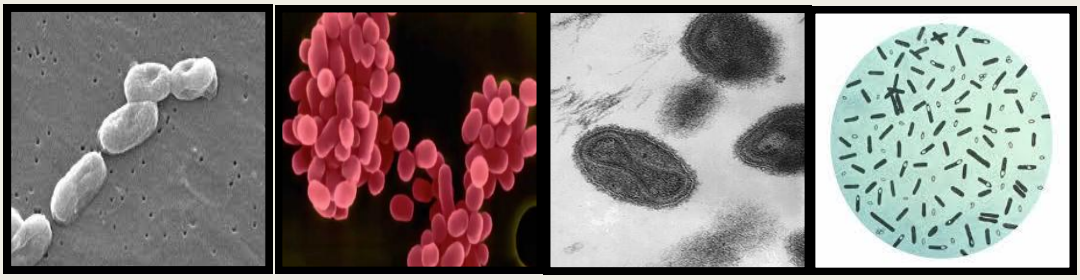
AUGUST 30, 2016

AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

Stakeholder Panel on Agent Detection Assays [SPADA]



Stakeholder Panel Meeting
2275 Research Boulevard
Conference Room #110
Rockville, Maryland, United States

AUGUST 30, 2016

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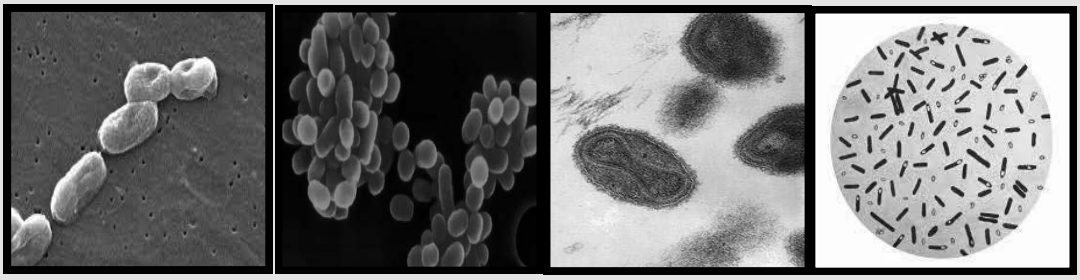
Stakeholder Panel on Agent Detection Assays [SPADA]



Stakeholder Panel Meeting
2275 Research Boulevard
Conference Room #110
Rockville, Maryland, United States

LINDA C. BECK, PhD, MT (ASCP)
LEAD SCIENTIST/MICROBIOLOGIST, CBR OFFICE
NAVAL SURFACE WARFARE CENTER

Co-Chair, AOAC Stakeholder Panel on Agent Detection Assays

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 Defense Division. Linda serves as the Navy Chem Bio Rad Nuclear (CBRN) Action Officer in the CBRN Defense T&E Navy Executive Policy Office. Her responsibilities include working on the joint service CBRN Test &Evaluation Capabilities and Methodology effort chaired by the Deputy Under Secretary of the Amy, Test and Evaluation (DUSA-T&E).

Prior to her current position, she worked for the Department of Homeland Security (DHS) 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. As Deputy, she provided technical oversight, guidance, and management of the BioWatch Program's daily laboratory operations, National Security Special Events, and surge capability.

Preceding her DHS position, Dr. Beck worked at the 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 as a professor in the School of Allied Health Professions at the Medical College of Virginia/Virginia Commonwealth University. During her academic tenure, she mentored numerous undergraduate and graduate students through her research in the areas of genetics, microbiology, and cellular biology.

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.

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

Co-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.

Jay E. Gee, PhD
Research Biologist, Bacterial Special Pathogens Branch, DHCPP, NCEZID
United States Centers for Disease Control and Prevention

SPADA *BURKHOLDERIA PSEUDOMALLEI* WORKING GROUP CHAIR

Jay E. Gee earned his BS in Microbiology at Mississippi State University in 1987 and his PhD in Biochemistry in 1992 at the University of Alabama at Birmingham School of Medicine. He studied antisense oligonucleotide technology in his first postdoctoral position at Baylor College of Medicine in Houston, TX. He later studied antiviral therapy strategies using chemically modified oligonucleotides in a vesicular stomatitis virus model at L'Institut de Génétique Moléculaire de Montpellier (The Institute of Molecular Genetics of Montpellier) in France in a second postdoctoral position.
He has been with the CDC for almost 14 years. During his research at CDC, he designed real-time PCR assays to identify pathogenic *Leptospira* spp. and *Burkholderia pseudomallei* and has performed molecular genetic subtyping on a variety of pathogens such as *Bacillus* spp. (e.g. *B. anthracis* and *B. cereus*) and *Burkholderia* spp. (e.g. *B. pseudomallei* and *B. mallei*) in support of epidemiological case investigations. He has served on the CDC Environmental Microbiology Work Group and serves on the CDC Next Generation Sequencing Quality Workgroup. He is currently a subject matter expert on *Burkholderia pseudomallei* and *B. mallei*.

Frank F. Roberto, PhD, SM (NRCM)
Directorate Fellow, Energy and Environment
Idaho National Laboratory

SPADA BRUCELLA WORKING GROUP CHAIR

Frank Roberto received his BS and PhD in biochemistry from the University of California, Davis, and University of California, Riverside. After a postdoctoral fellowship in molecular plant pathology at UC Davis, he moved to the US Dept. of Energy's Idaho National Laboratory, where he has conducted and directed R&D programs ranging from biomining with acidophilic bacteria and archaea to rapid detection of priority bacterial pathogens such as Brucella. For nearly ten years he worked closely with wildlife biologists studying interspecies transmission of brucellosis to develop field-deployable DNA assays to address bison and elk management issues in the Greater Yellowstone Area. He is a Specialist Microbiologist in biological safety (National Registry of Certified Microbiologists) and has held the Certified Biological Safety Professional (CBSP)certification (American Biological Safety Association).

Shashi Sharma, Ph.D.

SPADA Botulinum neurotoxin A Chair

Dr. Sharma received Ph.D. in Microbiology from University of Bhopal, Bhopal India. After Ph.D., he joined Lupin Biotechnology as a Scientist where he worked on development monoclonal antibodies and immunodiagnostics of HIV, Typhoid and Syphilis. He did posdoc from Department of Biochemistry, University of Massachusetts Dartmouth, where he worked on the structure and function of *Clostridium botulinum* neurotoxin and its associated proteins. Dr. Sharma joined FDA/ CFSAN, in May 2002. His research focuses on the development and validation of an effective and sensitive detection system for *Clostridium botulinum* in foods. He has over 22 years of experience in *C. botulinum* research and published several research papers in peer reviewed journals and holds an US patent on *C. botulinum* toxin associated proteins.

Dr. Victoria Olson
Microbiologist
United States Centers for Disease Control and Prevention

CHAIR, SPADA VARIOLA WORKING GROUP

Victoria Olson obtained her Ph.D. in Biochemistry from the University of Wisconsin – Madison in 2001. Her dissertation focused on understanding transcriptional regulation by the baculovirus *Autographa californica multicapsid nucleopolyhedrovirus* immediate early protein (IE1). Dr. Olson then joined the Poxvirus Program at the Centers for Disease Control and Prevention as an Oak Ridge Institute for Science and Education postdoctoral fellow in 2002. Her postdoctoral research focused on understanding how *Orthopoxviruses* interact with their hosts. While studying *Orthopoxviruses*, Dr. Olson completed training and certification for work at multiple biosafety levels, including work with *variola virus* within the Biosafety level 4 laboratories. In 2008, Dr. Olson became lead of the Virus-Host Molecular Interactions Unit within the Poxvirus Team at the Centers for Disease Control and Prevention. She supervises 4 masters-level researchers, 1 post-doctorate, 1 veterinarian, and 1 technician. The Virus-Host Molecular Interactions Unit focuses on research aimed at understanding how *Orthopoxviruses* interact with their hosts and what measures are effective at abrogating disease progression and mitigating morbidity. Since 2005, Dr. Olson has been closely involved in the validation of real-time PCR diagnostic assays for use in clinical settings, with particular focus on obtaining regulatory approvals. During her 12 years within the Poxvirus Team, she has contributed to some 39 peer-reviewed publications.

The Scientific Association Dedicated to Analytical Excellence®

STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Tuesday, August 30, 2016

AOAC INTERNATIONAL Headquarters
Conference Room 110
2275 Research Blvd., Rockville, Maryland, 20850
9:00 a.m. – 3:30 p.m.

STAKEHOLDER PANEL AGENDA – AUGUST 30, 2016

I. Introductions and Call to Order (**9:00 a.m. – 9:05 a.m.)**
Jim Bradford, AOAC INTERNATIONAL

II. Meeting Overview and Objectives (**9:05 a.m. – 9:30 a.m.)**
Linda Beck, DoD NSWC, SPADA Co-Chair
   a. SPADA Accomplishments
   b. Current Initiative

III. Future SPADA Projects
Scott Coates, AOAC INTERNATIONAL **(9:30 a.m. – 9:50 a.m.)**

IV. Draft Standard Method Performance Requirements (SMPR) (**9:50 a.m. – 1:45 p.m.)**
   a. AOAC Policies and Procedures for Adopting an SMPR – *Deborah McKenzie, AOAC INTL.* **(9:50 a.m. – 10:10 a.m.)**
   b. *Variola majora* – Victoria Olson, CDC* **(10:10 a.m. – 11:10 a.m.)**
   c. *Brucella* – Frank Roberto, Idaho National Laboratory* **(11:10 a.m. – 12:10 p.m.)**
   d. *Burkholderia pseudomallei,* Jay Gee, CDC* (**1:10 p.m. – 2:10 p.m.)**
   e. *Botulinum neurotoxin A* – Shashi Sharma, FDA* **(2:10 p.m. – 3:10 p.m.)**

V. Next Steps and Adjourn **(3:10 p.m. – 3:30 p.m.)**

Lunch: 12:10 pm – 1:10 pm

NO GOVERNMENT FUNDS HAVE BEEN USED IN THE PROVISION OF FOOD FOR THIS MEETING

**Item requires a vote*

Stakeholder Panel on Agent Detection Assays

AOAC INTERNATIONAL Headquarters
Rockville, Maryland
August 30, 2016

Linda C. Beck, PhD (NSWCDD) SPADA Co-Chair
Matthew Davenport, PhD (DHS) SPADA Co-Chair

1

AOAC Staff

- James Bradford, Chief Executive Officer, jbradford@aoac.org.
- Krystyna McIver, SPADA Project Lead, kmciver@aoac.org.
- Scott Coates, Chief Scientist, scoates@aoac.org.
- Deb McKenzie, Sr. Dir., Standards Development, dmckenzie@aoac.org.
- Christopher Dent, Standards Development Project Coordinator, cdent@aoac.org.
- Jonathon Goodwin, Senior Director, Membership, HR & Administration, jgoodwin@aoac.org.

2

Agenda

- History
- Current Projects
- Background on SMPRs
- Organization
- Meeting Goals

3

SPADA Sets Standards 2007 - 2013

Original Objectives in 2007

- Establish standards to validate Polymerase Chain Reaction (PCR)-based technologies that detect aerosolized *Bacillus anthracis*, *Yersinia Pestis*, or *Francisella tularensis*
- Pilot the validation process with an assay that detects *B. anthracis*

2009

- Develop standards to validate immunoassay-based Hand-Held Assays (HHAs) that detect *B. anthracis* or Ricin in suspicious powders
- Test commercially-available HHAs

2010

- Develop standards to validate PCR-based technologies that detect aerosolized *Burkholderia psuedomallei* and *Burkholderia mallei*
- Develop standards to validate PCR-based technologies that detect *B. anthracis* in suspicious powders

2011

- Develop recommendations on controls needed for field-based assays

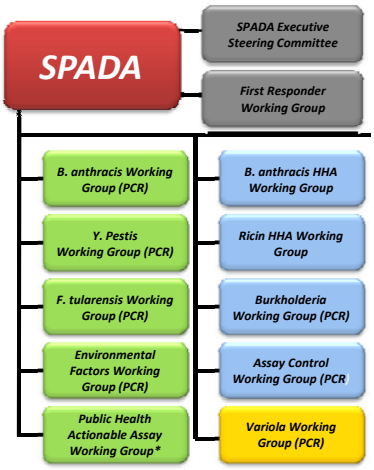
2013

- Develop standards to validate PCR-based technologies that detect aerosolized *Variola*
- Establish First Responder Working Group
- Maintain a SPADA Executive Steering Committee

4

SPADA Sets Standards 2007 - 2013

- A voluntary consensus standards body originally established via a DHS S&T contract with AOAC INTERNATIONAL
- Includes representatives from DHS, CDC, DoD, DoJ, FDA, EPA, USPS, NIST, State & Local Public Health, First Responders, Industry, and Academia
- Establishes method performance requirements and panels of reference materials (and validation protocols)



❖ *All SPADA members volunteer their time and expertise*

SPADA Working Group Chairs 2007 - 2013

***B. anthracis* Working Group (BaWG)**
Paul Jackson, LLNL and Ted Hadfield, MRI

***Y. pestis* Working Group (YpWG)**
Luther Lindler , DHS

***F. tularensis* Working Group (FtWG)**
Peter Emanuel, DoD
Mark Wolcott, DoD

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 and Alex Hoffmaster, CDC

Assay Controls Working Group (ACWG)
Christina Egan, NYSDH and Larry Blyn, Ibis

Variola Working Group (VWG)
Victoria Olson, CDC and Ted Hadfield, MRI

SPADA Objectives & History 2014 - 2016

- Under Contract with Deputy Undersecretary of the Army- Test and Evaluation through The Johns Hopkins University, Applied Physics Laboratory

2014

- Establish standards to validate technologies that detect Venezuelan Equine Encephalitis Virus, Staphylococcus Entertoxin B, and *Coxiella burnetti* (Q-fever) with emphasis on the warfighter.

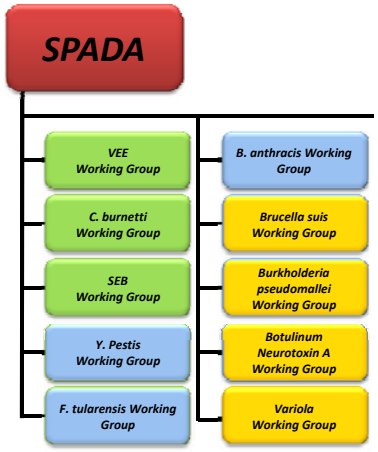
2015 – 2016

- Establish standards to validate technologies that detect *Bacillus anthracis*, *Yersinia Pestis*, *Francisella tularensis, Brucella suis, Burkholderia pseudomallei, Variola,* and Botulinum Neurotoxin A with emphasis on the warfighter.

7

SPADA - 2014 - 2016

- A voluntary consensus standards body established via DUSA-TE sponsored project through JHU/APL
- Includes representatives from DHS, CDC, DoD, DoJ, FDA, EPA, USPS, NIST, State & Local Public Health, First Responders, Industry, and Academia
- Establishes Standard Method Performance Requirements (SMPRs) that include inclusivity/exclusivity panels



❖ *All SPADA members volunteer their time and expertise*

8

SPADA Working Group Chairs 2014 - 2016

Approved at September 2015 SPADA Mtg:

Venezuelan Equine Encephalitis WG
James Samuel, U of Texas, A&M

***C. Burnetti* WG**
Eileen Ostlund, USDA, ARS

SEB WG
Sandra Tallent , FDA

Approved at March 22 – 23, 2016 Mtg:

***B. anthracis* WG**
Paul Jackson, LLNL and Ted Hadfield, Hadeco

***Y. pestis* WG**
Luther Lindler, DHS

***F. tularensis* WG**
Paul Keim, Northern Arizona University

Up for approval at August 30, 2016 SPADA Mtg:

***Burkholderia pseudomallei* WG**
Jay Gee, CDC

***Brucella suis* WG**
Frank Roberto, Idaho Natl. Laboratory

***Variola* WG**
Victoria Olson, CDC

Botulinum Neurotoxin A WG
Sashi Sharma, FDA, HHS

Background on Standard Methods Performance Requirements

- Commonly referred to as:
  - SMPRs
  - "Smipper"s

AOAC SMPR 2011.006

Standard Method Performance Requirements for Folate in Infant Formula and Adult/Pediatric Nutritional Formula

Approved by: Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN)

Final Version Date: April 5, 2011

Effective Date: April 5, 2011

Intended Use:

1. Applicability

2. Analytical Technique

3. Definitions

4. Method Performance Requirements

5. System Suitability Tests and/or Analytical Quality Control

6. Reference Material(s)

7. Validation Guidance

8. Maximum Time-to-Signal

Standard Methods Performance Requirements

- A standard for analytical methodology.
  - the traditional standard was a description of a method.
  - an SMPR specifies the minimum performance requirements for a methodology.
- Documents a community's analytical needs.
- Description of the analytical requirements.
- Includes method acceptance requirements.

Standard Methods Performance Requirements

General Format

- Intended Use
- Applicability
- Analytical technique
- System suitability
- Reference materials
- Validation guidance
- Maximum time-to-determination
- Method performance requirements table
- Inclusivity/exclusivity/environmental contaminants

Standard Methods Performance Requirements

Use of SMPRs

- Guidance to developers for the development of new assays.
- Advance the state-of-the-art in a particular direction.
- Address specific analytical needs.
- Specifications for acquisition.
- Vendor self-qualification.
- Basis for method acceptance and AOAC approval.

Organization: Stakeholder Panel

- Populate and oversee working groups.
- Standard adopting bodies for AOAC.
- Meetings are open to all interested parties.
- 50+ members.
- About 20 voting members.
  - Vetted based on:
    - Expertise.
    - Perspective:
    - Developers, users, industry, regulators, etc.

14

August 2016 Meeting Goals

SPADA review and approval of SMPRs for:

1. *Burkholderia*
2. *Brucella*
3. *Variola* (Small pox)
4. Botulinum toxin

Discussion of Future Projects

15

Questions?

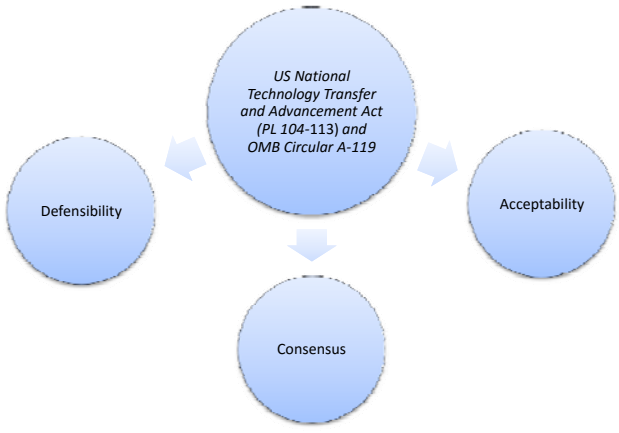
16

AOAC Stakeholder Panel on Agent Detection Assays

AOAC Standards Development Process

Approval of an AOAC *SMPR*®

AOAC Standard Development Process



AOAC Standards Development

- AOAC develops voluntary consensus standards using the following principles:

Transparency
Openness
Balance
Due Process
Consensus
Appeals

Stakeholder Panel Activity

March 22, 2016
- Define specific analytical issues
- WG chairs for *Brucella*, *Burkholderia*, *Variola*, and *Botulinum* Neurotoxin A provided background & proposed fit for purpose statements

March 23, 2016
- Form working groups to begin draft standard method performance requirements
- Working groups met on second day of SPADA meeting in March to begin their work and continued via teleconference

July 13-August 12, 2016
- Comment on draft standard method performance requirements
- Comment period for all four SMPRs began on July 13, 2016 through Friday, August 12, 2016.

August 30, 2016
- Deliberate and reach consensus on a final versions of the standard method performance requirements
- WG chairs will present draft SMPRs for stakeholder deliberation and consensus.

AOAC 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 Regulators
- Contract Research Organizations
- Reference Materials Developers
- Ingredient Manufacturers
- Method End Users
- Academia & Research
- Non Governmental Organizations
- Other as identified

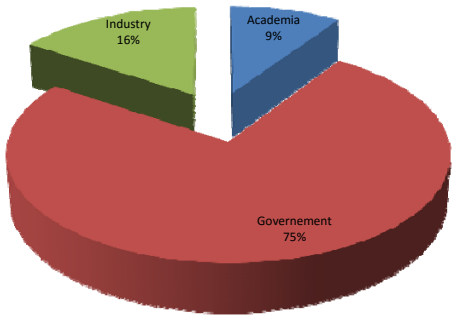
Anyone with a material interest can participate
Balanced group of representative voting stakeholders
Chair and voting stakeholders vetted by AOAC Official Methods Board

Organizational Meeting Registrants

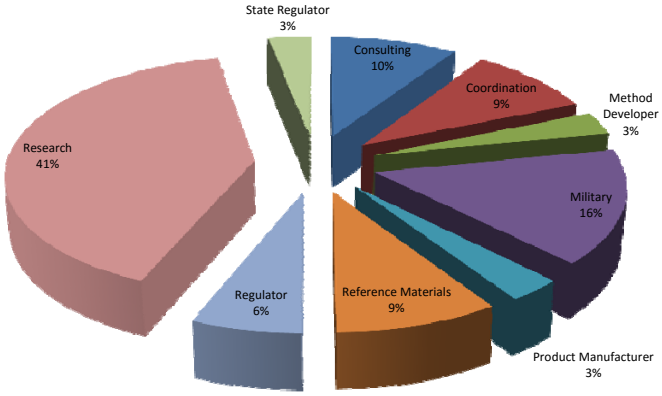
- ATCC
- CENSEO Insight
- Centers for Disease Control and Prevention
- Critical Reagents Program
- Defense Threat Reduction Agency
- Department Of Homeland Security
- DHS/OHA
- DoD ECBC
- EPA - National Homeland Security Research Center
- FDA - CFSAN
- FDA - CFSAN (Retired)
- InterAgency Board (IAB)
- Ibis Biosciences
- Idaho National Laboratory
- J. Craig Venter Institute
- Lawrence Livermore National Lab (Retired)
- MD Department Of Agriculture
- Naval Surface Warfare Center
- NBFAC
- NIH/NIAID
- NIST
- Northern Arizona University
- Northrop Grumman Electronic Systems
- Pacific Northwest National Laboratory
- Tunnell Government Services
- University of Florida
- US EPA (ret)
- USAMRIID
- USDA/ARS
- US FDA
- Walter Reed Army Institute of Research

As of August 11, 2016

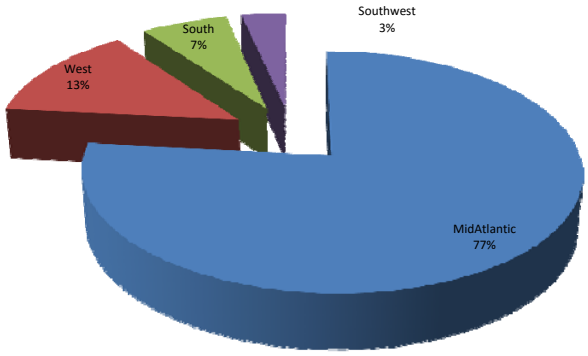
Registrants by Broad Perspectives



Registrants by Specific Perspectives



Registrants by Region



SPADA Voting Members – August 2016

ECBC	EPA
Navy Surface Warfare Center	Ibis Biosciences
USAMRIID or Walter Reed Army Institute of Research	Northrop Grumman
DHS/OHA	ATCC
DTRA	IAB
State of MD	University of Florida
Censeo Insight	DoD Critical Reagents Program
LLNL (Retired)	USDA/ARS
CDC	PNNL or Idaho National Laboratory
NIST	J. Craig Venter Institute
FDA-CFSAN	

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 stakeholders will be able to vote in favor of the motion, against the motion, abstain from voting
- 2/3 vote in favor required to approve/adopt an AOAC SMPR

Documentation and Communication

- AOAC carefully documents the actions of the 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 standard
  - 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 Method Board
  - Vet and approve stakeholder panel chair and representative voting stakeholders
  - 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

QUESTIONS?

