AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

AOAC INTERNATIONAL Presents...

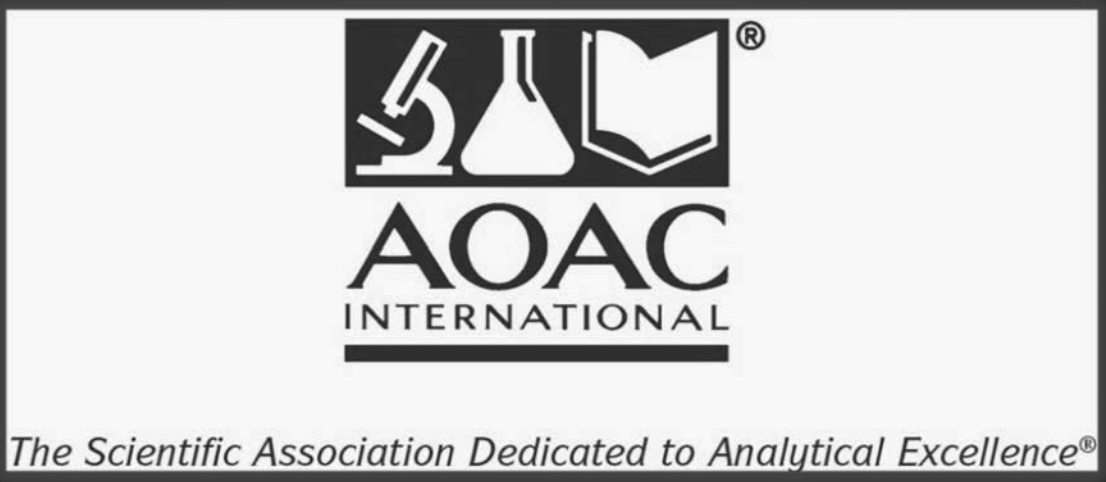
the Stakeholder Panel on Agent Detection Assays

(SPADA)

Tuesday, April 11, 2017, 9:00 a.m. – 3:30 p.m.
Conference Room 110

AOAC HEADQUARTERS
2275 RESEARCH BOULEVARD
ROCKVILLE, MARYLAND
UNITED STATES

contact: SPADA@aoac.org



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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.

The Scientific Association Dedicated to Analytical Excellence®

APRIL 11, 2017
AOAC INTERNATIONAL HEADQUARTERS
2275 RESEARCH BOULEVARD
ROCKVILLE, MARYLAND, 20850
CONFERENCE ROOM: 110

9:00am – 3:30pm Eastern Standard Time
Registration Opens at 8:00am

STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS (SPADA)

Chair: Linda Beck, NSWC - Dahlgren | Co-Chair: Matthew Davenport, DHS

A G E N D A

I. **Welcome and Introductions (9:00 – 9:15 a.m.)**
Linda Beck, NSWC – Dahlgren, SPADA Co-Chair and Jonathan Goodwin, AOAC Interim Executive Director

II. **Standards Development at AOAC: Voting and Balance (9:15 – 9:30 a.m.)**
Deborah McKenzie, Senior Director, Standards Development

III. **Environmental Organisms Panel Presentation & Consensus* (9:30 a.m. – 12:00 p.m.)**
Linda Beck, NSWC – Dahlgren, SPADA Co-Chair

IV. **Related NIST Initiatives (1:00 – 2:00 p.m.)**
Nancy Lin, NIST
   a. Surrogate Materials for Challenging On-site Biological Assessment Processes
   b. Mixed Microbiological Reference Materials

V. **Bacterial Pathogen Screening of Suspicious Visible Powders (2:00 – 2:30 p.m.)**
Jennifer Arce, PNNL

VI. **Considerations for Implementing Sequencing for BioWatch Laboratory Operations (2:30 p.m. – 3:00 p.m.)**
Vikram Munikoti, US Department of Homeland Security

VII. **Potential Future SPADA Activities (3:00 – 3:30 p.m.)**
Linda Beck, NSWC – Dahlgren, SPADA Co-Chair

VIII. **Adjourn**

Breaks:
MORNING: 10:15 a.m. – 10:30 a.m.
LUNCH: 12:00 p.m. – 1:00 p.m.

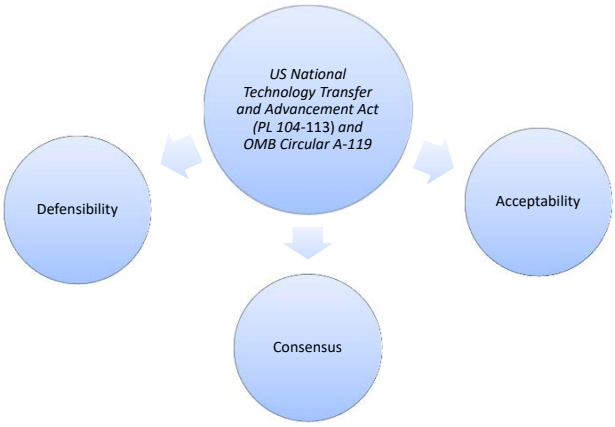
**Item(s) requires a vote by SPADA*

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

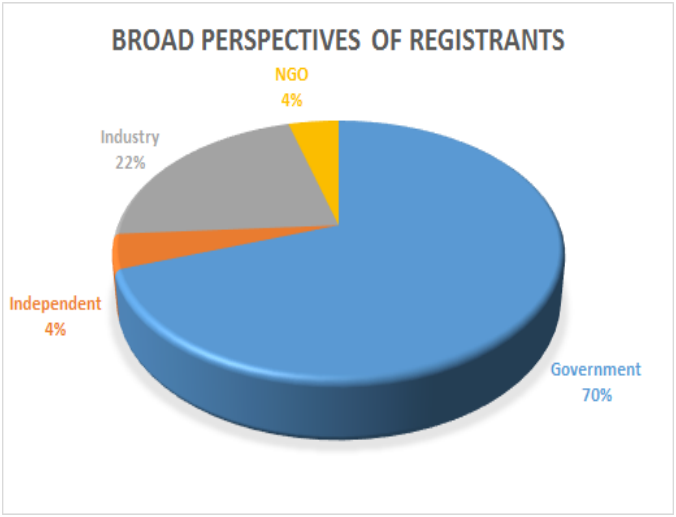
- Product Manufacturers
- Analyte/Method Subject Matter Experts
- Technology Providers
- Method Developers
- Government and Regulators
- Contract Research Organizations
- Reference Materials Developers
- Ingredient Manufacturers
- Method End Users
- Academia & Research
- Non Governmental Organizations
- Other as identified

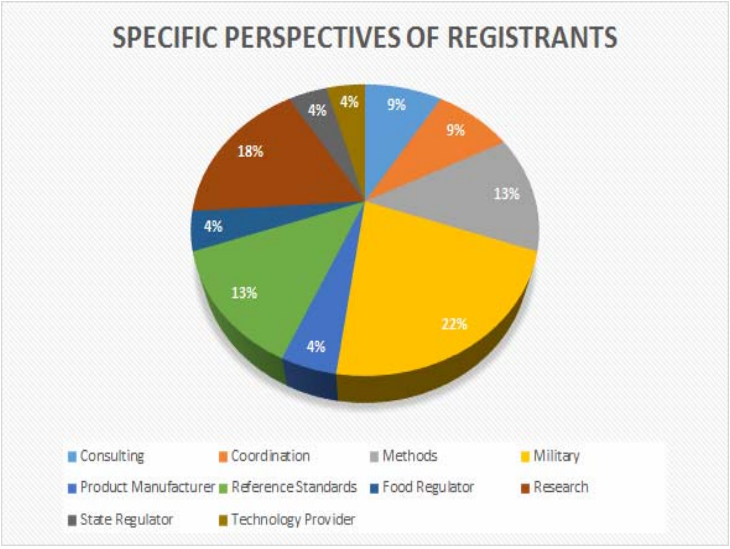
Anyone with a material interest can participate
Balanced group of representative voting stakeholders
Chair and voting stakeholders vetted by AOAC Official Methods Board

Organizational Meeting Registrants

ATCC	Medical Countermeasure Systems - Diagnostics
CBR Defense Concepts and Experimentation Branch, Naval Surface Warfare Center	Neogen Corporation
DoD - DBPAO (Formerly Critical Reagents Program)	NIH/NIAID
DHS	NIST
DHS/OHA	Northrop Grumman Electronic Systems
DuPont Nutrition & Health	PNNL
FDA - CFSAN (Retired)	R-Biopharm Rhone Ltd
FDA Division Of Microbiology	US Army Edgewood Chemical Biological Center
HADECO, LLC	US ARMY MEDCOM USAMRIID
JPdM BDS	US FDA
Lawrence Livermore National Lab (Retired)	USAMRIID
MD Department of Agriculture	

Registrants as of March 1, 2017


BROAD PERSPECTIVES OF REGISTRANTS
NGO
4%
Industry
22%
Independent
4%
Government
70%


SPECIFIC PERSPECTIVES OF REGISTRANTS
9%
9%
13%
22%
4%
13%
4%
18%
4%
4%
Consulting
Coordination
Methods
Military
Product Manufacturer
Reference Standards
Food Regulator
Research
State Regulator
Technology Provider

SPADA Voting Members – March 2017

Broad Perspective	Specific Perspective	Region	Organization (s)
Government	Military	US	JPdM BDS
Government	Military	US	US ARMY MEDCOM USAMRIID
Government	Military	US	DoD - DBPAO (Formerly Critical Reagents Program)
Government	Research	US	Lawrence Livermore National Lab (Retired)
Government	Research	US	Pacific Northwest National Laboratory (PNNL)
Government	Reference Standards	US	US NIST / Critical Reagents Program
Government	Coordination	US	US DHS OHA
Government	Food Regulator	US	US FDA
Government	Methods	US	Medical Countermeasure Systems - Diagnostics
Industry	Consulting	US	HADECO
Industry	Product Manufacturer	US	Northrop Grumman Electronic Systems
Industry	Method Developer	US	Neogen
NGO	Reference Standard	US	ATCC

Approving the Environmental Organisms Panel

- SPADA Chair/Working Group Chair will present on the draft environmental organisms panel including reconciled comments received on behalf of the working group and moves for SPADA to adopt the environmental organisms panel as presented
- SPADA chair will entertain deliberation on the draft panel
- After due deliberation, SPADA chair will call for a vote
- 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 the panel

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*
  - Publish revisions to the relevant sections of the SMPRs developed under this contract
- 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

Environmental Organisms Panel Working Group

Chair: Linda C. Beck, PhD; NSWC Dahlgren, CBR Defense Division

March 15, 2017

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

SPADA Meeting Morning Agenda

Welcome and Introductions (9:00 – 9:15 a.m.)
Linda Beck, NSWC – Dahlgren, SPADA Co-Chair

Standards Development at AOAC: Voting and Balance (9:15 – 9:30 a.m.)
Deborah McKenzie, Senior Director, Standards Development

Environmental Organisms Panel Presentation & Consensus* (9:30 a.m. – 12:00 p.m.)
Linda Beck, NSWC – Dahlgren, SPADA Co-Chair

Breaks:
MORNING: 10:15 a.m. – 10:30 a.m.
LUNCH: 12:00 p.m. – 1:00 p.m.

SPADA Meeting Afternoon Agenda

Related NIST Initiatives (1:00 – 2:00 p.m.)

Nancy Lin, NIST

Surrogate Materials for Challenging On-site Biological Assessment Processes
Mixed Microbiological Reference Materials

Bacterial Pathogen Screening of Suspicious Visible Powders (2:00 – 2:30 p.m.)

Jennifer Arce, PNNL

Considerations for Implementing Sequencing for BioWatch Laboratory Operations (2:30 p.m. – 3:00 p.m.)

Vikram Munikoti, US Department of Homeland Security

Potential Future SPADA Activities (3:00 – 3:30 p.m.)

Linda Beck, NSWC – Dahlgren, SPADA Co-Chair

Adjourn

Meeting Goal

- Approval of the Environmental Organism Panel, which will be published as a standard in the peer-reviewed *Journal of AOAC INTERNATIONAL* and the *Official Methods of Analysis* Compendium
  - All relevant existing SPADA Standard Method Performance Requirements will be updated to incorporate the revised Environmental Organism.
- Discussion of Future Projects

Background:
Development of Standards for Threat Agent Detection

- DUSA-TE sponsored SPADA Project
- Johns Hopkins University, Applied Physics Laboratory is prime contractor
- AOAC is the executing organization
- Stakeholder Panel on Agent Detection Assays (SPADA) is the standards adoption body

Background:
Development of Standards for Threat Agent Detection

Leads:

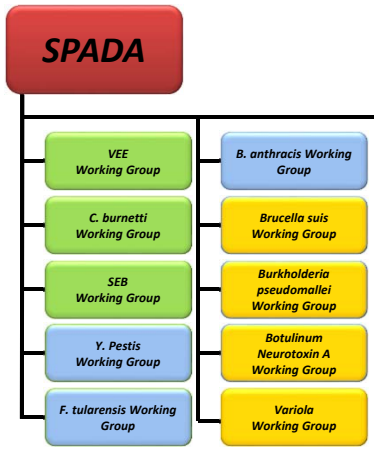
SPADA Co-Chairs:	Linda C. Beck, PhD; NSWCD
	Matthew Davenport, PhD; DHS
Project Lead:	Krystyna McIver; AOAC
Chief Scientist:	Scott Coates; AOAC
Program Manager:	Chris Dent; AOAC
DUSA-TE:	Ryan Cahall; Censeo Insight, INC., CTR Support

Background:
Over Arching DUSA TE Project Summary

- Mission: Create standards for 10 threat agents
- Started in November 2014
- 3 threat agents initially identified
- 7 additional threat agents identified
- 10 standards created and published
- Project completed on time in Sept 2016

SPADA - 2014 - 2016

- A voluntary consensus standards body currently supported by a 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*

Background on Standard Methods Performance Requirements

- Commonly refer
  - SMPRs
  - "Smipper"s

STANDARD METHOD PERFORMANCE REQUIREMENTS AOAC INTERNATIONAL (2011)

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

Determination of total folate [supplemental folic acid (CAS 59-30-3) or 5-methyl-tetrahydrofolate (CAS 68792-52-9), and endogenous 5-methyl-tetrahydrofolate polyglutamate] in all forms (powders, ready-to-feed liquids, and liquid concentrates) of infant, adult, and pediatric nutritional formula.

2. Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

3. Definitions

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment (AOAC SPIFAN, 2010), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant Formula

Breast-milk substitute specially manufactured to satisfy ... by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72-1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r), or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (%RSD_R).

Recovery

The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

4. Method Performance Requirements

Analytical range	0.50–300[a]	
Limit of detection (LOD)	≤0.10[a]	
Limit of quantitation (LOQ)	≤0.50[a]	
Repeatability (RSD_r)	0.50[a]	≤11%
	21.5[a]	
	43.0[a]	≤7%
	64.0[a]	
	85.0[a]	
Recovery	0.5	
	21.5[a]	
	43.0[a]	80–110%
	64.0[a]	
	85.0[a]	
Reproducibility (RSD_R)	0.5[a]	≤32%
	21.5[a]	
	43.0[a]	≤18%
	64.0[a]	
	85.0[a]	

Concentrations apply to (1) "ready-to-feed" liquids "as-is", (2) reconstituted powders (29 g into 200 g water), and (3) liquid concentrates diluted 1:1 by weight.

[a] µg/100 g expressed as folic acid in reconstituted final product.

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the applicability range.

6. Reference Material(s)

NIST Standard Reference Material ® (SRM) 1849 Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849 consists of 10 packets, each containing approximately 10 g of material. Certified value of folic acid in NIST 1849 is 2.11 (±0.13) mg/kg.

Note: The reference value for NIST 1849 is defined in terms of folic acid. The performance parameters in this SMPR are intended for folate and 5-methyl-tetrahydrofolate polyglutamate. Some discrepancy may be expected.

7. Validation Guidance

Recommended Level of validation: *Official Methods of Analysis*℠

8. Maximum Time-to-Signal

No maximum time.

v8: March 13, 2011

Standard Methods Performance Requirement

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

Environmental Factors Annex-evaluation study

Three parts:

- Part 1: Environmental Matrix Samples—Aerosol
- Part 2: Environmental Panel Organisms
- Part 3: Potential Interferents

Proposed Follow-On Project

- Review the Environmental Organism Panel (EOP) (Part 2 of the Environmental Factor Annex) with a focus on streamlining the panel by removing low incidence organisms and possibly incorporating the use of bio-informatics.
- The EOP is included as an appendix in 15 different *Standard Methods Performance Requirements (SMPR);* 86 environmental organisms (initiated in 2008)

Proposed Follow-On Project

Benefits to DoD:

- Every PCR assay under consideration for DoD acquisition would be evaluated using the streamlined environmental panel of organisms. Reduction in the number of evaluations needed will result in considerable savings in time and costs to DoD.

- Proposed Follow-On Project Accepted: October 2016; Working Group on Environmental Organism Panel organized and launched

Working Group Members

Working Group Chair, Linda Beck, NSWC Dahlgren

- Jessica Appler, HHS BARDA
- Ryan Cahall, Censeo Insight
- Ted Hadfield, HADECO, LLC.
- Sofi Ibrahim, USAMRIID
- Paul Jackson, LLNL (Retired)
- Katalin Kiss, ATCC
- Nancy Lin, NIST
- Stephen Morse, CDC (Retired)
- Tom Philips, MD Department of Agriculture
- Michael Retford, JPdM BDS
- Frank Schaefer, US EPA (Retired)
- Sanjiv Shah, US EPA
- Shanmuga Sozhamannan, Tauri Group, DBPAO JPM Guardian

Scope of Work

- Review the previous list of 86 environmental organisms
- Create a section on soil testing providing guidance on testing for cross-reactivity
- Modify and include a section on bioinformatics
- Deliverable:
- The approved Environmental Organism Panel will be published as a standard in the peer-reviewed *Journal of AOAC INTERNATIONAL* and the *Official Methods of Analysis* Compendium.
- All relevant existing SPADA Standard Method Performance Requirements will be updated to incorporate the revised Environmental Organism.

