
- Definitions
- Other useful information
  - ISO or other Guidelines for testing and reference material production

Test Design

- Three Distinct Phases
  - Developer Testing
  - Independent Laboratory Testing
  - Multicenter Testing



Developer Study

- This study will evaluate PCR-based methods for detection of *Bacillus anthracis* spores intended for use with aerosol collection samples. The PCR-based methods are systems using either filter or liquid air collection technology and are intended for laboratory use only. Candidate method instructions must include procedures for recovery of spores from the claimed matrix, DNA extraction and PCR. Filter recovery DNA extraction of spores may be done with surrogate organisms as the developer will likely not have access to *Bacillus anthracis.* PCR testing will likely be done with DNA from *Bacillus anthracis* obtained by the developer from a select agent laboratory

Independent Laboratory Validation

- The independent validation verifies the method performance observed in the method developer's laboratory using viable Bacillus anthracis cultures. The independent laboratory assessed method performance including
  - 1) probability of detection (POD) at the acceptable minimum detection level (AMDL) or lower
  - 2) inclusivity
  - 3) exclusivity
  - 4) environmental interference
    - Certified reference materials were used for POD, repeatability, dynamic range and specificity testing. Other reference materials are used for inclusivity, exclusivity and environmental factors testing.
- Robustness testing (instruments and people)
- Shelf life testing of Developer Kits

Multicenter Validation Testing

- Identify test centers
- Assure compliance with CDC regulations and Federal law
- Identify individuals not associated with developer or independent laboratory to do the testing.
  - Determine the acceptable skill of testers
  - Determine when testing will be done
  - Assure administrators, safety officers and support staff are qualified, aware and support the project
  - Have a well vetted test plan in place with easy data recording instructions
  - Assure samples can be sent to receiving laboratories
  - level Randomize and blind code the subsamples
  - Conduct testing over a short period of time if possible
  - Record results (pos or neg) for each primer pair and each sample

Reference Materials

- Any material being used to qualify the "assay(s)"
  - Filters or liquid from liquid samplers (if not standardized)
  - Soils used to challenge prospective "assay(s)"
  - Other matrices identified as a matrix for which the assay will be validated
  - Cultures used to challenge the assay(s)
- Certified or Noncertified Reference Materials
- What should be a reference material
  - Key organisms in the inclusivity panel and exclusivity panel
  - Matrices of interest (air samples or air sample equivalents)
- Suggested Reference Material for Air sample testing-
  - Dust from air conditioners, air conditioner chases, filters from commercial and residential buildings in multiple locations. Dust should be tested by existing test methods to determine cross reactivity for any *B. anthracis* targets
  - Accepted dust was sieved to get multiple samples of dust from distinct origins
  - Each was tested by culture and PCR currently being used for BA detection (3 distinct reactions)
  - Equal volumes of each dust were homogenized for 72 hours in a rolling drum
  - Homogenized dust was tested using PCR and culture for BA
  - Homogenized dust was placed in 100 ml serum bottles, frozen and lyophilized
  - The dust was characterized for particle size and background organisms
  - This SMPR had
    - 15 B. anthracis reference cultures (1 certified)
    - 20 other Bacillus cultures (1 certified)
    - 87 environmental biological sample DNAs (0 certified)
    - 25 soils (0 certified)
    - 22 powders and chemicals (0 certified)

Bacillus anthracis Inclusivity Strains

Number	Strain	Characteristics
BA1	Canadian bison	pXO1+ , pXO2+, VNTR genotype group A1a
BA2	V770-NP-1R	pXO1+ , pXO2-, VNTR genotype group A1b
BA3	PAK-1	pXO1+ , pXO2+, VNTR genotype group A2
BA4	BA1015	pXO1+ , pXO2+, VNTR genotype group A3a
BA5	Ames	pXO1+ , pXO2+, VNTR genotype group A3b
BA6	K3	pXO1+, pXO2+, VNTR genotype group A3c
BA7	Ohio ACB	pXO1+, pXO2+, VNTR genotype group A3d
BA8	SK-102 (Pakistan)	pXO1+ , pXO2+, VNTR genotype group A4
BA9	Vollum 1B	pXO1+ , pXO2+, VNTR genotype group A4
BA10	BA1035	pXO1+ , pXO2+, VNTR genotype group B1
BA11	RA3	pXO1+, pXO2+, VNTR genotype group B2
BA12	2002013094 (240)	pXO1+, pXO2+, VNTR genotype group C
BA13	Pasteur	pXO1- , pXO2+; VNTR genotype group A1a
BA14	Sterne	pXO1+ , pXO2-, VNTR genotype group A3b
BA15	Turkey #32	pXO1+, pXO2+, VNTR genotype group A1b