THANK YOU

☺

AOAC INTERNATIONAL STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

***Variola* Virus Working Group**
Chair: Victoria Olson, CDC
SMPR Presentation
August 30, 2016

Rockville, Maryland, USA

Fitness for Purpose from 3/22/16

"**Detection of *Variola virus* DNA in collection buffers from aerosol collection devices for DoD applications.**"

SPADA *Variola Virus* Working Group
Working Group Members

Victoria Olson, CDC (Chair)
Jennifer Arce, PNNL
Linda Beck, NSWC-Dahlgren
Larry Blyn, Ibis Biosciences
Amanda Clark, NSWC-Dahlgren
Ryan Cahall, Censeo Insight
Kenneth Damer, NGC
Paul Jackson, LLNL (Ret.)
Katalin Kiss, ATCC
Timothy Moshier, XX
Pejman Naraghi-Arani, InSilixa
Denise Pettit, NC DoH
Frank Schaefer, EPA (Ret.)
Mark Scheckelhoff, DHS
Shanmuga Sozhamannan, DoD CRP
Elizabeth Vitalis, LLNL

Variola virus Work to Date

- Working Group Launch (March, 2016)
- Three (3) teleconferences (May 2016 – July 2016)
- 1 SMPR Drafted
- Public comment period (July 15, 2016 – August 12, 2016)
- SMPRs made ready for SPADA review and approval

Mission of the SPADA Variola Working Group (2016)

- The Variola Working Group of the Stakeholder Panel on Agent Detection Assays (SPADA) was tasked to develop voluntary consensus standards required for evaluation of tools that detect *Variola virus* DNA from aerosol collection devices for DoD applications. … The standards will :
  - Support test and evaluation of Variola-detection tools for DoD applications
  - Provide guidance to industry and other capability developers for development of future detection tools that DoD may solicit

It is expected that any detection result from a tool validated against the SPADA Variola standards will be confirmed by the Poxvirus Laboratory at the Centers for Disease Control and Prevention.

Variola Working Group SMPR: Tailor Panel to Assay Based on Bioinformatics

Controls:

- Positive control:
  - Low but easily detectable concentration
  - Monitor performance of entire assay
  - Recommended include a technique to confirm positive control is not cause of positive signal generated by sample
- Negative control:
  - Confirm assay does not produce false positives
- Inhibition control:
  - Specifically confirms sample or sample matrix does not prevent assay to detect target organism

Variola Working Group SMPR:
Tailor Panel to Assay Based on Bioinformatics

Sensitivity analysis:

- AMDL = 50,000 copies/mL target region of *Variola virus*
  - ≥ 500 bp must receive permission from WHO
  - insertion into another *Orthopoxvirus* is prohibited

➢ Establish Probability of Detection at AMDL w/in collection buffer (≥ 95%)
➢ Establish Probability of Detection at AMDL w/in aerosol environmental matrix (≥ 95%)

- Inclusivity panel – *Variola virus*: ≥ 2 strain target regions
  - at least one from each primary clade *(Li, et. al. PNAS (2007) Oct. 2;104(40):15787-15792.)*
  - encompass differences in target region
    - Based on bioinformatic analysis

➢ Ensure all inclusivity strains are detected at 2X AMDL in collection buffer
➢ Ensure all inclusivity strains are detected at 2X AMDL in environmental matrix
➢ Ensure target is detected at 2X AMDL w/in pool of environmental panel organisms (pools of up to 10 organisms at 10X AMDL for each)

Variola Working Group SMPR:
Tailor Panel to Assay Based on Bioinformatics

Specificity analysis:

- Exclusivity panel – near neighbor (*Orthopoxvirus*):
  - All poxvirus strains listed in the table (one from each major clade)
    - See AOAC Website for the most updated list

Species	Strain	Commercial availability
Vaccinia	Elsree	ATCC VR-1549
Cowpox	Brighton	ATCC VR-302
Ectromelia	Moscow	ATCC VR-1374
Monkeypox	V79-I-005	BEI NR-2324
Monkeypox	USA-2003	BEI NR-2500
Raccoonpox	Herman	ATCC VR-838
Skunkpox		ATCC
Volepox		ATCC
Camelpox		BEI
Taterapox		BEI
Parapoxvirus Orf	Vaccine	Colorado Serum Company

  - Any additional strains with greater similarity to the assay's target region(s) than the strains listed above in the table
    - Based on bioinformatic analysis

➢ Ensure all exclusivity strains are NOT detected at 10X AMDL in collection buffer

Variola Working Group SMPR:
Tailor Panel to Assay Based on Bioinformatics

Specificity analysis (cont.):

- Environmental aerosol matrix samples:
  - Method developers should obtain environmental matrix samples that are representative/consistent with the collection method to be used
    - Considerations include:
      - Collection medium
      - Duration of collection
      - Diversity of geographical areas to be sampled
      - Climatic/environmental conditions
      - Seasonal changes
      - Ensure sufficient replicates to represent environmental condition
  - Ensure aerosol matrix samples do NOT cross-react
- Environmental panel organisms:
  - Organisms can be pooled (up to 10 per pool)
  - Method developer must justify exclusion of specific panel organisms
  - Ensure all organisms are NOT detected at 10X AMDL
    - If unexpected result, each individual organisms from failed pool must be tested individually at 10X AMDL

Variola Working Group SMPR:
Tailor Panel to Assay Based on Bioinformatics

Bioinformatic analysis:

- *In silico* screening on signature sequences
  - Suggestive of potential performance issues
  - Guide necessary additions to wet lab screening panels
- Potential tools for *in silico* screening:
  - http://sourceforge.net/projects/simulatepcr/files/?source=navbar
  - NCBI tools
- Method developer submission should include:
  - Description of sequence databases used in the *in silico* analysis
  - Description of conditions used for *in silico* analysis
    - Stringency of *in silico* analysis must match bench hybridization conditions
  - Description of tool used for bioinformatics evaluation
    - Data confirming selected tool performance based on wet-lab testing
      - Can be generated retrospectively using published assays
  - List of additional strains to be added to inclusivity or exclusivity panels

Comments Submitted (if any)

- No comments received

Motion

- Motion to accept the Standard Method Performance Requirements for *Variola virus* as presented.

Acknowledgements

All members of the SPADA *Variola Virus* Working Group

Poxvirus and Rabies Branch members 1999-present

"TNTC" CDC and external partners

For more information please contact Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 30333
Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: www.cdc.gov

National Center for Emerging and Zoonotic Infectious Diseases
Division of High Consequence Pathogens and Pathology

Current Recommendations:

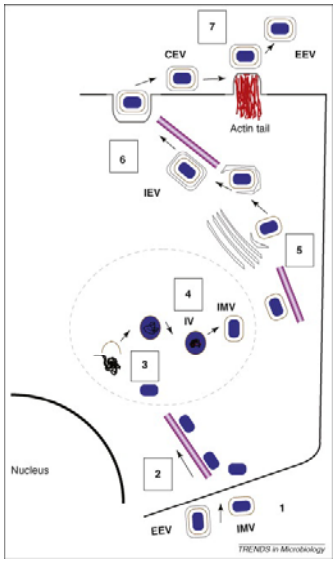
Exclusivity panels
100 pg/µl

Exclusivity panel - near neighbors (*Orthopoxviruses*)	
Species	**Strain Name**
Ectromelia	ECTV Moscow
Monkeypox	MPXV RCG 2003 358
Monkeypox	MPXV USA 2003 044
Camelpox	CMLV-78-I-2379
Cowpox	CPXV-NOR1995-MAN
Cowpox	CPXV GER1980-EP4
Cowpox	CPXV GER1991-3
Cowpox	CPXV FIN-2000-MAN
Cowpox	CPXV_GER1998_2
Taterapox (gerbilpox)	TATV-71-I-016
Vaccinia	Copenhagen
Vaccinia	WR
Vaccinia	ACAM 2000
Vaccinia	BRZ SERRO
Raccoonpox	RACV V71-I-84
Skunkpox	SKPV 1991
Volepox	VPXV 2004-CA-007

Exclusivity panel - other rash-causing illnesses	
Species	**Strain Name**
Varicella-zoster virus	pOKA (J clade)
Varicella-zoster virus	Webster (E1 clade)
Herpes simplex virus type 1	F
Herpes simplex virus type 2	G
Rickettsia conorii	CDC
Rickettsia akari	CDC
Parapoxvirus Orf	Vaccine for sheep

Exclusivity panel - Negatives	
Species	**ID number**
Enterococcus faecalis	ATCC 29212
Eschericia coli	ATCC 25922
Klebsiella pneumoniae	ATCC 33495
Peptostreptococcus anaerobius	ATCC 27337
Propionibacterium acnes	ATCC 6919
Pseudomonas aeruginosa	ATCC 27853
Staphylococcus aureus (strain1)	ATCC 12600
Staphylococcus aureus (strain 2)	ATCC 25923
Staphylococcus epidermidis (strain 1)	ATCC 49134
Staphylococcus epidermidis (strain 2)	ATCC 12228
Staphylococcus epidermidis (strain 3)	ATCC 14990
Streptococcus gallylyticus	ATCC 49147
Streptococcus pyogenes	ATCC 49117
Water	

Background: Poxvirus 101

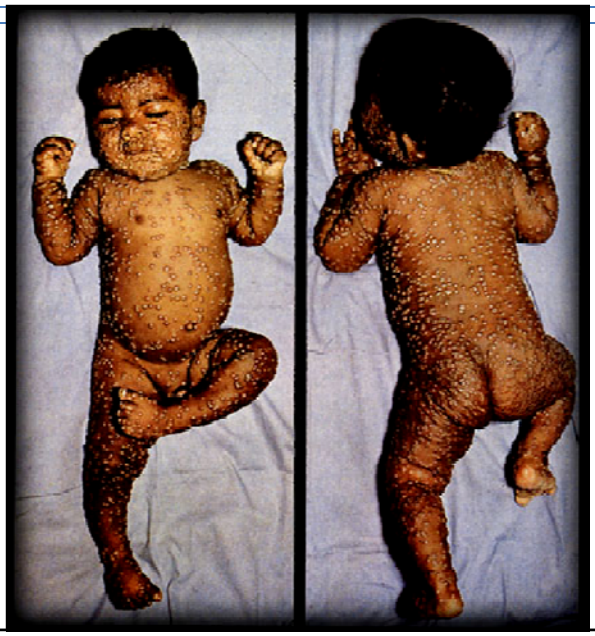


- **Family of large, double stranded DNA viruses**
  - Within genera, antigenic similarity: cross protection
- **Complex viruses, cytoplasmic lifecycle**
- **Genus *Orthopoxvirus:***
  - 90-98% nucleotide identity across species
  - *Variola, Vaccinia, Cowpox, Monkeypox* – all can cause human disease
    - *Variola* evolved to be a sole human pathogen: SMALLPOX
  - *Camelpox, Ectromelia, Taterapox* are NOT known to cause human disease

Objectives

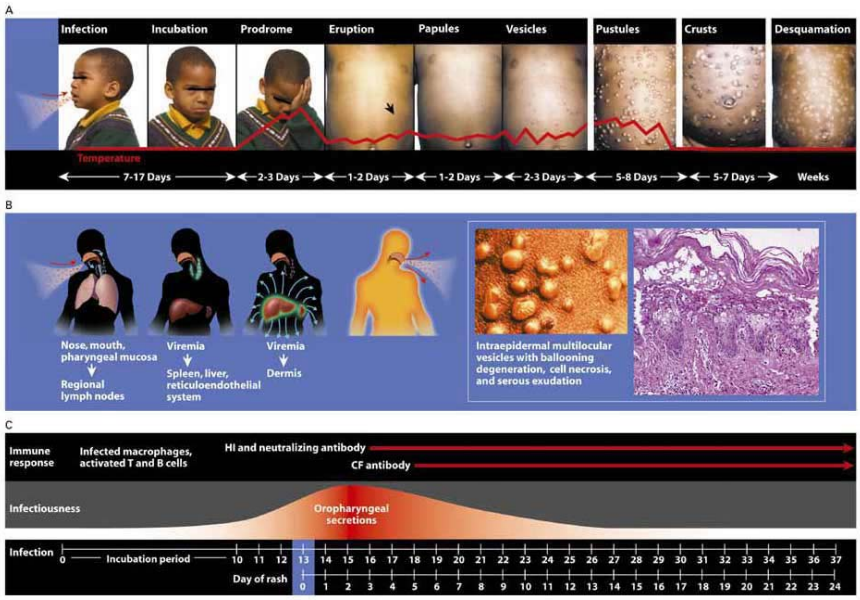
- Smallpox introduction
- Development of smallpox clinical diagnostics
- Developing a framework for considering environmental smallpox detection/testing
  - Results of 2016 Standard Method Performance Requirements for Detection of *Variola virus* DNA in collection buffers from aerosol collection devices for DoD applications

SMALLPOX History and Background



Disease

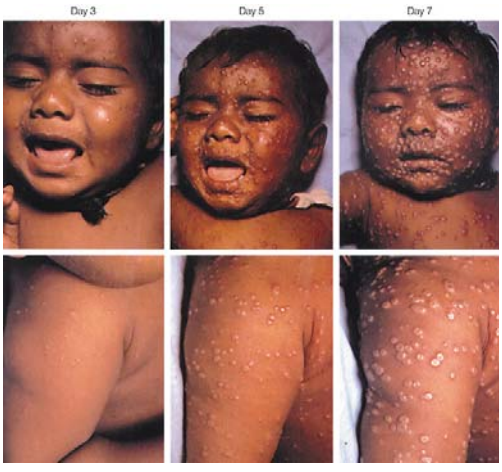
- **Sole human pathogen**
- **No known non-human animal reservoir**
- **Transmitted by respiratory route (largely airborne droplets)**
  - Rare, but notable occurrences of airborne transmission in some hospitals
- **Transmitted by percutaneous exposure**
- **Fomites – rare cause of transmission**
- **Not foodborne or waterborne**



Source: Breman JD, Henderson DA. N Engl J Med 2002;346:1300-8.

Progression of Smallpox

- **Incubation Period**
- **Pre-eruptive Stage:**
  - **abrupt onset high fever/constitutional symptoms**
- **Macules**
- **Papules**
- **Vesicles**
- **Pustules**
- **Scabs**
- **Scars**



Smallpox - Disease Elimination/Eradication

- **Disease – viral exanthem**
  - Major (“avg” 30% CFR) and minor disease (<1% CFR)
- **Disease prevention**
  - Childhood vaccination – variable rates; +/-variolation
- **Global Smallpox Eradication program - 1958**
  - Intensification of Smallpox eradication program 1967
    - Surveillance -> contact tracing, vaccination of contacts (and contacts)
    - Isolation of cases, observation of contacts
    - Lyophilized vaccine, semi-standardized vaccine production
      - *Vaccinia virus*
      - “Take” ~ protection
  - No non-human animal reservoir
- **Elimination in all countries by 1977**
  - Commission to Certify Smallpox eradication activities
    - Certified as eradicated in 1979
  - WHA : Declaration of smallpox eradication 1980

* Smallpox and its Eradication WHO 1988

History- *Variola virus* Elimination/Eradication

Consolidation of laboratory-held virus materials*

- 1975 survey by WHO, post lab exposure in 1973 (LSTMH)
  - 74 labs report *Variola virus* materials
- 1976 voluntary consolidation
  - 1978 – Birmingham, England smallpox “lab”: 1 death, illness
- 1979 – WHO Committee of Experts recommends to preserve *Variola virus* stocks in a few collaborating center (CC) laboratories, review in 1982:->19 recommendations by the Global Commission
  - 1979 – 7 labs report *Variola virus* stocks
  - 1981 – 4 CC laboratories with *Variola virus*
  - Periodic inspections for safe and secure use virus
- 1984 – consolidation of stocks to 2 WHO CCs – BSL-4 facilities (WHA 33.4)

* Smallpox and its Eradication 1988

Virus Eradication – Considerations to 1999

- **Additional Global Commission sanctioned research – reflect (new) technologies of the time**
  - Cloning of *Variola virus* genomes – in representative segments
  - Hybrid viruses* (1981): proof of recombination/"transfection"
    - Scientific Advisory Group of Experts (1984)
      - Vaccine research using *Vaccinia virus* vector
  - Sequencing of virus genomes –
    - 1993 - Two complete *Variola virus* "major" genomes available
- **Bioterrorism threat once vaccination program ceases**
- **Decision to prohibit genetic manipulation of *Variola virus*, restrict access to genomic elements and genome**
  - Reports that Russia had attempted to "weaponize" *Variola virus*

* Sam and Dumbell Expression of poxvirus DNA in coinfected cells and marker rescue of thermosensitive mutants by subgenomic fragments of DNA Annales de virologie, 1981

Smallpox Research Agenda: Focused on Preparedness Needs

- Institute of Medicine (IOM) Report recommendations for "Assessment of Future Scientific Needs for Live *Variola Virus*" (1999) have helped to frame the research agenda.
  - Protocols approved by WHO technical subcommittee
  - Research updates provided annually to the WHO Advisory Committee for *Variola Virus* Research
  - Collaborative HHS (largely CDC) and DoD (largely USAMRIID)
- All U.S. work with live *Variola virus* occurs within the BSL-4 containment laboratory at the CDC
  - Inspected regularly by U.S. security and biosafety authorities and WHO biosafety teams
    - Genetic manipulation of *Variola virus* not authorized by WHO
      - 1994 Ad hoc *Orthopoxvirus* Advisory Committee recommendation
    - **Full genomes of *Variola virus* can only be maintained at the 2 WHO CCs**
    - **No lab can have more than 20% of the *Variola virus* genome, except a WHO Collaborating Center**
- All research findings to be made available to the international scientific community

IOM Recommendations* 1999→WHO Sanctioned Research Agenda

- Molecular characterization of *Variola virus* for more sensitive and specific diagnostic development
  - Sequencing entire genomes and specific genes
- Antiviral
- Less reactogenic vaccine development
- Animal model – pathogenesis, model system for antiviral & novel vaccine evaluation
- *Fundamental research – host pathogen interaction*

* Assessment of Future Scientific Needs for Live *Variola Virus*; N.A.Press (1999)

WHA Resolutions and WHO Protocol Approval Process

- 1999 WHA resolution - postpone decision on destruction until 2002
- 2002 WHA resolution - postpone decision on destruction
- 2005 WHA
  - Increased focus on “essential” public health research
    - Interpreted by WHO committee to preclude fundamental research
  - Major review of the research to the WHA in 2011
    - Advisory Group of Independent Experts (AGIES) review *Variola virus* research in 2010
- 2011 WHA
  - Resolution to revisit in 3 years
    - AGIES conduct second review of *Variola virus* research in 2013
- 2013 WHA
  - Request to consider question of synthetic biology
    - Report shared 2016

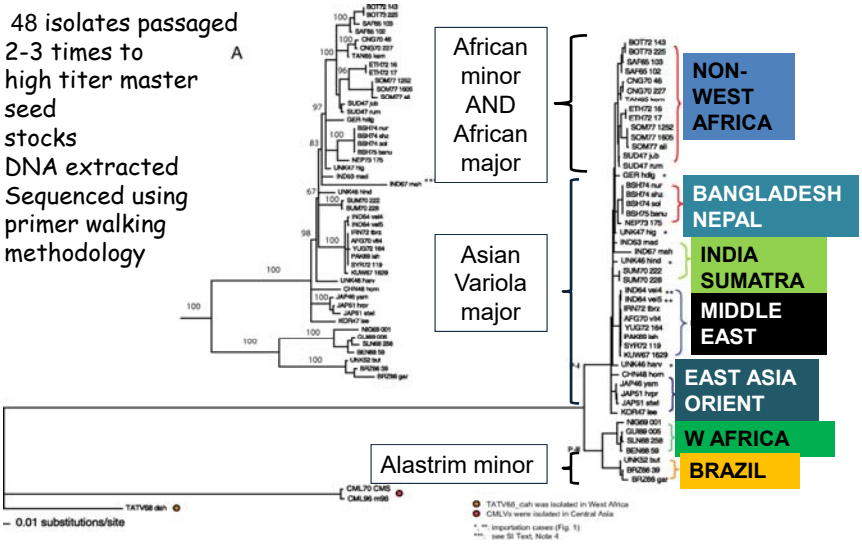
Virus Characterization

"Genomic sequencing and limited study of *Variola virus* surface proteins derived from geographically dispersed specimens is an essential foundation for important future work. Such research could be carried out now, and could require a delay in the destruction of known stocks, but would not necessitate their indefinite retention.*"

* Assessment of Future Scientific Needs for Live *Variola Virus*; N.A.Press (1999)

Maximum Likelihood Analysis of Single Nucleotide Matrices (*Variola virus*)

48 isolates passaged 2-3 times to high titer master seed stocks
DNA extracted
Sequenced using primer walking methodology



Li Y. et.al. PNAS 2007;104:15787-15792 Esposito et. al Science 2006

Diversity of *Variola virus* ~200 kb dsDNA, ~200 ORFs

- Diversity of *Variola virus* strains is associated with geographic distance
- Alastrim minor (South America) / Variola major (Asia): ~600 SNPs, ~80 Indels
- Alastrim minor / Variola intermediate (West Africa): ~350 SNPs, ~45 Indels
- Variola African minor/major / Variola major (Asia): ~150 SNPs, ~30 Indels

- Central region: virion structural proteins, enzymes - 30 gene sequences are perfectly conserved or have only synonymous SNPs, highly conserved, essential function.
- Left and Right end regions: Host range and immunomodulatory genes - majority of Indels/frameshift mutations, fragmented sequences, additional/absent of ORFs, - likely reflecting selection pressures.
- **Versus other *Orthopoxviruses***:
- *Variola / Camelpox-Taterapox viruses*: ~3200 SNPs, ~380 Indels
- *Variola / Monkeypox virus*: ~7500 SNPs, ~600 Indels

Diagnostics/Environmental Detection

"If further development of procedures for the environmental detection of *Variola virus* or for diagnostic purposes were to be pursued, more extensive knowledge of the genome variability, predicted protein sequences, virion surface structure, and functionality of *Variola virus* from widely dispersed geographic sources would be needed.*"

* Assessment of Future Scientific Needs for Live *Variola Virus*; N.A.Press (1999)

Diagnostics

- Why “if?”
- Proponents, in 1999, that EM and standard PCR techniques were sufficient for smallpox diagnostics
  - As of 2002 – survey of EM capacity in state health departments reveals only 3-8 with skilled capability to any capability
  - Newer technology: real time PCR

Diagnostics: Nucleic Acid Testing Real Time PCR Assays

- Platform supported at Laboratory Response Network (LRN)
- High throughput
- Sensitive/specific
  - Sensitive to 1-50 genome copies
  - Historically lesion samples contained 10^4-10^7 infectious virions
  - Assays validated against authentic *Variola virus* genomic material
- Limitations:
  - time to get samples to reference labs

