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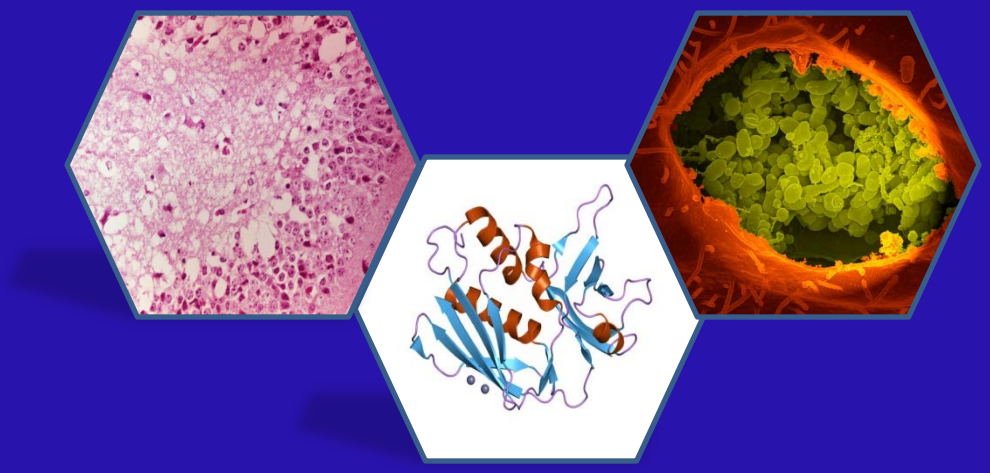
Stakeholder Panel on Agent Detection Assays: Working Groups Meeting



AOAC INTERNATIONAL Headquarters
Suite 300
2275 Research Boulevard
Rockville, Maryland, 20850

April 23-24, 2015

contact: spada@aoac.org

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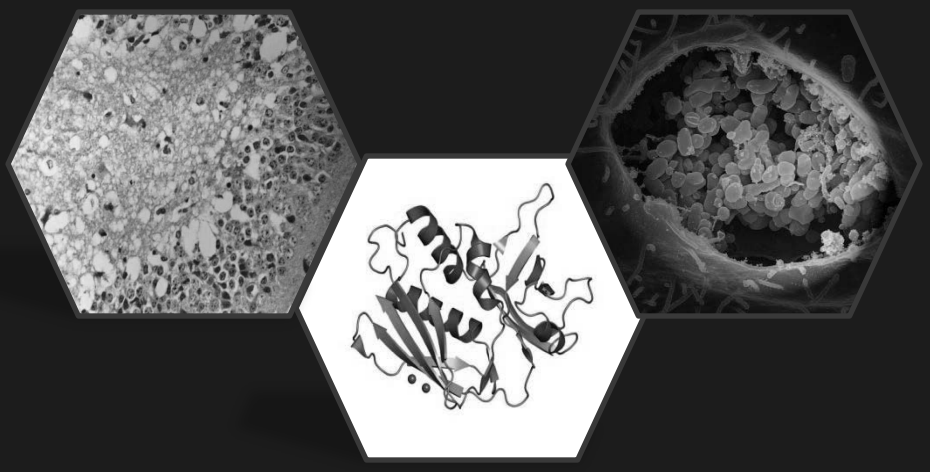
The Scientific Association Dedicated to Analytical Excellence®

Stakeholder Panel on Agent Detection Assays: Working Groups Meeting



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STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

April 23 - 24, 2015

AOAC INTERNATIONAL Headquarters, Suite 300
2275 Research Blvd., Rockville, Maryland, 20850

Working Group Sessions – Thursday, February 23, 2014

I. Staphylococcus enterotoxin b (8:30 a.m. – 12:30 p.m.)
Chair: Sandra Tallent, FDA
a. Review of SMPRs and AOAC Process
b. SMPR Development Session
c. Next Steps

II. SPADA sub-group to develop recommendations for determining the authenticity of strains and species for testing purposes (1:00 p.m. – 1:15 p.m.)

III. Q-Fever (1:15 p.m. – 5:15 p.m.)
Co-Chairs: Linda Beck, Naval Surface Warfare Center and *James Samuel, Texas A&M*
a. Review of SMPRs and AOAC Process
b. SMPR Development Session
c. Next Steps

Working Group Sessions – Friday, February 24, 2014

IV. Venezuelan Equine Encephalitis (8:30 a.m. – 12:30 p.m.)
Chair: Eileen Ostlund, USDA
a. Review of SMPRs and AOAC Process
b. SMPR Development Session
c. Next Steps

SPADA SEB Working Group

Meeting Minutes
Wednesday, March 4, 2015; 2:00 p.m. – 3:00 p.m. EST

Attendees

Panel Members *(Present during all or part of the meeting)*:

Sandra Tallent, FDA (Chair)
Ryan Cahall, Censeo Insight
Martha Hale, USAMRIID
Malcolm Johns, DHS
Saleem Khan, University of Pittsburgh
Katalin Kiss, ATCC
Matthew Lesho, Luminex
Stephen Morse, CDC
Roberto Rebeil, ECBC
Reinhardt Witzenberger, R-Biopharm

AOAC Staff
(Present during all or part of the meeting):

Scott Coates
Christopher Dent
Krystyna McIver

Meeting Minutes

I. Welcome and Introductions

All were welcomed, roll call was taken and the meeting commenced at approximately 11:00 a.m. EST.