Key Points

- Working group met by teleconferences:
  November, twice in December, January
- Reviewed the current list of environmental organisms
- 37 species DNA were identified as unnecessary for inclusion based on improvements to technology, more knowledge, and experience
- Remaining organisms divided into two groups:
  Group 1: 12 arthropods and mammalian species DNA

  Group 2: 37 cultivatable air, soil, and water bacteria

Group 1

- *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)
- *Mus musculus* (ATCC/HB-123) mouse
- *Rattus norvegicus* (ATCC/CRL-1896) rat
- *Homo sapiens* (HeLa cell line ATCC/CCL-2) human

Group 2

- *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

Optional Bioinformatics

- Instructions for application of bioinformatics analysis were developed for the *Variola* and *Fransicella* standards
- These instruction were refined and added as an option to wet testing of Group 2 organisms (cultivatable soil, water, and air bacteria)
- Only DNA from Group 1 are recommended for evaluation regardless of bioinformatics analysis

Streamlined EOP

- Number of species DNA required for wet testing:
  reduced from 86 to 49 species (reduced by 43%)
- Number of species DNA required for wet testing in tandem with bioinformatic analysis:
  reduced from 86 to 12 species (reduced by 86%)

Next Step - Soil Testing

•Soils contain genomic materials or nucleic acid fragments of countless archaebacterial, bacterial, and eukaryotic organisms

•Soils may also contain unanticipated inhibitors that interfere with extraction, denaturation, polymerization, or annealing reactions.

•Therefore, an investigative challenge of a PCR assay to variety of representative soils is an important first step to establish the specificity of the primers/probes, and the robustness of PCR assay against potential interfering compounds.

Soil Testing

- Using the primers/probe, and amplicon sequences specific for any given assay evaluate each regional soil type for any signs of positive response

- Samples of each regional soil type should be spiked at 2x, 5x and 10x AMDL with the archetype organism (usually specified in the SMPR for AMDL testing, such as strain CO92 for *Yersinia pestis*) and then the samples evaluated for inhibition

Summary

- Reviewed Environment Organisms Panel
  - Reduced number of species by 43% w/o bioinformatics.
  - Reduced number of species by 86% w bioinformatics.
- Included updated guidance on bioinformatic analysis
- Added guidance on soil testing
- A more focused document with additional guidance

Comments

- Submitted for review and comments: February 1 – March 2, 2017
- Grammatical Changes Recommended: all were accepted
- Technical suggestions: to be discussed

Technical Suggestion

- While line 8 is true it is not part of the specificity testing which should be done early on in testing. This soil should be tested with other gold standard methods to insure the target sequence is absent. Then and only then can it be tested with the assay being developed. If the assay is positive in a soil declared negative the assay fails because it is giving a false positive response. Testing with the target spiked at 2X plus a native DNA control without soil DNA will identify the presence of inhibitors.

Technical Suggestion

- DNA containing soil inhibitors can be tested at 1X and dilutions up to 50X to overcome any inhibitor response. The assay could be very specific (and sensitive) allowing field samples to be diluted prior to testing to overcome those inhibitory substances. Removal of inhibitors is different than testing for specificity and is a function of the DNA extraction method.

Technical Suggestion

- 83-86: the size of the eukaryotic genomes makes using a 10X concentration of the genome difficult as the quantity of DNA is huge. Using a concentration equivalent to the 10X of the bacterial genome still provides a background but is not so viscous. Alternatively, you can place a maximum DNA concentration on testing such as 100ug of background DNA.

Proposed Change

- 83-86:Organisms may be tested as pools containing ten organisms each represented at 10 times the AMDL. Cell lines should be tested individually at a DNA concentration equivalent to 10X AMDL. These tests should be negative for the target sequence. The same pools can be spiked with 2X the AMDL for the target organism, mixed and tested. These tests should be positive for the target in the high DNA background.

Discussion

Comments and discussion on proposed changes

Additional Suggested Modifications

Propose: Move soil testing to Part 1 of the panel (rather than Part 2)

Dr. Nancy Lin, NIST

Propose: Move soil testing to Part 1 of the panel (rather than Part 2)

Current Environmental Factors Panel

- Part 1: Environmental Matrix Samples—Aerosol Environmental Matrices
- Part 2: Environmental Panel Organisms *(DNA testing)*
- Part 3: Potential Interferants Study *(DOD specific interferants)*

The Working Group focused on changes to Part 2

Part 1: Environmental Matrix Samples

- 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.

Results are applied via Table 1 on method performance requirements

- System **false-negative** rate using spiked environmental matrix materials ≤5%
- System **false-positive** rate using environmental matrix materials ≤5%

Reason 1: Soil testing aligns directly with Part 1

Current Part 1		Proposed revisions in Part 2
– Interference testing (false negatives)	↔	• Soil testing for robustness against potential interferants
– Cross-reactivity testing (false positives)	↔	• Soil testing for assay specificity

Soil testing for interferants and cross-reactivity fits much better in Part 1 vs Part 2 on DNA testing.

Reason 2: Soil testing should apply to all SPADA SMPRs

- Soil testing is relevant for non-nucleic acid analytical technologies (e.g., protein detection).
- Current SMPRs for proteins omit Part 2 of the Environmental Panel (e.g., SMPR 2016.011 for Botulinum neurotoxins A1 and A2).
- The introductory paragraph for the panel currently states, "Part 2 is not applicable to techniques that do not detect nucleic acid."

Moving soil testing to Part 1 would enable soil testing to apply to all SMPRs, regardless of analytical approach.

Reason 3: SMPRs already contains performance requirements for environmental matrix testing

- System false-negative rate using spiked environmental matrix materials ≤5%
- System false-positive rate using environmental matrix materials ≤5%

Is a footnote needed to recognize that some soils or matrices might actually contain the target organism? Such as:

"Rates of ≤5% are expected. All discrepancies are to be…"
(reported? Retested? Other?)

If soil testing moves to Part 1, we must reconcile:

Current

Interferants/inhibition

- "target biological threat agent sufficient to achieve **95% probability of detection**"

Cross-reactivity/specificity

- "include **sufficient samples and replicates** to ensure each environmental condition is adequately represented"

Proposed

Interferants/inhibition

- "spiked at **2x, 5x and 10x AMDL** with the archetype organism"
- Specifies use of "**intact target organisms**"

Cross-reactivity/specificity

- Evaluate "for any **signs of positive response**"

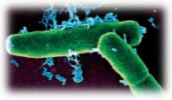
Neither has language for results reporting, such as: "Full results from all soils and matrices tested shall be submitted."

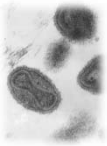
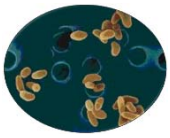
Motion

Motion to accept the Environmental Organisms Panel

Additional Discussion

Thank you!



Memo

To: SPADA

From: Scott Coates, AOAC International

Date: March 27, 2017

Re: Environment Organism Panel (EOP)

Two sets of comments and two versions of the draft Environment Organism Panel (EOP) (version 4 and version 5) are attached.

Version 5 of the EOP and the second set of comments contain some somewhat significant changes to the direction of the draft EOP, and so we thought it would be easier to present these changes separately.

Summary of proposed changes in version 5:

1) Move the soil interference study from Part 2 to Part 1 as it is more consistent with the aims of Part 1 than Part 2. We haven't reviewed Part 1 together because we were primarily focused on the organisms (in Part 2). So, version 5 includes Part 1 of the standard.

2) Make the SMPR more generic to *assays* instead of *PCR assays* so the EOP applies to all assays as well as PCR assays.

3) A series of editorial changes that support the suggestion to make the EOP more generic such as " assays" in stead of "PCR assays"; and remove references to "primers", "probes", and "DNA".

Please call me (301 924 7077 ext 137) or email me (scoates@aoac.org) if you have questions or concerns.

Part 2: Environmental Panel Organisms

2.1 Soil Testing

Airborne soil particles may constitute a significant challenge to the analysis of collected aerosol samples by **polymerase chain reaction** (**PCR**) assays. Soils contain genomic materials or nucleic acid fragments of countless archaebacterial, bacterial, and eukaryotic organisms. Some of the more common soil organisms can be anticipated. Soils may also contain unanticipated inhibitors that interfere with extraction, denaturation, polymerization, or annealing reactions. Therefore, determining the effect of a variety of representative soils on the PCR assay is an important first step in establishing the specificity of the primers/probes, and the robustness of a PCR assay in the presence of interfering compounds.

Using the primers/probe, and amplicon sequences specific for any given assay evaluate each regional soil type*† for any signs of positive response.

Samples of each regional soil type* should be spiked at **2x, 5x and** 10x AMDL with the archetype organism (usually specified in the SMPR for AMDL testing, such as strain CO92 for *Yersinia pestis*) and then the samples evaluated for inhibition. Inhibition testing should be done using intact target organisms so that potential interference with the DNA extraction can be determined.

* Arizona Test Dust is available as a baseline starting point.

† See section 2.2 “Bioinformatics Analysis of Signature Sequences” on probing all available data bases including those containing soil metagenome sequences generated from specific regions of operations (if available) for In Silico Analysis and further validation of the signature sequences.

2.2 Bioinformatics Analyses of Signature Sequences

In silico screening will be performed on signature sequences (eg: oligo primers/probes and amplicon) to demonstrate specificity to the target biological threat agent.

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 organism/strain be included in the exclusivity or inclusivity panels, respectively, if available.

A method developer-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, databases and/or published documents describing the genetic sequences found in soils that are representative of the regions of operation. 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 into predictions. The program should detect possible amplicons from any selected database of sequences.

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 sequences.

- NCBI tools

The 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 the tool(s) used for bioinformatics evaluation
    - Data demonstrating the selected tool(s) 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 organisms and/or strains to be added to the inclusivity (Annex II) or exclusivity (Annex III) panels based on the bioinformatics evaluation

2.3 Environmental Organisms

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.

If bioinformatic analysis is completed then DNA from Group 1 organisms should be tested. If bioinformatic analysis is not completed then DNA from Group 1 and 2 organisms should be tested.

Organisms may be tested as pools containing ten organisms each represented at 10 times the AMDL. Cell lines should be tested individually at a DNA concentration equivalent to 10X AMDL. These tests should be negative for the target sequence. The same pools can be spiked with 2X the AMDL for the target organism, mixed and tested. These tests should be positive for the target in the high DNA background. ~~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 from organisms on this list that already appears in the inclusivity or exclusivity panel does not need to be tested again as part of the environmental factors panel.

Group 1

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)[1]
Mus musculus (ATCC/HB-123) mouse
Rattus norvegicus (ATCC/CRL-1896) rat
Homo sapiens (HeLa cell line ATCC/CCL-2) human

Group 2

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

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.[1]

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.

Interference from Soil

To evaluate robustness to soil interferents, samples of each regional soil types* should be spiked at **2x, 5x and** 10x AMDL with the archetype organism (usually specified in the SMPR for AMDL testing, such as strain CO92 for *Yersinia pestis* in SMPR 2016.008) and then the samples evaluated for inhibition. Inhibition testing should be done using intact target organisms so that potential interference can be determined.

* Arizona Test Dust is available as a baseline starting point.

[1] Added in June 2015 for the Department of Defense project.

Part 2: Environmental Panel Organisms

2.1 Soil Testing

Airborne soil particles may constitute a significant challenge to assays for analysis of aerosol collection filters and/or liquids. Soils contain genomic materials or nucleic acid fragments of countless archaebacterial, bacterial, and eukaryotic organisms. Some of the more common soil organisms can be anticipated. Soils may also contain unanticipated inhibitors that interfere with sample processing and detection.

Therefore, determining the effect of a variety of representative soils on an assay is an important first step in establishing the specificity and robustness of an assay in the presence of interfering compounds.

To challenge assay specificity, use the assay to evaluate each regional soil type *† for positive responses.

There is no consensus on a course of action if a positive response is detected. Therefore, it is incumbent on the method developer to determine the appropriate course of action if a positive response is detected.

† See section 2.2 “Bioinformatics Analysis” on probing all available data bases including those that contain soil metagenome sequences generated from specific regions of operations (if available) for *In-Silico Analysis* and further validation of the signature sequences.

2.2 Bioinformatics Analyses

In silico screening shall be performed on all nucleic acid signature sequences used in the assay (e.g., primers, probes, amplicons, etc.) to demonstrate specificity to the target biological threat agent.

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 organism/strain be included in the exclusivity or inclusivity panels, respectively, if the strains are available.

A method developer-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, databases and/or published documents describing the genetic sequences found in soils that are representative of the regions of operation. 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 into predictions. The program should detect possible amplicons from any selected database of sequences.

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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



Standards and Metrics to Support Field Biodetection

Nancy J. Lin

April 11, 2017

For SPADA

NIST National Institute of Standards and Technology U.S. Department of Commerce

MATERIAL MEASUREMENT LABORATORY

Acknowledgement

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Promote US innovation and industrial competitiveness thru

Measurement Science *Standards* *Technology*

to enhance economic security and improve quality of life

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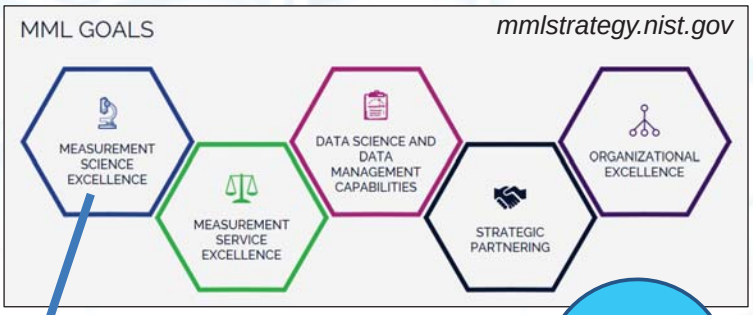
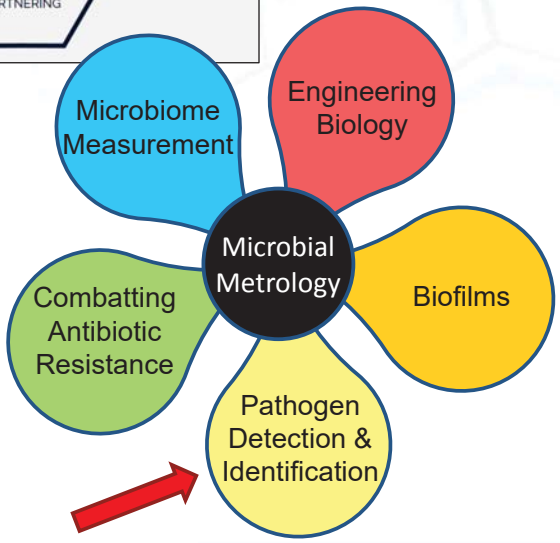
Material Measurement Laboratory (MML) Strategic Plan 2015-2020



Measurement Science Excellence

→ Biological Sciences

→ Strategy 3 – Microbial Metrology



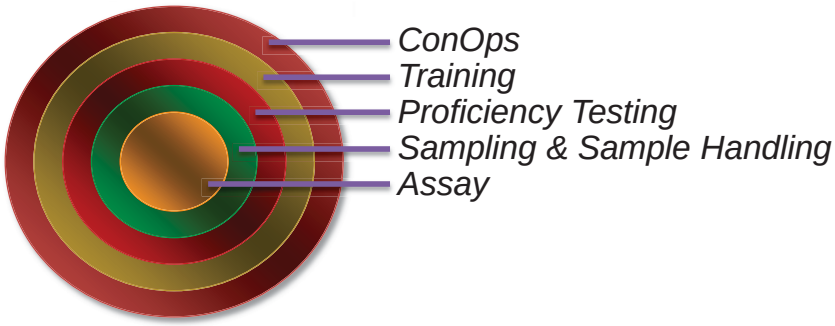
Standards to Support Biological Agent Detection

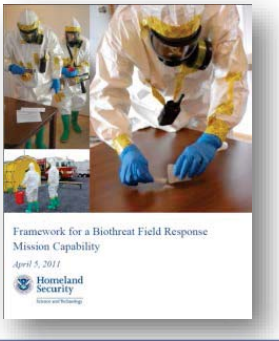
DHS S&T – NIST Interagency Agreement

Goal: Develop standards and methods to support field biothreat detection and biosurveillance

- Surrogate reference materials and related documentary standards for training
- Methods, metrics and standards for biological test material characterization
- Documentary standards to support field response mission capability

Impact: Increased confidence in field results and an improved National ability to detect and respond to suspected biological incidents





Overview

- Yeast as a surrogate for biothreat agents
- Mixed microbial reference materials
- Documentary standards

Overview

- **Yeast as a surrogate for biothreat agents**
- Mixed microbial reference materials
- Documentary standards

Challenges in Training with Real or Attenuated Biothreat Agents

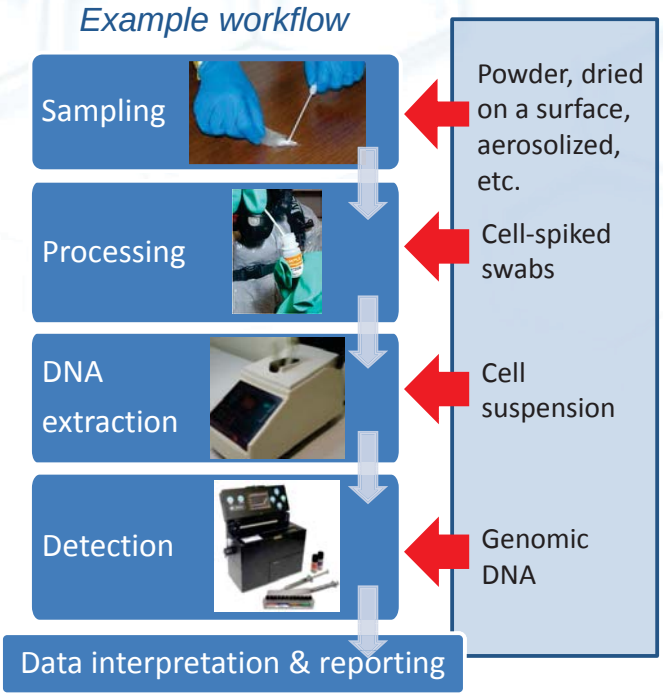
- Health and safety risks (real and perceived)
- False positives due to equipment contamination
- Limited material availability
- Need for specialized facilities

Surrogates: Non-threat, Biological Materials

Evaluate, challenge, and establish confidence in biological assessment in the field

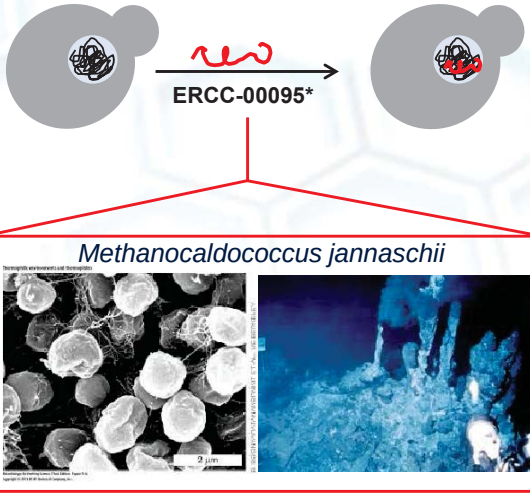
Potential Formats for a Surrogate Material

Example workflow



Saccharomyces cerevisiae NE095

- Designed to challenge nucleic-acid based detection technologies
- Target DNA sequence inserted via homologous recombination
  - Conveys specificity
  - Eliminates false positives from near-neighbors
  - Sequencing confirms one copy per genome
- Lyophilized format



* External RNA Controls Consortium (ERCC)
- NIST Standard Reference Material (SRM) 2374: DNA Sequence Library for External RNA Controls
- ERCC-00095 corresponds to the latter three (of eight) open reading frames in the phosphate specific transport complex component of *M. jannaschii*

S. cerevisiae NE095 in lieu of Biothreat Agents

Minimized real and perceived risk

Readily available, can use almost anywhere

No false positives in real events from equipment contamination

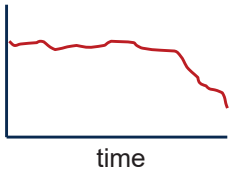
No green light for threat assay validation

Low DNA extraction efficiency, similar to *Bacillus* spores

DNA target eliminates environmental false positives

Lyophilized yeast can be crushed into a powder

Quantitative material can track performance



Stakeholder Input on the Use of the Yeast

The yeast could "serve as a viable resource for the first phase of training programs" and "be used periodically as a 'confidence checker' to allow first responders, under controlled conditions, to review or practice their skills."