- The study included DNA from 15 *B. anthracis* strains tested at the AMDL (2000 genome equivalents/mL or 11 pg/mL) or lower and DNA from 20 near neighbor organisms tested at 10 times the AMDL, or 110 pg/mL

Bacillus anthracis Exclusivity Near Neighbors

Number	Species	Strain	Plasmid Status
NN1	B. cereus	S2-8	pXO1-, pXO2-
NN2	B. cereus	3A	pXO1-, pXO2-
NN3	B. thuringiensis	HD1011	pXO1-, pXO2-
NN4	B. thuringiensis	97-27	pXO1-, pXO2-
NN5	B. thuringiensis	HD682	pXO1-, pXO2-
NN6	B. cereus	E33L	pXO1-, pXO2-
NN7	B. cereus	D17	pXO1-, pXO2-
NN8	B. thuringiensis	HD571	pXO1-, pXO2-
NN9	Bacillus Al Hakam		pXO1-, pXO2-
NN10	B. cereus	ATCC 4342	pXO1-, pXO2-
NN11	B. cereus	FM1	pXO1-, pXO2-
NN12	B. cereus	G9241	pBCXO1+*, pXO2-
NN13	B. cereus	03BB102	pXO1+**, capA+, capB+, capC+***
NN14	B. cereus	03BB108	pXO1+**, capA+, capB+, capC+***
NN15	B. thuringiensis	subsp. Israelensis	pXO1-, pXO2-
NN16	B. thuringiensis	subsp. kurstaki	pXO1-, pXO2-
NN17	B. thuringiensis	Subsp. Morrisoni	pXO1-, pXO2-
NN18	B. coagulans	ATCC 7050	pXO1-, pXO2-
NN19	B. mycoides	ATCC 6462	pXO1-, pXO2-
NN20	B. megaterium	ATCC 12872	pXO1-, pXO2-

*pBCXO1 is pXO1-like, but not identical.
** Not all of the pXO1 plasmid is present in these two isolates. *B. cereus* 03BB102 is missing parts of the plasmid near ORF's 12 through 37, near ORF's 84 through 89 and near ORF 115 among others. *B. cereus* 03BB108 is missing even more extensive regions of pXO1.
***capA, B and C are contained within the pXO2 plasmid of *B. anthracis*, and are found in *B. cereus* strains 03BB102 and 03BB108.

Environmental Panel

7 Agents	37 "Air and Soil" (continued)	5 Viruses	4 Plants
Brucella melintensis	*Clostridium perfringens*	Herpes simplex 1[3]	Pine
Burkholderia pseudomallei	*Clostridium sardiniense*	Adenovirus 14	Corn
Clostridium botulinum	*Deinococcus radiodurans*	Gypsy moth virus (Gypcheck)	Cotton
Coxiella brunetii	*Delftia acidovorans*	Vaccinia virus (pox)	Ricin
Yersinia pestis Colorado-92	*Escherichia coli*	Cyd-X Product	**9 Arthropods**
Francisella tularensis Schu-S4	*Fusobacterium nucleatum*	**2 Amoebae**	Cockroach
Rickettsia prowazekii[1]	*Lactobacillus plantarum*[2]	*Acanthamoeba castellanii*	*Aedes albopictus* (mosquito)
37 "Air and Soil"	*Legionella pneumophila*	*Naegleria fowleri*	Fruit fly
Acinetobacter lwoffii	*Listeria monocytogenes*	**11 Fungi[4]**	Gypsy moth
Agrobacterium tumefaciens	*Moraxella nonliquefaciens*	*Alternaria alternata*	Tick
Bacillus benzoevorans	*Mycobacterium smegmatis*	*Aspergillus fumigatus*	Flea
Bacillus cohnii	*Neisseria lactamica*	*Aureobasidium pullulans*	House fly
Bacillus horikoshii	*Pseudomonas aeroginosa*	*Cladosporium cladosporioides*	Dust mite
Bacillus macroides[2]	*Rhodobacter sphaeroides*[2]	*Cladosporium sphaerospermum*	*Aedes aegypti* (mosquito)
Bacillus megaterium	*Riemerella anatipestifer*	*Epicoccum nigrum*	**6 Mammalian/Avian Cell Lines**
Bacillus psychrosaccharolyticus	*Shewanella oneidensis*[2]	*Eurotium amstelodami*	Mouse
Bacteroides fragilis[2]	*Staphylococcus aureus*	*Mucor racemosus*	Rat
Burkholderia cepacia	*Stenotophomonas maltophilia*	*Paecilomyces variotii*	Dog
Burkholderia gladioli	*Streptococcus pneumoniae*	*Penicillium chrysogenum*	Cat
Burkholderia plantarii	*Streptomyces coelicolor*	*Wallernia sebi*	Human
Burkholderia stabilis	*Synechocystis*		Chicken
Chryseobacterium indologenes	*Vibrio cholerae*		
Clostridium butyricum[2]			