Diagnostics Developed

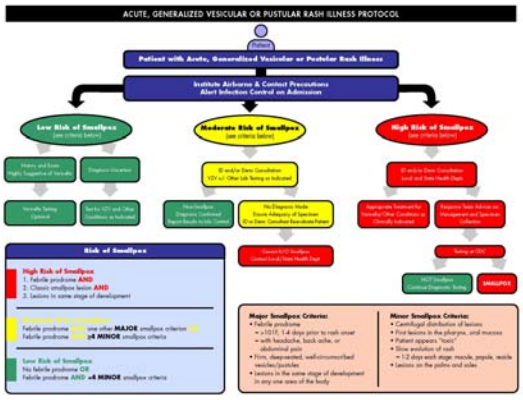
- CDC developed/evaluated (real time) PCR assays targeting *Orthopoxvirus* genus and various species (*Variola, Monkeypox, Vaccinia*, etc.)
  - Provide reagents/facilities for others to evaluate assays
    - ~Thirteen peer-reviewed publications evaluate PCR assays against authentic *Variola virus* genomic material
- Subset used in LRN (*Variola, Monkeypox, Cowpox* and *Vaccinia virus* detection)
  - 2002 onward: vaccine AE identification
  - 2003: response to monkeypox outbreak
- Regulatory agency approval
  - De novo 510K submitted on *Orthopoxvirus* non-variola assay
    - Approved September 2012
  - Discussion initiated with FDA (2002) on *Variola virus* assay
    - Submission on newly validated assays in 2016
- Initiating, technology transfer to other countries
  - Monkeypox
  - Smallpox laboratory diagnostics network (WHO sponsored)

Clinical Diagnostic Approaches used at the WHO CC at CDC

- Nucleic acid testing
- Viral isolation
- Serologic assays
- Protein based/virus detection
  - In development
  - Commercial assay available
- Only one *Orthopoxvirus* diagnostic assay has achieved regulatory approval
  - LRN *Orthopoxvirus* non-variola real-time PCR assay
    - FDA de novo 510(k) approved September 2012
    - Dependent upon LRN algorithm

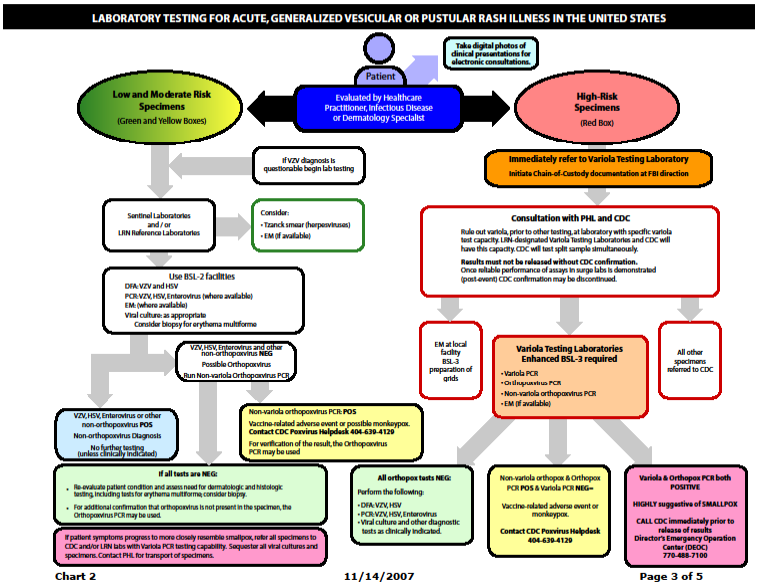
Clinical Laboratory Algorithm Development and Successes

- Focuses clinical attention to most serious look-a-likes
- Focuses lab attention to most serious contenders
- Helps define/remind what conditions are most frequently confused with possible smallpox
- Frames logic for approaching diagnostics
- Minimizes false positives
- Use of the algorithm in 2002*



Seward et. al. CID 2004

LRN



Clinical Diagnostic Performance Related to Disease Prevalence: Test Parameters

- **Sensitivity:**
  - the ability to identify as positive all those with the disease
- **Specificity:**
  - the ability to identify as negative all those without the disease
- **Predictive value positive (PV+):**
  - the proportion of true positives among those testing positive
- **Predictive value negative (PV-):**
  - the proportion of true negatives among those testing negative

Clinical Diagnostic Performance Related to Disease Prevalence: Test Parameters

		Disease		
		present	absent	total
Test result	POS	a=true positives	b=false positives	a+b
	NEG	c=false negatives	d=true negatives	c+d
	TOT	a+c	b+d	

PV+ = a/ (a+b)

PV- = d/ (c+d)

Sensitivity = a/ (a+c)

Specificity = d/ (b+d)

Pre-event/Post-event

- Test > 90% sensitivity
- Pre-event prevalence of smallpox is zero:
  - If test has 95% specificity, 10 tests done per month, in 6 to 8 sites, every month there will be 3 or 4 false positives
- Post-event prevalence of smallpox is finite:
  - If test has 95% specificity, 1000 tests done per week, every week 50 results will be false positives
  - If test has 99% sensitivity, 1000 tests done per week, 10 results will be false negatives

Test Parameters

- Sensitivity and specificity are independent of the prevalence of disease
- Predictive value positive and negative vary with disease prevalence (Bayes' Theorem)
- Implications for smallpox testing "pre-event" and "post-event": use of tests for decision making

Test Parameters - Examples

- Sensitivity 95%
- Specificity 95%

PREV	PV+	PV-
50%	95%	95%
10%	67.80%	99%
1%	16%	99.95%
0.10%	1.80%	99.99%
0.01%	0.20%	99.99%

Test Parameters - Examples

- Sensitivity 99%
- Specificity 99%

PREV	PV+	PV-
10.00%	92.80%	99.80%
1%	50%	99.99%
0.10%	9%	100.00%

"Pre-event" Ex.: Increase PV+ by using > 1 Test

Individual with clinical scenario with fever, followed by centifugal rash:

Test 1:

sensitivity 99%,

specificity 99%

PREV	PV+	PV-
10.00%	92.80%	99.80%
1%	50%	99.99%
0.10%	9%	100.00%

Test 2:

sensitivity 95%, specificity 95%

PREV	PV+	PV-
50.00%	95.00%	95.00%
10%	67%	99.00%
1.00%	16%	99.50%

Pre-event: Prevalence of Smallpox is Zero
Peri-event: Prevalence of Smallpox is Low

- Clinical scenario should be consistent
  - Use febrile rash algorithm; validate algorithm
- Wide availability of other key diagnostic tests especially rapid VZV testing to rule in VZV
- Limit the number of laboratories performing *Variola virus* testing
  - Establish confirmatory testing protocols
- Approaches to improving predictive value positive: implications for result use (rule in, and institution of vaccination campaign vs. rule out)
  - Use more than 1 test (different targets)
  - Use tests without common sources of false positives

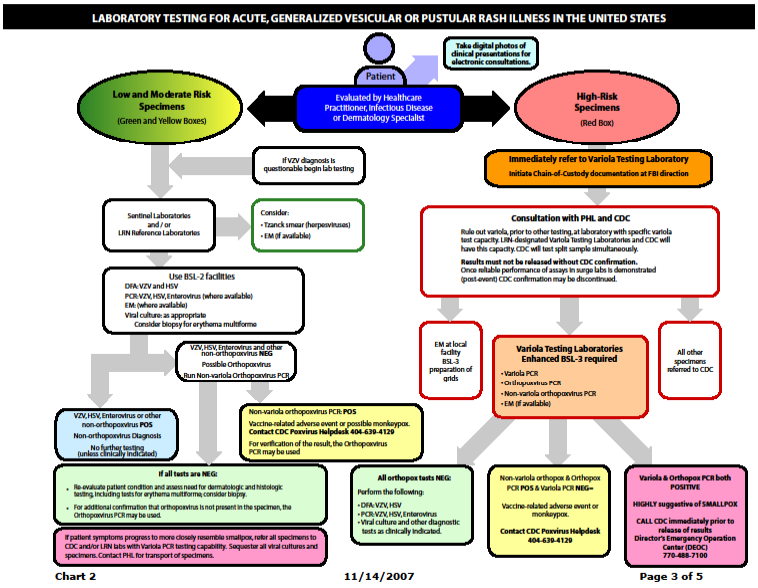
Post-event

- Need to assess what needs for testing will be:
  - One scenario: greatest needs in beginning, and near end of smallpox re-eradication
- May need to test more “low suspicion samples”
  - Sensitivity vs. specificity

Issues Relevant to Implementation of Smallpox Diagnostics

- Biosafety:
  - containment;
  - Standard vs. Universal vs. Airborne precautions;
  - Vaccination
- Biosecurity
- Reagents
  - WHO, CDC, select agent recommendations on use of *Variola virus* DNA
- Centralized/regionalized testing
  - Transportation of specimens
- QA/QC, Proficiency testing: implementation
- Communication between clinician/epidemiologists/laboratory
  - Clinical history, case patient photos
- Development of a disease confirmatory algorithm
  - Screening tests, confirmatory tests: regional vs. centralized
  - Presumed positive, Confirmed Positive
  - Communication of results, and public health response
  - Role of viral isolation by culture

LRN



Iterative Approaches to NA Test Validations: Previous Validations

Sensitivity analysis: Serial 100-fold dilutions (1ng/μl to 1 fg/μL) of DNA

Inclusivity panel – *Variola virus* DNA:

2 purified viral stocks
33 crude viral stocks
2 Human scab samples

Specificity analysis: Serial 100-fold dilutions (1ng/μl to 1 fg/μL) of DNA

Exclusivity panel – near neighbor (*Orthopoxvirus*):

Eurasian: 1 *Ectromelia virus*
2 *Monkeypox viruses*
2 *Camelpox viruses*
1 *Cowpox virus*
1 *Taterapox virus*
5 *Vaccinia viruses*
North American: 1 *Skunkpox virus*

Exclusivity panel – other rash-causing illnesses:

1 *Varicella Zoster virus*
1 *Herpes simplex virus* (type 1)
1 Rickettsia strain

Exclusivity panel – negatives:

Myxoma & tissue culture (2)

Inclusivity Panel		
Species	**Strain**	**Sample**
Variola virus	102	Crude
Variola virus	103	Crude
Variola virus	66-39	Crude
Variola virus	7124	Crude
Variola virus	7125	Crude
Variola virus	72-119	Crude
Variola virus	73-175	Crude
Variola virus	77-1605	Crude
Variola virus	Bombay	Crust
Variola virus	Brazil-Garcia	Crude
Variola virus	Congo	Crude
Variola virus	Eth-17	Crude
Variola virus	Harper	Crude
Variola virus	Harvey	Crude
Variola virus	Heidelberg	Crude
Variola virus	Higgins	Crude
Variola virus	Hinton	Crude
Variola virus	Horn	Crude
Variola virus	Horn	Pure
Variola virus	K1629	Crude
Variola virus	Kali Mathu	Crude
Variola virus	Kembula	Crude
Variola virus	Minnesota 124	Crude
Variola virus	MS Lee	Crude
Variola virus	Nepal	Pure
Variola virus	New Dehli	Crude
Variola virus	Nigeria Kuclano	Crust
Variola virus	Nur Islam	Crude
Variola virus	Rumbec	Crude
Variola virus	Shahzamon	Crude
Variola virus	Solaiman	Crude
Variola virus	Stillwell	Crude
Variola virus	V68-59	Crude
Variola virus	V70-222	Crude
Variola virus	V70-228	Crude
Variola virus	Variolator-4	Crude
Variola virus	Yamada	Crude

Complementary Set:

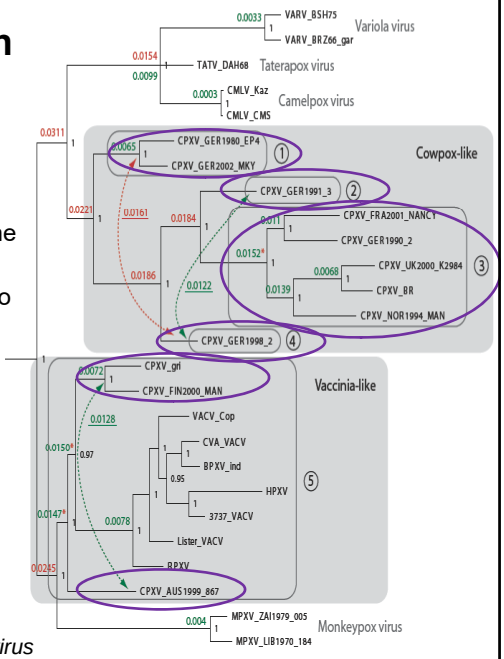
Exclusivity Panel - near neighbor (*Orthopoxviruses*)	
Species	**Strain**
Ectromelia virus	Moscow
Monkeypox virus	79-0266
Monkeypox virus	79-0005
Camelpoxvirus	LLC
Camelpoxvirus	V78-I-903
Cowpoxvirus	Brighton
Taterapoxvirus	(Gerbilpox)
Vaccinia virus	Lister
Vaccinia virus	VTH
Vaccinia virus	Wyeth
Vaccinia virus	WYH pGS62-9-v1-1-1
Vaccinia virus	Rabbitpoxvirus
Skunkpovirus	

Exclusivity Panel - other rash-causing illnesses	
Species	**Strain**
Varicella Zoster Virus	Webster
Herpes Simplex Virus-1	HFEM
Rickettsia	conorii

Exclusivity Panel - other negatives	
Species	**Strain**
Myxoma	
Human tissue culture cells	Sup-T
Monkey kidney tissue culture cells	BSC-40

Variola virus : Diagnostic Creation and Validation

- Validated diagnostic real-time PCR assays
  - Target multiple regions of the genome
  - Target sequences specific to *Variola virus*
- *Cowpox virus* sequences acquired after validation
  - Exhibit extensive phylogenetic diversity
  - Contain certain regions previously thought to be specific to *Variola virus*
    - Now assays predicted to cross-react with *Cowpox virus*



D. Carroll, et. al PLoS One. 2011;6(8):e23086

Variola virus Signature (Eroded Specificity): Assay Cross-reacts with *Cowpox virus*

Exclusivity panel Species	Strain Name	Assay 1 ct for 5 ng DNA	Assay 2 ct for 5 ng DNA
Vaccinia	Copenhagen	Negative	Negative
Vaccinia	WR	Negative	Negative
Vaccinia	ACAM 2000	Negative	Negative
Vaccinia	BRZ SERRO	Negative	Negative
Cowpox	CPXV-NOR1995-MAN	Negative	Negative
Cowpox	CPXV GER1980-EP4	19	Negative
Cowpox	CPXV GER1991-3	18	Negative
Cowpox	CPXV_GER1998_2	17	Negative
Cowpox	CPXV FIN 2000		Negative
Ectromelia	ECTV Moscow	Negative	Negative
Monkeypox	MPXV RCG 2003 358	Negative	Negative
Monkeypox	MPXV USA 2003 044	Negative	Negative
Raccoonpox	RACV V71-I-84	Negative	Negative
Skunkpox	SKPV 1991	Negative	Negative
Volepox	VPXV 2004-CA-007	Negative	Negative
Camelpox	CMLV-78-I-2379	17	Negative
Taterapox (gerbilpox)	TATV-71-I-016	16	Negative
Parapoxvirus Orf	Vaccine for sheep	Negative	Negative

What We Have Learned About *Variola virus* Diagnostic Assay Development/Validation

- Bioinformatic analysis should lead design of validation panels
  - Inclusivity panel include all *Variola virus* strains with differences in assay target region
  - Exclusivity panel (near neighbor *Orthopoxvirus*) contain viruses with assay target regions most similar to *Variola virus*
  - Exceedingly difficult to construct uniform panels for all assays due to high similarity between *Orthopoxvirus*es
- Simultaneous identification of multiple *Variola virus* signatures will increase confidence in initial identification/ verification of the pathogen with real-time PCR

Current Recommendations:
Tailor Panel to Assay Based on Bioinformatics

Sensitivity analysis: Two low dilutions (100 and 10 fg/μL) of DNA

Inclusivity panel – *Variola virus*: ≥ 2 purified viral stocks

(at least one from each primary clade)

(encompass differences in target region)

Specificity analysis: One high dilution (100 pg/μl) of nucleic acid

Exclusivity panel – near neighbor (*Orthopoxvirus*):

Eurasian: 1 *Ectromelia virus*

2 *Monkeypox viruses* (one from each clade)

1 *Camelpox virus*

5 *Cowpox viruses* (one from each predicted clade)

1 *Taterapox virus*

4 *Vaccinia viruses* (one from each clade)

North American: 1 *Raccoonpox virus*

1 *Skunkpox virus*

1 *Volepox virus*

Current Recommendations:
Tailor Panel to Assay Based on Bioinformatics

Specificity analysis (cont.): One high dilution (100 pg/μl) of DNA

Exclusivity panel (cont.) – other rash-causing illnesses:

2 *Varicella Zoster viruses* (J clade and E1 clade)

2 *Herpes simplex viruses* (type 1 and 2)

2 Rickettsia strains

Chordopoxviruses:

1 *Parapoxvirus* (*Orf*)

Negatives:

13 bacteria strains (skin flora)

water

Environmental Detection: Additional Considerations to Clinical Diagnostics

- **How to verify authentic agent?**
  - Sufficient versus necessary
  - Culture?
  - Nucleic acid tests
    - How much of the genome?
- **How to verify infectious risk?**
  - Absent a clinically ill human, what is sufficient, what is necessary?
- **How and where to evaluate/validate**
  - WHO considerations what is sufficient, necessary to have a public safety/health actionable assay result;
  - What should that action be?

Discussion?

AOAC SMPR 2016.XXX; Version 3

Method Name: **Detection and Identification of *Variola Virus***

Approved Body: *AOAC Stakeholder Panel on Agent Detection Assays*
Approval Date:
Final version date:

1. Intended Use: Laboratory use by trained technicians.

2. Applicability: Detection of *Variola virus* DNA in collection buffers from aerosol collection devices for DoD applications

Note: Method developers are advised to check the AOAC website for the most up to date version of this SMPR before initiating a validation.

3. Analytical Technique: Polymerase Chain Reaction (PCR) Methods.

4. Definitions:

Acceptable Minimum Detection Level (AMDL)
The predetermined minimum level of an analyte, as specified by an expert committee that must be detected by the candidate method at a specified probability of detection (POD). The AMDL is dependent on the intended use. (Draft ISO 16140) [1]

Exclusivity
Study involving pure non-target strains, that are potentially cross-reactive, that shall not be detected or enumerated by the teste~~d~~ method. (Draft ISO 16140)[2]

Inclusivity
Study involving pure target strains that shall be detected or enumerated by the alternative method. (Draft ISO 16140)[3]

Maximum Time-To-Assay Result
Maximum time to complete an analysis starting from the collection buffer 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. [4] .

System False-Negative Rate

[1] Draft EN ISO/CD 16140-1: Microbiology of food and animal feeding stuffs - Method validation - Part 1: Terminology of method validation, *vs 17-03-2011*
[2] *Ibid*.
[3] *Ibid*.
[4] Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL, 19th edition, 2012.

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.

Variola virus
A member of the genus *Orthopoxvirus* and the causative agent of smallpox.

5. System suitability tests and/or analytical quality control:
The controls listed in Annex I shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded 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).

64 **7. Method Performance Requirements**:

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Parameter	Minimum Performance Requirement
Acceptable Minimal Detection Level (AMDL)	50,000 copies/ml of *Variola virus* target DNA in the candidate method sample collection buffer. Copies/ml refers to number of viral genomes or equivalent plasmid copies containing target viral gene or gene fragment.
Probability of Detection at AMDL within sample collection buffer	≥ 0.95
Probability of Detection at AMDL in an aerosol environmental matrix	≥ 0.95 (Annex V; part 1)
Inclusivity panel purified DNA	All inclusivity strains (Annex II) must test positive at 2x the AMDL†
Exclusivity panel purified DNA	All exclusivity strains (Annex III and Annex V; part 2) must test negative at 10x the AMDL†
System False-Negative Rate using spiked aerosol environmental matrix	≤ 5% (Annex V; Part 1)
System False-Positive Rate using aerosol environmental matrix	≤ 5% (Annex V; Part 1)
Maximum Time to Assay Result	≤ 4 hours
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[5]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.	

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- [5] 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 **ANNEX 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. It is recommended that a technique (ie unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	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

Annex II: Inclusivity Panel

The inclusivity panel shall include:

- Sequences from at least two representative strains, one strain from each major clade of *Variola virus (Li, et. al. On the origin of smallpox: correlating variola phylogenics with historical smallpox records. PNAS (2007) Oct. 2;104 (40):15787-15792.)*
- Any other strain with differences in the assay primer and/or probe target sequences based on bioinformatic analysis. See Annex IV.

Note: The World Health Organization (WHO) restricts access to *Variola virus* genomic material; use of any genomic sequences greater than 500 bp requires written permission/approval from the WHO. Insertion of *Variola virus* DNA into other *Orthopoxviruses* is prohibited.

More details can be found at:

WHO Advisory Committee on Variola Virus Research: Report of the Seventeenth Meeting

Annex 5: WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA
http://apps.who.int/iris/bitstream/10665/205564/1/WHO_OHE_PED_2016.1_eng.pdf

WHO Recommendations concerning the distribution, handling and synthesis of Variola virus DNA
http://www.who.int/csr/disease/smallpox/SummaryrecommendationsMay08.pdf

Annex III: Exclusivity Panel (near-neighbor)

The exclusivity panel shall include:

- All poxvirus strains listed in the table below (Note: See AOAC Website for the most recent list.)
- Any additional strains determined through the bioinformatics analysis, performed in accordance with Annex IV, with greater similarity to the assay's target region(s) than the strains listed in the table below.

CORE EXCLUSIVITY PANEL

Species	Strain	Commercial availability
Vaccinia	Elstree (Lister vaccine)	ATCC VR-1549
Cowpox	Brighton	ATCC VR-302
Ectromelia	Moscow	ATCC VR-1374
Monkeypox	V79-I-005	BEI NR-2324
Monkeypox	USA-2003	BEI NR-2500
Raccoonpox	Herman	ATCC VR-838
Skunkpox	SKPV-USA-1978-WA	ATCC VR-1830
Volepox	VPXV-USA-1985-CA	ATCC VR-1831
Camelpox	V78-I-2379	BEI NR-49736 NR-49737
Taterapox	V71-I-016	BEI
Parapoxvirus Orf	vaccine	Colorado Serum Company

Annex IV: Bioinformatics Analyses of Signature Sequences underlying *Variola* Virus Assays

In silico screening will be performed on signature sequences (eg: oligo primers) to demonstrate specificity to *Variola virus* and inclusivity across all sequenced *Variola virus* strains.

In silico results are suggestive of potential performance issues, so will guide necessary additions to the wet screening panels. *In silico* identification of potential cross-reactions (false positives) or non-verifications (false negatives) would require the affected strains be included in the exclusivity or inclusivity panels, respectively, if available.

A vendor-selected tool to carry out the bioinformatics evaluation should be able to predict hybridization events between signature components and a sequence in a database including available genomic sequence data, using public genbank nt [http://www.ncbi.nlm.nih.gov/genbank/]. The selected tool should be able to identify predicted hybridization events based on platform annealing temperatures, thus ensuring an accurate degree of allowed mismatch is incorporated in predictions. The program should detect possible amplicons from any selected database of sequence.

Potential tools for *in silico* screening of real-time PCR signatures include:

- http://sourceforge.net/projects/simulatepcr/files/?source=navbar
  - This program will find all possible amplicons and real time fluorescing events from any selected database of sequence.

- NCBI tools

The vendor submission should include:

- Description of sequence databases used in the *in silico* analysis
- Description of conditions used for *in silico* analysis
  - Stringency of *in silico* analysis must match bench hybridization conditions
- Description of tool used for bioinformatics evaluation
  - Data demonstrating the selected tool successfully predicts specificity that has been confirmed by wet-lab testing on designated isolates
    - These data can be generated retrospectively using published assays
- List of additional strains to be added to the inclusivity (Annex II) or exclusivity (Annex III) panels based on the bioinformatics evaluation

Annex V: Environmental Factors For Validating Biological Threat Agent Detection Assays

[Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]

The Environmental Factors Studies supplement the biological threat agent near-neighbor exclusivity testing panel. It is critical to understand the performance of the method in the presence of these environmental factors. This panel is used to characterize assay performance in the presence of these factors.There are three parts to Environmental Factors studies: part 1 - environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the potential interferents applicable to Department of Defense applications.[6]

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

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. This includes considerations that may be encountered when the collection system is deployed operationally such as collection medium, duration of collection, diversity of geographical areas that will be sampled, climatic/environmental conditions that may be encountered and seasonal changes in the regions of deployment.