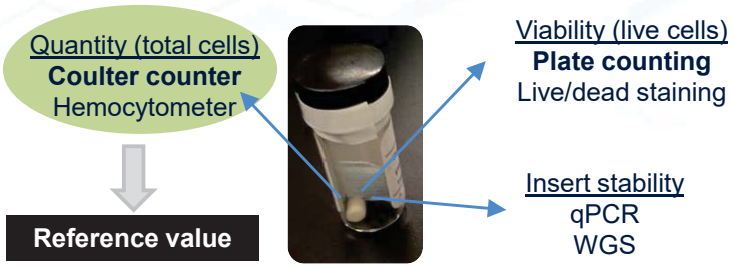
– APHL Public Health Preparedness and Response (PHPR) Committee

Yeast can "provide responders the ability to exercise and evaluate sample collection… with material that safeguards against the potential of cross contamination" and "be used as a competency assessment tool to evaluate the process of sample collection/submission."

– CPT Bryon Marsh (ret), 4th CST

Prototype Batch of Lyophilized Yeast

- **Homogeneity** – Determine vial-to-vial variability in terms of total cells per vial and colony forming units (CFUs) per vial
- **Stability –** Assess total and viable cells per vial for real-time and accelerated test conditions; Also evaluate DNA stability/integrity



- **Fitness for purpose** – Evaluate suitability for end-user requirements and applications

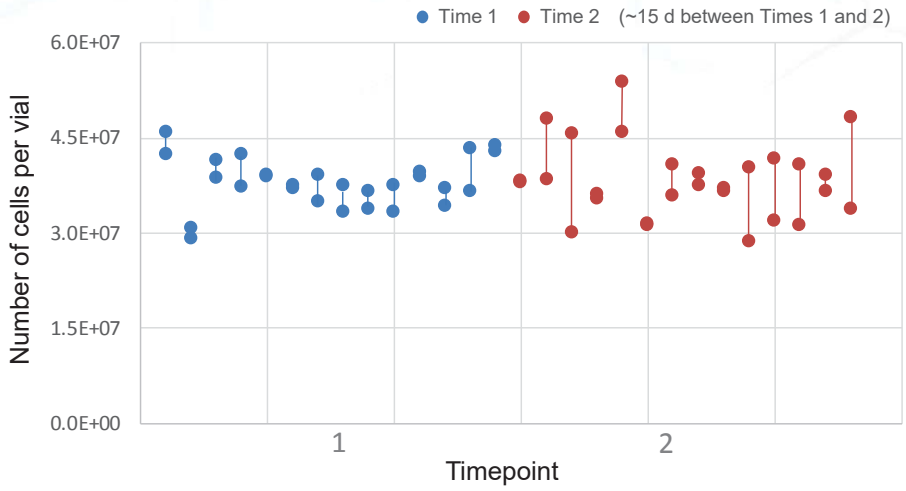
Microbiologics®

Homogeneity Study

Technique	Cells per vial* x 10^7	Vials	Reps
Coulter – Total cells	3.81 ± 0.51 (13.3 %)	28	2
Plating – CFUs	0.095 ± 0.018 (18.9 %)	14	1

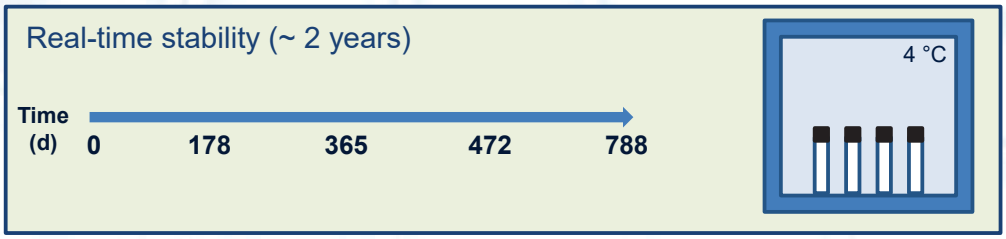
Viability = 2.50 ± 0.58 %

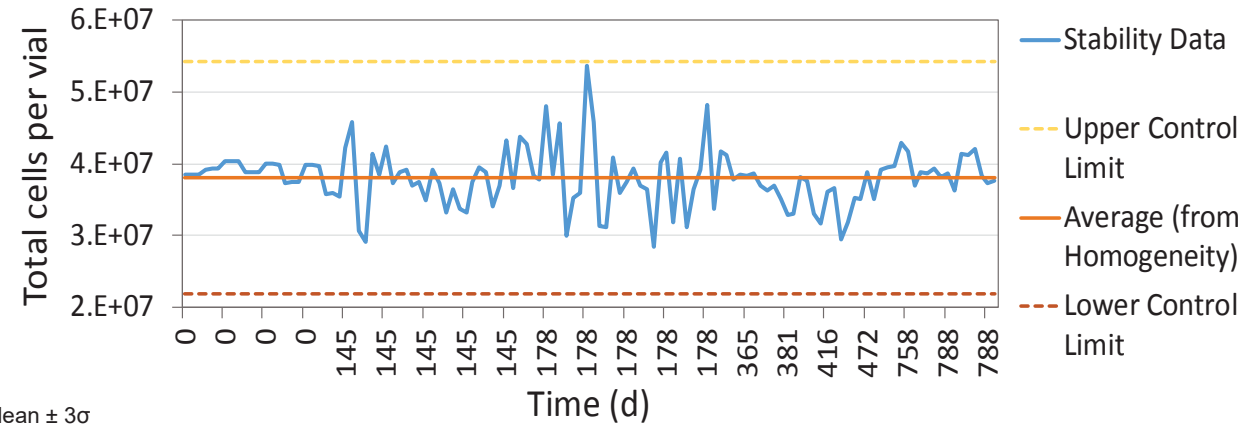
*± 95 % expanded uncertainty (coefficient of variation)



Long-term Stability Study

To establish shelf-life

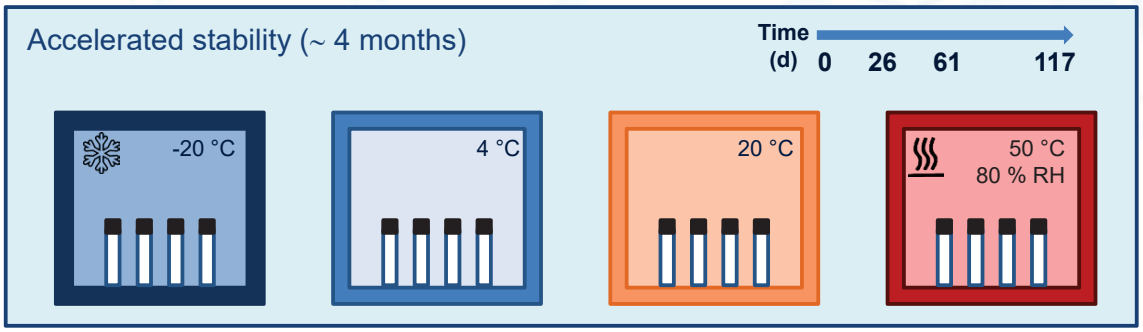




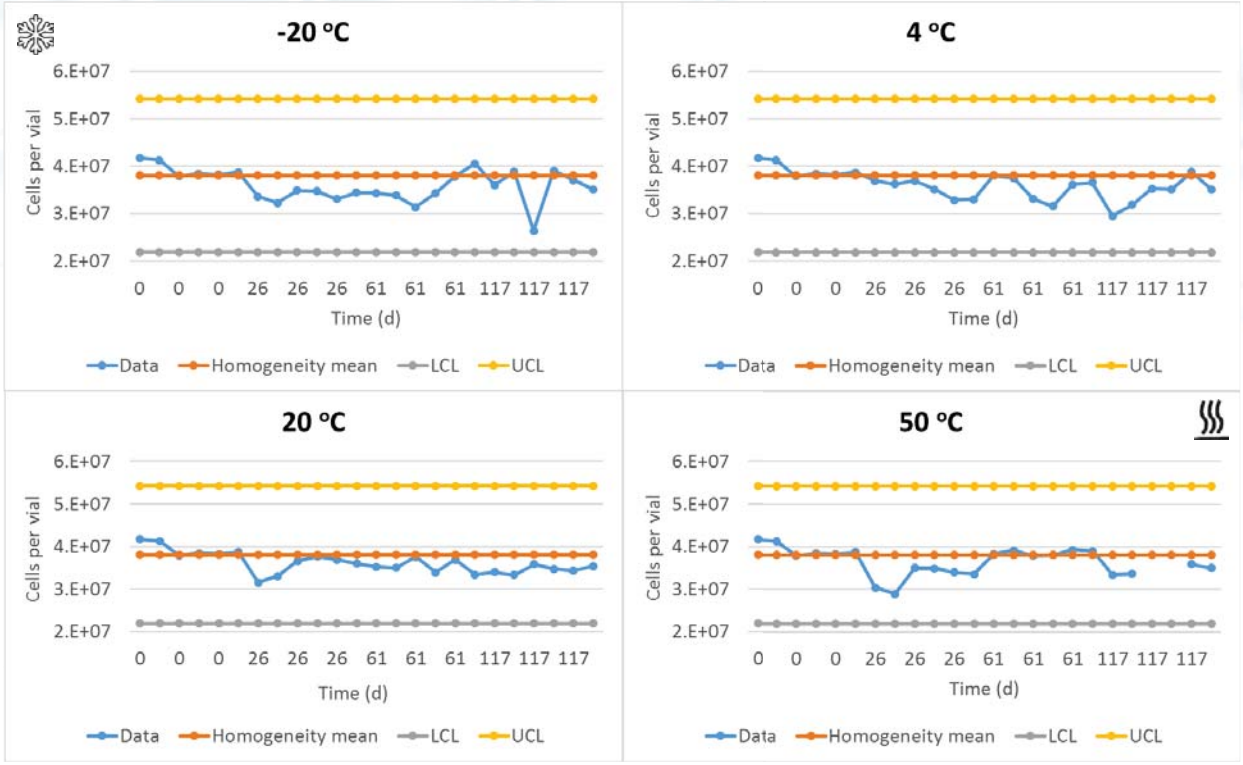
Mean ± 3σ
UCL: upper control level
LCL: lower control level

Accelerated Stability Study

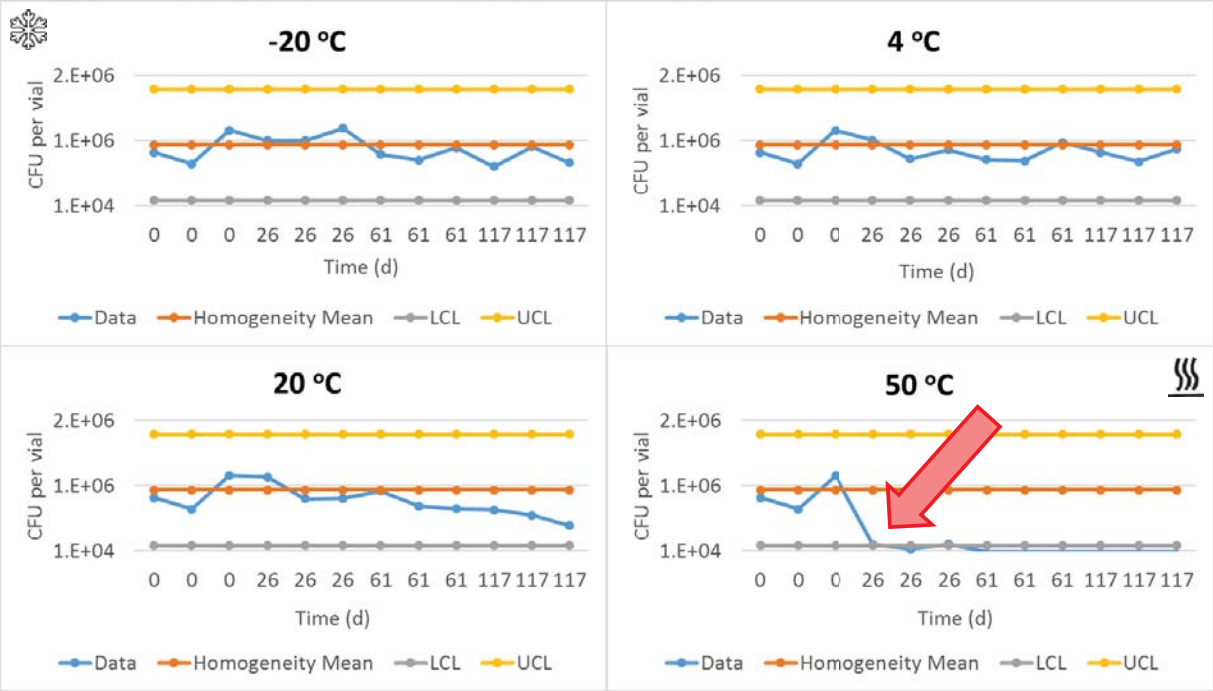
Gather information about RM degradation as an effect of temperature (e.g., short-term exposures during shipping)



Cell Count is Within Control Limits for All Temperatures

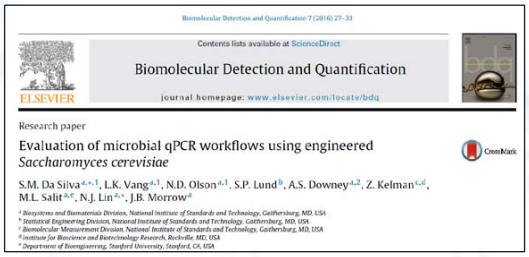


Storage at 50 °C Reduces Viable Cells to ≈ 0



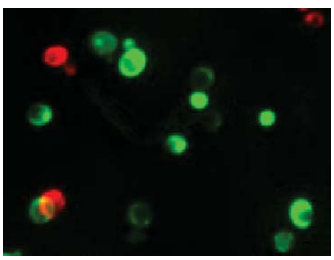
Real World Application

- Previous interlaboratory test of yeast cells was successful – in the lab
- Evaluate fitness for purpose in a real-time, functional full-scale exercise



Da Silva et al, Biomol Detect Quant, 2016

- If we provide materials and a protocol, can the yeast be applied and detected by exercise participants?



Functional Exercise – Operation Vigilant Sample IV (OVS IV), July 2015

- Led by CPT Bryon Marsh, 4th CST
- Participants included
  - 4th and 48th NGB CSTs
  - FL and GA State Dept. of Health, APHL
  - DHS BioWatch
  - Local first responders: Calhoun, Cherokee, Cobb, and Dalton County Fire Depts.
  - GA BioWatch Advisory Committee
  - EPA and FBI Atlanta Field Offices
  - Signature Science

OVS IV Overview

- Conducted in real-time, using state response plans and local responders
- Designed to help define a national exercise template for CST Commanders
- Exercise components included
  - Initial suspected threat event (putative *Yersinia pestis*)
  - BioWatch Actionable Result (BAR)
  - Phase I sampling
  - Phase II sampling
- EPA approval obtained to use yeast (TERA)
- Sampling protocol based on CDC/NIOSH protocol[1]

[1] Surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces, 2012. http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html

Yeast Incorporation into the Exercise

Day 1

On-site yeast prep by CSTs

Day 2

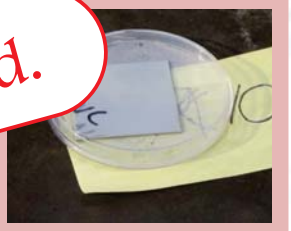
Team assembly and JIT training

Plume model

Yeast successfully detected.