Total of 81 biological entities in the Environmental panel

Dust Characterization

- Dust has been checked for cross reactive signatures with MRI QA assays (LF, CapB, DHP7, chromosomal marker H1)-non-reactive
- Dust was checked for inhibition effects-not inhibitory at 0.1 g using MRI extraction method
- LOD of spiked *B. anthracis* in dust extract is 600 genome equivalent copies (LF assay,)
- Dust spiked with 600 spores tested positive for target (Ct= 36.7±0.45, N=50)

Reference Dust (SRD)

Dust Application Device

0.1g ±0.023g for more than 300 filters

Reference Dust

•Dust was pooled and dispensed into 244 $\times$ 50g aliquots. Aliquots were lyophilized, labeled and stored.

Filters for Testing
Vacuum sealed in Mylar Bags

Filter Stability Testing

- Sterne spores (10,000 cfu, 20,000 cfu) spiked on matrix loaded filters
  - sealed and stored at room temperature or at 45°C
  - baseline established (DNA signature and CFU counts)
  - testing monthly-stable for 12 months +

Soils

- Soils were collected from five geographic locations across the United States
  - Washington DC/Silver Spring
  - El Paso
  - Houston
  - Seattle
  - Kansas
  - Not all sites had all types of soils (loam, sandy loam, sand, clay, subsoil)

Soil Characterization

- Soils were sifted to remove debris
  - Tested for PCR inhibition extract from a 1 g extraction of soil- spiked with 1 pg; Spike blank N=1; Ct=34.9, soil extract N=24; Ct=34.4±0.23
  - No signature cross reactivity in the soils using MRI QA PCR reagents for *Bacillus anthracis*
  - DNA sent to University of Houston for Metagenomic sequencing
  - Soils analysis characterization was completed
  - Soils were blended to make Master Lots

Composition Testing of Pooled Soils

Sample ID	soil pH	% H2O	% TOC	CEC (me/100g)	density (g/cc)	Ca mg/kg	K mg/kg	Mg mg/kg
Sand	7.65	1.12	0.53	2.9	1.27	3266	102	132
Silt	7.20	1.56	0.41	8.4	1.01	1007	164	402
Clay	6.65	4.17	1.15	24.1	0.96	4164	341	742
Loam	7.65	3.79	1.47	10.6	1.00	8425	200	308
Subsoil	7.65	2.71	0.78	7.8	1.13	4117	113	207
Check Soil SS09								
measured->	5.25	1.75	------	11.5	1.08	800	250	84
normal range ->	5.1-5.3	------	------	*11-12*	*1.07-1.12*	*780-840*	*235-265*	*80-95*

Sample ID	Na mg/kg	Al mg/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	P mg/kg	S mg/kg
Sand	21	8.0	0.27	3.1	12.4	1.2	6.2	12
Silt	27	39	2.88	36.1	6.4	1.1	3.5	118
Clay	179	32	0.54	18.2	90.5	9.0	9.4	344
Loam	28	22	1.07	4.3	52.8	1.3	9.2	44
Subsoil	35	21	1.01	2.6	7.9	11.2	1.5	37
Check Soil SS09								
measured->	14.3	30	0.94	1.3	164	1.0	9.1	75
normal range ->	*13-19*	*29-34*	*0.8-1.0*	*0.9-1.9*	*160-180*	*1.0-1.3*	*8.5-10.0*	*68-78*