II. Review of Last Meeting and Fitness for Purpose

Dent advised that minutes from the in-person meeting are still in the approval process but will be released very soon. Coates provided a verbal summary of changes made to the draft SMPR at the last meeting.

III. Consideration of Method Performance Criteria

Tallent then led the group in further revising the SMPR document. Coates asked if it is detrimental to write a standard that allows for detection of other toxins but concenterates on SEA, B and C? All agreed that the other toxins do not create aerosol problems and therefore do not need to be included. The method name remained "Detection of SEA-C."

Definitions were then reviewed. The group discussed the definition for Maximum Time to Determination. The group agreed that time to result should start from recovery of the toxins. The definition was modified to read "Maximum time to complete an analysis starting with recovery of toxins from the collection matrix and ending with the assay result." A minor change was also made to the Selectivity Study to include "related toxins." All definitions were agreed and the group moved

on to "Validation Guidance," where the word "roughly" was removed and SEC was clarified include to SEC 1, SEC 2, and SEC 3. The group agreed that this assay needs to be specific to SEA-C and does not detect D and onwards. Witzenberger highlighted that SEE is one of the stronger cross reactions there is, although it is very rare.

IV. Adjourn

The meeting adjourned at 3:00 p.m. EST and the group agreed to meet again on March 25 at 2:00 p.m. EDT.

MARCH 4, 2015 SPADA SEB WG MEETING: ACTION ITEMS	
Action	**Owner**
Schedule follow up call or March 25.	AOAC
Incorporate today's changes into SMPR	AOAC / Tallent

AOAC Stakeholder Panel on Agent Detection Assays:
SEB Working Group Telecon

Meeting Minutes
Thursday, March 25, 2015; 2:00 p.m. – 3:00 p.m. EST

Attendees

Panel Members *(Present during all or part of the meeting)*:

Sandra Tallent, FDA (Chair)
Linda Beck, Naval Surface Warfare Center
Ryan Cahall, Censeo Insight
Martha Hale, USAMRIID
Malcolm Johns, DHS
Liz Kerrigan, ATCC
Katalin Kiss, ATCC
Mathew Lesho, Luminex
Stephen Morse, CDC
Roberto Rebeil, ECBC
Reinhardt Witzenberger, R-Biopharm

AOAC Staff
***(Present during all or part of the meeting)*:**

Scott Coates
Christopher Dent
Krystyna McIver

Meeting Minutes

I. Welcome and Introductions

- All were introduced and roll call was taken.

II. SMPR Development

- The group had a discussion on sample collection and filter spiking. Coates recommended adding a paragraph clarifying this to "Validation Guidance" once the call is complete.
- The group continued through the SMPR and made various changes, detailed in Attachment 1.

III. Adjourn

- AOAC Staff advised that this working group will not meet again until the in-person meeting scheduled for April 23 at AOAC Headquarters.

FEBRUARY 26, 2015 SPSFAM AP MEETING: ACTION ITEMS	
Action	**Owner**
Add paragraph about sample collection to "Validation Guidance" section of document.	CD

Attachments:

Attachment 1: Draft SEB SMPR v5.1

AOAC SMPR 2015.XXX; Version 5, March 4, 2015

Method Name: **Detection of ~~SE~~Staphylococcal enterotoxin A-C**

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

1. **Intended Use**: Laboratory or field use by trained operators.

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

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

4. **Definitions**:

Acceptable Minimum Detection Level (AMDL)
The predetermined minimum level of an analyte, as specified by an expert committee which must be detected by the candidate method at a specified probability of detection (POD). ~~For the purposes of this SMPR, SPADA established the AMDL as 0.25 ng/mL.~~

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

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

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

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

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

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

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

7. Method Performance Requirements

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

8. Maximum Time for Assay Results: Four hours

Approval Date:
Final version date:

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

Table I: Controls

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

74 **Table II: Nontarget Compounds (near-neighbors)**

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Compound	Commercial availability
SED	
SEE	
SEH	
SEG	
SEI	
SEJ	
SEK	
SEU	

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Table III: Powders and Chemicals

[From SMPR 2010.004; Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of Bacillus anthracis Spores in Visible Powders]