Day 3

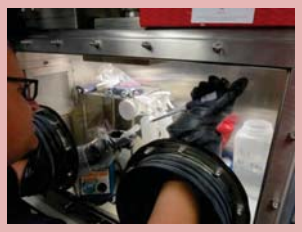
Sample Placement



Sample collection

Decon and chain of custody

On-site DNA extraction and qPCR



On-site results

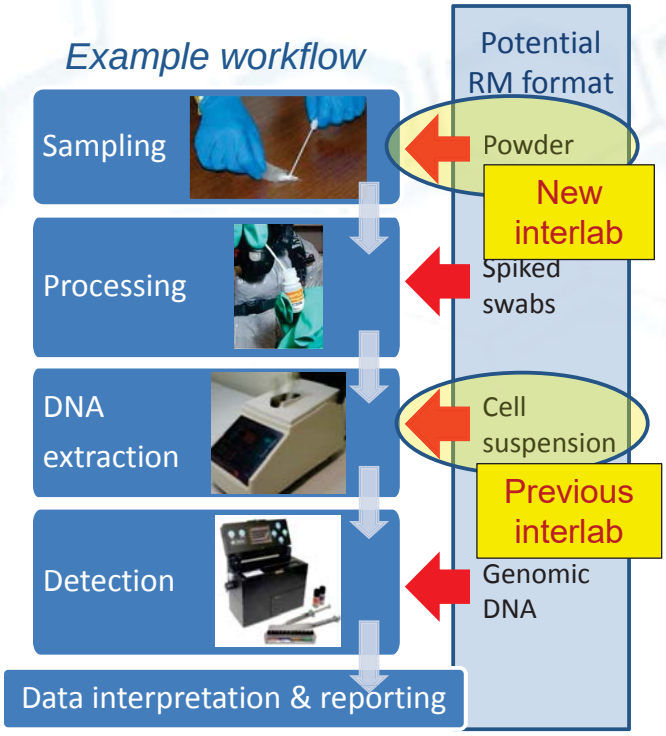
Next – Yeast as a Surrogate Powder

Objective: Establish a protocol for first responder training in sample collection and field assessment using yeast as a surrogate powder

Part 1: Confirm the limit of detection for yeast in PHLs ✓

Part 2: Evaluate a protocol for sampling and detection of yeast powder

Example workflow



Summary of Yeast Surrogate Material

- Homogeneity, stability, and fitness-for-purpose of the lyophilized format support development of the yeast as a reference material
- Heat inactivation may be appropriate if needed
- Next steps
  - Yeast powder interlaboratory study
  - Production batch for NIST RM

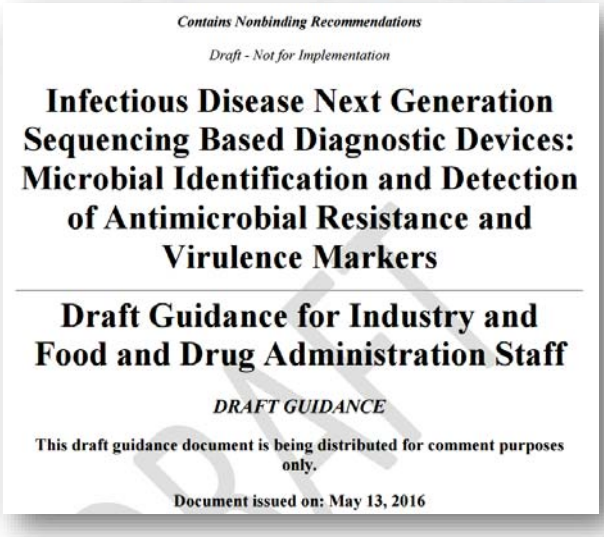
The yeast material is a critical part of the developing Quality Assurance infrastructure to support reliable, consistent results from the First Responder Community

Overview

- Yeast as a surrogate for biothreat agents
- **Mixed microbial reference materials**
- Documentary standards

Known Mixtures of DNA or Cells are Needed

- Technologies are advancing from targeted detection of one or a few agents to untargeted detection of multiple unknowns
- "Ground truth" samples are needed for these technologies to realize their potential to revolutionize biological detection



Contains Nonbinding Recommendations

Draft - Not for Implementation

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

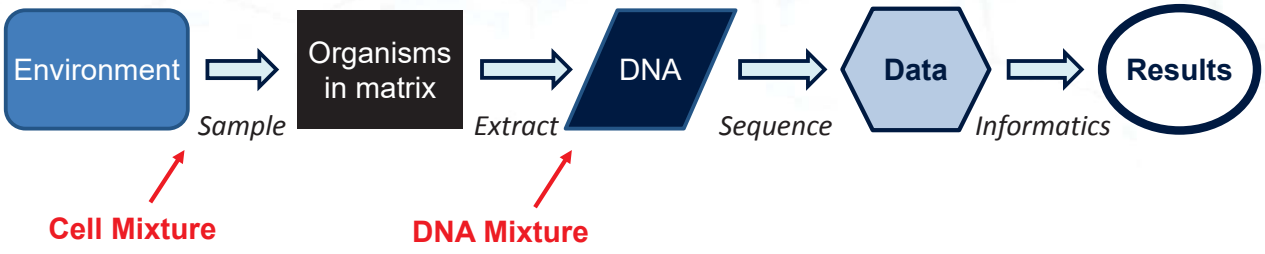
Document issued on: May 13, 2016

"FDA intends to regulate Infectious Disease NGS Dx devices as systems, including all of the components necessary to generate a result."

"Microbial standard reference materials…will be a valuable tool for use in this evaluation."

Mixtures of Microbial DNA or Whole Cells

- **Goal:** Develop (or enable development of) control and reference materials based on DNA or cell mixtures to increase confidence in biological detection technologies



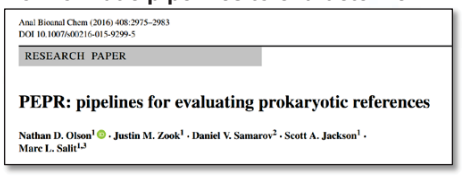
Approach – Build on Existing NIST Efforts

Toward a mixed DNA material

RM 8375: DNA from 4 microbes

SRM 2374 for RNA spike-in mixtures

Bioinformatic pipelines to characterize DNA



Anal Bioanal Chem (2016) 408:2975–2983
DOI 10.1007/s00216-015-9299-5

RESEARCH PAPER

PEPR: pipelines for evaluating prokaryotic references

Nathan D. Olson[1] · Justin M. Zook[1] · Daniel V. Samarov[2] · Scott A. Jackson[1] · Marc L. Salit[1,3]

Initial efforts toward a mixed pathogen DNA RM

NIST Candidate RM for whole yeast cells

Cell quantification methods for yeast

Toward a mixed cell material

Standards for Pathogen Identification via Next-Generation Sequencing (SPIN) Workshop

October 20-21, 2014 at NIST

- Identify measurement challenges and potential solutions associated with using NGS for pathogen identification
- On the wish list
  - DNA, RNA, or cell mixtures at known ratios
  - Guidelines to develop in-house mixtures

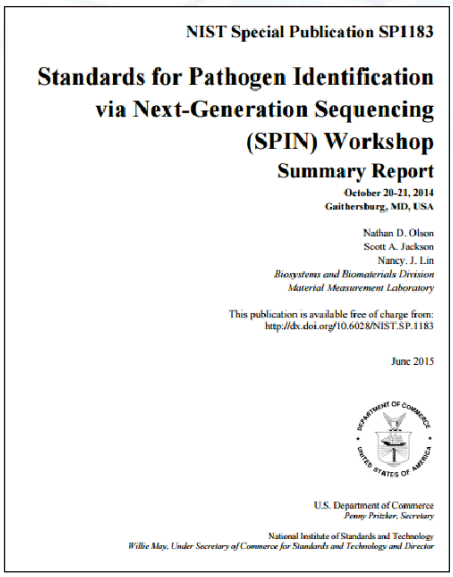
Olson et al. Standards in Genomic Sciences (2015) 10:119
DOI 10.1186/s40793-015-0112-z

Standards in Genomic Sciences

MEETING REPORT Open Access

Report from the Standards for Pathogen Identification via Next-Generation Sequencing (SPIN) Workshop

Nathan D. Olson*, Scott A. Jackson and Nancy J. Lin



NIST Special Publication SP1183

Standards for Pathogen Identification via Next-Generation Sequencing (SPIN) Workshop

Summary Report

October 20-21, 2014
Gaithersburg, MD, USA

Nathan D. Olson
Scott A. Jackson
Nancy. J. Lin

Biosystems and Biomaterials Division
Material Measurement Laboratory

This publication is available free of charge from:
http://dx.doi.org/10.6028/NIST.SP.1183

June 2015

U.S. Department of Commerce
Penny Pritzker, Secretary

National Institute of Standards and Technology
Willie May, Under Secretary of Commerce for Standards and Technology and Director

NIST-FDA Workshop: Standards for Pathogen Detection via Next-Generation Sequencing

October 27-28, 2015 at NIST

#NISTpathogen

NIST-FDA Workshop: Standards for Pathogen Detection via Next-Generation Sequencing

October 27-28, 2015
NIST Gaithersburg, MD

Report

Organizers: Scott Jackson[1], Heike Sichtig[2], Brittany Goldberg[2], Jason Kralj[1]

[1] National Institute of Standards and Technology, Gaithersburg, MD USA
[2] Food and Drug Administration, Silver Spring, MD USA

Purpose of the Workshop
The purpose of this NIST-FDA workshop was to seek input on defining reference materials, reference data and reference methods for assessing analytical sensitivity, specificity, and

Action item: Develop a mixed pathogen DNA material

Prototype Mixed Pathogen DNA Material

Tube Rack – 25 Tubes

Tubes 1-24: Clinically-relevant microbial pathogens

Tube 25: Human DNA as "background"

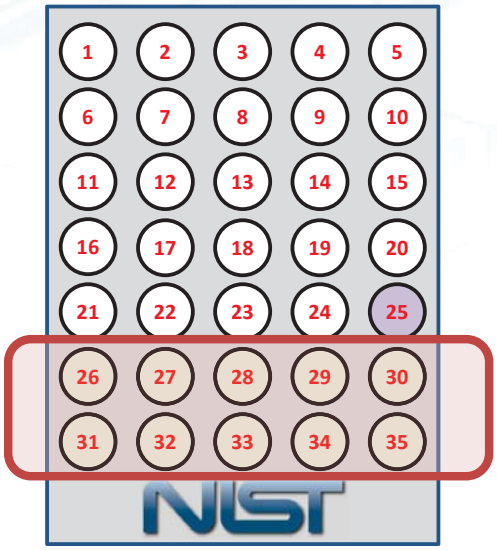
Each tube will contain a single genome and will be characterized:

- Genome assembly
- Base level purity (e.g. rare variants)
- Genomic contaminants
- Quantity (~ 50-100 ng/µL), to infer genomes per µL
- Stability

Potential Expansion to Address Biosurveillance

In the future…

Tube Rack – 35 Tubes



Cell characteristics to consider

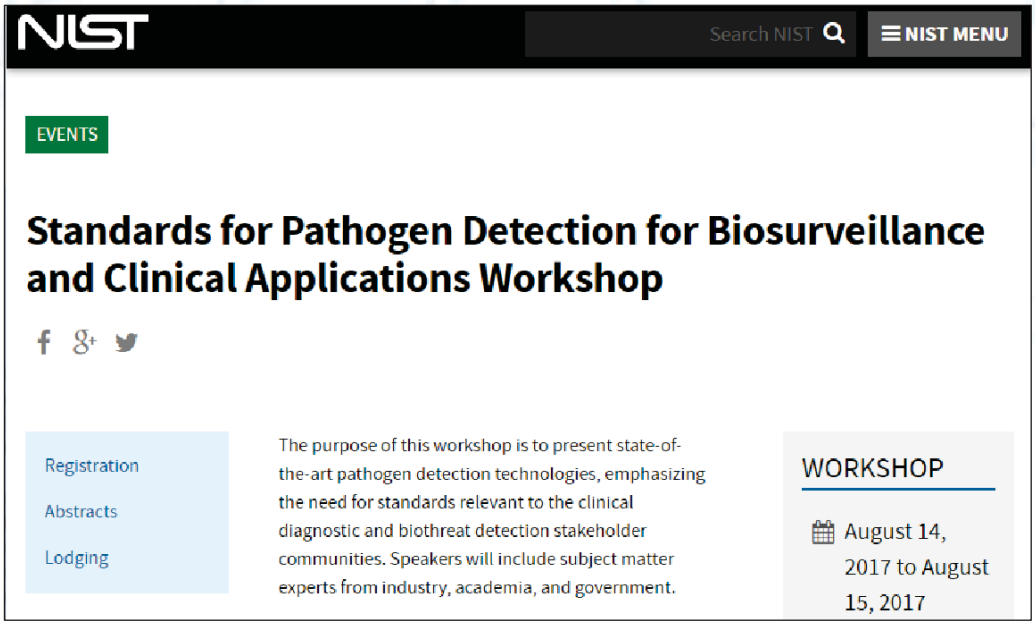
- GC content
- Cell wall physiology
- Genetically similar organisms
- Microbes
- Other organisms (e.g., viruses, human, plants, animals)

Strains to consider

- Environmental background organisms (e.g., SPADA panel?)
- Biothreat near neighbors
- Select agents

YOUR INPUT NEEDED!

Please Join Us:
NIST-DHS Workshop – Aug 14-15, 2017 @ NIST



#NISTpathogen

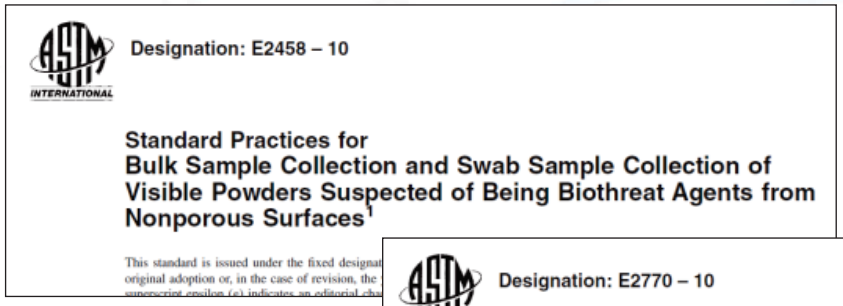
Overview

- Yeast as a surrogate for biothreat agents
- Mixed microbial reference materials
- **Documentary standards**

Documentary Standards

ASTM Committee E54 on Homeland Security Applications, Subcommittee E54.01 on CBRNE Sensors and Detectors

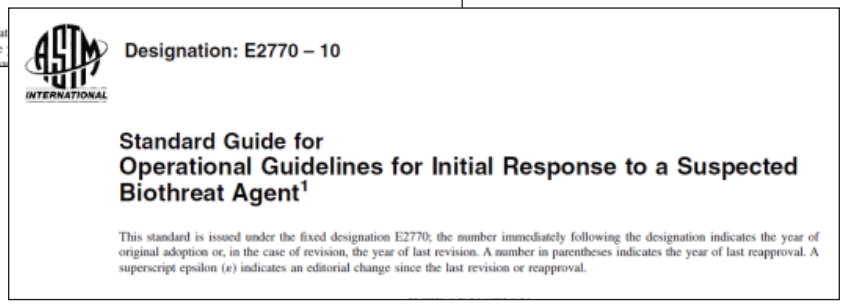
Revised standards accepted with editorial changes!



Designation: E2458 – 10

Standard Practices for
Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat Agents from Nonporous Surfaces[1]

This standard is issued under the fixed designat
original adoption or, in the case of revision, the
superscript epsilon (ε) indicates an editorial cha



Designation: E2770 – 10

Standard Guide for
Operational Guidelines for Initial Response to a Suspected Biothreat Agent[1]

This standard is issued under the fixed designation E2770; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

Possible Standard Guidance on Prokaryotic Strain Characterization and/or Lineage Documentation

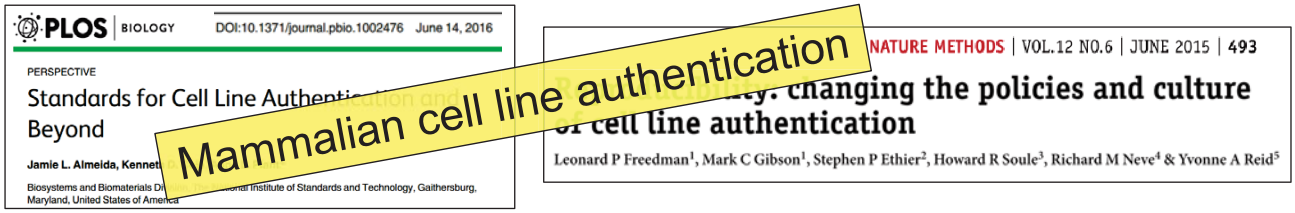
Microbial materials used for R&D, T&E, and training on biothreat detection technologies should be appropriately and consistently characterized.

"NIH expects that key biological and/or chemical resources will be regularly authenticated to ensure their identity and validity for use in the proposed studies."

--NIH, Enhancing Reproducibility through Rigor and Transparency

Strains "must be characterized and documented to truly be the species and strains they are purported to be."

--AOAC. SMPR® 2016.006. Standard Method Performance Requirements for DNA-Based Methods of Detecting *Bacillus anthracis* in Field-Deployable, Department of Defense Aerosol Collection Devices, 2016.