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.

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

[6] Added in June 2015 for the Department 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 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 gene or gene fragment at 2x the AMDL in the candidate method DNA elution buffer.

DNA in this list that already appear in the inclusivity or exclusivity panel do not need to be tested again as part of the environmental factors panel.

- **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 soil or water**
  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
Legionella pneumophilas
Listeria monocytogenes
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae

- **Microbial eukaryotes**

<u>Freshwater amoebae</u>
Acanthamoeba castellanii
Naegleria fowleri

<u>Fungi</u>
Alternaria alternata
Aspergillus fumagatis
Aureobasidium pullulans
Cladosporium cladosporioides
Cladosporium sphaerospermum
Epicoccum nigrum
Eurotium amstelodami
Mucor racemosus
Paecilomyces variotii
Penicillum chrysogenum
Wallemia sebi

- **DNA from higher eukaryotes**

  Plant Pollen[7]
  Zea mays (corn)
  Pinus spp . (pine)
  Gossypium spp. (Cotton)

  Arthropods
  Aedes aegypti (ATCC /CCL-125(tm) 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* and *Dermacentor* tick species for *F. tularensis* detection assays)[8]

  Vertebrates
  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)
  Capri hirca (Goat[9])

- **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)

[7] If pollen is unavailable, vegetative DNA is acceptable
[8] Added by SPADA on March 22, 2016.
[9] Added by SPADA on September 1, 2015.

Part 3: Potential Interferents Study

The Potential Interferents Study supplements the Environmental Factors Study, and is applicable to all biological threat agent detection assays for Department of Defense applications. Table 1a provides a list of potential interferents that are likely to be encountered in various Department of Defense applications.

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

Spiked / inoculated sample collection buffers shall be tested using the procedure specified by the candidate method. A candidate method that fails at the 1 microgram per ml level may be reevaluated at lower concentrations until the inhibition level is determined.

It is expected that all samples are correctly identified as positive.

337 **Table 5a: Potential Interferents**

338

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

339

340 Table 1a is offered for guidance and there are no mandatory minimum requirements for the number
341 of potential interferents to be tested.

342

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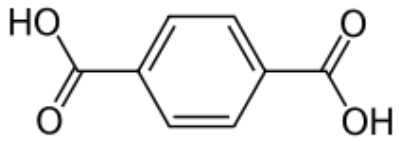
[1] **JP-8**. Air Force formulation jet fuel.

[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.

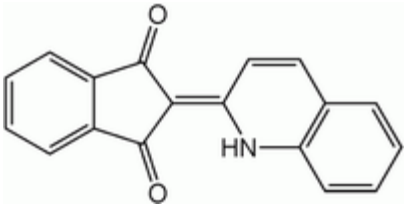
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead;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

***Brucella suis* Working Group**
Chair: Frank Roberto, Idaho National Laboratory
SMPR Presentation
August 30, 2016

Rockville, Maryland, USA

Fitness for Purpose from 3/22/16

"**Detection and identification of *B. abortus*, *B. suis* (including *B. canis*) and *B. melitensis* DNA in aerosol collection filters and/or liquids for DoD applications.**"

SPADA *Brucella* Working Group
Working Group Members

Frank Roberto, INL (Chair)
Jennifer Arce, PNNL
Linda Beck, NSWC – Dahlgren
Thomas Blank, NBACC
Larry Blyn, Ibis Biosciences
Ryan Cahall, Censeo Insight
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David Rozak, USAMRIID
Frank Schaefer, EPA (Ret)
Sanjiv Shah, EPA
Shanmuga Sozhamannan, DoD CRP
Rebekah Tiller, CDC
Richard Warren, HHS
Edward Young, VA Medical Center

Brucella SMPR Work to Date

- Working Group Launch (March, 2016)
- Four (4) teleconferences (May 2016 – July 2016)
- One (1) SMPR Drafted
- Public comment period (July 15, 2016 – August 12, 2016)
- SMPRs made ready for SPADA review and approval

Background

- Brucellosis, aka Malta Fever, undulant fever, Bang's Disease, fistulous withers (in horses)
- The causal agent is an intracellular bacterial pathogen, first isolated after cases of the disease in garrison troops on the island of Malta who drank infected goat's milk (Sir David Bruce, "Micrococcus melitensis")
- Pasteurization of dairy products and culling of infected animals have reduced the prevalence of the disease in many domesticated species, but wildlife reservoirs remain throughout the world including US

Background

- *B. suis* anecdotally first pathogen developed as a biological weapon by former US offensive program (W. Patrick, III, personal communication)
- Infectious dose of 10-100 organisms validated in historic "8-ball" chamber at Ft. Detrick with human subjects
- Aerosol transmission (first noted in swine abattoirs), but also via fomites and sexual route documented in animals and humans
- Serious acute and chronic symptoms of brucellosis often complicate correct diagnosis

Background

- α-proteobacteria, Order Rhizobiales, Family Brucellaceae
- Other Brucellaceae include Mycoplana, Ochrobactrum, Pseudochrobactrum, Paenochrobactrum, and Crabtreella.
- Ten currently recognized species (often with strong host-specificity) further divided into biovars
- Members of the Rhizobiales are similar enough to generate false positives (eg *Agrobacterium tumefaciens*)

SMPR Key Points

Table 1: Method Performance Requirements

Parameter	Minimum Performance Requirement
AMDL	2,000 genomic equivalents of *Brucella suis* (Biovar 1, Type Strain 1330) per mL liquid 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-Negative Rate using spiked environmental matrix materials.	≤ 5%
System False-Positive Rate using environmental matrix materials.	≤ 5%
Inclusivity	All inclusivity strains (Table 3) must test positive at 2x the AMDL †
Exclusivity	All exclusivity strains (Table 4 and Annnex I; part 2) must test negative at 10x the AMDL †

Notes:
† 100% correct analyses are expected. All discrepancies are to be retested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures. [1]

[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.

SMPR Key Points

- Inclusivity panel includes available type strains for *B. suis* biovars 1 to 4, and genomic sequences for biovars 5 and 6
- Exclusivity panel includes 27 strains of Brucella - biovars of *B. abortus, melitensis, canis, microti, neotomae, ovis, ceti, pinnipedialis, inopinata, papionis, vulpis,* vaccine strains - and 3 strains of related genera Agrobacterium and Ochrobactrum

Many of the exclusivity strains are virulent in human and animal hosts







Zoonotic transmission also possible

Comments Submitted

- No comments received

Motion

- Motion to accept the Standard Method Performance Requirements for *Brucella suis* as presented

Discussion?

AOAC SMPR 2016.XXX; Version 6.0

Standard Method Performance Requirements (SMPRs®) for
DNA-based methods of detecting *Brucella suis* in field-deployable, Department of Defense aerosol collection devices

Intended Use: *Field-deployed use for analysis of aerosol collection filters and/or liquids*

1. **Applicability**: Detection of *Brucella suis* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

2. **Analytical Technique**: Molecular detection of nucleic acid.

3. **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).

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

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

Maximum Time-To- Result
Maximum time to complete an analysis starting from the collection buffer 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.

4. **Method Performance Requirements**:
See Table I.

5. **System suitability tests and/or analytical quality control:**
The controls listed in Table II shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded 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).

Inclusivity and exclusivity panel organisms used for evaluation must be characterized and documented to truly be the species and strains they are purported to be.

7. Maximum time-to-results: Within four hours.

Table 1: Method Performance Requirements

Parameter	**Minimum Performance Requirement**
AMDL	2,000 genomic equivalents of *Brucella suis* (Biovar 1, Type Strain 1330) per mL liquid 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-Negative Rate using spiked environmental matrix materials.	≤ 5%
System False-Positive Rate using environmental matrix materials.	≤ 5%
Inclusivity	All inclusivity strains (Table 3) must test positive at 2x the AMDL †
Exclusivity	All exclusivity strains (Table 4 and Annnex I; part 2) must test negative at 10x the AMDL †
Notes: † 100% correct analyses are expected. All discrepancies are to be retested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures. [1]	

[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.

68 **Table 2: Controls**

69

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. It is recommended that a technique (i.e. unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	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 (or sample set) run

Table 3: Inclusivity Panel

No.	Strain designation	Biovar	ATCC/BEI/GB accession #	Available from	Comments
1	*B. suis 1330*	1	ATCC 23444 BEI NR-302	BEI Resources	Swine, USA
2	*B. suis Thomsen*	2	ATCC 23445 BEI NR-303	BEI Resources	Hare, Denmark
3	*B. suis 686*	3	ATCC 23446 BEI NR-304	BEI Resources	swine, USA
4	*B. suis 40*	4	ATCC 23447 BEI NR-305	BEI Resources	Reindeer, Russia
5	*B. suis 513*	5	ACBK00000000*	Gen Bank	mouse, Russia
6	*B. suis S2*	N/A	ALOS00000000.1*	Gen Bank	naturally attenuated vaccine strain used in China

Notes:

1) The *Brucella* Working Group recognizes that *B.suis* biovar 5 is difficult to distinguish from the other *B. suis* biovars. The working group concluded that *B.suis* biovar 5 should be included as a part of the *B.suis* inclusivity panel with caution that *B.suis* biovar 5 may be very difficult to differentiate from other *B. suis* biovars. However, the SMPR does not require candidate assays to differentiate biovars.

*Available in the whole genome database at Genbank.

Table 4: Exclusivity Panel

No.	Strain designation	Biovar	ATCC/BEI/ Accession #	Available from	Comments
1	*B. abortus S19*	1		NVSL	S19 vaccine strain, smooth
2	*B. abortus RB51*	1	BEI NR-2552	NVSL BEI Resources	RB51 vaccine strain, rough
3	*B. abortus 86/8/59*	2	ATCC 23449 BEI NR-231	BEI Resources	Bovine, England
4	*B. abortus 12*	3	ATCC 17385 BEI NR-229	BEI Resources	
5	*B. abortus Tulya*	3	ATCC 23450		Human, Uganda
6	*B. abortus 292 (39/94)*	4	ATCC 23451 BEI NR-233	BEI Resources	Bovine, England
7	*B. abortus B3196*	5	ATCC 23452 BEI NR-234	BEI Resources	Bovine, England
8	*B. abortus 870*	6	ATCC 23453 BEI NR-261	BEI Resources	Bovine, Africa
9	*B. abortus 63/75*	7	ATCC 23454		Bovine, Africa
10	*B. abortus C68*	9	ATCC 23455 BEI NR-263	BEI Resources	Bovine, England
11	*B. abortus 544*	1	ATCC 23448 BEI NR-69	BEI Resources	Bovine, England
12	*B. melitensis 16M*	1	ATCC 23456 BEI NR-256	BEI Resources	Goat, USA
13	*B. melitensis 63/9*	2	ATCC 23457		Goat, Turkey
14	*B. melitensis Ether*	3	ATCC 23458		Goat, Italy
15	*B. melitensis bv. 1 str. Rev.1*	1	ACEG00000000		Elberg origin, *B. melitensis vaccine strain*
16	*B. canis* RM-666	N/A	ATCC 23365 NR-683	ATCC	Dog
17	*B. neotomae* 5K33	N/A	ATCC 23459 BEI NR-684	ATCC BEI Resources	Desert Wood Rat
18	*B. ovis* 63-390	N/A	ATCC 25840 BEI NR-682	ATCC BEI Resources	Ram, Australia
19	*B. ceti* B1/94	N/A	AZBH02000000		Porpoise, Scotland

20	*B. pinnipedialis* B2/94	N/A	ACBN00000000		Seal, Scotland
21	*Brucella* spp. 83/13	N/A	ACBQ00000000		Rat, Australia
22	*B. inopinata* BO1	N/A	ADEZ00000000		Human, Oregon
23	*Brucella* sp. BO2	N/A	ADFA00000000		Human, Australia
24	*B. papionis* F8/08-60(T)	N/A	ACXD00000000		Novel Brucella associated with primates(NVSL 07-0026)
26	*B. microti* CCM 4915	N/A	CP001578,CP001579		Cvole, Czech Republic
27	*B. vulpis*	N/A	LN997863-LN997864		Red fox, Austria
31	*Agrobacterium tumefaciens*	N/A	ATCC 4452	ATCC	
33	*Ochrobactrum anthropi*	N/A	ATCC 49188	ATCC	
34	*Ochrobactrum intermedium* LMG 3301	N/A	2010022371	CDC	

Notes:

1) The Brucella Working Group is aware that *B. canis* can infect humans, causing approximately 100 cases of human brucellosis annually. The working group is also aware of the close relationship between *B. suis* and *B. canis.* In fact, the taxonomic classification of all *Brucella* spp has undergone debate during the last few decades, with some scientists proposing that all *Brucella* spp should be re-classified as *B melitensis* on the basis of results of DNA-DNA hybridization, and that the current species should be re-classified as biovars. However, the classic taxonomic scheme for the *Brucella* spp and existing biovars was reapproved in 2003 (Osterman B, Moriyon I. International Committee on Systematics of Prokaryotes: Subcommittee on the taxonomy of Brucella. Int J Syst Evol Microbiol 2006;56:1173–1175) on the basis of host specificity, phenotypic characteristics, varying virulence, and genotyping data. For these reasons as well as directions from DoD to focus on *B. suis,* the working group determined to develop this SMPR for the specific detection of *B. suis*.

2) The Brucella Working Group is aware of Russian vaccines using *B. abortus* SR82 and *B. abortus* 7579, and other strains may also be in use. These vaccine strains were not available at the time this SMPR was adopted. Consequently the working group decided not to include these vaccine strains in the exclusivity panel.

Guidance

Organisms may be tested as isolated DNA, or combined to form pooled isolated DNA. Isolated DNA may be combined into pools of up to 10 exclusivity panel organisms, with each panel organism represented at 10 times the AMDL. If an unexpected result occurs, each of the exclusivity organisms from a failed pool must be individually re-tested at 10 times the AMDL.

Annex I: Environmental Factors For Validating Biological Threat Agent Detection Assays

[Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]

The Environmental Factors Studies supplement the biological threat agent near-neighbor exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 - environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the potential interferents applicable to Department of Defense applications.[2]

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

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. This includes considerations that may be encountered when the collection system is deployed operationally such as collection medium, duration of collection, diversity of geographical areas that will be sampled, climatic/environmental conditions that may be encountered and seasonal changes in the regions of deployment.

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.

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

[2] Added in June 2015 for the Department 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 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 gene or gene fragment at 2x the AMDL in the candidate method DNA elution buffer.

DNA in this list that already appear in the inclusivity or exclusivity panel do not need to be tested again as part of the environmental factors panel.

- **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 soil or water**
  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
Legionella pneumophilas
Listeria monocytogenes
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae

- **Microbial eukaryotes**

Freshwater amoebae
Acanthamoeba castellanii
Naegleria fowleri

Fungi
Alternaria alternata
Aspergillus fumagatis
Aureobasidium pullulans
Cladosporium cladosporioides
Cladosporium sphaerospermum
Epicoccum nigrum
Eurotium amstelodami
Mucor racemosus
Paecilomyces variotii
Penicillum chrysogenum
Wallemia sebi

- **DNA from higher eukaryotes**

  Plant Pollen[3]

  Zea mays (corn)
  Pinus spp . (pine)
  Gossypium spp. (Cotton)

  Arthropods

  Aedes aegypti (ATCC /CCL-125(tm) 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* and *Dermacentor* tick species for *F. tularensis* detection assays)[4]

  Vertebrates

  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)
  Capri hirca (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)

[3] If pollen is unavailable, vegetative DNA is acceptable
[4] Added by SPADA on March 22, 2016.
[5] Added by SPADA on September 1, 2015.

Part 3: Potential Interferents Study

The Potential Interferents Study supplements the Environmental Factors Study, and is applicable to all biological threat agent detection assays for Department of Defense applications. Table 1a provides a list of potential interferents that are likely to be encountered in various Department of Defense applications.

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

Spiked / inoculated sample collection buffers shall be tested using the procedure specified by the candidate method. A candidate method that fails at the 1 microgram per ml level may be reevaluated at lower concentrations until the inhibition level is determined.

It is expected that all samples are correctly identified as positive.

274 **Table 5a: Potential Interferents**

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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

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277 Table 1a is offered for guidance and there are no mandatory minimum requirements for the
278 number of potential interferents to be tested.

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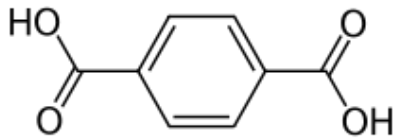
[1] **JP-8**. Air Force formulation jet fuel.

[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.

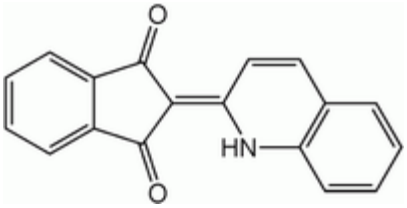
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead;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

***Burkholderia pseudomallei* Working Group**
Chair: Jay E. Gee, CDC
SMPR Presentation
August 30, 2016

Rockville, Maryland, USA

Fitness for Purpose from 3/22/16

"Detection and identification of *Burkholderia pseudomallei* DNA in aerosol collection filters and/or liquids for DoD applications. Detection and identification of *Burkholderia mallei* would also be desirable as long as it does not detract from the specificity for *Burkholderia pseudomallei*."

SPADA *Burkholderia pseudomallei* Working Group Members

Jay Gee, CDC (Chair)
Jennifer Arce, PNNL
Linda Beck, NSWC – Dahlgren
Thomas Blank, DHS
Larry Blyn, Ibis Biosciences
Ryan Cahall, Censeo Insight
Amanda Clark, NSWC – Dahlgren
Kenneth Damer, NGC
David Deshazer, USAMRIID
Martha Hale, USAMRIID
Malcolm Johns, DHS
Paul Keim, NAU
Katalin Kiss, ATCC
Matthew Lesho, Luminex
Nancy Lin, NIST
Steven Morse, CDC (Ret.)
Pejman Naraghi-Arani, InSilixa
Richard Ozanich, PNNL
David Rozak, USAMRIID
Jason Sahl, NAU
Frank Schaefer, EPA (Ret)
Herbert Schweizer, University of Florida
Sanjiv Shah, EPA
Shanmuga Sozhamannan, DoD CRP

Burkholderia pseudomallei Work to Date

- Working Group Launch (March, 2016)
- Three (3) teleconferences (May 2016 – July 2016)
- 1 SMPR Drafted
- Public comment period (July 15, 2016 – August 12, 2016)
- SMPRs made ready for SPADA review and approval

Background

Burkholderia pseudomallei

- Tier 1 Select Agent
- Causes disease melioidosis
- Aerobic, gram-negative bacillus
- Naturally in water and soil in endemic areas
- Opportunistic pathogen
- Causes human and animal disease
- Incubation period: 1 to 21 days, mean of 9 days
- Can be latent for decades before causing illness

Background

- Improvements in rapid detection are desirable
- A uniform panel of isolates will allow standardization to better compare analytical test results

SMPR Key Points

- **Detection of *B. pseudomallei* in field-deployable, DoD aerosol collection devices**
- **Analytical Technique**
  - Molecular detection of nucleic acid.

SMPR Key Points

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).

SMPR Key Points

Acceptable Minimum Detection Level (AMDL)

- 2,000 standardized cells of B. pseudomallei strain 1026b per mL liquid in the candidate method sample collection buffer.

SMPR Key Points

- **Inclusivity panel based on genetic diversity**
- **Exclusivity panel composed of *Burkholderia* spp that have potential to cause diagnostic confusion based on close relationship to *B. pseudomallei***

SMPR Key Points

Inclusivity Panel

Species	Isolate
B. pseudomallei	MSHR668
B. pseudomallei	MSHR1655
B. pseudomallei	K96243
B. pseudomallei	MSHR305
B. pseudomallei	1026b
B. pseudomallei	7894
B. pseudomallei	MSHR840
B. pseudomallei	576
B. pseudomallei	HBPUB10134a

SMPR Key Points

Exclusivity Panel

Species	Isolate
B. mallei	Strain 6
B. mallei	NCTC10247
B. thailandensis	CDC3015869 (aka TXDOH)
B. thailandensis	H0587
B. thailandensis	Malaysia20 (aka Bp7046)
B. thailandensis	E1 (aka Bp7045)
B. humptydooensis (proposed)	MSMB43 (aka Bp5365)
B. humptydooensis (proposed)	MSMB1589 (aka Bp7270)
MSMB264	MSMB0265 (aka Bp7063)
B. oklahomensis	1974002358 (aka Bp0072)

SMPR Key Points

Exclusivity Panel

Species	Isolate
B. oklahomensis-like	BDU8 (aka Bp7004)
MSMB175	TSV85 (aka Bp7000)
B. ubonensis	MSMB2036 (aka Bp7062)
B. ubonensis	MSMB1189 (aka Bp7434)
B. multivorans	AU1185 (aka Bp7344)
B. stagnalis	MSMB735 (aka Bp7657)
B. cepacia	MSMB1824 (aka Bp7307)
B. vietnamiensis	FL-2-3-30-S1-D0 (aka Bp7021)
B. vietnamiensis	AU1233 (aka Bp7345)

Comments Submitted (if any)

- No comments submitted

Motion

- Motion to accept the Standard Method Performance Requirements for *Burkholderia pseudomallei* as presented.

Discussion?

AOAC SMPR 2016.XXX; Version 6

Standard Method Performance Requirements (SMPRs®) for
DNA-based methods of detecting *Burkholderia pseudomallei* in field-deployable, Department of Defense aerosol collection devices

Intended Use: *Field-deployed use for analysis of aerosol collection filters and/or liquids*

1. **Applicability**: Detection of *Burkholderia pseudomallei* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

2. **Analytical Technique**: Molecular detection of nucleic acid.

3. **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).

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

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

Maximum Time-To- Result
Maximum time to complete an analysis starting from the collection buffer 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.

4. **Method Performance Requirements**:
See Table I.

5. **System suitability tests and/or analytical quality control:**
The controls listed in Table II shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded 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).

Inclusivity and exclusivity panel organisms used for evaluation must be characterized and documented to truly be the species and strains they are purported to be.

7. Maximum time-to-results: Within four hours.

Table I: Method Performance Requirements

Parameter	**Minimum Performance Requirement**
AMDL	2,000 standardized cells of *Burkholderia pseudomallei* 1026b per mL liquid 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-Negative Rate using spiked environmental matrix materials.	≤ 5%
System False-Positive Rate using environmental matrix materials.	≤ 5%
Inclusivity	All inclusivity strains (Table III) must test positive at 2x the AMDL †
Exclusivity	All exclusivity strains (Table IV and Annex I; part 2) must test negative at 10x the AMDL†.
Notes: † 100% correct analyses are expected. All discrepancies are to be retested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures. [1]	

[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.