Soil Processing

- Approximately 45 Lbs. of usable soils from each state
- Stored at 4C

Powders and Chemicals

Interferent	Ames	E33L	Blank	Ames	E33L	Blank	Ames	E33L	Blank	Ames	E33L	Blank
Expected Response	**Pos**	**Neg**	**Neg**	**Pos**	**Neg**	**Neg**	**Pos**	**Neg**	**Neg**	**Pos**	**Neg**	**Neg**
Bacillus thuringiensis	10	10	2	10	0	2	10	0	2	10	0	2
Powdered Milk	10	10	2	10	0	2	10	0	2	10	0	2
Powered Coffee Creamer	10	10	2	10	0	2	10	0	2	10	0	2
Powdered Sugar	10	10	2	10	0	2	10	0	2	7	0	2
Talcom Powder	10	10	2	10	0	2	10	0	2	9	0	2
Flour	10	10	2	10	0	2	10	0	2	9	0	2
Baking Soda	10	10	2	10	0	2	10	0	2	8	0	2
Chalk Dust	10	10	2	10	0	2	10	0	2	9	0	1
Brewer's Yeast	10	10	2	10	0	2	10	0	2	9	0	2
Dry Wall Dust	10	10	2	10	0	2	10	0	2	7	0	2
Cornstarch	10	10	2	10	0	2	10	0	2	7	0	2
Baking Powder	10	10	2	10	0	2	10	0	2	10	0	2
GABA	10	10	2	10	0	2	10	0	2	10	0	2
L-Glutamic acid	10	10	2	10	0	2	10	0	2	8	0	2
Kaolin	10	10	2	10	0	2	10	0	2	7	0	0
Chitin	10	10	2	10	0	2	10	0	2	6	0	2
Chitosan	10	10	2	10	0	2	10	0	2	9	0	2
MgSO4	10	10	2	10	0	2	10	0	2	6	0	2
Boric Acid	10	10	2	10	0	2	10	0	2	3	1	2
Powdered Toothpaste	10	10	2	10	0	2	10	0	2	6	1	2
Popcorn Salt	10	10	2	10	0	2	10	0	2	5	0	2
Standardized Dust	10	10	2	10	0	2	10	0	2	9	3	2

Data Recording

- What type of data will be recorded
- How many markers must be positive to call the sample positive
- How many markers can be present for in a negative sample
    - Considerations for pX01 positive, pX02 negative and vice versa

Lessons Learned

- Its easy to make a big list of “reference materials” but you need to be practical
  - Decide on the type of reference materials needed for the project
  - Are the materials available
  - How difficult are they to grow and store
  - Can you obtain sufficient quantities to use them as a reference material
  - What acceptance criteria will be used to qualify the reference material
  - Do you need multiple forms of the reference material (cells and DNA)
  - How stable will the reference material be
  - What steps will be taken to replace reference materials when they are exhausted or no longer give acceptable results

Questions and Discussion

SEPTEMBER 1-2, 2015
STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

RESOURCES

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AOAC Website: http://www.aoac.org

SPADA Website: http://bit.ly/1Hmf6ba

SPADA Working Group Sign Up: http://www.jotform.us/form/52364156405149

AOAC Acronyms and abbreviations

AMDL	acceptable minimum detection level
AOAC	AOAC INTERNATIONAL (AOAC formerly stood for *Association of Official Analytical Chemists*, but long-name no longer used)
CSO	chief scientific officer
ERP	expert review panel
ISO	International Organization for Standardization
LOD	limit of detection
LPOD	laboratory probability of detection
NGO	non-governmental organization
OMA	*Official Methods of Analysis*, frequently pronounced like “o maa”
POD	probability of detection
SPADA	Stakeholder Panel on Agent Detection Assays
SMPR	Standard Method Performance Requirements, frequently pronounced as in “smipper”.

Name	Organization
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David Hodge, SPADA Member	Department Of Homeland Security
Jeffery Hogan, SPADA Member	ATEC, Life Sciences Division, Dugway Proving Ground
Sally Hojvat, SPADA Member	FDA, CDRH
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