Conclusions

- Modified yeast strains have potential to be used as training materials in lieu of biothreats in a variety of applications
  - *Opportunity for involvement in participating (and helping design) future interlaboratory studies and field tests*
- Mixed microbial RMs and characterization methods can help increase confidence in sequencing and other technologies
  - *Opportunity to provide input on strains to include for biosurveillance applications*
- Documentary standards continue to be needed
  - *In addition to SPADA, there are opportunities to be involved in standards development activities*

Acknowledgements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Lindsay Harris


Jim Filliben


Scott Jackson


Jason Kralj

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MN State Dept of Health
Rich Ozanich (PNNL)
DHS S&T Office of Standards
And others…

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Proposed ASTM Standard Specification for Nucleic Acid-Based Systems for Bacterial Pathogen Screening of Suspicious Visible Powders

Work Item: WK46895

Draft Biothreat Panel Review and Discussion

Brucella species
Coxiella burnetii

Bacillus anthracis
Francisella tularensis
Yersinia pestis

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- This project is sponsored by the U.S. Department of Homeland Security Science and Technology Directorate, Office of Standards
  - Yonas Nebiyeloul-Kifle, Sr. Technical Advisor
  - Phil Mattson, Director
- Pacific Northwest National Laboratory (PNNL) is managing this project under the direction of DHS S&T
  - Rich Ozanich, Project Manager
- Legal Statement:

Project Team

- **Rich Ozanich**
- **Alejandro Heredia Langner**
- **Jennifer Arce**
- **Rachel Bartholomew**
- **Cindy Bruckner-Lea**

Why an ASTM Standard?

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- There are no AOAC SMPR standards for field PCR detection of visible powders.
  - Two immunoassay standards (*Bacillus anthracis* and ricin) do exist for this specific application (field use for visible powders)
- Other AOAC standards have a stated intended use, “Laboratory use for analysis of aerosol collection filters and/or liquids”, or “Field-deployed use for analysis of aerosol collection filters and/or liquids”
  - Emphasis on laboratory analysis and environmental monitoring using aerosol collection filters
  - Inclusivity/exclusivity panels members differ from the application of suspicious visible powder screening
- The ASTM standard will:
  - Have two performance tiers rather than pass/fail
  - Have three levels of testing rigor (diversity of inclusivity/exclusivity panels)

ASTM Committee E54 on Homeland Security Applications & Subcommittee E54.01 on CBRNE Sensors and Detectors

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Designation: E2458 – 10
Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat Agents from Nonporous Surfaces[1]

Designation: E2933 – 13
Standard Specification for Stationary Point Chemical Vapor Detectors (SPCVD) for Homeland Security Applications[1]

Designation: E2677 – 14
Standard Test Method for Determining Limits of Detection in Explosive Trace Detectors[1]

Designation: E2520 – 15
Standard Practice for Measuring and Scoring Performance of Trace Explosive Chemical Detectors[1]

Designation: XXXXX-XX
Standard Specification for Polymerase Chain Reaction Systems for Biothreat Agent Screening of Suspicious Visible Powders[1]

Designation: E2770 – 10
Standard Guide for Operational Guidelines for Initial Response to a Suspected Biothreat Agent[1]

Designation: E2800 – 11
Standard Practice for Characterization of *Bacillus* Spore Suspensions for Reference Materials[1]

Designation: E2852 – 13
Standard Guide for Acquisition, Maintenance, Storage, and Use of Hazardous Material Detection Instrumentation[1]

Designation: E2894 – 13
Standard Test Method for Applying Aerosolized *Bacillus* Spores as Dry Inocula to Inanimate Surfaces[1]

Designation: X XXXX-XX
Standard Practice for Field Evaluation of On-Site Biological Assessment Technologies

Designation: E2885 – 13
Standard Specification for Handheld Point Chemical Vapor Detectors (HPCVD) for Homeland Security Applications[1]

Designation: E2805 – 11
Standard Practice for Measurement of the Biological Activity of Ricin[1]

Designation: E2851/E2851M – 13
Standard Specification for Ruggedness Requirements for HAZMAT Instrumentation[1]

Designation: XXXXX-XX
Standard Specification for The Characterization of Systems and Instruments with Binary Responses[1]

Designation: X XXXX-XX
Work Item Number: WK46944
Date: January 5, 2016
Guide for Standards-Based Testing of Radiation-Detection Systems[1]

Preliminary Work Item Draft Standards:

- Standard Specification for Immunoassays for Biothreat Agent Screening of Suspicious Visible Powders
- A Statistical Method for Establishing Bioassay Equivalency between Active and Inactive Organisms
- Draft protocol for determining LOD when the response is binary

A growing body of ASTM standards support first responders and the evaluation and use of CBRNE Sensors and Detectors

Background and Guiding Principles for this Standard

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- Field biodetection products widely used, but performance largely unknown
- Standards written only for one application: *field screening of visible suspicious powders*
- Allow flexibility in the rigor of testing (cost/time)
  - Two LCB/confidence level options
  - 3 levels of testing rigor
- Once the standard is approved, market forces may decide the balance between testing rigor/costs and user acceptance of certain validated performance levels
- Revisions and refinements to the standard can be made in the future – but we need something as a starting point

Some level of performance testing is better than none

Test Modules (TM) for Nucleic Acid Testing

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- TM1: Inclusivity
  - Samples expected to give a positive result
- TM2: Exclusivity
  - "Near-neighbor" samples expected to give a negative result
  - Tested at 10X inclusivity test concentration
- TM3: Commonly Encountered Suspicious Powders
  - Assessment of whether powders generate a false positive result
- TM4: Biothreat-spiked powders
  - Assessment of whether the presence of powder causes a false negative result

If nucleic acid testing (TM1 and TM2) and powder testing (TM3) achieve target performance criteria, go on to test a single inclusivity whole organism (the "representative" organism) spiked into powders and no whole exclusivity organisms

TM Rigor for Nucleic Acid Testing

- Level 1
  - Full AOAC SMPR inclusivity and exclusivity nucleic acid panels (where applicable)
- Level 2
  - ~1/2 AOAC SMPR inclusivity and full exclusivity nucleic acid panels
- Level 3
  - A single representative organism nucleic acid inclusivity strain
  - Full exclusivity panel

Test Sample Concentrations for Nucleic Acid Testing

- Inclusivity test sample concentration: 10,000 GE/mL
  - Must meet or be lower than the concentration required to meet the needs of the end user/application (e.g., field screening of ***visible*** suspicious powder)
  - Can be set lower than needed for the application, based on the capabilities of the technology, but should not be set so low that otherwise acceptable products can not achieve metrics
- Exclusivity test sample concentration: 100,000 GE/mL (10X inclusivity concentration)
- Powder test sample concentration: 0.1 mg/mL

Brucella Inclusivity Panel - Questions

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- Included 12 *Brucella* species in both Tier 1 and 2 Inclusivity panels
  - *melitensis* (1-3)
  - *suis* (1-5)
  - *abortus (1-6,[7?],9)*
  - *neotomae*
  - *ovis*
  - *canis*
  - *ceti*
  - *pinnipedialis*
  - *microti*
  - *inopinata*
  - *vulpis*
  - *papionis*

- Included all species and biovars in Tier 1 panel
- Included one of each species in Tier 2 panel

***1) B. abortus* biovar 7?**

2) What is the "representative strain" for *Brucella* – a *melitensis* or *suis* (or other)?

Brucella Inclusivity Panel – Tier 1

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A4.1.2 *Brucella*. If any listed strain is not available, an alternate strain shall be selected from 1) the same biovar for *B. abortus, B. melitensis* and *B. suis* species, 2) a different strain for *B. canis, B. ceti, B. inopinata, B. microti, B. neotomae, B. ovis, B. papionis, B. pinipedialis* and *B. vulpis* species. If an alternate strain is not available, a strain different than those listed below shall be selected if available;

Tier 1 *Brucella* Inclusivity Panel

Note: This panel is still under development and strain selection has not been finalized. The following needs to be addressed:

1. **Inclusion of all species and Biovars in Tier 1 panel?**
2. **Include B. abortus Biovar 7 if widely accepted (only a few references in lit)?**
3. **Determine representative strain for Brucella species.**

Species	Strain	Notes
Brucella abortus	292 (39/94)	Biovar 4
Brucella abortus	B3196	Biovar 5
Brucella abortus	C68	Biovar 9
Brucella abortus	86/8/59	Biovar 2
Brucella abortus	12	Biovar 3
Brucella abortus	544	Biovar 1
Brucella abortus	870	Biovar 6
Brucella canis	RM-666	N/A
Brucella ceti	B1/94 or other?	N/A
Brucella inopinata	BO1	N/A
Brucella melitensis	Ether	Biovar 3
Brucella melitensis	63/9	Biovar 2
Brucella melitensis	16M	Biovar 1
Brucella microti	CCM 4915 or other?	N/A
Brucella neotomae	5K33	N/A
Brucella ovis	63-390	N/A
Brucella papionis	F8/08-60(T) or other?	N/A
Brucella pinipedialis	B2/94 or other	N/A
Brucella suis	S2	Biovar 1
Brucella suis	Thomsen	Biovar 2
Brucella suis	40	Biovar 4
Brucella suis	513	Biovar 5
Brucella suis	686	Biovar 3
Brucella suis	1330	Biovar 1
Brucella vulpis	F60 or F965 if available?	N/A

Adapted from *Brucella* 2016.009-Journal of AOAC International Volume 100, Number 1, January-February 2017, pp. 255-260.

N/A: These *Brucella* species are not further subdivided into biovars.

Brucella Inclusivity Panel – Tier 2

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A4.2.2 *Brucella*. If any listed strain is not available, an alternate strain shall be selected from 1) the same biovar for *B. abortus, B. melitensis* and *B. suis* species, 2) a different strain for *B. canis, B. ceti, B. inopinata, B. microti, B. neotomae, B. ovis, B. papionis, B. pinipedialis* and *B. vulpis* species. If an alternate strain is not available, a strain different than those listed below shall be selected if available;

Tier 2 *Brucella* Inclusivity Panel

Species	Strain	Notes
Brucella abortus	544	Biovar 1
Brucella canis	RM-666	N/A
Brucella ceti	B1/94	N/A
Brucella inopinata	BO1	N/A
Brucella melitensis	16M	Biovar 1
Brucella microti	CCM 4915 (or other?)	N/A
Brucella neotomae	5K33	N/A
Brucella ovis	63-390	N/A
Brucella papionis	F8/08-60(T) (or other?)	N/A
Brucella pinnipedialis	B2/94 (or other?)	N/A
Brucella suis	1330	Biovar 1
Brucella vulpis	F60 or F965 if available?	N/A

Adapted from *Brucella* 2016.009-Journal of AOAC International Volume 100, Number 1, January-February 2017, pp. 255-260.

N/A: These *Brucella* species are not further subdivided into biovars.

Brucella Exclusivity Panel - Questions

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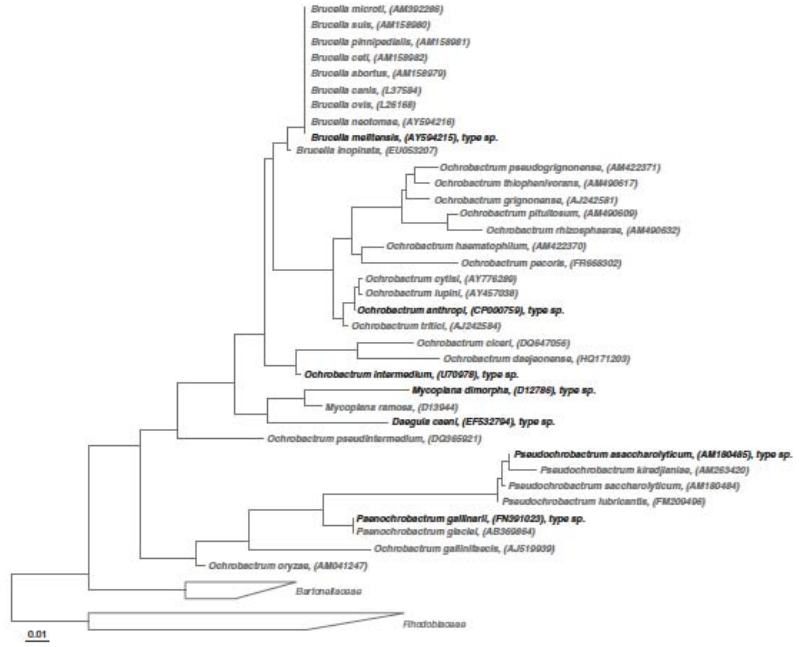

Fig. 6.1
Phylogenetic reconstruction of the family *Brucellaceae* based on 16S rRNA and created using the maximum likelihood algorithm PhyML (Guindon et al. 2010). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as out-groups. Scale bar indicates estimated sequence divergence

- Tier 1 = 13 strains
- Tier 2 & 3 = 7 strains

1) **Which *Bartonella* species and strain would be most appropriate?**
2) **Do we include a *Rhizobium* species?**
3) **Do we include other Brucellaceae genera?**
4) **Do we include other Rhizobiales families?**

DeLong, E. F., & Rosenberg, E. (2014). The Prokaryotes. Berlin: Springer Reference.

Brucella Exclusivity - Tier 1

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A5.1.2 *Brucella*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 1 *Brucella* Exclusivity Panel

Note: This panel is still under development and strain selection has not been finalized. The following needs to be addressed:

1. **Which Bartonella species and strain would be most appropriate?**
2. **Include a Rhizobium species?**
3. **Can we reduce number of Ochrobactrum species?**
4. **Any other organisms we should include from closely related genera?**

Species	Strain	Notes
Agrobacterium tumefaciens	ATCC 4452	
Bartonella spp. *Bartonella henselae*	TBD Houston-1	Could have one or none, or more. Need consensus on this species in exclusivity panel.
Bradyrhizobium japonicum	ATCC 10324	
Ochrobactrum anthropi	ATCC 49188	Do we need multiple *Ochrobactrum?*
Ochrobactrum gallinifaecis	Iso196	Do we need multiple *Ochrobactrum?*
Ochrobactrum grignonense	Oga9a	Do we need multiple *Ochrobactrum?*
Ochrobactrum intermedium	CNS 2-75 (NCTC 12171)	Do we need multiple *Ochrobactrum?*
Ochrobactrum lupini	LUP 21	Do we need multiple *Ochrobactrum?*
Ochrobactrum oryzae	DSM 17471	Do we need multiple *Ochrobactrum?*
Ochrobactrum pseudintermedium	ADV31	Do we need multiple *Ochrobactrum?*
Ochrobactrum tritici	SCII24 (DSM 13340)	Do we need multiple *Ochrobactrum?*
Pseudochrobactrum ***kiredjianiae***	SS5	Maybe pick one strain
Paenochrobactrum gallinari	Sa-25	Maybe pick one strain
Rhizobium spp.	TBD	Should we include one from this genus?
Other Rhizobiales members	TBD	Similar family, do we include more?

Adapted from *Brucella* 2016.009-Journal of AOAC International Volume 100, Number 1, January-February 2017, pp. 255-260.

Brucella Exclusivity - Tier 2/3

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A5.2.2 *Brucella*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 2 and Tier 3 *Brucella* Exclusivity Panel

Species	Strain	Notes
Agrobacterium tumefaciens	ATCC 4452	
Bartonella spp. *Bartonella henselae*	TBD Houston-1	Could have one or none. Need consensus on this species.
Bradyrhizobium japonicum	ATCC 10324	
Ochrobactrum anthropi	ATCC 49188	Which neighbors should we use?
Ochrobactrum intermedium	LMG 3301	Which neighbors should we use?
Pseudochrobactrum kiredjianiae	SS5	Which neighbors should we use?
Paenochrobactrum gallinari	Sa-25	Which neighbors should we use?

Adapted from *Brucella* 2016.009-Journal of AOAC International Volume 100, Number 1, January-February 2017, pp. 255-260.

Coxiella burnetii Inclusivity Panel - Questions

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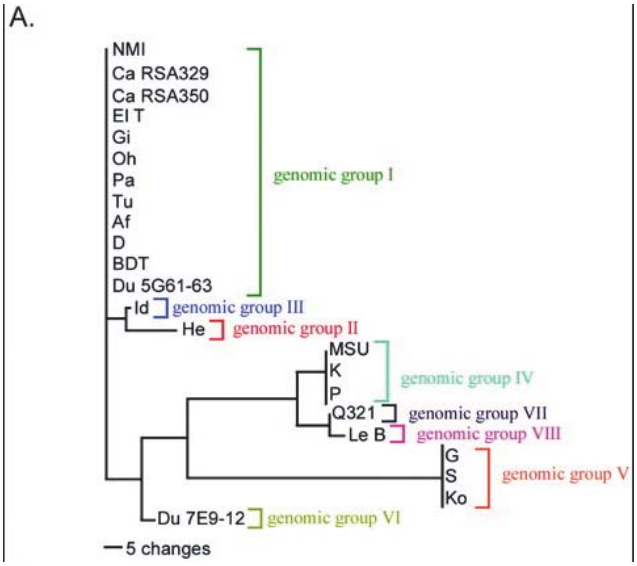
1) **For Genomic Group 1 strains, is there a preference for either of the strains listed (RSA493 or RSA 439)?**

2) **There is a reference to GG 7 and 8 in some of the literature, should these be included and if so what would the representative strains be for these groups?**

- References for GG 7 and 8:

Jado, I., et al. 2012. Molecular method for the characterization of Coxiella burnetii from clinical and environmental samples: variability of genotypes in Spain. BMC Microbiology 12:91.

Beare, P. A., J. E. Samuel, D. Howe, K. Virtaneva, S. F. Porcella, and R. A. Heinzen. 2006. Genetic diversity of the Q fever agent, Coxiella burnetii, assessed by microarray-based whole-genome comparisons. J. Bacteriol. 188:2309-2324.



Beare, P. A., J. E. Samuel, D. Howe, K. Virtaneva, S. F. Porcella, and R. A. Heinzen. 2006. Genetic diversity of the Q fever agent, Coxiella burnetii, assessed by microarray-based whole-genome comparisons. J. Bacteriol. 188:2309-2324.

Coxiella burnetii Inclusivity Panel – Tier 1

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A4.1.3 *Coxiella burnetii*. If any listed strain is not available, an alternate strain shall be selected from the same genomic group. If an alternate strain from the same genomic group is not available, a strain different than those listed below shall be selected if available;

Tier 1 *Coxiella burnetii* Inclusivity Panel

Note: This panel is still under development and strain selection has not been finalized. The following needs to be addressed:

1. **Is there a preference for Nine Mile strain RSA493 or 439?**
2. **Should strains from genomic groups 7 & 8 be included in panel (there are two references to these in Jado et al 2012 and Beare et al 2006)?**

Species	Strain	Notes
Coxiella burnetii	Nine Mile RSA493 or RSA439	Genomic group I
Coxiella burnetii	Henzerling	Genomic group II
Coxiella burnetii	Idaho Goat	Genomic group III
Coxiella burnetii	K	Genomic group IV
Coxiella burnetii	G	Genomic group V
Coxiella burnetii	Dugway	Genomic group VI

Adapted from *Coxiella burnetii* 2015.011 - Journal of AOAC International, Number 1, January-February 2016, pp. 298-302.