66 **TABLE II: Controls**

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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. It is recommended that a technique (ie unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	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 (or sample set) run

Table III: Inclusivity Panel

Species	Isolate
B. pseudomallei	MSHR668
B. pseudomallei	MSHR1655
B. pseudomallei	K96243
B. pseudomallei	MSHR305
B. pseudomallei	1026b
B. pseudomallei	7894
B. pseudomallei	MSHR840
B. pseudomallei	576
B. pseudomallei	HBPUB10134a

Table IV: Exclusivity Panel (near-neighbor)

	Species	Isolate
1	B. mallei	Strain 6
2	B. mallei	NCTC10247
3	B. thailandensis	CDC3015869 (aka TXDOH)
4	B. thailandensis	H0587
5	B. thailandensis	Malaysia20 (aka Bp7046)
6	B. thailandensis	E1 (aka Bp7045)
7	B. humptydooensis (proposed)	MSMB43 (aka Bp5365)
8	B. humptydooensis (proposed)	MSMB1589 (aka Bp7270)
9	MSMB264	MSMB0265 (aka Bp7063)
10	B. oklahomensis	1974002358 (aka Bp0072)
11	B. oklahomensis-like	BDU8 (aka Bp7004)
12	MSMB175	TSV85 (aka Bp7000)
13	B. ubonensis	MSMB2036 (aka Bp7062)
14	B. ubonensis	MSMB1189 (aka Bp7434)
15	B. multivorans	AU1185 (aka Bp7344)
16	B. stagnalis	MSMB735 (aka Bp7657)
17	B. cepacia	MSMB1824 (aka Bp7307)
18	B. vietnamiensis	FL-2-3-30-S1-D0 (aka Bp7021)
19	B. vietnamiensis	AU1233 (aka Bp7345)

Note: Strains and species from item 3 to 19 can be used as an exclusivity panel for *B. mallei* assays.

Guidance

Organisms may be tested as isolated DNA, or combined to form pooled isolated DNA. Isolated DNA may be combined into pools of up to 10 exclusivity panel organisms, with each panel organism represented at 10 times the AMDL. If an unexpected result occurs, each of the exclusivity organisms from a failed pool must be individually re-tested at 10 times the AMDL.

Annex I: Environmental Factors For Validating Biological Threat Agent Detection Assays

[Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]

The Environmental Factors Studies supplement the biological threat agent near-neighbor exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 - environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the potential interferents applicable to Department of Defense applications.[2]

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

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. This includes considerations that may be encountered when the collection system is deployed operationally such as collection medium, duration of collection, diversity of geographical areas that will be sampled, climatic/environmental conditions that may be encountered and seasonal changes in the regions of deployment.

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.

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

[2] Added in June 2015 for the Department 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 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 gene or gene fragment at 2x the AMDL in the candidate method DNA elution buffer.

DNA in this list that already appear in the inclusivity or exclusivity panel do not need to be tested again as part of the environmental factors panel.

- **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 soil or water**
  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
Legionella pneumophilas
Listeria monocytogenes
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae

- **Microbial eukaryotes**

<u>Freshwater amoebae</u>
Acanthamoeba castellanii
Naegleria fowleri

<u>Fungi</u>
Alternaria alternata
Aspergillus fumagatis
Aureobasidium pullulans
Cladosporium cladosporioides
Cladosporium sphaerospermum
Epicoccum nigrum
Eurotium amstelodami
Mucor racemosus
Paecilomyces variotii
Penicillum chrysogenum
Wallemia sebi

- **DNA from higher eukaryotes**

  Plant Pollen[3]

  Zea mays (corn)
  Pinus spp . (pine)
  Gossypium spp. (Cotton)

  Arthropods

  Aedes aegypti (ATCC /CCL-125(tm) 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* and *Dermacentor* tick species for *F. tularensis* detection assays)[4]

  Vertebrates

  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)
  Capri hirca (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)

[3] If pollen is unavailable, vegetative DNA is acceptable
[4] Added by SPADA on March 22, 2016.
[5] Added by SPADA on September 1, 2015.

Part 3: Potential Interferents Study

The Potential Interferents Study supplements the Environmental Factors Study, and is applicable to all biological threat agent detection assays for Department of Defense applications. Table 1a provides a list of potential interferents that are likely to be encountered in various Department of Defense applications.

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

Spiked / inoculated sample collection buffers shall be tested using the procedure specified by the candidate method. A candidate method that fails at the 1 microgram per ml level may be reevaluated at lower concentrations until the inhibition level is determined.

It is expected that all samples are correctly identified as positive.

Table 5a: Potential Interferents

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

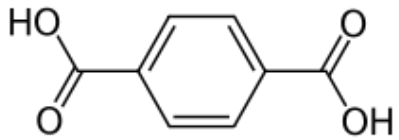
Table 1a is offered for guidance and there are no mandatory minimum requirements for the number of potential interferents to be tested.

[1] **JP-8**. Air Force 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.

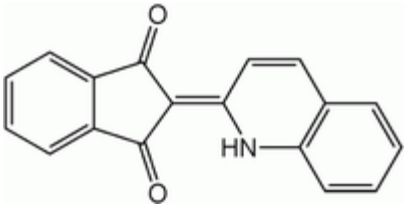
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead;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

Botulinum neurotoxins Working Group
Chair: Shashi Sharma, FDA
SMPR Presentation
August 30, 2016

Rockville, Maryland, USA

Fitness for Purpose from 3/22/16

"Detection and identification of Botulinum neurotoxin A in aerosol collection filters and/or liquids for DoD applications. Detection and/or identification of Botulinum neurotoxins B-G would also be desirable."

SPADA Botulinum neurotoxin Working Group Working Group Members

Shashi Sharma, FDA (Chair)

Linda Beck, NSWC Dahlgren

Ryan Cahall, Censeo Insight

Kenneth Damer, Northrop Grumman

Paul Jackson, LLNL

Matthew Lesho, Luminex

Richard Ozanich, PNNL

Mark Poli, USAMRIID

David Rozak, USAMRIID

Sanjiv Shah, US EPA

Theresa Smith, USAMRIID

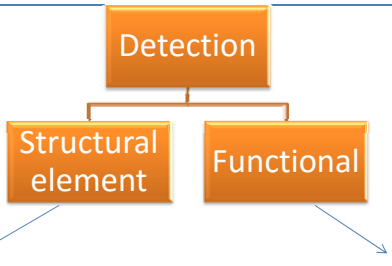
Shanmuga Sozhamannan, DoD CRP

Nagarajan Thirunavukkarasu, FDA

Botulinum neurotoxin Work to Date

- Working Group Launch (March, 2016)
- Three (3) teleconferences (May 2016 – July 2016)
- 1 SMPR Drafted
- Public comment period (July 15, 2016 – August 12, 2016)
- SMPRs made ready for SPADA review and approval

Background



- Relatively easy to detect
- Presence or absence
- Often qualitative
- Cost-effective

- Relatively Not-easy to detect
- Active or inactive
- Qualitative and quantitative
- Expensive
- Representative of BoNT intoxication

SMPR Key Points

- Botulinum neurotoxin type A
- Field deployable assay
- Complexities in detecting di-chain and complex toxins, culture filtrates
- Subtypes A2-A8; degree of confidence (address in SMPR)
- Characterization of B-G

Comments Submitted (if any)

- No comments submitted

Motion

- Motion to accept the Standard Method Performance Requirements for *Botulinum neurotoxins* as presented.

Discussion?

AOAC SMPR 2016.XXX; Version 4

Method Name: **Detection of Botulinum Neurotoxins A1 and A2**

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 Botulinum neurotoxins A1 and A2 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.

Selectivity Study
A study designed to demonstrate a candidate method's ability to detect the various forms of botulium neurotoxin A, and at the same time, demonstrate that a candidate method does not detect nontarget compounds and related nontarget 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).

- Equal numbers of botulinum neurotoxin A1 and A2 and botulinum neurotoxin A1 and A2 complex samples must be represented in the selectivity study. Use pristine buffer

48 solution. Samples with target and nontarget compounds must be: 1) blind coded; 2)
49 randomly mixed together; 3) evaluated at the same time, and 4) masked, so that the
50 sample identity remains unknown to the analysts. Batches are permissible provided 6.1,
51 6.2, 6.3, and 6.4 are followed.
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53 • Information on other subtypes is desirable but not required.
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56 **7. Method Performance Requirements**
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Parameter	Minimum Performance Requirement
AMDL	1.25 ng /mL recovered Botulinum neurotoxin A1 and A2 complexes in liquid
Selectivity Study	POD ≥ 0.95 at AMDL for Botulinum neurotoxin A1 and A2 complex
	Tetanus toxin must test negative at 10x the AMDL†
System False-Negative Rate using spiked aerosol environmental matrix at the AMDL	≤ 5% (Annex I, Part 1)
System False-Positive Rate using aerosol environmental matrix at the AMDL	≤ 5% (Annex I, 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.	

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59 **8. Maximum Time for Assay Results:** Four hours
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[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.

61 **Table I: Controls**

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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. It is recommended that a technique (i.e. unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	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
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 (or sample set) run

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Annex I: Environmental Factors For Validating Biological Threat Agent Detection Assays

[Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]

The Environmental Factors Studies supplement the biological threat agent near-neighbor exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 - environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the potential interferants applicable to Department of Defense applications.[2] Part 2 is not applicable to techniques that do not detect nucleic acid; and therefore not included in this SMPR.

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

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. This includes considerations that may be encountered when the collection system is deployed operationally such as collection medium, duration of collection, diversity of geographical areas that will be sampled, climatic/environmental conditions that may be encountered and seasonal changes in the regions of deployment.

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.

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

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

Part 2: Environmental Panel Organisms -

Not applicable to this SMPR and therefore removed.

Part 3: Potential Interferants Study

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

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

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

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

Table 1A: 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

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

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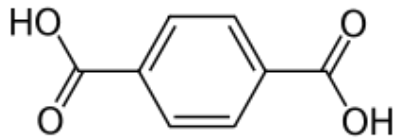
[1] **JP-8**. Airforce formulation jet fuel.

[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.

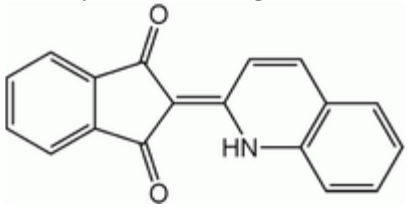
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead;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

August 30, 2016
STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

RESOURCES

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Deborah McKenzie	Sr. Director, Standards Development and Method Approval Processes	dmckenzie@aoac.org	301.924.7077 x 157

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

SPADA Website: http://tinyurl.com/z479wgl

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

Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

Standard method performance requirements (SMPRs) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods. In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable. Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria. However, as the *Eurachem Guide* points out: " . . . the judgment of method suitability for its intended use is equally important . . ." (1) to the evaluation process.

International Voluntary Consensus Standards

An SMPR is a form of an international, voluntary consensus standard. A standard is an agreed, repeatable way of doing something that is published as document that contains a technical specification or other precise criteria designed to be used consistently as a rule, guideline, or definition. SMPRs are a *consensus* standards developed by stakeholders in a very controlled process that ensures that users, research organizations, government departments, and consumers work together to create a standard that meets the demands of the analytical community and technology. SMPRs are also *voluntary* standards. AOAC cannot, and does not, impose the use of SMPRs. Users are free to use SMPRs as they see fit. AOAC is very careful to include participants from as many regions of the world as possible so that SMPRs are accepted as *international* standards.

Guidance for Standard Method Performance Requirements

Commonly known as the "SMPR Guidelines." The first version of the SMPR Guidelines were drafted in 2010 in response to the increasing use and popularity of SMPRs as a vehicle to describe the analytical requirements of a method. Several early "acceptance criteria" documents were prepared for publication in late 2009, but the format of the acceptance criteria documents diverged significantly from one another in basic format. AOAC realized that a guidance document was needed to promote uniformity.

An early version of the SMPR Guidelines were used for a project to define the analytical requirements for endocrine disruptors in potable water. The guidelines proved to be extremely useful in guiding the work of the experts and resulted in uniform SMPRs. Subsequent versions of the SMPR Guidelines were used in the Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN) project with very positive results. The SMPR Guidelines are now published for the first time in the *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis*.

Users of the guidelines are advised that they are: (*1*) a *guidance* document, not a statute that users must conform to; and (*2*) a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using these guidelines.

The SMPR Guidelines are intended to provide basic information for working groups assigned to prepare SMPRs. The guidelines consist of the standard format of an SMPR, followed by a series of informative tables and annexes.

SMPR Format

The general format for an SMPR is provided in *Annex A*.

Each SMPR is identified by a unique SMPR number consisting of the year followed by a sequential identification number (YYYY.XXX). An SMPR number is assigned when the standard is approved. By convention, the SMPR number indicates the year a standard is approved (as opposed to the year the standard is initiated). For example, SMPR 2010.003 indicates the third SMPR adopted in 2010.

The SMPR number is followed by a method name that must include the analyte(s), matrix(es), and analytical technique (unless the SMPR is truly intended to be independent of the analytical technology). The method name may also refer to a "common" name (e.g., "Kjeldahl" method).

The SMPR number and method name are followed by the name of the stakeholder panel or expert review panel that approved the SMPR, and the approval and effective dates.

Information about method requirements is itemized into nine categories: (*1*) intended use; (*2*) applicability; (*3*) analytical technique; (*4*) definitions; (*5*) method performance requirements; (*6*) system suitability; (*7*) reference materials; (*8*) validation guidance; and (*9*) maximum time-to-determination.

An SMPR for qualitative and/or identification methods may include up to three additional annexes: (*1*) inclusivity/selectivity panel; (*2*) exclusivity/cross-reactivity panel; and (*3*) environmental material panels. These annexes not required.

Informative tables.—The SMPR Guidelines contain seven informative tables that represent the distilled knowledge of many years of method evaluation, and are intended as guidance for SMPR working groups. The informative tables are not necessarily AOAC

policy. SMPR working groups are expected to apply their expertise in the development of SMPRs.

Table A1: Performance Requirements. Provides recommended performance parameters to be included into an SMPR. Table A1 is organized by five method classifications: (*1*) main component quantitative methods; (*2*) trace or contaminant quantitative methods; (*3*) main component qualitative methods; (*4*) trace or contaminant quantitative methods; and (*5*) identification methods. The table is designed to accommodate both microbiological and chemical methods. Alternate microbiological/chemical terms are provided for equivalent concepts.

Table A2: Recommended Definitions. Provides definitions for standard terms in the SMPR Guidelines. AOAC relies on *The International Vocabulary of Metrology Basic and General Concepts and Associated Terms* (VIM) and the International Organization for Standadization (ISO) for definition of terms not included in Table A2.

Table A3: Recommendations for Evaluation. Provides general guidance for evaluation of performance parameters. More detailed evaluation guidance can be found in *Appendix D, Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2); *Appendix I, Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (3); *Appendix K, AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (4); Codex Alimentarius Codex Procedure Manual (5); and ISO Standard 5725-1-1994 (6).

Table A4: Expected Precision (Repeatability) as a Function of Analyte Concentration. The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation (RSD) of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides target RSDs for a range of analyte concentrations.

Table A5: Expected Recovery as a Function of Analyte Concentration. Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 1 ppb to 100%.

Table A6: Predicted Relative Standard Deviation of Reproducibility ($PRSD_R$). This table provides the calculated $PRSD_R$ using the Horwitz formula:

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction.

Table A7: POD and Number of Test Portions. This table provides the calculated probability of detection (POD) for given sample sizes and events (detections). A method developer can use this table to determine the number of analyses required to obtain a specific POD.

Informative annexes.—The SMPR Guidelines contain informative annexes on the topics of classification of methods, POD model, HorRat values, reference materials, and method accuracy and review. As with the informative tables, these annexes are intended to provide guidance and information to the working groups.

Initiation of an SMPR

See Figure 1 for a schematic flowchart diagram of the SMPR development process.

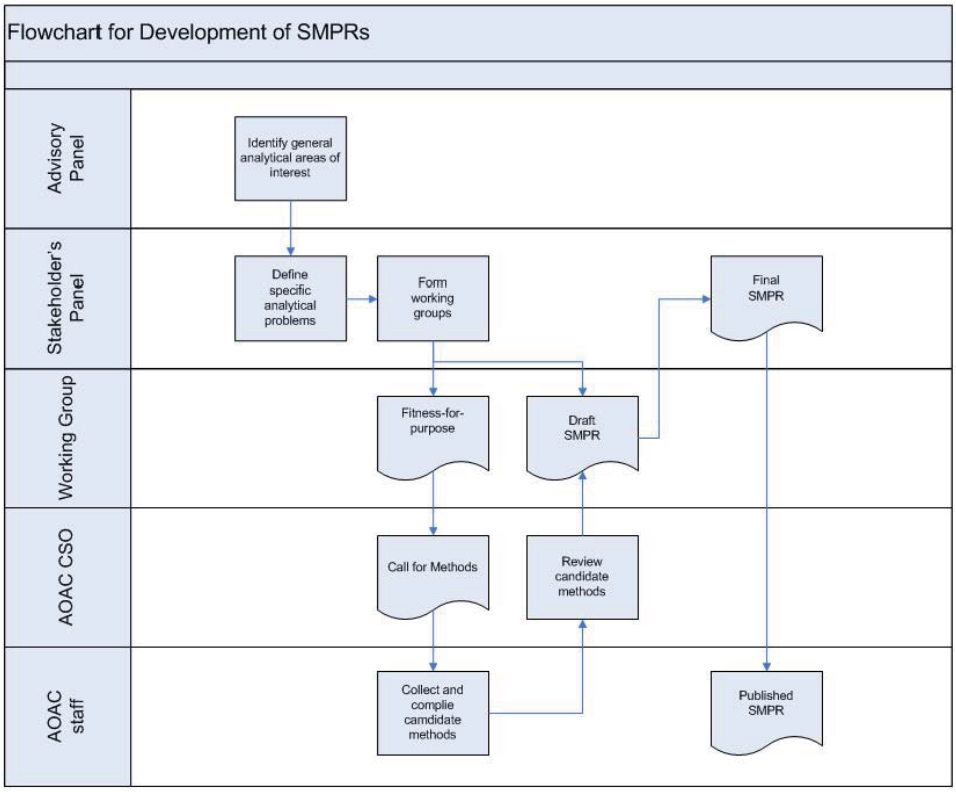


Figure 1. Schematic flowchart diagram of the SMPR development process.

Advisory panels.—Most commonly, an SMPR is created in response to an analytical need identified by an advisory panel. Advisory panels normally consist of sponsors and key stakeholders who have organized to address analytical problems. Usually, the advisory panel identifies general analytical problems, such as the need to update analytical methods for determination of nutrients in infant formula. An advisory panel, with the input of appropriate subject matter experts, also prioritizes the specific analytical problems within the general topic. This panel is critical in planning for the stakeholder panel meeting.

Stakeholder panels.—After an advisory panel has identified a general analytical problem, AOAC announces the standards development activity, identifies stakeholders, and organizes a stakeholder panel. Membership on a stakeholder panel is open to anyone materially affected by the proposed standard. AOAC recruits scientists to participate on stakeholder panels on the basis of their expertise with the analytical problem identified by the advisory panel. Experts are recruited from academia, government, nongovernmental organizations (such as ISO), industry, contract research organizations, method developers, and instrument/equipment manufacturers. AOAC employs a representative voting panel model to ensure balance with regards to stakeholder perspective, and to ensure that no particular stakeholder perspective dominates the proceedings of the stakeholder panel. All stakeholder candidates are reviewed by the AOAC Chief Scientific Officer (CSO) for relevant qualifications, and again by the Official Methods Board to ensure that the stakeholder panel is balanced and all stakeholders are fairly represented.

Stakeholder panels are extremely important as they serve several functions: (*1*) identify specific analytical topics within the general analytical problem described by the advisory panel; (*2*) form working groups to address the specific analytical topics; (*3*) identify additional subject matter experts needed for the working groups; (*4*) provide oversight of the SMPR development; and (*5*) formally adopt SMPRs originally drafted by working groups.

Working groups.—Working groups are formed by the stakeholder panel when a specific analytical topic has been identified. The primary purpose of a working group is to draft an SMPR. Working groups may also be formed to make general recommendations, such as developing a common definition to be used by multiple working groups. For example, SPIFAN formed a working group to create a definition for "infant formula" that could be shared and used by all of the SPIFAN working groups.

The process of drafting an SMPR usually requires several months, and several meetings and conference calls. An SMPR drafted by a working group is presented to a stakeholder panel. A stakeholder panel may revise, amend, or adopt a proposed SMPR on behalf of AOAC.

Fitness-for-Purpose Statement and Call for Methods

One of the first steps in organizing a project is creating a fitness-for-purpose statement. In AOAC, the fitness-for-purpose statement is a very general description of the methods needed. It is the responsibility of a working group chair to draft a fitness-for-purpose statement. A working group chair is also asked to prepare a presentation with background information about the analyte, matrix, and the nature of the analytical problem. A working group chair presents the background information and proposes a draft fitness-for-purpose statement to the presiding stakeholder panel. The stakeholder panel is asked to endorse the fitness-for-purpose statement.

The AOAC CSO prepares a call for methods based on the stakeholder panel-approved fitness-for-purpose statement. The call for methods is posted on the AOAC website and/or e-mailed to the AOAC membership and other known interested parties. AOAC staff collects and compiles candidate methods submitted in response to the call for methods. The CSO reviews and categorizes the methods.

Creating an SMPR

Starting the process of developing an SMPR can be a daunting challenge. In fact, drafting an SMPR should be a daunting challenge because the advisory panel has specifically identified an analytical problem that has yet to be resolved. Completing an SMPR can be a very rewarding experience because working group members will have worked with their colleagues through a tangle of problems and reached a consensus where before there were only questions.

It is advisable to have some representative candidate methods available for reference when a working group starts to develop an SMPR. These methods may have been submitted in response to the call for methods, or may be known to a working group member. In any case, whatever the origin of the method, candidate methods may assist working group members to determine reasonable performance requirements to be specified in the SMPR. The performance capabilities of exisiting analytical methodologies is a common question facing a working group.

Normally, a working chair and/or the AOAC CSO prepares a draft SMPR. A draft SMPR greatly facilitates the process and provides the working group with a structure from which to work.

Working group members are advised to first consider the "intended use" and "maximum time-to-determination" sections as this will greatly affect expectations for candidate methods. For example, methods intended to be used for surveillance probably need to be quick but do not require a great deal of precision, and false-positive results might be more tolerable. Whereas methods intended to be used for dispute resolution will require better accuracy, precision, and reproducibility, but time to determination is not as important.

Once a working group has agreed on the intended use of candidate methods, then it can begin to define the applicability of candidate methods. The applicability section of the SMPR is one of the most important, and sometimes most difficult, sections of the SMPR. The analyte(s) and matrixes must be explicitly identified. For chemical analytes, International Union of Pure and Applied Chemistry (IUPAC) nomenclature and/or Chemical Abstracts Service (CAS) registry numbers should be specified. Matrixes should be clearly identified including the form of the matrix such as raw, cooked, tablets, powders, etc. The nature of the matrix may affect the specific analyte. It may be advantageous to fully identify and describe the matrix before determining the specific analyte(s). It is not uncommon for working groups to revise the initial definition of the analyte(s) after the matrix(es) has been better defined.

Table 1. Example of method performance table for a single analyte

Analytical range	7.0–382.6 µg/mL	
Limit of quantitation (LOQ)	≤7.0 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%
	≥10 µg/mL	≤6%

Table 2. Example of method performance table for multiple analytes

	Analyte 1		Analyte 2		Analyte 3	
Analytical range	10–20 µg/mL		100–200 µg/mL		200–500 µg/mL	
Limit of quantitation (LOQ)	≤10 µg/mL		≤100 µg/mL		≤200 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%	<10 µg/mL	≤8%	<200 µg/mL	≤10%
	≥10 µg/mL	≤6%	≥10 µg/mL	≤6%	≥200 µg/mL	≤8%

For projects with multiple analytes, for example, vitamins A, D, E, and K in infant formula, it may be useful to organize a separate working group to fully describe the matrix(es) so that a common description of the matrix(es) can be applied to all of the analytes.