Coxiella burnetii Inclusivity Panel – Tier 2

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A4.2.3 *Coxiella burnetii*. If any listed strain is not available, an alternate strain shall be selected from the same genomic group. If an alternate strain from the same genomic group is not available, a strain different than those listed below shall be selected if available;

Tier 2 *Coxiella burnetii* Inclusivity Panel		
Species	**Strain**	**Notes**
Coxiella burnetii	Nine Mile RSA493 or RSA439	Genomic group I
Coxiella burnetii	Henzerling	Genomic group II
Coxiella burnetii	Idaho Goat	Genomic group III
Coxiella burnetii	K	Genomic group IV
Coxiella burnetii	G	Genomic group V
Coxiella burnetii	Dugway	Genomic group VI
Adapted from *Coxiella burnetii* 2015.011 - Journal of AOAC International, Number 1, January-February 2016, pp. 298-302.		

Coxiella burnetii Exclusivity Panel - Questions

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- Tier 1 & 2/3 all have 4 strains listed

1) **Do we include any from the *Rickettsiella* genus (if available)?**

2) **Should we include any other *Legionella* species?**

Coxiella burnetii Exclusivity - Tier 1

Pacific Northwest NATIONAL LABORATORY
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A5.1.3 *Coxiella burnetii*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 1 *Coxiella burnetii* Exclusivity Panel

Note: This panel is still under development and strain selection has not been finalized. The following needs to be addressed:
1. Include any other Legionella species?
2. Include any from the Rickettsiella genus (mostly found in ticks, but doesn't appear to be much available)?

Species	Strain	Notes
Legionella pneumophilia	Philadelphia 1	
Legionella pneumophila	Wadsworth 1	
Legionella pneumophila	Sg6	
Legionella longbeachae	ATCC No. 33462	
Rickettsiella spp.		If available. I can't find any.

Adapted from *Coxiella burnetii* 2015.011 - Journal of AOAC International, Number 1, January-February 2016, pp. 298-302.

Coxiella burnetii Exclusivity - Tier 2/3

Pacific Northwest NATIONAL LABORATORY
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A5.2.3 *Coxiella burnetii*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 2 and Tier 3 *Coxiella burnetii* Exclusivity Panel

Species	Strain	Notes
Legionella pneumophilia	Philadelphia 1	
Legionella pneumophilia	Wadsworth 1	
Legionella pneumophilia	Sg6	
Legionella longbeachae	ATCC No. 33462	
Rickettsiella spp.		If available

Adapted from *Coxiella burnetii* 2015.011 - Journal of AOAC International, Number 1, January-February 2016, pp. 298-302.

Possible Discussion Topic

Pacific Northwest NATIONAL LABORATORY
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Bacillus anthracis Inclusivity Panel – Tier 1

A4.1.1 *Bacillus anthracis.* If any listed strain is not available, an alternate strain shall be selected from the same VNTR group. If an alternate strain from the same VNTR group is not available, a strain from the same genotype shall be selected. If an alternate strain from the same genotype is not available, a strain different than those listed below shall be selected if available;

Tier 1 *Bacillus anthracis* Inclusivity Panel		
Species	**Strain**	**Notes**
Bacillus anthracis	Ames	pXO1+, pXO2+; VNTR group A3b; genotype 62
Bacillus anthracis	BA1035	pXO1+, pXO2+, VNTR group B1; genotype 82
Bacillus anthracis	BA0018	pXO1+, pXO2+; VNTR group A1a; genotype 7
Bacillus anthracis	K3	pXO1+, pXO2+; VNTR group A3c; genotype 67
Bacillus anthracis	Ohio ACB	pXO1+, pXO2+; VNTR group A3d; genotype 68
Bacillus anthracis	PAK-1	pXO1+, pXO2+; VNTR group A2; genotype 29
Bacillus anthracis[A]	Pasteur	pXO1-, pXO2+; VNTR group A1a; genotype 8
Bacillus anthracis	RA3	pXO1+, pXO2+; VNTR group B2; genotype 80
Bacillus anthracis	SK-102	pXO1+, pXO2+; VNTR group A4; genotype 69
Bacillus anthracis[B]	Sterne	pXO1+, pXO2-; VNTR group A3b; genotype 59, 61
Bacillus anthracis	Turkey 32	pXO1+, pXO2+; VNTR group A1b; genotype 23
Bacillus anthracis[B]	V770-NP-1R	pXO1+, pXO2-; VNTR group A3A; genotype 45
Bacillus anthracis	Vollum 1B	pXO1+, pXO2+; VNTR group A4; genotype 77

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.003, www.eoma.aoac.org,and *Bacillus anthracis* 2016.006 - Journal of AOAC International, Volume 99, Number 4, July-August 2016, pp. 1084-1089. https://doi.org/10.5740/jaoacint.SMPR2016.006.

[A] This strain should not be included in the inclusivity panel if the product being tested only utilizes a pXO1 assay, as this strain is expected to be pXO1 negative.

[B] These two strains should not be included in the inclusivity panel if the product being tested only utilizes a pXO2 assay, as these strains are expected to be pXO2 negative.

A4.2.1 *Bacillus anthracis*. If any listed strain is not available, an alternate strain shall be selected from the same VNTR group. If an alternate strain from the same VNTR group is not available, a strain from the same genotype shall be selected. If an alternate strain from the same genotype is not available, a strain different than those listed below shall be selected if available;

Tier 2 *Bacillus anthracis* Inclusivity Panel		
Species	**Strain**	**Notes**
Bacillus anthracis	Turkey 32	pXO1+, pXO2+; VNTR group A1b; genotype 23
Bacillus anthracis	K3	pXO1+, pXO2+; VNTR group A3c; genotype 67
Bacillus anthracis	Ohio ACB	pXO1+, pXO2+; VNTR group A3d; genotype 68
Bacillus anthracis	Ames	pXO1+, pXO2+; VNTR group A3b; genotype 62
Bacillus anthracis	BA1035	pXO1+, pXO2+; VNTR group B1; genotype 82
Bacillus anthracis	BA0018	pXO1+, pXO2+; VNTR group A1a; genotype 7
Bacillus anthracis	PAK-1	pXO1+, pXO2+; VNTR group A2; genotype 29
Bacillus anthracis	RA3	pXO1+, pXO2+; VNTR group B2; genotype 80
Bacillus anthracis	SK-102	pXO1+, pXO2+; VNTR group A4; genotype 69
Bacillus anthracis[A]	Sterne	pXO1+, pXO2-; VNTR group A3b; genotype 59, 61
Bacillus anthracis[A]	V770-NP-1R	pXO1+, pXO2-; VNTR group A3a; genotype 45
Bacillus anthracis	Vollum 1B	pXO1+, pXO2+; VNTR group A4; genotype 77

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.003, www.eoma.aoac.org, and *Bacillus anthracis* 2016.006 - Journal of AOAC International, Volume 99, Number 4, July-August 2016, pp. 1084-1089.

[A] These two strains should not be included in the inclusivity panel if the product being tested only utilizes a pX02 assay, as these strains are expected to be pXO2 negative.

A5.1.1 *Bacillus anthracis*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 1 *Bacillus anthracis* Exclusivity Panel		
Species	**Strain**	**Notes**
Bacillus cereus	S2-8	pXO1-, pXO2-
Bacillus cereus	3A	pXO1-, pXO2-; toxigenic
Bacillus thuringiensis	HD1011	pXO1-, pXO2-
Bacillus thuringiensis	97-27	pXO1-, pXO2-
Bacillus thuringiensis	HD682	pXO1-, pXO2-
Bacillus cereus	E33L	pXO1-, pXO2-
Bacillus cereus	D17	pXO1-, pXO2-
Bacillus thuringiensis	HD571	pXO1-, pXO2-
Bacillus cereus	Al Hakam	pXO1-, pXO2-
Bacillus cereus	ATCC 4342	pXO1-, pXO2-
Bacillus cereus	FM1	pXO1-, pXO2-
Bacillus cereus[B]	G9241	pBCXO1+, pXO2-; toxigenic
Bacillus cereus[A]	3BB102	pXO1+, capA+, capB+, capC+; toxigenic
Bacillus cereus[A]	03BB108	pXO1+, capA+, capB+, capC+; toxigenic
Bacillus thuringiensis	HD 1002	pXO1-, pXO2-
Bacillus thuringiensis	HD 1	pXO1-, pXO2-
Bacillus thuringiensis	HD 600	pXO1-, pXO2-

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.003, www.eoma.aoac.org, and *Bacillus anthracis* 2016.006 - Journal of AOAC International, Volume 99, Number 4, July-August 2016, pp. 1084-1089.

[A] These three strains should not be included in the exclusivity panel if the product being tested only utilizes a pXO1 assay, as these strains are expected to be pXO1 positive .

[B] The pBCXO1 plasmid is similar to pXO1 and may generate a positive result if the product being tested only utilizes a pXO1 assay.

Bacillus anthracis Exclusivity - Tier 2/3

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A5.2.1 *Bacillus anthracis*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 2 and Tier 3 *Bacillus anthracis* Exclusivity Panel		
Species	**Strain**	**Notes**
Bacillus cereus	S2-8	pXO1-, pXO2-
Bacillus cereus	3A	pXO1-, pXO2-
Bacillus thuringiensis	97-27	pXO1-, pXO2-
Bacillus cereus	E33L	pXO1-, pXO2-
Bacillus cereus	Al Hakam	pXO1-, pXO2-
Bacillus cereus[B]	G9241	pBCXO1+, pXO2-; toxigenic
Bacillus cereus[A]	03BB102	pXO1+, capA+, capB+, capC+; toxigenic
Bacillus cereus[A]	03BB108	pXO1+, capA+, capB+, capC+; toxigenic
Bacillus thuringiensis	HD 1002	pXO1-, pXO2-
Bacillus thuringiensis	HD 1	pXO1-, pXO2-
Bacillus thuringiensis	HD 600	pXO1-, pXO2-

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.003, www.eoma.aoac.org, and *Bacillus anthracis* 2016.006 - Journal of AOAC International, Volume 99, Number 4, July-August 2016, pp. 1084-1089.
[A] These three strains should not be included in the exclusivity panel if the product being tested only utilizes a pXO1 assay, as these strains are expected to be pXO1 positive .
[B] The pBCXO1 plasmid is similar to pXO1 and may generate a positive result if the product being tested only utilizes a pXO1 assay.

Francisella tularensis Inclusivity Panel - Tier 1

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A4.1.4 *Francisella tularensis*. If any listed strain is not available, an alternate strain shall be selected from the same type (if applicable). If an alternate strain from the same type is not available, a strain different than those listed below shall be selected if available;

Tier 1 *Francisella tularensis* Inclusivity Panel		
Species	**Strain**	**Notes**
Francisella tularensis subsp. *holarctica*	VT68	Type B
Francisella tularensis subsp. *holarctica*	JAP Cincinnati	Type B
Francisella tularensis subsp. *holarctica*	LVS	Type B
Francisella tularensis subsp. *holarctica*	425	Type B
Francisella tularensis subsp. *mediasiatica*	FSC147	N/A
Francisella tularensis subsp. *tularensis*	NM99-1823	Type A2
Francisella tularensis subsp. *tularensis*	Scherm	Type A1
Francisella tularensis subsp. *tularensis*	SCHU S4	Type A1
Francisella tularensis subsp. *tularensis*	WY96-3418	Type A2

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.001, www.eoma.aoac.org, and *Francisella tularensis* 2016.007-Journal of AOAC, Volume 99, Number 4, July-August 2016, pp. 1090-1094.
N/A: This subspecies is not further subdivided into types.

Francisella tularensis Inclusivity Panel - Tier 2

Pacific Northwest NATIONAL LABORATORY
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A4.2.4 *Francisella tularensis*. If any listed strain is not available, an alternate strain shall be selected from the same type (if applicable). If an alternate strain from the same type is not available, a strain different than those listed below shall be selected if available;

Tier 2 *Francisella tularensis* Inclusivity Panel		
Species	**Strain**	**Notes**
Francisella tularensis subsp. *holarctica*	LVS	Type B
Francisella tularensis subsp. *mediasiatica*	FSC147	N/A
Francisella tularensis subsp. *tularensis*	SCHU S4	Type A1
Francisella tularensis subsp. *tularensis*	WY96	Type A2
Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.001, www.eoma.aoac.org, and *Francisella tularensis* 2016.007-Journal of AOAC, Volume 99, Number 4, July-August 2016, pp. 1090-1094. N/A: This subspecies is not further subdivided into types.		

Francisella tularensis Exclusivity - Tier 1

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A5.1.4 *Francisella tularensis*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 1 *Francisella tularensis* Exclusivity Panel		
Species	**Strain**	**Notes**
Francisella hispaniensis	DSM 22475	
Francisella novicida	D9876, GA993548	
Francisella novicida	F6168, GA993549	
Francisella novicida	U112, GA993550	
Francisella philomiragia	D7533, GA012794	
Francisella philomiragia	E9923, GA012801	
Francisella philomiragia	Jensen O#319-029 ATCC 25016	
Francisella philomiragia	Jensen O#319-036 ATCC 25017	
Francisella philomiragia	Jensen O#319-067 ATCC 25018	
Francisella philomiragia	Jensen O#319L ATCC 25015	
Francisella opportunistica or *Francisella persica*	MA067296 ATCC VR-331	
Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.001, www.eoma.aoac.org, and *Francisella tularensis* 2016.007-Journal of AOAC, Volume 99, Number 4, July-August 2016, pp. 1090-1094.		

Francisella tularensis Exclusivity - Tier 2/3

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A5.2.4 *Francisella tularensis*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 2 and Tier 3 *Francisella tularensis* Exclusivity Panel

Species	Strain	Notes
Francisella hispaniensis	DSM 22475	
Francisella novicida		*More if we can find them
Francisella novicida	D9876, GA993548	
Francisella novicida	F6168, GA993549	
Francisella novicida	U112, GA993550	
Francisella philomiragia	D7533, GA012794	
Francisella philomiragia	Jensen O#319-067 ATCC 25018	
Francisella philomiragia	Jensen O#319L ATCC 25015	

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.001, www.eoma.aoac.org, and *Francisella tularensis* 2016.007-Journal of AOAC, Volume 99, Number 4, July-August 2016, pp. 1090-1094.

N/A: This subspecies is not further subdivided into types.

Yersinia pestis Inclusivity Panel – Tier 1

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A4.1.5 *Yersinia pestis*. If any listed strain is not available, an alternate strain shall be selected from the same biovar. If an alternate strain from the same biovar is not available, a strain different than those listed below shall be selected if available;

Tier 1 *Yersinia pestis* Inclusivity Panel

Species	Strain	Notes
Yersinia pestis	A1122	Biovar Orientalis
Yersinia pestis	Angola	Biovar Antiqua
Yersinia pestis	Antiqua	Biovar Antiqua
Yersinia pestis	CO92	Biovar Orientalis
Yersinia pestis	Harbin35	Biovar Mediaevalis
Yersinia pestis	Java9	Biovar Orientalis
Yersinia pestis	KIM	Biovar Mediaevalis
Yersinia pestis	Nairobi	Biovar Antiqua
Yersinia pestis	Nicholisk 41	Biovar Mediaevalis
Yersinia pestis	PBM19	Biovar Orientalis
Yersinia pestis	Pestoides B	Biovar Mediaevalis
Yersinia pestis	Pestoides F	Biovar Antiqua
Yersinia pestis	Pestoides G	Biovar Antiqua

Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.002, www.eoma.aoac.org, and *Yersinia pestis* 2016.008-Journal of AOAC InternationalVolume 99, Number 4, July-August 2016, pp. 1095-1100.

Yersinia pestis Inclusivity Panel – Tier 2

Pacific Northwest NATIONAL LABORATORY
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A4.2.5 *Yersinia pestis*. If any listed strain is not available, an alternate strain shall be selected from the same biovar. If an alternate strain from the same biovar is not available, a strain different than those listed below shall be selected if available;

Tier 2 *Yersinia pestis* Inclusivity Panel		
Note: This panel is still under development and strain selection has not been finalized. The following needs to be addressed: **1) Can we reduce this panel to fewer strains and still have representative coverage of Yersinia pestsis?** **2) If so, which do we keep and which do we remove?**		
Species	**Strain**	**Notes**
Yersinia pestis	Angola A	Biovar Antiqua
Yersinia pestis	Antiqua	Biovar Antiqua
Yersinia pestis	CO92	Biovar Orientalis
Yersinia pestis	Harbin35	Biovar Mediaevalis
Yersinia pestis	KIM	Biovar Mediaevalis
Yersinia pestis	Nairobi	Biovar Antiqua
Yersinia pestis	Nicholisk 41	Biovar Mediaevalis
Yersinia pestis	Pestoides B	Biovar Mediaevalis
Yersinia pestis	Pestoides F	Biovar Antiqua
Yersinia pestis	Pestoides G	Biovar Antiqua
Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.002, www.eoma.aoac.org, and *Yersinia pestis* 2016.008-Journal of AOAC InternationalVolume 99, Number 4, July-August 2016, pp. 1095-1100.		

Yersinia pestis Exclusivity - Tier 1

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5.1.5 *Yersinia pestis*. If any listed strains are not available, an alternate strain shall be selected from the same species if available. If alternate strains from the same species are not available, a strain different than those listed below shall be selected, if available.

Tier 1 *Yersinia pestis* Exclusivity Panel		
Species	**Strain**	**Notes**
Yersinia enterocolitica	2516-87	Serotype O:9
Yersinia enterocolitica	8081	Serotype O:8,
Yersinia enterocolitica	WA	Serotype O:8
Yersinia pseudotuberculosis	1	Serotype 1a
Yersinia pseudotuberculosis	EP2/+	Serotype 1
Yersinia pseudotuberculosis	IB	Serotype 1b
Yersinia pseudotuberculosis	IP32953	Serotype 1
Yersinia pseudotuberculosis	MD67	Serotype 1
Yersinia pseudotuberculosis	Pa3606	Serotype 1b
Yersinia pseudotuberculosis	PB1/+	Serotype 1
Yersinia pseudotuberculosis	YPIII	Serotype 3
Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.002, www.eoma.aoac.org, and *Yersinia pestis* 2016.008-Journal of AOAC InternationalVolume 99, Number 4, July-August 2016, pp. 1095-1100.		

Yersinia pestis Exclusivity - Tier 2/3

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A5.2.5 *Yersinia pestis*. If any listed strains are not available, an alternate strain shall be selected from the same serotype if available. If alternate strains from the same serotype are not available, a strain different than those listed below shall be selected, if available.

Tier 2 and Tier 3 *Yersinia pestis* Exclusivity Panel		
Species	**Strain**	**Notes**
Yersinia enterocolitica	2516-87	Serotype O:9
Yersinia enterocolitica	WA	Serotype O:8
Yersinia pseudotuberculosis	1	Serotype 1a
Yersinia pseudotuberculosis	IB	Serotype 1b
Yersinia pseudotuberculosis	IP32953	Serotype 1
Yersinia pseudotuberculosis	Pa3606	Serotype 1b
Yersinia pseudotuberculosis	PB1/+	Serotype 1,
Yersinia pseudotuberculosis	YPIII	Serotype 3
Adapted from Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, AOAC SMPR 2010.002, www.eoma.aoac.org, and *Yersinia pestis* 2016.008-Journal of AOAC InternationalVolume 99, Number 4, July-August 2016, pp. 1095-1100.		

Questions/comments?

Pacific Northwest NATIONAL LABORATORY
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Thank you!

Acknowledgments

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  - Steve Stein

Considerations for Implementing Sequencing for BioWatch Laboratory Operations

Dr. Vikram V. Munikoti
LT, U.S. Public Health Service
Deputy Director of BioWatch Laboratory Operations
Office of Health Affairs
Department of Homeland Security
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March 15, 2017

Homeland Security

BioWatch is a Critical Layer of Our Nation's BioDefense

The BioWatch Story

- The BioWatch Program's critical mission is to operate a nationwide, aerosol detection system providing **early warning** across all levels of government to support public health and emergency management communities **to prepare for and respond to a biological incident**
- The program was started in **2003**
- The program's success is due to an **extraordinary network** composed of federal, state and local stakeholders

BioWatch Overview

- Operates a network of aerosol collectors at more than **600 sites** (including **50+** indoor sites) working 24x7x365
- Analyzes more than **230,000 samples** per year across more than 30 major metropolitan areas that have deemed to be at the greatest risk for a bioterrorism event
- Creates a forum for **local, state and federal** stakeholders to **share all types of relevant data** and information during a biological event
- Provides a critical layer of biodefense to National Special Security Events and Special Events (Supported **114** in 2015-2016)
- Conducts an extensive Quality Assurance Program, enhancing defensibility and confidence in BioWatch results. **> 35,800 QA samples have been analyzed** since **2011**

Provides early warning to the majority of the US population in 30+ jurisdictions from a bioterrorism attack

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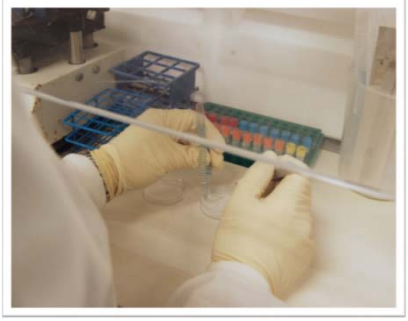
Technology Refresh: National Academy of Sciences Discussion

- "Detection Technology for the BioWatch Program: Experts Discussion" — August 22, 2016; The National Academy of Sciences
- Lessons Learned from Multiplexed Assay: Panel Discussion
  - General approach for the multiplex project was sound
  - Recommended earlier engagement with laboratories
  - Recommended inclusion of operational suitability criteria earlier in the process
  - The approach may need to be tailored to reflect differences across technologies
- Technology on the Horizon: Panel Discussion
  - High level perspectives from a diverse set of experts
  - Consensus that amplicon sequencing most likely to benefit the BioWatch Program within five years

Homeland Security

3

Path Forward: Initial Assessment of Sequencing



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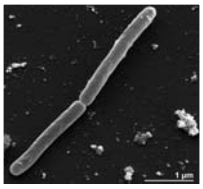
Technical Assessment: Microbial Diversity

- Environmental background
  - Biodiversity across jurisdictions has not been defined
  - BioWatch target agents and near-neighbors may occur naturally in the environment
  - Assessment requires a focused approach
- BioWatch Agents
  - Example: *F. tularensis* subspeciation
  - Potential weaponization
  - Signature evolution and erosion



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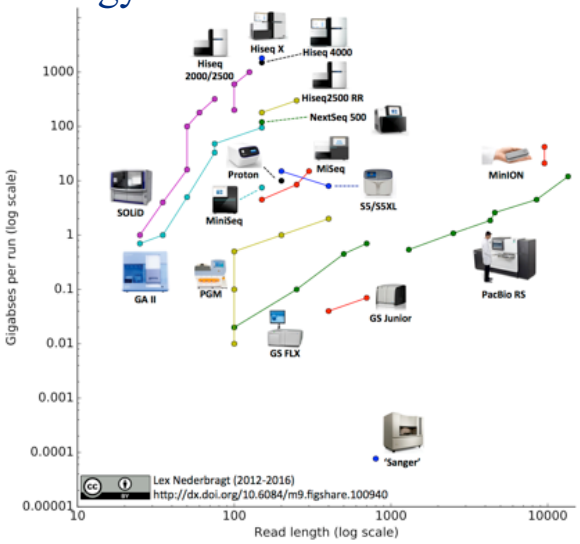
Technical Assessment: Rapidly Changing Technology



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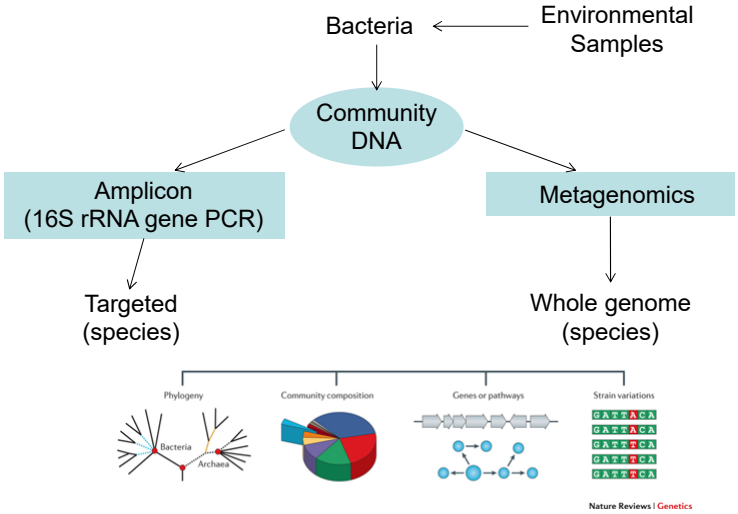
Technical Assessment: Metagenomics Approaches



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Technical Assessment: Sequencing Comparison

	Targeted Methods (amplicon or enrichment)	Shotgun Metagenomics
Cost	• Cheaper	• Expensive
Bioinformatics	• Signature selection and PCR design • Analysis	• Database construction • Database screening
Bias	• All methods biased in some way • Based on target selected • Need to minimize PCR or hybridization bias	• Minimally biased
Focus	• Operational Suitability	• DHS S&T (research)

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Technical Assessment: Operational Suitability

- Time-consuming multi-step process
  - May take hours to days
  - Time dependent on the sample, protocols, selected platform and analysis complexity
- Protocol Complexity
  - Multiple step process (e.g., DNA/RNA extraction, nucleic acids fragmented and end-repaired, sequencing, analysis)
- Operational suitability for any approach will be assessed with BioWatch laboratories and other SMEs before being considered for operational use

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Technical Assessment: Laboratory Suitability

- **How would we optimize protocols for BioWatch**?
  - What type of sample prep is needed?
  - How can time requirements be minimized?
    - Example: How much time does it take per run?
  - How much sample is needed?
  - What is the depth and quality?
  - What are the sequencing biases and errors?
- **Can we trust the analytical results**?
  - When do I call something present?
    - One read? Many? Stacked reads or broad coverage?
    - Contamination and host cross-interaction?
    - Determine abundance from read counts?
  - How do I evaluate the probability that the result is accurate?

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Technical Assessment: Data Interpretation

- **What are the data interpretation challenges and how will BioWatch address these challenges?**
  - Want to detect both knowns and unknowns
    - What "distance" from knowns?
    - Desire to characterize functional potential
  - Incomplete databases with biased and incorrect entries
  - Biological diversity challenges

False Negatives	False Positives
Horizontal gene transfer (misclassification)	Horizontal gene transfer (misclassification)
Evolution and diversifying selection	Similar species
	Different species (and host material) with some shared genomic sequences

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Technical Assessment: The Path Forward

Overall Study Goals

- Bioinformatics for complex samples to best characterize metagenomic 'background'
- Examining potential "dual use" with additional clinical applicability
- Exploring potential bio-informatics capability to enable straightforward analysis in an operational laboratory

Naval Medical Research Center (NMRC): Metagenomics assessment of jurisdictional background

- Conducting in-depth analysis of one jurisdiction

Centers for Disease Control and Prevention (CDC): Conducting sequencing of jurisdictional filters

Los Alamos National Laboratory (LANL)

- Initial Assessment: What would be needed to incorporate sequencing into the BioWatch Program?
- Studies
  - Characterize the environmental background found in BioWatch jurisdictions
  - Develop/confirm laboratory protocols and processes needed to support sequencing
  - Inform the Program about platform options (pros and cons)

Sequencing webinar: Spring 2017

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Technical Assessment: Sequencing Working Group

- Discussion and analysis based on August 2016 National Academy of Sciences discussion regarding the potential for use of amplicon sequencing
- Focused on technical assessment of metagenomic and amplicon sequencing
  - Document and/or leverage existing sequencing activities across multiple agencies and laboratories
  - Assess strengths and weaknesses of approaches
- Membership — Sequencing Experts
  - Federal agencies
  - BioWatch laboratories
  - Other laboratory experts
- Meetings or teleconferences on a monthly or bi-weekly basis

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Informing the Sequencing Working Group

Development of Program Requirements

- "Value to stakeholder" sequencing discussion with local laboratory directors/designees
- Identify level of characterization required to inform public health decisions
- Develop stakeholder questionnaire
  - Effectiveness criteria
  - Suitability criteria
- Feedback generated will inform the Program requirement development and discussions of the Sequencing Working Group

Evaluate targeted amplicon sequencing as well as metagenomics

Identify opportunities to leverage clinical and non-clinical applications; areas of overlap as well as separation

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Which Model Should BioWatch Adopt?

FDA ARGOS

Curated database of microbial reference-grade genomes for regulatory use

- Common clinical agents and near neighbors
- Supporting the development of medical countermeasures
- US-initiated

Livermore Metagenomic Analysis Toolkit (LMAT)

Comprehensive searchable database for strain level typing

- 116 billion bases of genomic data; ~5X more bacterial and viral genomic data than other databases
- Microbial background characterization

FDA: Food and Drug Administration
ARGOS: dAtabase for Regulatory Grade micrObial Sequences

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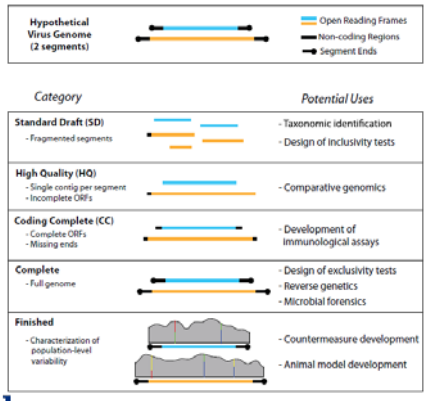
Quality Standards for Sequencing

TABLE 1 Overview of viral genome standards

Feature	Standard draft[a]	High quality[a]	Coding complete[a]	Complete	Finished
No. of contigs	>1 for some segments	1 per segment	1 per segment	1 per segment	1 per segment
Open reading frames	Incomplete	Incomplete	Complete	Complete	Complete
Estimated % of genome covered[b]	≥50%	~80-90%	~90-99%	100%	100%
Population-level characterization	Optional	Optional	Optional	Optional	Required
Contaminant analysis	Optional	Optional	Optional	Optional	Optional

[a] It is suggested that all bases included in any incomplete genome meet a minimum quality standard, with ≥5 reads supporting the consensus base call with individual base qualities of ≥20 on the Phred scale.
[b] Percentages of genome covered are not meant to serve as criteria for categorizing a genome; they are simply estimates of expected levels of coverage.



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Ladner et al. Mbio. 2014

Discussion

- How do the different agencies currently validate their sequencing methods?
- Can we validate amplicon sequencing as well as metagenomic sequencing assays using the same set of standards? Or should there be different standards for each?
- Would the model proposed by Ladner et al. work for organisms more complex than viruses?
  - Is it just a matter of scale?
  - Are there other considerations?
- Development of standards for testing:
  - Should they be developed internally?
  - Is there a benefit to developing an overarching set of standards across organizations and sequencing efforts? If so, what are the challenges? What criteria/variables must be considered?

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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.

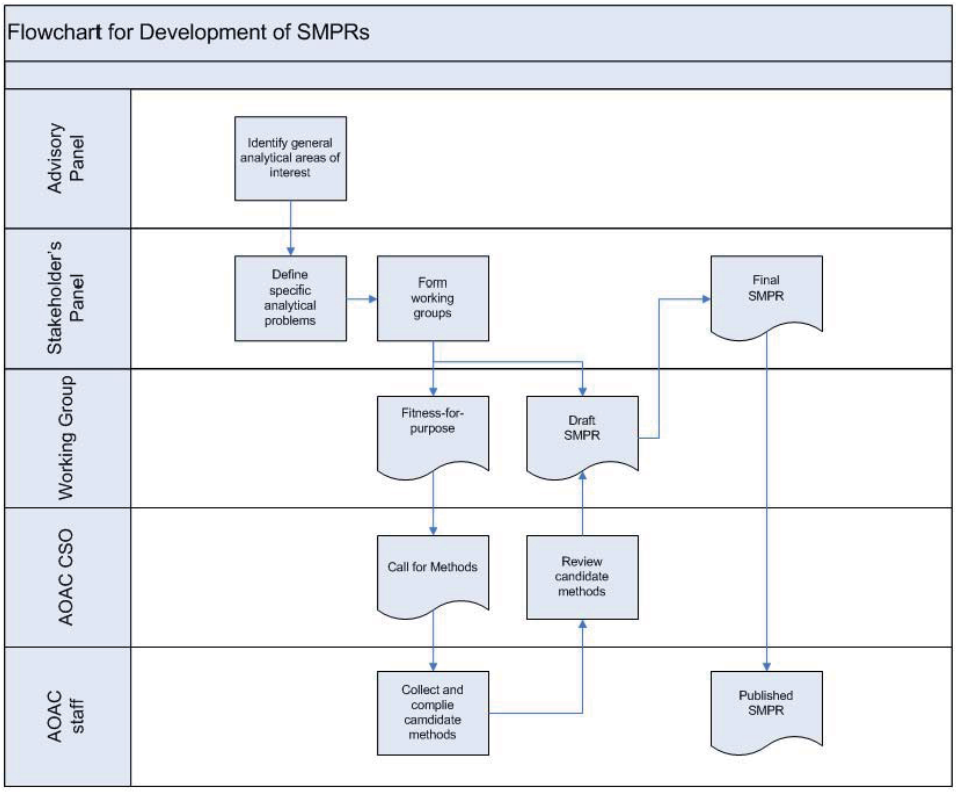


Figure 1. Schematic flowchart diagram of the SMPR development process.

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.

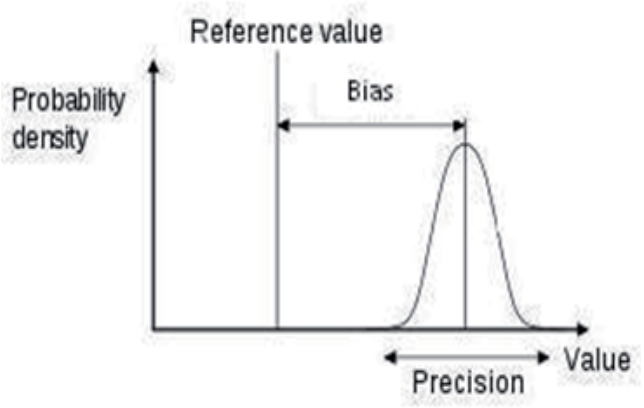


Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

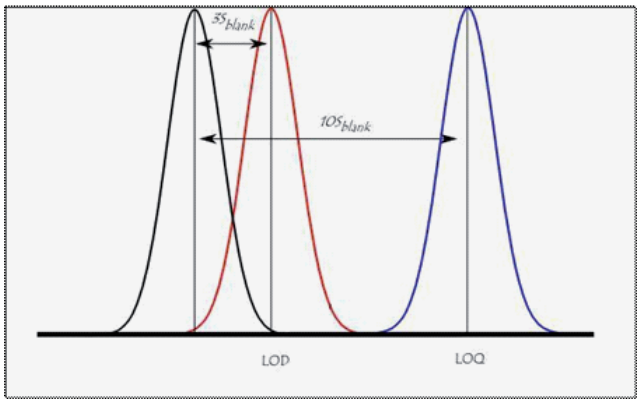


Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

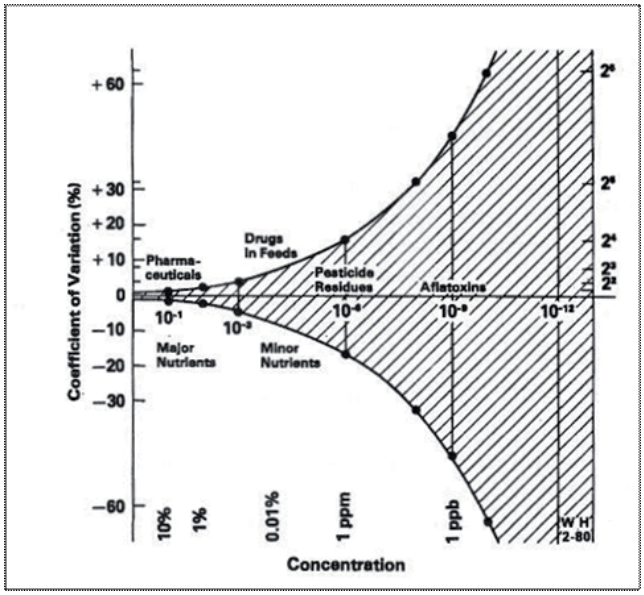


Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

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.