For single analyte SMPRs, it is most common to organize the method performance requirements into a table with 2–3 columns as illustrated in Table 1. For multiple analyte SMPRs, it is often convenient to present the requirements in an expanded table with analytes forming additional columns as illustrated in Table 2.

Once the intended use, analytical techniques, and method performance requirements have been determined, then a working group can proceed to consider the quality control parameters, such as the minimum validation requirements, system suitability procedures, and reference materials (if available). It is not uncommon that an appropriate reference material is not available. *Annex F* of the SMPR Guidelines provides comprehensive guidance for the development and use of in-house reference materials.

Most working groups are able to prepare a consensus SMPR in about 3 months.

Open Comment Period

Once a working group has produced a draft standard, AOAC opens a comment period for the standard. The comment period provides an opportunity for other stakeholders to state their perspective on the draft SMPR. All collected comments are reviewed by the AOAC CSO and the working group chair, and the comments are reconciled. If there are significant changes required to the draft standard as a result of the comments, the working group is convened to discuss and any unresolved issues will be presented for discussion at the stakeholder panel meeting.

Submission of Draft SMPRs to the Stakeholder Panel

Stakeholder panels meet several times a year at various locations. The working group chair (or designee) presents a draft SMPR to the stakeholder panel for review and discussion. A working group chair is expected to be able to explain the conclusions of the working group, discuss comments received, and to answer questions from the stakeholder panel. The members of the stakeholder panel may revise, amend, approve, or defer a decision on the proposed SMPR. A super majority of 2/3 or more of those voting is required to adopt an SMPR as an AOAC voluntary consensus standard.

Publication

Adopted SMPRs are prepared for publication by AOAC staff, and are published in the *Journal of AOAC INTERNATIONAL* and in the AOAC *Official Methods of Analysis*℠ compendium. Often, the AOAC CSO and working group chair prepare a companion article to introduce an SMPR and describe the analytical issues considered and resolved by the SMPR. An SMPR is usually published within 6 months of adoption.

Conclusion

SMPRs are a unique and novel concept for the analytical methods community. SMPRs are voluntary, consensus standards developed by stakeholders that prescribe the minimum analytical performance requirements for classes of analytical methods. The SMPR Guidelines provide a structure for working groups to use as they develop an SMPR. The guidelines have been employed in several AOAC projects and have been proven to be very useful. The guidelines are not a statute that users must conform to; they are a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using the guidelines.

References

(1) Eurachem, *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, Validation*, http://www.eurachem.org/guides/pdf/valid.pdf, posted December 1998, accessed March 2012

(2) *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis, Appendix D*, AOAC INTERNATIONAL, Gaithersburg, MD

(3) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (2012) *Official Methods of Analysis,* 19th Ed., *Appendix I, Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*, AOAC INTERNATIONAL, Gaithersburg, MD

(4) *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD

(5) Codex Alimentarius Codex Procedure Manual

(6) International Organization for Standardization, Geneva, Switzlerland

ANNEX A
Format of a
Standard Method Performance Requirement

AOAC SMPR YYYY.XXX
(YYYY = Year; XXX = sequential identification number)

Method Name: Must include the analyte(s), matrix(es), and analytical technique [unless the standard method performance requirement (SMPR) is truly intended to be independent of the analytical technology]. The method name may refer to a "common" name (e.g., "Kjeldahl" method).

Approved By: Name of stakeholder panel or expert review panel

Final Version Date: Date

Effective Date: Date

1. Intended Use: Additional information about the method and conditions for use.

2. Applicability: List matrixes if more than one. Provide details on matrix such as specific species for biological analytes, or International Union of Pure and Applied Chemistry (IUPAC) nomenclature and Chemical Abstracts Service (CAS) registry number for chemical analytes. Specify the form of the matrix such as raw, cooked, tablets, powders, etc.

3. Analytical Technique: Provide a detailed description of the analytical technique if the SMPR is to apply to a specific analytical technique; or state that the SMPR applies to any method that meets the method performance requirements.

4. Definitions: List and define terms used in the performance parameter table (*see* Table A2 for list of standard terms).

5. Method Performance Requirements: List the performance parameters and acceptance criteria appropriate for each method/analyte/matrix. *See* Table A1 for appropriate performance requirements.

If more than one analyte/matrix, and if acceptance criteria differ for analyte/matrix combinations then organize a table listing each analyte/matrix combination and its minimum acceptance criteria for each performance criteria.

6. System Suitability Tests and/or Analytical Quality Control: Describe minimum system controls and QC procedures.

7. Reference Material(s): Identify the appropriate reference materials if they exist, or state that reference materials are not available. Refer to *Annex E* (*AOAC Method Accuracy Review*) for instructions on the use of reference materials in evaluations.

8. Validation Guidance: Recommendations for type of evaluation or validation program such as single-laboratory validation (SLV), *Official Methods of Analysis*℠ (OMA), or *Performance Tested Methods*℠ (PTM).

9. Maximum Time-to-Determination: Maximum allowable time to complete an analysis starting from the test portion preparation to final determination or measurement.

Annex I: Inclusivity/Selectivity Panel. Recommended for qualitative and identification method SMPRs.

Annex II: Exclusivity/Cross-Reactivity Panel. Recommended for qualitative and identification method SMPRs.

Annex III: Environmental Materials Panel. Recommended for qualitative and identification method SMPRs.

Table A1. Performance requirements

Classifications of methods[a]				
Quantitative method		Qualitative method		
Main component[b]	Trace or contaminant[c]	Main component[b]	Trace or contaminant[c]	Identification method
Parameter				
Single-laboratory validation				
Applicable range	Applicable range	Inclusivity/selectivity	Inclusivity/selectivity	Inclusivity/selectivity
Bias[d]	Bias[d]	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity
Precision	Precision	Environmental interference	Environmental interference	Environmental interference
Recovery	Recovery	Laboratory variance	Laboratory variance	
Limit of quantitation (LOQ)	LOQ			
		Probability of detection (POD)[e]	POD at AMDL[f]	Probability of identification (POI)
Reproducibility				
RSD_R or target measurement uncertainty	RSD_R or target measurement uncertainty	POD (0)	POD (0)	POI (c)
		POD (c)	POD (c)	
		Laboratory POD[g]	Laboratory POD[g]	Laboratory POI

[a] *See Annex B* for additional information on classification of methods.

[b] ≥100 g/kg.

[c] <100 g/kg.

[d] If a reference material is available.

[e] At a critical level.

[f] AMDL = Acceptable minimum detection level.

[g] LPOD = CPOD.

Table A2. Recommended definitions

Bias	Difference between the expectation of the test results and an accepted reference value. Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias.
Environmental interference	Ability of the assay to detect target organism in the presence of environmental substances and to be free of cross reaction from environmental substances.
Exclusivity	Strains or isolates or variants of the target agent(s) that the method must not detect.
Inclusivity	Strains or isolates or variants of the target agent(s) that the method can detect.
Laboratory probability of detection (POD)	Overall fractional response (mean POD = CPOD) for the method calculated from the pooled POD_j responses of the individual laboratories (j = 1, 2, ..., L).[a] *See Annex C*.
Limit of quantitation (LOQ)	Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.
POD (0)	Probability of the method giving a (+) response when the sample is truly without analyte.
POD (c)	Probability of the method giving a (–) response when the sample is truly without analyte.
POD	Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. Consult *Annex C* for a full explanation.
Probability of identification (POI)	Expected or observed fraction of test portions at a given concentration that gives positive result when tested at a given concentration. Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*.[c]
Precision (repeatability)	Closeness of agreement between independent test results obtained under stipulated conditions. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results.[d]
Recovery	Fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method. There are two types of recovery: (*1*) Total recovery based on recovery of the native plus added analyte, and (*2*) marginal recovery based only on the added analyte (the native analyte is subtracted from both the numerator and denominator).[e]
Repeatability	Precision under repeatability conditions.
Repeatability conditions	Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.
Reproducibility	Precision under reproducibility conditions.
Reproducibility conditions	Conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.
Relative standard deviation (RSD)	$RSD = s_i \times 100/\bar{x}$
Standard deviation (s_i)	$s_i = [\Sigma(x_i - \bar{x})^2/n]^{0.5}$

[a] *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (*Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*), *J. AOAC Int*. **94**, 1359(2011) and *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., *Appendix I*.

[b] *International Vocabulary of Metrology (VIM)—Basic and General Concepts and Associated Terms* (2008) JCGM 200:2008, Joint Committee for Guides in Metrology (JCGM), www.bipm.org

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int*. **95**, 273–285.

[d] ISO 5725-1-1994.

[e] *Official Methods of Analysis* (2012) *Appendix D* (*Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*), AOAC INTERNATIONAL, Gaithersburg, MD.

Table A3. Recommendations for evaluation

Bias (if a reference material is available)	A minimum of five replicate analyses of a Certified Reference Material.[a]
Environmental interference	Analyze test portions containing a specified concentration of one environmental materials panel member. Materials may be pooled. Consult with AOAC statistician.
Exclusivity/cross-reactivity	Analyze one test portion containing a specified concentration of one exclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Inclusivity/selectivity	Analyze one test portion containing a specified concentration of one inclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Limit of quantitation (LOQ)	Estimate the LOQ = average (blank) + 10 × s_0 (blank). Measure blank samples with analyte at the estimated LOQ. Calculate the mean average and standard deviation of the results. Guidance[b]: For ML ≥ 100 ppm (0.1 mg/kg): LOD = ML × 1/5. For ML < 100 ppm (0.1 mg/kg): LOD = ML × 2/5.
Measurement uncertainty	Use ISO 21748: *Guidance for the use of repeatability, reproducibility, and trueness estimates in measurement uncertainty estimation to analyze data collected for bias, repeatability, and intermediate precision to estimate measurement uncertainty*.
POD(0)	Use data from collaborative study.
POD (c)	
Repeatability	Prepare and homogenize three unknown samples at different concentrations to represent the full, claimed range of the method. Analyze each unknown sample by the candidate method seven times, beginning each analysis from weighing out the test portion through to final result with no additional replication (unless stated to do so in the method). All of the analyses for one unknown sample should be performed within as short a period of time as is allowed by the method. The second and third unknowns may be analyzed in another short time period. Repeat for each claimed matrix.
Probability of detection (POD)	Determine the desired POD at a critical concentration. Consult with Table A7 to determine the number of test portions required to demonstrate the desired POD.
Probability of identification (POI)	Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*[c].
Recovery	Determined from spiked blanks or samples with at least seven independent analyses per concentration level at a minimum of three concentration levels covering the analytical range. Independent means at least at different times. If no confirmed (natural) blank is available, the average inherent (naturally containing) level of the analyte should be determined on at least seven independent replicates. Marginal % recovery = $(C_f - C_u) \times 100/C_A$ Total % recovery = $100(C_f)/(C_u + C_A)$ where C_f = concentration of fortified samples, C_u = concentration of unfortified samples, and C_A = concentration of analyte added to the test sample.[d] Usually total recovery is used unless the native analyte is present in amounts greater than about 10% of the amount added, in which case use the method of addition.[e]
Reproducibility (collaborative or interlaboratory study)	Quantitative methods: Recruit 10–12 collaborators; must have eight valid data sets; two blind duplicate replicates at five concentrations for each analyte/matrix combination to each collaborator.
	Qualitative methods: Recruit 12–15 collaborators; must have 10 valid data sets; six replicates at five concentrations for each analyte/matrix combination to each collaborator.

[a] *Guidance for Industry for Bioanalytical Method Validation* (May 2001) U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).

[b] Codex Alimentarius Codex Procedure Manual.

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int.* **95**, 273–285.

[d] *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix D,* AOAC INTERNATIONAL, Gaithersburg, MD.

[e] *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD.

Table A4. Expected precision (repeatability) as a function of analyte concentration[a]

Analyte, %	Analyte ratio	Unit	RSD, %
100	1	100%	1.3
10	10^{-1}	10%	1.9
1	10^{-2}	1%	2.7
0.01	10^{-3}	0.1%	3.7
0.001	10^{-4}	100 ppm (mg/kg)	5.3
0.0001	10^{-5}	10 ppm (mg/kg)	7.3
0.00001	10^{-6}	1 ppm (mg/kg)	11
0.000001	10^{-7}	100 ppb (µg/kg)	15
0.0000001	10^{-8}	10 ppb (µg/kg)	21
0.00000001	10^{-9}	1 ppb (µg/kg)	30

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Gaithersburg, MD.

The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides targets RSDs for a range of analyte concentrations.

Table A5. Expected recovery as a function of analyte concentration[a]

Analyte, %	Analyte ratio	Unit	Mean recovery, %
100	1	100%	98–102
10	10^{-1}	10%	98–102
1	10^{-2}	1%	97–103
0.01	10^{-3}	0.1%	95–105
0.001	10^{-4}	100 ppm	90–107
0.0001	10^{-5}	10 ppm	80–110
0.00001	10^{-6}	1 ppm	80–110
0.000001	10^{-7}	100 ppb	80–110
0.0000001	10^{-8}	10 ppb	60–115
0.00000001	10^{-9}	1 ppb	40–120

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Gaithersburg, MD.

Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 100% to 1 ppb.

Table A6. Predicted relative standard deviation of reproducibility ($PRSD_R$)[a]

Concentration (C)	Mass fraction (C)	$PRSD_R$, %
100%	1.0	2
1%	0.01	4
0.01%	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

[a] Table excerpted from *Definitions and Calculations of HorRat Values from Intralaboratory Data*, HorRat for SLV.doc, 2004-01-18, AOAC INTERNATIONAL, Gaithersburg, MD.

Predicted relative standard deviation = $PRSD_R$. Reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD_R = 2C^{-0.15}, \text{ where C is expressed as a mass fraction}$$

This table provides the calculated $PRSD_R$ for a range of concentrations. *See Annex D* for additional information.

Table A7. POD and number of test portions[a,b]

	Sample size required for proportion						
Assume	1. Binary outcome (occur/not occur). 2. Constant probability rho of event occurring. 3. Independent trials (e.g., simple random sample). 4. Fixed number of trials (*N*)						
Inference	95% Confidence interval lies entirely at or above specified minimum rho						
Desired	Sample size *N* needed						
Minimum probability rho, %	Sample size (*N*)	Minimum No. events (*x*)	Maximum No. nonevents (*y*)	1-Sided lower confidence limit on rho[c], %	Expected lower confidence limit on rho, %	Expected upper confidence limit on rho, %	Effective AOQL[d] rho, %
50	3	3	0	52.6	43.8	100.0	71.9
50	10	8	2	54.1	49.0	94.3	71.7
50	20	14	6	51.6	48.1	85.5	66.8
50	40	26	14	52.0	49.5	77.9	63.7
50	80	48	32	50.8	49.0	70.0	59.5
55	4	4	0	59.7	51.0	100.0	75.5
55	10	9	1	65.2	59.6	100.0	79.8
55	20	15	5	56.8	53.1	88.8	71.0
55	40	28	12	57.1	54.6	81.9	68.2
55	80	52	28	55.9	54.1	74.5	64.3
60	5	5	0	64.9	56.5	100.0	78.3
60	10	9	1	65.2	59.6	100.0	79.8
60	20	16	4	62.2	58.4	91.9	75.2
60	40	30	10	62.4	59.8	85.8	72.8
60	80	56	24	61.0	59.2	78.9	69.1
65	6	6	0	68.9	61.0	100.0	80.5
65	10	9	1	65.2	59.6	100.0	79.8
65	20	17	3	67.8	64.0	94.8	79.4
65	40	31	9	65.1	62.5	87.7	75.1
65	80	59	21	65.0	63.2	82.1	72.7
70	7	7	0	72.1	64.6	100.0	82.3
70	10	10	0	78.7	72.2	100.0	86.1
70	20	18	2	73.8	69.9	97.2	83.6
70	40	33	7	70.7	68.0	91.3	79.7
70	80	63	17	70.4	68.6	86.3	77.4
75	9	9	0	76.9	70.1	100.0	85.0
75	10	10	0	78.7	72.2	100.0	86.1
75	20	19	1	80.4	76.4	100.0	88.2
75	40	35	5	76.5	73.9	94.5	84.2
75	80	67	13	75.9	74.2	90.3	82.2
80	11	11	0	80.3	74.1	100.0	87.1
80	20	19	1	80.4	76.4	100.0	88.2
80	40	37	3	82.7	80.1	97.4	88.8
80	80	70	10	80.2	78.5	93.1	85.8
85	20	20	0	88.1	83.9	100.0	91.9
85	40	38	2	86.0	83.5	98.6	91.1
85	80	74	6	86.1	84.6	96.5	90.6
90	40	40	0	93.7	91.2	100.0	95.6
90	60	58	2	90.4	88.6	99.1	93.9
90	80	77	3	91.0	89.5	98.7	94.1
95	60	60	0	95.7	94.0	100.0	97.0
95	80	80	0	96.7	95.4	100.0	97.7
95	90	89	1	95.2	94.0	100.0	97.0
95	96	95	1	95.5	94.3	100.0	97.2
98	130	130	0	98.0	97.1	100.0	98.6
98	240	239	1	98.2	97.7	100.0	98.8
99	280	280	0	99.0	98.6	100.0	99.3
99	480	479	1	99.1	98.8	100.0	99.4

[a] Table excerpted from Technical Report TR308, *Sampling plans to verify the proportion of an event exceeds or falls below a specified value*, LaBudde, R. (June 4, 2010) (not published). The table was produced as part of an informative report for the Working Group for Validation of Identity Methods for Botanical Raw Materials commissioned by the AOAC INTERNATIONAL Presidential Task Force on Dietary Supplements. The project was funded by the Office of Dietary Supplements, National Institutes of Health.

[b]

[c] Based on modified Wilson score 1-sided confidence interval.

[d] AOQL = Average outgoing quality level.

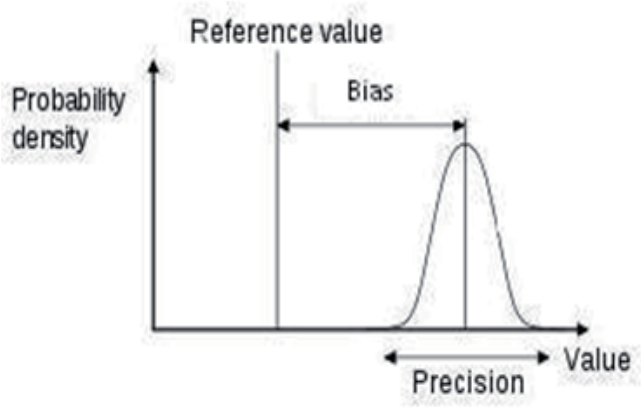


Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

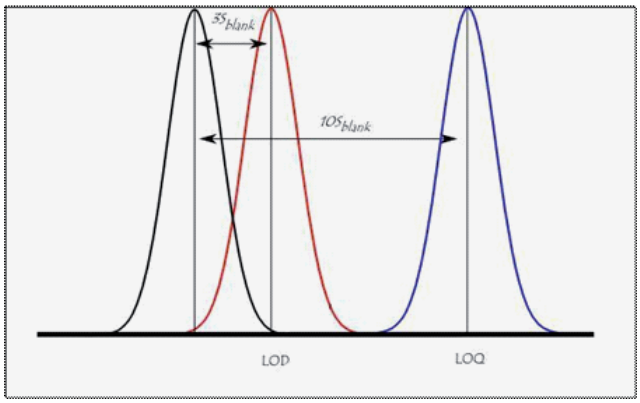


Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

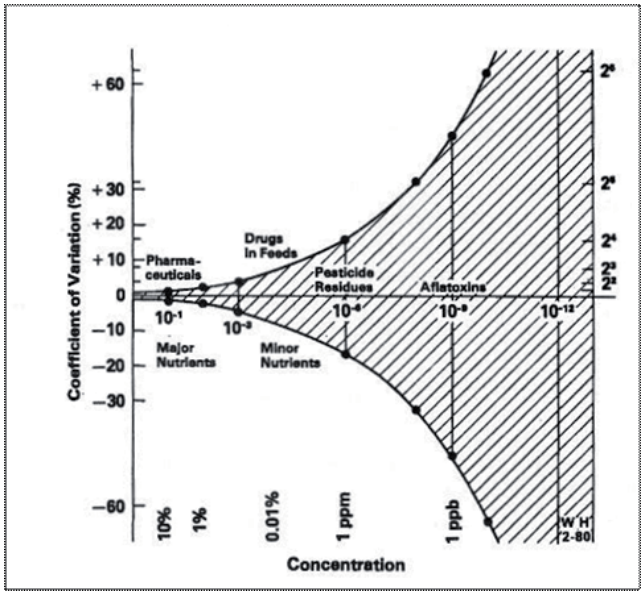


Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

ANNEX B
Classification of Methods

The following guidance may be used to determine which performance parameters in Table A1 apply to different classifications of methods. AOAC INTERNATIONAL does not recognize the term "semiquantitative" as a method classification. Methods that have been self-identified as semiquantitative will be classified into one of the following five types:

Type I: Quantitative Methods

Characteristics: Generates a continuous number as a result.

Recommendation: Use performance requirements specified for quantitative method (main or trace component). Use recovery range and maximum precision variation in Tables A4 and A5.

In some cases and for some purposes, methods with less accuracy and precision than recommended in Tables A4 and A5 may be acceptable. Method developers should consult with the appropriate method committee to determine if the recommendations in Tables A4 and A5 do or do not apply to their method.

Type II: Methods that Report Ranges

Characteristics: Generates a "range" indicator such as 0, low, moderate, and high.

Recommendation: Use performance requirements specified for qualitative methods (main component). Specify a range of POD for each range "range" indicator.

Type III: Methods with Cutoff Values

Characteristics: Method may generate a continuous number as an interim result (such as a CT value for a PCR method), which is not reported but converted to a qualitative result (presence/ absence) with the use of a cutoff value.

Recommendation: Use performance requirements specified for qualitative methods.

Type IV: Qualitative Methods

Characteristics: Method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a specified test portion.

Recommendation: Use performance requirements specified for qualitative methods.

Type V: Identification Methods

Characteristics: Method of analysis whose purpose is to determine the identity of an analyte.

Recommendation: Use performance requirements specified for identification methods.

ANNEX C
Understanding the POD Model

Excerpted from AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures, J. AOAC Int. ***94****, 1359(2011) and Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix I.*

The Probability of Detection (POD) model is a way of characterizing the performance of a qualitative (binary) method. A binary qualitative method is one that gives a result as one of two possible outcomes, either positive or negative, presence/absence, or +/–.

The single parameter of interest is the POD, which is defined as the probability at a given concentration of obtaining a positive response by the detection method. POD is assumed to be dependent on concentration, and generally, the probability of a positive response will increase as concentration increases.

For example, at very low concentration, the expectation is that the method will not be sensitive to the analyte, and at very high concentration, a high probability of obtaining a positive response is desired. The goal of method validation is to characterize how method response transitions from low concentration/low response to high concentration/high response.

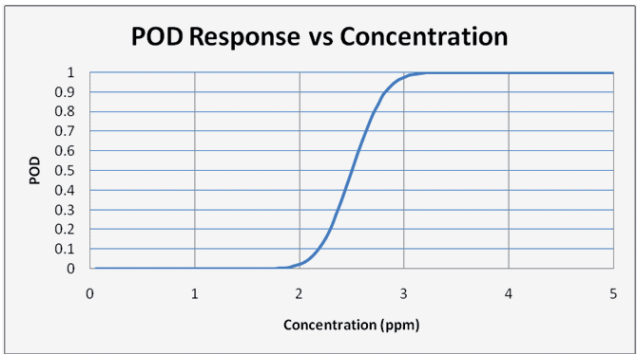


Figure C1. Theoretical POD curve for a qualitative detection method.

POD is always considered to be dependent upon analyte concentration. The POD curve is a graphical representation of method performance, where the probability is plotted as a function of concentration (*see*, for example, Figure C1).

The POD model is designed to allow an objective description of method response without consideration to an a priori expectation of the probabilities at given concentrations. The model is general enough to allow comparisons to any theoretical probability function.

The POD model is also designed to allow for an independent description of method response without consideration to the response of a reference method. The model is general enough to allow for comparisons between reference and candidate method responses, if desired.

Older validation models have used the terms "sensitivity," "specificity," "false positive," and "false negative" to describe method performance. The POD model incorporates all of the performance concepts of these systems into a single parameter, POD.

For example, false positive has been defined by some models as the probability of a positive response, given the sample is truly negative (concentration = 0). The equivalent point on the POD curve for this performance characteristic is the value of the curve at Conc = 0.

Similarly, false negative has sometimes been defined as the probability of a negative response when the sample is truly positive (concentration >0). In the POD curve, this would always be specific to a given sample concentration, but would be represented as the distance from the POD curve to the POD = 1 horizontal top axis at all concentrations except C = 0.

The POD model incorporates all these method characteristics into a single parameter, which is always assumed to vary by concentration. In other models, the terms "false positive," "false negative," "sensitivity," and "specificity" have been defined in a variety of ways, usually not conditional on concentration. For these reasons, these terms are obsolete under this model (*see* Table C1).

The terms "sensitivity," "specificity," "false positive," and "false negative" are obsolete under the POD model (*see* Figure C2).

Table C1. Terminology

Traditional terminology	Concept	POD equivalent	Comment
False positive	Probability of the method giving a (+) response when the sample is truly without analyte	POD(0) POD at conc = 0	POD curve value at conc = 0; "*Y*-intercept" of the POD curve
Specificity	Probability of the method giving a (-) response when the sample is truly without analyte	1-POD(0)	Distance along the POD axis from POD = 1 to the POD curve value
False negative (at a given concentration)	Probability of a (–) response at a given concentration	1-POD(c)	Distance from the POD curve to the POD = 1 "top axis" in the vertical direction
Sensitivity (at a given concentration)	Probability of a (+) response at a given concentration	POD(c)	Value of the POD curve at any given concentration
True negative	A sample that contains no analyte	C = 0	Point on concentration axis where c = 0
True positive	A sample that contains analyte at some positive concentration	C > 0	Range of concentration where c > 0

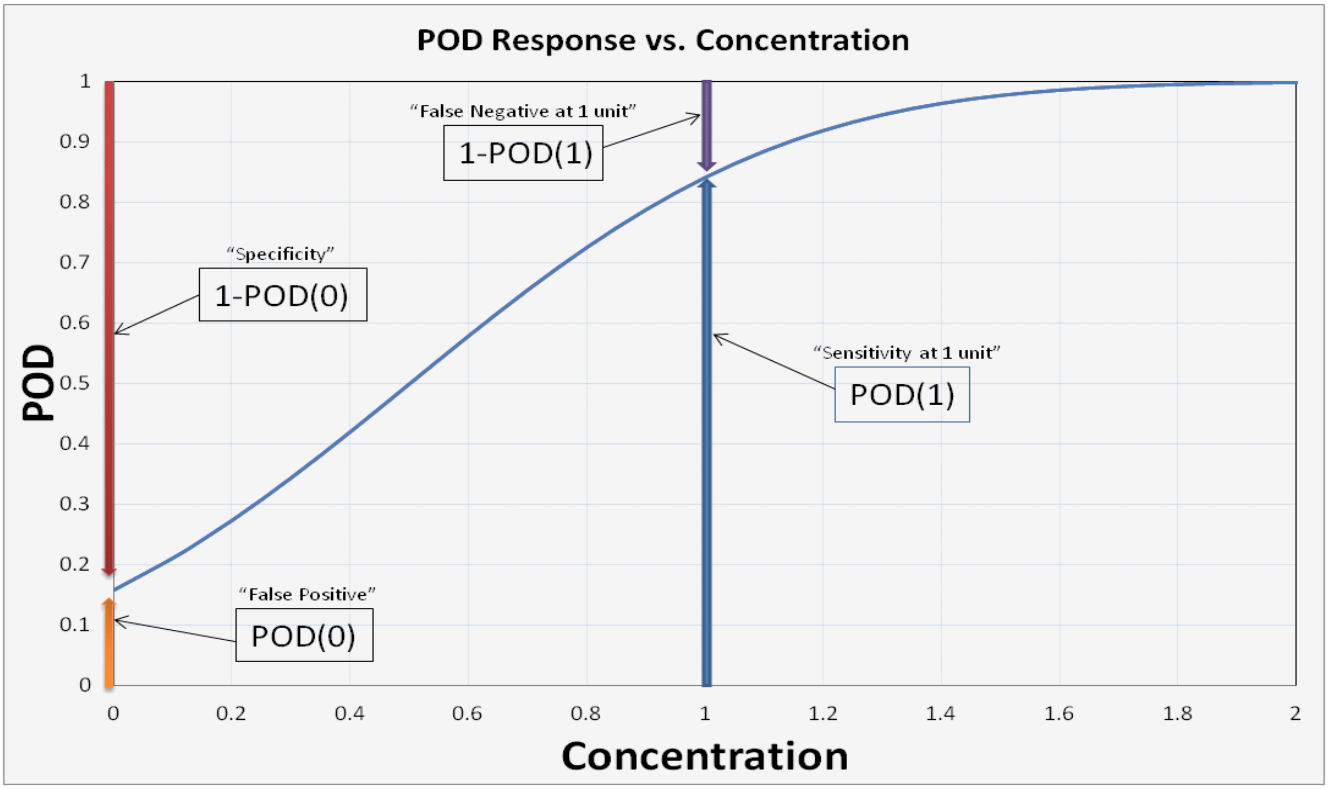


Figure C2. Comparison of POD model terminology to other obsolete terms.

ANNEX D
Definitions and Calculations of HorRat Values from Intralaboratory Data

Excerpted from Definitions and Calculations of HorRat Values from Intralaboratory Data, AOAC INTERNATIONAL, HorRat for SLV.doc, 2004-01-18.

1. Definitions

1.1 Replicate Data

Data developed under common conditions in the same laboratory: simultaneous performance, or, if necessary to obtain sufficient values, same series, same analyst, same day. Such data provides "repeatability statistical parameters."

1.2 Pooled Data

Replicate data developed in the same laboratory under different conditions but considered sufficiently similar that, for the purpose of statistical analysis, they may be considered together. These may include different runs, different instruments, different analysts, and different days.

1.3 Average

0 = Sum of the individual values, x_i, divided by the number of individual values, *n*.

$$0 = (\Sigma\, x_i)/n$$

1.4 Standard Deviation

$$s_i = [\Sigma(x_i - (\bar{x})^2/n]^{0.5}$$

1.5 Relative Standard Deviation

$$RSD = s_i \times 100/\bar{x}$$

1.5.1 Repeatability Relative Standard Deviation [RSD(r) or RSD_r]

The relative standard deviation calculated from within-laboratory data.

1.5.2 Reproducibility Relative Standard Deviation [RSD(R) or RSD_R]

The relative standard deviation calculated from among-laboratory data.

Table D1. Predicted relative standard deviations

Concentration (C)	Mass fraction (C)	$PRSD_R$, %
100%	1.0	2
1%	0.01	4
0.01%	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

1.6 Mass Fraction

Concentration, C, expressed as a decimal fraction. For calculating and reporting statistical parameters, data may be expressed in any convenient units (e.g., %, ppm, ppb, mg/g, µg/g; µg/kg; µg/L, µg/µL, etc.). For reporting HorRat values, data must be reported as a mass fraction where the units of the numerator and denominator are the same: e.g., for 100% (pure materials), the mass fraction C = 1.00; for 1 µg/g (ppm), C = 0.000001 = (E-6). *See* Table D1 for other examples.

1.7 Predicted Relative Standard Deviation [PRSD(R) or $PRSD_R$]

The reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD(R) = 2C^{-0.15}$$

where C is expressed as a mass fraction. *See* Table D1.

In spreadsheet notation: PRSD(R) = 2 * C ^(–0.15).

1.8 HorRat Value

The ratio of the reproducibility relative standard deviation calculated from the data to the PRSD(R) calculated from the Horwitz formula:

$$HorRat = RSD(R)/PRSD(R)$$

To differentiate the usual HorRat value calculated from reproducibility data from the HorRat value calculated from repeatability data, attach an R for the former and an r for the latter. But note that the denominator always uses the PRSD(R) calculated from reproducibility data because this parameter is more predictable than the parameter calculated from repeatability data:

$$HorRat(R) = RSD_R/PRSD(R)$$

$$HorRat(r) = RSD_r/PRSD(R)$$

Some expected, predicted relative standard deviations are given in Table D1.

2 *Acceptable HorRat Values*

2.1 For Interlaboratory Studies

HorRat(R): The original data developed from interlaboratory (among-laboratory) studies assigned a HorRat value of 1.0 with limits of acceptability of 0.5 to 2.0. The corresponding within-laboratory relative standard deviations were found to be typically 1/2 to 2/3 the among-laboratory relative standard deviations.

Table D2. Predicted relative standard deviations

Concentration (C)	$PRSD_R$, %	$PRSD_r$, %
100%	2	1
1%	4	2
0.01%	8	4
1 ppm	16	8
10 ppb	32	16
1 ppb	45	22

2.1.1 Limitations

HorRat values do not apply to method-defined (empirical) analytes (moisture, ash, fiber, carbohydrates by difference, etc.), physical properties or physical methods (pH, viscosity, drained weight, etc.), and ill-defined analytes (polymers, products of enzyme reactions).

2.2 For Intralaboratory Studies

2.2.1 Repeatability

Within-laboratory acceptable predicted target values for repeatability are given in Table D2 at 1/2 of PRSD(R), which represents the best case.

2.2.2 HorRat(r)

Based on experience and for the purpose of exploring the extrapolation of HorRat values to SLV studies, take as the minimum acceptability 1/2 of the lower limit ($0.5 \times 0.5 \approx 0.3$) and as the maximum acceptability 2/3 of the upper limit ($0.67 \times 2.0 \approx 1.3$).

Calculate HorRat(r) from the SLV data:

$$HorRat(r) = RSD(r)/PRSD(R)$$

Acceptable HorRat(r) values are 0.3–1.3. Values at the extremes must be interpreted with caution. With a series of low values, check for unreported averaging or prior knowledge of the analyte content; with a series of high values, check for method deficiencies such as unrestricted times, temperatures, masses, volumes, and concentrations; unrecognized impurities (detergent residues on glassware, peroxides in ether); incomplete extractions and transfers and uncontrolled parameters in specific instrumental techniques.

2.3 Other Limitations and Extrapolations

The HorRat value is a very rough but useful summary of the precision in analytical chemistry. It overestimates the precision at the extremes, predicting more variability than observed at the high end of the scale (C > ca 0.1; i.e., >10%) and at the low end of the scale (C < E-8; i.e., 10 ng/g; 10 ppb).

ANNEX E
AOAC Method Accuracy Review

Accuracy of Method Based on Reference Material

Reference material (RM) used.—The use of RMs should be seen as integral to the process of method development, validation, and performance evaluation. RMs are not the only component of a quality system, but correct use of RMs is essential to appropriate quality management. RMs with or without assigned quantity values can be used for measurement precision control, whereas only RMs with assigned quantity values can be used for calibration or measurement trueness control. Method development and validation for matrices within the scope of the method is done to characterize attributes such as recovery, selectivity, "trueness" (accuracy, bias), precision (repeatability and reproducibility), uncertainty estimation, ruggedness, LOQ or LOD, and dynamic range. RMs should be chosen that are fit-for-purpose. When certified reference materials (CRMs) are available with matrices that match the method scope, much of the work involved in method development has already been completed, and that work is documented through the certificate. RMs with analyte values in the range of test samples, as well as "blank" matrix RMs, with values below or near detection limits, are needed.

Availability of RM.—Consideration needs to be given to the future availability of the chosen RM. Well-documented methods that cannot be verified in the future due to lack of material may lose credibility or be seen as inferior.

Fit to method scope.—Natural matrix CRMs provide the greatest assurance that the method is capable of producing accurate results for that matrix. When selecting an RM to perform a method validation, analysts should consider the method to material fit. An example of a good fit would be a method for specified organic molecules in infant formula and using an infant formula or powder milk RM. A poor fit would be a method for specified organic molecules in infant formula and using a sediment material.

Stability.—Providing a stable RM can be challenging where analytes are biologically active, easily oxidized, or interactive with other components of the matrix. CRM producers provide assurance of material stability, as well as homogeneity.CRMs are accompanied by a certificate that includes the following key criteria:

(*1*) Assigned values with measurement uncertainty and metrological traceability
(*2*) Homogeneity
(*3*) Stability, with the expiration date for the certificate
(*4*) Storage requirements
(*5*) Information on intended use
(*6*) Identity of matrix

For some RMs, such as botanical RMs, the source and/or authenticity can be a very important piece of information that should be included with the certificate. Even under ideal storage conditions, many analytes have some rate of change. Recertification may be done by the supplier, and a certificate reissued with a different expiration date and with certain analyte data updated or removed.

Definition of CRM.—Refer to the AOAC TDRM document for definitions from ISO Guide 30, Amd. 1 (2008), http://www.aoac.org/divisions/References.pdf.

Information on source of RM is available.—It is the responsibility of the material producer to provide reliable authentication of the RM and make a clear statement in the accompanying documentation. This should be an as detailed listing as possible, including handling of ingredients, identification of plant materials as completely as feasible (species, type, subtype, growing region), etc. This is comparable to other required information on an RM for judging its suitability for a specific application purpose (e.g., containing how much of the targeted analyte, stabilized by adding acid—therefore not suited for certain parameters/procedures, etc.).

Separate RM used for calibration and validation.—A single RM cannot be used for both calibration and validation of results in the same measurement procedure.

Blank RM used where appropriate.—Blank matrix RMs are useful for ensuring performance at or near the detection limits. These are particularly useful for routine quality control in methods measuring, for instance, trace levels of allergens, mycotoxins, or drug residues.

Storage requirements were maintained.—Method developers should maintain good documentation showing that the RM producer's recommended storage conditions were followed.

Cost.—The cost of ongoing method checks should be considered. Daily use of CRMs can be cost prohibitive. Monthly or quarterly analysis of these materials may be an option.

Concentration of analyte fits intended method.—Concentration of the analyte of interest is appropriate for standard method performance requirements (SMPRs).

Uncertainty available.—Every measurement result has an uncertainty associated with it, and the individual contributions toward the combined uncertainty arise from multiple sources. Achieving the target measurement uncertainty set by the customer for his/her problem of interest is often one of the criteria used in selecting a method for a given application. Estimation of measurement uncertainty can be accomplished by different approaches, but the use of RMs greatly facilitates this part of a method validation.

Demonstration of Method Accuracy when No Reference Material Is Available

If an RM is not available, how is accuracy demonstrated?

There are many analytes for which a CRM with a suitable matrix is not available. This leaves the analyst with few options. For some methods, there may be proficiency testing programs that include a matrix of interest for the analyte. Proficiency testing allows an analyst to compare results with results from other laboratories, which may or may not be using similar methods. Spiking is another technique that may be used. When alternative methods are available, results may be compared between the different methods. These alternatives do not provide the same level of assurance that is gained through the use of a CRM.

Spike recovery.—In the absence of an available CRM, one technique that is sometimes used for assessing performance is the spiking of a matrix RM with a known quantity of the analyte. When this method is used, it cannot be assumed that the analyte is bound in the same way as it would be in a natural matrix. Nevertheless, a certified blank RM would be the preferred choice for constructing a spiked material.

When preparing reference solutions, the pure standards must be completely soluble in the solvent. For insoluble materials in a liquid suspension or for powdered forms of dry materials, validation is required to demonstrate that the analyte is homogeneously distributed and that the response of the detection system to the analyte is not affected by the matrix or preparation technique. When a matrix material is selected for spiking, it should be reasonably

The document, *AOAC Method Accuracy Review*, was prepared by the AOAC Technical Division on Reference Materials (TDRM) and approved by the AOAC Official Methods Board in June 2012.

characterized to determine that it is sufficiently representative of the matrix of interest. Spiked samples must be carried through all steps of the method. Many analytes are bound in a natural matrix and whether the spiked analyte will behave the same as the analyte in a natural matrix is unknown.

Other.—Use of a substitute RM involves the replacement of the CRM with an alternative matrix RM matching the matrix of interest as close as possible based on technical knowledge.

ANNEX F
Development and Use of In-House Reference Materials

The use of reference materials is a vital part of any analytical quality assurance program. However, you may have questions about their creation and use. The purpose of this document is to help answer many of these questions.

- What is a reference material?
- Why use reference materials?
- What certified reference materials are currently available?
- Why use an in-house reference material?
- How do I create an in-house reference material?
- How do I use the data from an in-house reference material?

What Is a Reference Material?

The International Organization for Standardization (ISO) defines a reference material as a "material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials" (1). In plain English, natural-matrix reference materials, such as those you might prepare for use in-house, can be used to validate an analytical method or for quality assurance while you're using your method to analyze your samples. (Natural-matrix materials are not generally used as calibrants because of the increased uncertainty that this would add to an analysis.) The assigned values for the target analytes of an in-house reference material can be used to establish the precision of your analytical method and, if used in conjunction with a CRM, to establish the accuracy of your method.

ISO defines a certified reference material (CRM) as a "reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence" (1).

Why Use Reference Materials?

Certified reference materials can be used across the entire scope of an analytical method and can provide traceability of results to the International System of Units (SI). During method development, CRMs can be used to optimize your method. During method validation, they can be used to ensure that your method is capable of producing the "right" answer, and to determine how close your result is to that answer. During routine use, they can be used to determine within-day and between-day repeatability, and so demonstrate that your method is in control and is producing accurate results every time it is used.

Natural-matrix reference materials should mimic the real samples that will be analyzed with a method. They should behave just as your samples would during a procedure, so if you obtain accurate and precise values for your reference material, you should obtain accurate and precise values for your samples as well.

What Certified Reference Materials Are Currently Available?

CRMs are available from a number of sources, including (but not limited to):

- American Association of Cereal Chemists (AACC)
- American Oil Chemists Society (AOCS)
- International Atomic Energy Agency (IAEA)
- Institute for Reference Materials and Measurements (IRMM)
- LGC Promochem
- National Institute of Standards and Technology (NIST)
- National Research Council Canada (NRC Canada)
- UK Food Analysis Proficiency Assessment Program (FAPAS)

A number of websites provide general overviews and catalogs of producers' and distributors' reference materials:

http://www.aocs.org/tech/crm/
http://www.comar.bam.de
http://www.erm-crm.org
http://www.iaea.org/oregrammeslaqcs
http://www.aaccnet.org/checksample
http://www.irmm·ire.be/mrm.html
http://www.lgcpromochem.com
http://www.naweb.iaea.org/nahu/nmrm/
http://www.nist.gov/srm
http://www.fapas.com/index. cfm
http://www.virm.net.

Because new reference materials are produced regularly, it is important to check these websites to determine what is currently available.

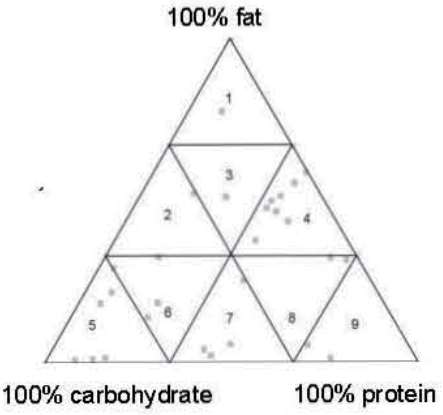
Why Use an In-House Reference Material?

There are many benefits to the use of a CRM. CRMs have been prepared to be homogeneous and, if stored under the proper conditions, stable. You are provided with a certified value as well as the statistical data for theconcentration of your analyte; this is about as close as you can come to knowing the true value of the concentration of the analyte. The material has been tested by experienced analysts in leading laboratories, so you have the security of knowing that your method is generating values similar to those generated in other competent laboratories. The CRMs from the sources mentioned above are nationally and/or internationally recognized, so when you obtain acceptable results for a CRM using your analytical method, you give credibility to your methodology and traceability to your results.

But there are some drawbacks associated with CRMs. Unfortunately, many analyte/matrix combinations are not currently available. When testing food products for nutrient content, for example, a laboratory can be asked to analyze anything that might be found in a kitchen or grocery store. Reference materials that represent all of the types of foods that need to be tested are not available, and most CRMs are certified for a limited number of analytes. It is important to match the reference material matrix to your sample matrix. (Food examples dominate the discussion below, but the same processes apply to the development of in-house RMs in other areas of analytical chemistry.)

To demonstrate the applicability of an analytical method to a wide variety of food matrices, AOAC INTERNATIONAL's Task

Excerpted from *Development and Use of In-House Reference Materials*, Rev. 2, 2009. Copyright 2005 by the AOAC Technical Division on Reference Materials (TDRM).



Force on Methods for Nutrition Labeling developed a triangle partitioned into sectors in which foods are placed based on their protein, fat, and carbohydrate content (2, 3). Since ash does not have a great impact on the performance of an analytical method for organic-material foods, and water can be added or removed, it can be assumed that the behavior of an analytical method is determined to large extent by the relative proportions of these proximates. AOAC INTERNATIONAL anticipated that one or two foods in a given sector would be representative of other foods in that sector and therefore would be useful for method assessment. Similarly, one or two reference materials in a given sector (or near each other in adjacent sectors) should be useful for quality assurance for analyses involving the other foods in the sector. The positions of many of the food-matrix CRMs from the sources listed above are shown in the triangle and are provided in the list.

These food-matrix reference materials are spread through all sectors of the triangle, thereby making it likely that you can find an appropriate CRM to match to your samples. Ultimately, however, the routine use of a CRM can be cost prohibitive, and is not really the purpose of CRMs. For example, in order to use NIST's Standard Reference Material (SRM) 2387 Peanut Butter for all mandatory nutrition labeling analyses, you could buy one sales unit (three jars, each containing 170 g material) for $649 (2009 price). If you charge your customer about $1000 for analysis of all mandatory nutrients in a test material, the control material would account for more than 60% of your fees. Therefore, many laboratories have found it more cost-effective to create in-house reference materials for routine quality control and characterize them in conjunction with the analysis of a CRM (4). You can prepare larger quantities of a reference material by preparing it in-house, and you have more flexibility in the types of matrices you can use. There are not many limitations on what can be purchased.

How Do I Create an In-House Reference Material?

There are basically three steps to preparing an in-house reference material: selection (including consideration of homogeneity and stability), preparation, and characterization. Additional guidance through these steps can be provided from TDRM as well as in ISO Guides 34 (5) and 35 (6).