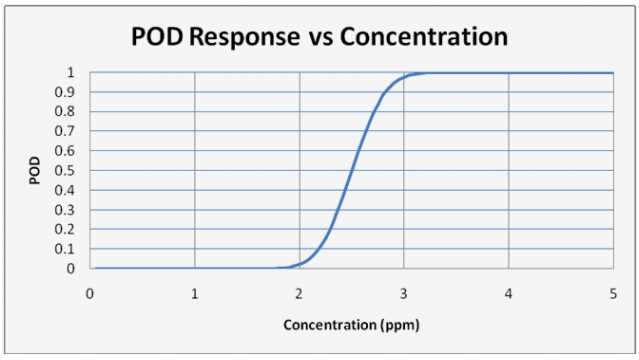


Figure C1. Theoretical POD curve for a qualitative detection method.

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

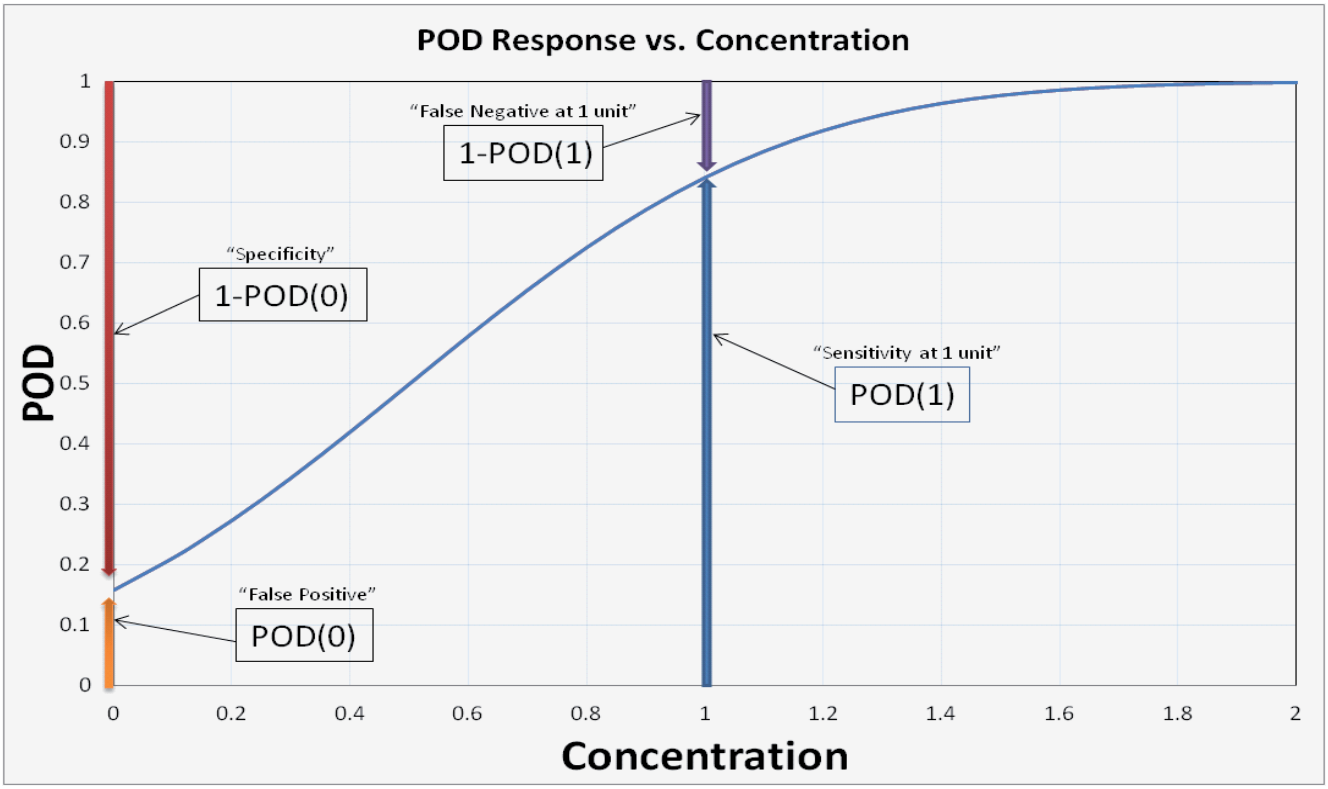


Figure C2. Comparison of POD model terminology to other obsolete terms.

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.

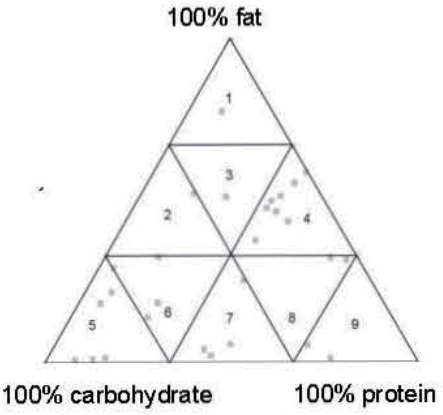
Why Use an In-House Reference Material?

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.

Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis

Expert Review Panels, Official Methods Board, First and Final Action *Official Methods*℠

In early 2011, an AOAC Presidential Task Force recommended that AOAC use Expert review panels (ERPs) to assess candidate methods against standard method performance requirements (SMPRs) to ensure that adopted First Action Official Methods℠ are fit for purpose.

Formation of an ERP

AOAC ERPs are authorized to adopt candidate methods as First Action *Official Methods* and to recommend adoption of these methods to Final Action *Official Methods* status. Scientists are recruited to serve on ERPs by a variety of ways. Normally, a call for experts is published at the same time as a call for methods is posted. Interested scientists are invited to submit their *curriculum vitae* (CV) for consideration. Advisory panel, stakeholder panel, and working group members may make recommendations to AOAC for ERP members. All CVs are reviewed and evaluated for expertise by the AOAC Chief Scientific Officer (CSO). The CVs and CSO evaluations are forwarded to the OMB for formal review. Both the CSO and OMB strive to ensure that the composition of a proposed ERP is both qualified and represent the various stakeholder groups. The recommended ERP members are submitted to the AOAC president who then appoints the ERP members.

Review of Methods

Methods submitted to AOAC in response to a call for methods are collected and compiled by AOAC staff. The AOAC CSO and working group chair perform a preliminary review of the methods and classify them into three categories: (*1*) fully developed and written methods that appear to meet SMPRs; (*2*) fully developed and written methods that may or may not meet SMPRs; and (*3*) incomplete methods with no performance data. Method submitters are apprised of the evaluation of their methods. Method developers with submissions that are classified as Category 2 or 3 are encouraged to provide additional information if available. A list of all the submitted methods and their classifications are posted for public review.

Usually, two ERP members (sometimes more) are assigned to lead the review of each Category 1 method. An ERP meeting is convened to review the methods. ERP meetings are open to all interested parties, and are usually well-attended events with about 50–60 attendees common. Each Category 1 method is reviewed and discussed by the ERP. If stakeholders have designated the method to be a dispute resolution method (as stated in the SMPR), then the ERP is asked to identify the single best candidate method to be adopted as a First Action *Official Method*. If the SMPR does not specify the need for a dispute resolution method, then the ERP may choose to adopt all methods that meet the SMPRs, or may choose to adopt the single best method in their collective, expert opinion.

In addition, an ERP may choose to require changes to a candidate method as part of its First Action adoption and/or identify issues that are required to be resolved prior to adoption as a Final Action *Official Method.*

Methods adopted by an ERP as First Action *Official Methods* may not be in AOAC *Official Methods* format. Method developers/authors are asked to assist AOAC to rewrite the method and accompanying manuscript into an AOAC-acceptable format.

Two-Year First Action Evaluation Period

Under the new pathway, a method may be designated as a First Action *Official Method* based on the collective judgment of an ERP. *Official Methods* remain as First Action for a period of about 2 years. During the First Action period, the method will be used in laboratories, and method users will be asked to provide feedback on the performance of the method.

As previously described, two (or more) ERP members are assigned to lead the review of candidate methods for adoption as First Action *Official Methods*. After a method has been adopted as First Action, these lead reviewers are expected to keep track of the use of and experience with the First Action *Official Method*. At the conclusion of the 2-year evaluation period, one or both of the lead reviewers will report back to the ERP on the experience of the First Action *Official Method.*

The presiding ERP will monitor the performance of the method, and, at the completion of the 2-year First Action evaluation period, determine whether the method should be recommended to the OMB for adoption as an AOAC Final Action *Official Method*.

It is also possible that First Action *Official Methods* are not recommended for Final Action. There are two possibilities for an ERP to decide not to proceed with a First Action method: (*1*) feedback from method users indicates that a First Action method is not performing as well in the field as was expected; or (*2*) another method with better performance characteristics has been developed and reviewed. In either case, the ERP may choose to repeal the First Action status of a method.

OMB Review

The OMB will review all methods recommended for Final Action or repeal by the ERP, and will consider a number of factors in their decision. A guidance document for factors to consider is provided on the AOAC website at http://www.aoac.org/vmeth/OMB_ERP_Guidance.pdf. Some of the factors identified by the guidance document for OMB consideration are (*1*) feedback from method users, (*2*) comparison to the appropriate SMPR, (*3*) results from single-laboratory validation, (*4*) reproducibility/uncertainty and probability of detection, (*5*) availability of reference materials, and (*6*) safety concerns.

Conclusion

The new pathway to *Official Methods*℠ is deliberately designed to avoid creation of elaborate review systems. The intent of the model is for method experts to use their scientific knowledge, experience, and good judgment to identify and adopt the best methods possible for the analytical need.

These methods are then published as First Action *Official Methods*, and used by analysts while additional information about the method is collected.

Method reviewers may consider other forms of information in lieu of the traditional collaborative study to demonstrate method reproducibility.

Additional Information

Coates, S. (2012) "Alternative Pathway," *Inside Laboratory Management* **16**(3), pp 10–12

Expert Review Panels, Policies and Procedures, AOAC INTERNATIONAL, http://www.aoac.org/News/EXPERT%20REVIEW%20PANELS%20final%20revision.pdf

Standard Format and Guidance for AOAC Standard Method Performance Requirement (SMPR) Documents, AOAC INTERNATIONAL, http://www.aoac.org/ISPAM/pdf/3.5%20SMPR%20Guideline%20v12.1.pdf

Guidance Documents

Requirements for First Action Official Methods℠ Status

See Figure 1 for process flowchart.

Expert Review Panels

(*1*) Supported by relevant stakeholders.

(*2*) Constituted solely for the ERP purpose, not for SMPR purposes or as an extension of an SMPR.

(*3*) Consist of a minimum of seven members representing a balance of key stakeholders.

(*4*) ERP constituency must be approved by the OMB.

(*5*) Hold transparent public meetings only.

(*6*) Remain in force as long as method in First Action status.

First Action Official Method℠ Status Decision

(*1*) Must be made by an ERP constituted or reinstated post March 28, 2011 for First Action *Official Method℠* status approval.

(*2*) Must be made by an ERP vetted for First Action *Official Method℠* status purposes by OMB post March 28, 2011.

(*3*) Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders.

(*4*) Method must be adopted by unanimous decision of ERP on first ballot. If not unanimous, negative votes must delineate scientific reasons.

(*5*) Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.

(*6*) Method becomes Official First Action on date when ERP decision is made.

(*7*) Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.

(*8*) Report of First Action *Official Method℠* status decision complete with ERP report regarding decision, including scientific background (references, etc.), to be published concurrently with method in traditional AOAC publication venues.

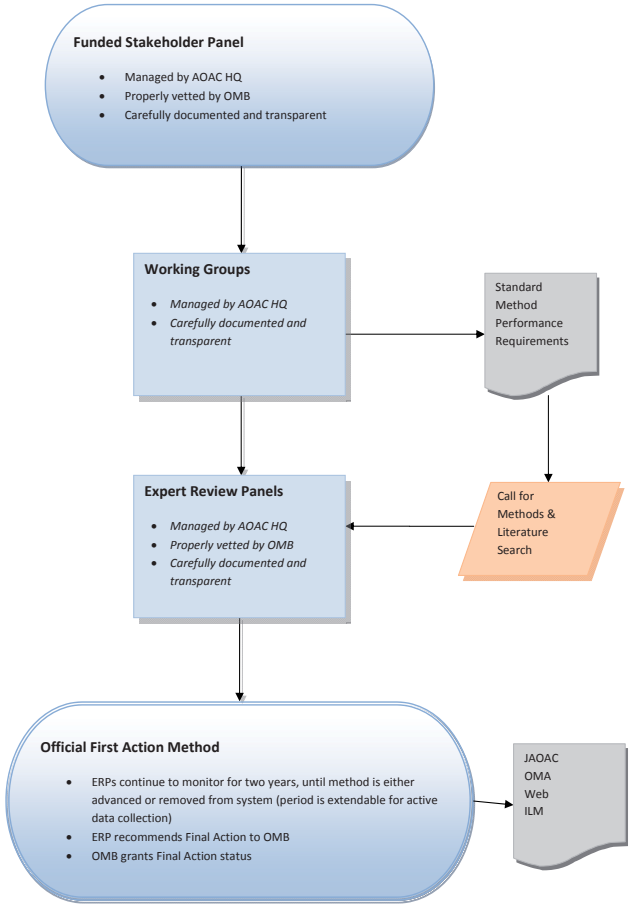


Figure 1. Summary of standards development through *Official Methods of Analysis*.

Method in First Action Status and Transitioning to Final Action Status

(*1*) Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

(*2*) Two years maximum transition time [additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress].

(*3*) Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

(*4*) Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

(*5*) ERP to recommend method to Final Action Official status to the OMB.

(*6*) OMB decision on First to Final Action status.

These guidance documents were approved by the AOAC Board of Directors on May 25, 2011.

First Action to Final Action Methods: Guidance for AOAC Expert Review Panels

In December 2011, the Official Methods Board (OMB) approved a guidance document for ERPs to support their work as they deliberate on methods, adopt methods as Official First Action, and, subsequently, track method usage and performance between First Action status and Final Action consideration. The guideline is based on parameters of a method that the OMB will consider when deliberating on methods recommended for Final Action status. ERPs are to use this guideline in their deliberations.

ERPs working within the AOAC process may recommend a First Action status method be elevated to Final Action status. Such a recommendation leverages the ERP's high level of expertise supported by data from the initial evaluation, and results from the subsequent 2-year method performance evaluation period.

The OMB receives the recommendation with supporting documentation, and determines if Final Action status is warranted. OMB's review verifies the method process was conducted in compliance with the guidelines and protocols of the Association.

For transparency and to expedite the review process, the main areas OMB will review when evaluating ERP recommendations to promote methods to Final Action are listed below. Documentation of the areas listed below will also increase confidence in method performance and assist users to properly and safely perform the methods at their locations.

A. Method Applicability

(**a**) A method's applicability to the identified stakeholder needs is best assessed by the stakeholder panel and should be a part of the process from the onset. OMB liaisons will remind stakeholder panels to maintain this focus point.

(**b**) OMB may ask ERPs and stakeholder panels for feedback to improve the applicability of the method, such as potential method scope expansions and potential points of concern.

B. Safety Concerns

(**a**) A safety review must be performed for a method to be recognized as First Action.

(**b**) All safety concerns identified during the 2-year evaluation period must be addressed.

(**c**) Guidance and support can be obtained from the AOAC Safety Committee.

C. Reference Materials

(**a**) Document efforts undertaken to locate reference materials. Methods may still progress to Final Action even if reference materials are not available.

(**b**) Guidance and support can be obtained from the AOAC Technical Division on Reference Materials.

D. Single-Laboratory Validation

(**a**) Data demonstrating response linearity, accuracy, repeatability, LOD/LOQ, and matrix scope must be present. Experimental designs to collect this data may vary with the method protocol and the intended use of the method.

(**b**) Resources can be identified by the AOAC Statistics Committee.

E. Reproducibility/Uncertainty and Probability of Detection

(**a**) For quantitative methods, data demonstrating reproducibility and uncertainty must be present. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method (i.e., collaborative studies, proficiency testing, etc.).

(**b**) For qualitative methods, data must be present demonstrating the probability of detection at specified concentration levels as defined by the SMPR. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method.

(**c**) Guidance and support can be obtained from the AOAC Statistics Committee.

F. Comparison to SMPR

(**a**) Document method performance versus SMPR criteria. Note which SMPR criteria are met. For SMPR criteria not met, the ERP documents the reasoning why the method is still acceptable.

(**b**) Data is present to assure the matrix and analyte scopes are covered. This is critical for methods used for dispute resolutions.

G. Feedback from Users of Method

(**a**) Document positive and negative feedback from users of the method during the trial period.

(**b**) Feedback from users demonstrating method ruggedness should be documented.

(**c**) Assess the future availability of vital equipment, reference materials, and supplies.

H. ERP Recommendations to Repeal First Action Methods

Recommendations to repeal First Action methods shall be accompanied with detailed reasons for the decision.

The First to Final Action guidance for ERPs was approved by the OMB in December 2011 and effective as of February 1, 2012.

APRIL 11, 2017
STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

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

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