References

(1) JCGM 200:2008, *International vocabulary of metrology—Basic and general concepts and associated terms (VIM)*, International Bureau of Weights and Measures (www.bipm.org)

Sector	RM No.	Matrix
	NIST 1563	Coconut oil
1	NIST 3274	Fatty acids in botanical oils
1	NIST 3276	Carrot extract in oil
1	LGC 7104	Sterilized cream
2	NIST 2384	Baking chocolate
3	NIST 2387	Peanut butter
4	NIST 1546	Meat homogenate
4	LGC 7106	Processed cheese
4	LGC 7000	Beef/pork meat
4	LGC 7150	Processed meat
4	LGC 7151	Processed meat
4	LGC 7152	Processed meat
4	SMRD 2000	Fresh meat
4	LGC 7101	Mackerel paste
4	LGC QC1001	Meat paste 1
4	LGC QC1004	Fish paste 1
5	BCR-382	Wleat flour
5	BCR-381	Rye flour
5	LGC 7103	Sweet digestive biscuit
5	LGC 7107	Madeira cake
5	LGC QC1002	Flour 1
6	NIST 1544	Fatty acids
6	NIST 1548a	Typical diet
6	NIST 1849	Infant/adult nutritional formula
6	LGC 7105	Rice pudding
7	LGC 7001	Pork meat
7	NIST 1566b	Oyster tissue
7	NIST 1570a	Spinach leaves
7	NIST 2385	Spinach
8	NIST 1946	Lake trout
8	LGC 7176	Canned pet food
9	NIST 1974a	Mussel tissue
9	NIST 3244	Protein powder

(2) Wolf, W.R., & Andrews, K.W. (1995) *Fresenius' J. Anal. Chem.* **352**, 73–76

(3) Wolf, W.R. (1993) *Methods of Analysis for Nutrition Labeling*, D.R. Sullivan & D.E. Carpenter (Eds), AOAC INTERNATIONAL, Gaithersburg, MD

(4) European Reference Materials (2005) *Comparison of a Measurement Result with the Certified Value*, Application Note 1

(5) *ISO Guide 34 General Requirements for the Competence of Reference Material Producers* (2009) 2nd, International Organization for Standardization, Geneva, Switzerland

(6) *Guide 35 Certification of Reference Materials—General and Statistical Principles* (2006) International Organization for Standardization, Geneva, Switzerland

For more information about the AOAC Technical Division on Reference Materials, visit http://aoac.org/divisions/tdrm.

AOAC Stakeholder Panel Voting Members

AOAC INTERNATIONAL (AOAC) assembles stakeholder panels to develop voluntary consensus standards. While AOAC maintains transparency and openness in accordance with national and international guidance and regulations for standards development and its policies and procedures for assembling stakeholder panels, its policies and procedures also ensures that there is a balance of interests and perspectives in achieving consensus of the stakeholder panel.

Due Process and Balance

All AOAC stakeholder panels are diverse and can vary in size. Where a stakeholder panel is not balanced or if it is significantly large whereby consensus of the general assembly may be impractical, a balanced representative voting panel will be used to demonstrate consensus. AOAC encourages ALL stakeholders to participate in deliberations during stakeholder panel meetings and working group meetings, in addition to participating during any posted comment periods. To ensure that there is a balance of interests and perspectives, a ***representative subset*** of the stakeholder panel, the voting members, is selected to reach consensus for the development of AOAC voluntary consensus standards.

Composition

Voting members represent the perspectives of the larger stakeholder panel. The voting members consist of no more than ¼ to 1/3 of the total number of stakeholders in registered. Primary and secondary representative voting members are approved. Every attempt is made to approve a panel of voting members that represents all perspectives of the stakeholder panel. In the event of a primary voting member is not able to attend, and no alternate has been approved, the stakeholder panel chair, working with AOAC can provisionally approve an alternate from those in attendance to assure balance and lack of dominance. For stakeholder panels with scopes including diverse topics, the voting member representatives may be rotated to include other stakeholders for successive meetings to ensure a lack of dominance by any particular stakeholder.

Approval Process

AOAC works with the chair of the stakeholder panel and potentially other key stakeholders to develop a proposed representative voting member panel. Following AOAC policies and procedures, the proposed voting members and documentation are submitted to the AOAC Official Methods Board (OMB) for review and approval. The OMB's review ensures that the proposed panel is balanced in interests and perspectives representing the stakeholder panel and a lack of dominance.

Roles and Responsibilities

Every stakeholder has a voice and every stakeholder is entitled to state his/her or organizational perspective(s). This is due process. In developing AOAC standards, stakeholder consensus is demonstrated by 2/3 vote (67%) in favor of a motion to adopt a standard. It is important to note: Individual voting members do not have any additional weight, voice or status in stakeholder deliberations than other stakeholders. The role of the voting members is to demonstrate the consensus of the stakeholder panel. Voting members may vote in favor or against any motion and/or they may abstain. Stakeholder panel chair will moderate voting process. AOAC carefully documents the vote. It is important for voting members to be in the room during the time for voting. It is also important for voting members to inform the chair of his/her inability to serve as a voting member.

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS (SPDS)
WORKING GROUP CHAIR & MEMBERS

VOLUNTEER ROLE DESCRIPTION

POSITION TITLE: **Working Group Chair and Members, AOAC SPDS Working Group**
POSITION CLASSIFICATION: **Volunteer**
REPORTS TO: **SPDS Chair**
DATE PREPARED: **March 13, 2014**

POSITION SUMMARY:
In keeping with the mission of AOAC INTERNATIONAL and the goals of the Stakeholder Panel on Dietary Supplements (SPDS), working group chairs will lead their working group in the development of standards (or other tasks as assigned by the SPDS chair) for specific priority ingredients as defined by the SPDS Advisory Panel. Working group chair(s) will work with AOAC staff and stakeholders to meet the working group's goals and disseminate recommendations to the stakeholder panel and community at-large. The working group may hold meetings in person and/or via teleconference (web and video) to complete its work. The chair of the working group will moderate the working group discussions, assist in scheduling the meetings, and report the working group's recommendation back to SPDS. Working group chairs will work with AOAC to formulate the working group's recommendations into motions for SPDS's consideration.

ELIGIBILITY CRITERIA FOR SPDS WORKING GROUP CHAIR:
- Must be a key expert and/or thought leader in dietary supplements and the technologies used for priority ingredients as assigned for the specific working group.
- Must have the recommendation of the SPDS Chair.

WORKING GROUP CHAIR RESPONSIBILITIES:
- Chair meetings of the working group, moderate discussions of the working group and work with AOAC staff to facilitate working group's work.
- Work with AOAC staff and SPDS chair to identify working group members, any additional expertise/resources needed facilitate the work of the working group.
- Work as a team member and also independently.
- Present an overview on the specified priority ingredient under consideration including, but not limited to, regulatory implications, and public health and public safety challenges with methodology.
- Prepare a draft fitness for purpose statement for specified priority ingredient and technology to present to SPDS for consideration.
- Work with AOAC staff to reconcile actions and outcomes of working group deliberations.
- Using AOAC guidance to reconcile comments and address questions on SMPR.
- Present working group recommended SMPR to SPDS for review and approval.
- Work with AOAC staff and stakeholders to draft and review relevant methodology and working group documentation.
- Draft SMPR white paper for publication.
- Perform duties and reviews in timely fashion.
- Other tasks as agreed upon by working group chair, SPDS chair and AOAC staff.

DUTIES AND RESPONSIBILITIES OF THE SPDS WORKING GROUP MEMBERS:
The working group will meet either in person and via teleconference, web conferencing or by other means of communication. All communication and meetings of the working group must be facilitated through AOAC

staff. The working group's tasks will include developing standard method performance requirements (SMPRs), review of methodology, identifying expertise and other as may be requested by the SPDS chair. Working groups are not required to vote, but to show general consensus for its recommendations. The groups should meet to discuss their objectives and complete their assigned tasks. Individuals on the working groups may be tasked with their own action items and responsibilities. More than one meeting and one round of communication may be required to complete the working group's tasks. All working group participants are expected to contribute and are expected to have completed the SMPR Education Session. AOAC staff will document all working group decisions and actions.

AOAC RESOURCES:

- Referencing AOAC guidance documentation to assist in drafting the fitness for purpose statement, standard method performance requirements (SMPR), and additional work as tasked.
    1) AOAC Fitness for Purpose Statement Guideline
    2) Appendix F: Guidelines for Standard Method Performance Requirements
    3) Appendix K: Guidelines for Dietary Supplements and Botanicals

STAFF LIASON:

AOAC will assign staff to facilitate the work of the working group.

TERMS OF REVIEW:

This document will be reviewed biannually by the SPDS Chair and AOAC staff.

DATES REVISED:

Stakeholder Panel

Voting Panel – A vetted, representative, and balanced subset of the assembled stakeholders. Ideally the number of voters represents $^1/_4$ to $^1/_3$ of the assembly.

Voting Guidelines – A. motions to create a consensus based standard (ex: voting on fitness for purpose statements or Standard Method Performance Requirements) require a 2/3 vote for the motion to carry.
B. Any other motion (ex: votes to clarify information for working groups, set priorities or direction, etc.) requires a majority vote to carry.

Expert Review Panel

Voting Panel – 7 – 10 vetted experts
Quorum - The presence of **7** members or **2/3** of total vetted ERP membership, whichever is greater.
Voting Guidelines – Motions to adopt a *First Action Official Method℠ of Analysis* carry by unanimous vote on first ballot. If not unanimous, negative votes must delineate scientific reasons, and can be overridden by 2/3 of voting ERP members after due consideration. Dissenting opinions are recorded.

Working Group

Voting Panel – There is no formal voting panel. Any interested and knowledgeable party may participate. Working groups sole purpose is to provide recommendations to stakeholder panels.

Voting Guidelines –majority vote carries all motions, dissenting opinions considered by assembly and recorded.

Helpful Definitions & Terminology

Quorum	The number of members who must be present in order to validly transact business. It is determined by the number of members present, not the number present and voting. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 151*).
Representative Voting Panel Members	Every member has an obligation to vote and the right to abstain.
Abstentions	Abstentions reduce the number required to obtain a majority of those present and voting. They are only counted to confirm the presence of a quorum. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 237).*
Order	Meetings should address only one item of business at one time (only one pending motion at a time). Chairs should not permit digression or introduction of different topics until the business at hand is resolved. No pending motions while changing topics. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 1).* All business must be conducted with order and should be done fairly and impartially. The presiding officer should impartially ensure that each member has an opportunity to speak. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. pp. 1-2).*
Equality	All members have equal opportunity to propose motions, to participate in debate, to vote, to serve on committees or as an officer, to share in activities according to the member's abilities. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Justice	All members have the right to ask questions, to be informed, to have complex motions explained by the chair. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Minority Rights	Dissenting members have equal rights to voice opposing or minority opinions and strive to become the majority. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Majority Rights	No members, board, or officers have the right to dictate or control decisions unless the member grant such rights Members may not take any action in conflict with federal, regional or organizational laws or policies. Decisions are based on the will of the majority. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*

SPADA Roster, August 2016

Don't see your name on the list? Let us know at cdent@aoac.org to ensure you receive SPADA emails!

Role	Name	Organization
Chair	Linda Beck	CBR Defense Concepts And Experimentation Branch, Naval Surface Warfare Center
Chair	Matthew Davenport	Department Of Homeland Security
SPADA Member	James Agin	Q Laboratories, Inc.
SPADA Member	Terrance Allen	Pentagon Force Protection Agency
SPADA Member	Amy Altman	Luminex Corporation
SPADA Member	Douglas Anders	Federal Bureau Of Investigation (FBI)
SPADA Member	Jessica Appler	HHS BARDA
SPADA Member	Jennifer Arce	PNNL
SPADA Member	Thomas Archibald	HazTech Systems, Inc
SPADA Member	Charles Asowata	Executive Office For Chemical And Biological Defense
SPADA Member	Les Baillie	School Of Pharmacy And Pharmaceutical Sciences, Cardiff University
SPADA Member	Ed Bailor	IAB
SPADA Member	Jeff Ballin	ECBC
SPADA Member	Timothy Bauer	Naval Surface Warfare Center Dahlgren
SPADA Member	Maureen Beanan	National Institutes Of Health
SPADA Member	Brian Bennett	West Desert Test Center, CAPAT, Dugway Proving Ground
SPADA Member	Thomas Blank	NBACC
SPADA Member	Steven Blanke	University of Illinois
SPADA Member	Jerold Blutman	DTRA
SPADA Member	Larry Blyn	Ibis Biosciences
SPADA Member	Donna Boston	HHS, ASPR/BARDA
SPADA Member	Julie Boylan	Defense Threat Reduction Agency
SPADA Member	Carrie Brennan	Austin Peay State University
SPADA Member	Paul Brett	University Of South Alabama
SPADA Member	Cindy Bruckner-Lea	Pacific Northwest National Lab
SPADA Member	Robert Bull	Department Of Homeland Security
SPADA Member	Mary Burtnick	University Of South Alabama
SPADA Member	Don Bushner	JS J8, JRO-CBRND
SPADA Member	Ryan Cahall	Censeo Insight
SPADA Member	Andrew Cannon	USF Center For Biological Defense
SPADA Member	Amanda Clark	Naval Surface Warfare Center Dahlgren Virginia
SPADA Member	Kenneth Cole	NIST
SPADA Member	Bart Currie	Tropical And Emerging Infectious Diseases Division, Menzies School Of Health Res

SPADA Roster, 3/22/2016
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Role	Name	Organization
SPADA Member	Kenneth Damer	Northrop Grumman Electronic Systems
SPADA Member	Inger Damon	Centers For Disease Control And Prevention
SPADA Member	David Dance	Health Protection Agency (South West)
SPADA Member	David DeShazer	USAMRIID
SPADA Member	Christina Egan	NYSDOH
SPADA Member	Phillip Elzer	LSU AgCenter
SPADA Member	Peter Emanuel	BioScience
SPADA Member	Thomas Ficht	TAMU
SPADA Member	Brandan Fisher	US Army Test And Evaluation Command
SPADA Member	Steven Fisher	The Office Of Justice Programs (OJP)
SPADA Member	Bill Folkerts	ATCC
SPADA Member	Mats Forsman	FOI Sweden
SPADA Member	Edward Gabriel	DHS - HHS
SPADA Member	Ken Gage	CDC
SPADA Member	Mike Gavin	Emergency Manager City Of Fort Collins
SPADA Member	Joan Gebhardt	Naval Medical Research Center
SPADA Member	Jay Gee	CDC
SPADA Member	Jennifer Gibbons	ECBC / Excet
SPADA Member	Michael Gillenwater	DHS - FEMA, Preparedness Grants Division
SPADA Member	Arthur Goff	CIV USA MEDCOM USAMRIID
SPADA Member	Bruce Goodwin	DoD CRP
SPADA Member	Robert Griffin	DHS S&T First Responders Group
SPADA Member	Ted Hadfield	HADECO, LLC
SPADA Member	Martha Hale	US ARMY MEDCOM USAMRIID
SPADA Member	Andrew Hebbeler	Office Of Science And Technology Policy
SPADA Member	Matthew Hickman	Department Of Homeland Security
SPADA Member	Steven Hinrichs	University Of Nebraska Medical Center
SPADA Member	Anthony Hitchins	FDA - CFSAN (Retired)
SPADA Member	Peyton Hobson	FDA HHS
SPADA Member	David Hodge	Department Of Homeland Security
SPADA Member	Alex Hoffmaster	Centers For Disease Control And Prevention
SPADA Member	Jeffery Hogan	ATEC, Life Sciences Division, Dugway Proving Ground
SPADA Member	Harvey Holmes	Centers for Disease Control

SPADA Roster, 3/22/2016
Don't see your name on this list? Let us know at cdent@aoac.org to ensure you receive SPADA emails!

Role	Name	Organization
SPADA Member	Kia Hopkins	ECBC
SPADA Member	Rosemary Humes	HHS, ASPR/BARDA
SPADA Member	Duane Hunt	Baltimore City Environmental Services
SPADA Member	Aaron Hyre	JBTDS/NBCCA
SPADA Member	Sofi Ibrahim	USAMRIID
SPADA Member	Paula Imbro	The Tauri Group
SPADA Member	Robert Ingram	FDNY
SPADA Member	Paul Jackson	Lawrence Livermore National Lab (Retired)
SPADA Member	Crystal Jaing	LLNL
SPADA Member	Malcolm Johns	DHS
SPADA Member	Frederick Johnson	DA DCS G-3/5/7
SPADA Member	Ronald Johnson	BioMérieux, Inc.
SPADA Member	Franca Jones	White House Office of Science and Technology Policy
SPADA Member	Brian Kamoie	DHS - FEMA - Grants Program Directorate
SPADA Member	Cecilia Kato	CDC
SPADA Member	Alexander Kayatani	Pentagon Force Protection Agency
SPADA Member	Paul Keim	Northern Arizona University
SPADA Member	Liz Kerrigan	ATCC
SPADA Member	Saleem Khan	University Of Pittsburgh School Of Medicine
SPADA Member	Katalin Kiss	ATCC
SPADA Member	Kristin Korte	ICx Technologies
SPADA Member	Matt Kramer	Qiagen
SPADA Member	Tom Labombarda	Aventura Police Department
SPADA Member	Markus Lacorn	R-Biopharm AG
SPADA Member	David Ladd	The Commonwealth Of MassachusettsDepartment Of Fire Services
SPADA Member	John Lednicky	University Of Florida
SPADA Member	Matthew Lesho	Luminex
SPADA Member	Direk Limmathurotsakul	Mahidol-Oxford Tropical Medicine Research Unit, Thailand
SPADA Member	Nancy Lin	NIST
SPADA Member	Luther Lindler	DHS
SPADA Member	Angelo Madonna	Dugway Proving Ground - Life Sciences Division
SPADA Member	Chris Mangal	Association Of Public Health Laboratories
SPADA Member	Laura Maple	NSWC

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Role	Name	Organization
SPADA Member	James Marks	UCSF
SPADA Member	Jim Marks	UCSF
SPADA Member	Bryon Marsh	Georgia National Guard
SPADA Member	Robert Massung	Centers For Disease Control And Prevention
SPADA Member	J. Clay McGuyer	National Guard Bureau
SPADA Member	Marian McKee	BioReliance Corporation
SPADA Member	Michael McLaughlin	US FDA - ORA/ORS
SPADA Member	Toby Merlin	Centers For Disease Control And Prevention
SPADA Member	Richard Meyer	
SPADA Member	Timothy Minogue	DoD, USAMRIID
SPADA Member	Jayne Morrow	NSTC
SPADA Member	Stephen Morse	Centers For Disease Control And Prevention(Retired)
SPADA Member	Timothy Moshier	Acumen Detection LLC
SPADA Member	Pejman Naraghi-Arani	InSilixa Corp.
SPADA Member	Dallas New	
SPADA Member	Ann Nguyen	BARDA
SPADA Member	Christopher Niblick	JPM NBC CA, PD CCAT&TI
SPADA Member	William Nierman	J. Craig Venter Institute
SPADA Member	Mikeljon Nikolich	Walter Reed Army Institute Of Research
SPADA Member	Sean O'Brien	DoD DUSA T&E
SPADA Member	Tom O'Brien	Tetracore, Inc.
SPADA Member	Catherine O'Connell	Life Technologies
SPADA Member	William O'Neill	US Postal Service
SPADA Member	Steven Olsen	USDA ARS
SPADA Member	Ted Olsen	PathSensors, Inc.
SPADA Member	Victoria Olson	Centers For Disease Control And Prevention
SPADA Member	Kate Ong	JPEO-CBD
SPADA Member	Palmer Orlandi	FDA Office of Foods and Veterinary Medicine
SPADA Member	Eileen Ostlund	USDA, APHIS, Veterinary Services
SPADA Member	Rich Ozanich	Pacific Northwest National Laboratory
SPADA Member	Traci Pals	DTRA
SPADA Member	Joseph Perrone	SRI International Bioscience Division
SPADA Member	Robert Perry	University Of Kentucky

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Role	Name	Organization
SPADA Member	Jeannine Petersen	Centers For Disease Control And Prevention (CDC)
SPADA Member	Denise Pettit	N. C. Department Of Health And Human Services
SPADA Member	Sally Phillips	DHS OHA
SPADA Member	Mark Poli	USAMRIID
SPADA Member	Mark Poli	US Army Medical Research Institute Of Infectious Diseases
SPADA Member	Ann Powers	CDC
SPADA Member	Lyle Probst	Microfluidic Systems, Inc.
SPADA Member	Richard Prouty	JRO-CBRND
SPADA Member	Amy Pullman	Association Of Public Health Laboratories
SPADA Member	Beena Puri	FDA Division Of Microbiology
SPADA Member	Amy Rasley	Lawrence Livermore National Laboratory
SPADA Member	Jon Rayner	Southern Research Institute
SPADA Member	Roberto Rebeil	ECBC
SPADA Member	Christopher Renner	JRO-CBRND
SPADA Member	Michael Retford	JBTDS JPM NBCCA
SPADA Member	Frank Roberto	Idaho National Laboratory
SPADA Member	Frank Roberto	Idaho National Laboratory
SPADA Member	Jason Roos	JPEO-CBD
SPADA Member	David Rozak	USAMRIID
SPADA Member	James Samuel	Texas A&M
SPADA Member	Stefan Saravia	Minnesota Department Of HealthPublic Health Laboratory Division
SPADA Member	Frank Schaefer	US EPA (ret)
SPADA Member	Mark Scheckelhoff	DHS/OHA
SPADA Member	Herbert Schweizer	University of Florida
SPADA Member	Sanjiv Shah	US EPA
SPADA Member	Shashi Sharma	FDA - CFSAN
SPADA Member	Deborah Shuping	DoD, DUSA-TE
SPADA Member	Russell Sillmon	CTTSO
SPADA Member	Tom Slezak	Lawrence Livermore National Lab
SPADA Member	Darci Smith	Southern Research Institute
SPADA Member	Theresa Smith	USAMRIID
SPADA Member	Sandra Smole	Massachusetts Department Of Public Health
SPADA Member	Shanmuga Sozhamannan	DoD ECBC

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Role	Name	Organization
SPADA Member	Darryl Sullivan	Covance Laboratories
SPADA Member	Maureen Sullivan	Minnesota Department Of Health
SPADA Member	Mary Beth Tabacco	Smiths Detection
SPADA Member	Sandra Tallent	FDA - ORS/DM
SPADA Member	Christina Thompson	Thompson Biosafety, LLC
SPADA Member	Maureen Thornton	MRI Global
SPADA Member	Rebekah Tiller	CDC
SPADA Member	Patrick Treado	ChemImage Corp
SPADA Member	David Trudil	New Horizons Diagnostics Corporation
SPADA Member	APICHAI TUANYOK	University of Florida
SPADA Member	Venkat Venkateswaran	Omni Array Biotechnology
SPADA Member	Elizabeth Vitalis	Lawrence Livermore National Laboratory
SPADA Member	David Wagner	Northern Arizona University
SPADA Member	Jennifer Walker	Tetracore Inc.
SPADA Member	Rodney Wallace	BARDA
SPADA Member	Mike Walter	Office Of Health AffairsDHS
SPADA Member	Richard Warren	BARDA/Contractor Tunnell Government Services
SPADA Member	David Watson	DTRA
SPADA Member	Scott Weaver	University Of Texas Medical Branch
SPADA Member	Clyde Webster	DoD DUSA T&E
SPADA Member	Susan Welkos	USAMRIID
SPADA Member	James Whelan	Alexeter Technologies
SPADA Member	Christian Whitchurch	DTRA
SPADA Member	Patrick Williams	Evogen
SPADA Member	Reinhardt Witzenberger	R-Biopharm AG
SPADA Member	Donald Woodbury	DHS CDB S&T
SPADA Member	Patricia Worsham	USAMRIID
SPADA Member	Emily Yost	ATEC, Life Sciences Division, Dugway Proving Ground
SPADA Member	Edward Young	VA Medical Center/Baylor College Of Medicine
OMB Advisor	Douglas Abbott	USDA - FSIS/OPHS/BD/MOSPB
AOAC Staff	Scott Coates	AOAC INTERNATIONAL
AOAC Staff	Christopher Dent	AOAC INTERNATIONAL
AOAC Staff	Krystyna McIver	AOAC INTERNATIONAL

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Role	Name	Organization
AOAC Staff	Deborah McKenzie	AOAC INTERNATIONAL
AOAC Staff	Tien Milor	AOAC INTERNATIONAL
OMB Advisor	Tom Phillips	MD Department Of Agriculture