Bacillus thuringiensis powders (e.g., Dipel)
Powdered milk
Powdered infant formula (Fe fortified)
Powdered infant formula (low Fe formulation)
Powdered coffee creamer
Powdered sugar
Talcum powder
Wheat flour
Baking soda
Chalk dust
Brewer's yeast
Dry wall dust
Cornstarch
Baking powder
GABA (Gama aminobutyric acid)
L-Glutamic acid
Kaolin
Chitin
Chitosan
MgSO4
Boric acid
Powdered toothpaste
Popcorn salt

AOAC Stakeholder Panel on Agent Detection Assays
Q-Fever Working Group Teleconference

Meeting Minutes
Friday, March 6, 2015; 11:00 a.m. ET

Attendees

Panel Members *(Present during all or part of the meeting)*:

James Samuel, Texas A&M (Chair)
Christina Egan, NYSDOH
Jeff Ballin, ECBC
Linda Beck, Naval Surface Warface Center
Ryan Cahall, Censeo Insight
Joan Gebhardt, NMRC
Ted Hadfield, Hadeco., LLC
Katalin Kiss, ATCC
John Lednicky, University of Florida
Kris Roth, FDA

AOAC Staff
(Present during all or part of the meeting):

Scott Coates
Christopher Dent
Krystyna McIver

Meeting Minutes

I. Welcome and Introductions

All were introduced and roll call was taken at 11:00 a.m. ET. Samuel asked AOAC to ensure that Cato and Massung are available for the next conference call.

II. Review of February Meeting

Coates reviewed the Fitness for Purpose statement for this working group as well as the draft SMPR. Minor editorial changes to the SMPR were made in real time.

III. SMPR Development

Maximum time to result was discussed – is four hours appropriate? ACTION for AOAC to check earlier SMPRs for the maximum time to result.

With regards to inclusivity panels, Samuel stated that phylogenetic diversity is the basis for the isolates he would choose and he recommended six (6) different isolate groups. Kiss asked if the recent Netherlands strain be covered by the six and Samuel said that it would. Samuel identified the Inclusivity groups as follows:

- Group 1: Nine Mile (RSA493 and RSA439)
- Group 2: Henzerling
- Group 3: Idaho Goat
- Group 4: K

- Group 5: G
- Group 6: Dugway

ACTION for AOAC to reference Samuel's presentation regarding the six groups in this part of the SMPR.

Lednicky asked about authenticity of the strains. Samuel advised that SPADA is forming a subcommittee to make recommendations on that issue and emphasized that these are not clonal isolates.

IV. Adjourn

The teleconference was adjourned at 12:00 p.m. EST.

MARCH 6, 2015 SPADA WORKING GROUP MEETINGS: ACTION ITEMS	
Action	**Owner**
Ensure the next teleconference time works for Cato and Massung.	AOAC
AOAC to investigate previous SMPR maximum time to result.	AOAC
Reference Samuel's presentation regarding the six groups within the SMPR	AOAC
Add sentence that isolates listed for each of the six groups discussed are examples.	AOAC
Begin to consider what should be on the Exclusivity Panel of the *Coxiella burnetii* SMPR.	ALL

AOAC SMPR 2015.XXX; Version 3, March 6, 2015

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

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

1. **Intended Use**: Laboratory or field use by trained operators.

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

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

4. **Definitions**:

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

For this SMPR, SPADA has established the AMDL at: 2,000 copies/ml of *Coxiella burnetii* target DNA in the candidate method sample collection buffer. Copies/ml refers to number of *Coxiella burnetii* genomes or equivalent plasmid copies containing target gene or gene fragment.

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

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

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

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

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

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

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

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

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

Inclusivity and exclusivity panel members must be characterized and documented to truly be the species and strains they are purported to be.

7. **Method Performance Requirements**:

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

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

8. **Time-to-results**: Four hours.

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

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68 **TABLE I: Controls**

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

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71 **Table II: Inclusivity Panel**

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Phylogenetic Group[2]	Isolate[3]	Commercial availability
Group 1	Nine Mile RSA493 Nine Mile RSA439	
Group 2	Henzerling	
Group 3	Idaho Goat	
Group 4	K	
Group 5	G	
Group 6	Dugway	

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[2] SNP and VNTR based trees for 25 worldwide isolates of *Coxiella burnetii.* Pearson, Keim et al. SM2005

[3] Isolates listed for each of the six groups discussed are examples only.

Table III: Exclusivity Panel (near-neighbor)

Species	Strain	Commercial availability

Table IV: Environmental Factors Panel For Validating PCR Detectors For Biothreat Agents

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

The Environmental Factors Panel is intended to supplement the biothreat agent near- neighbor exclusivity testing panel, and it should be applicable to all PCR biothreat agent detection assays. The panel criteria are divided into two main groups – the matrix panel of unknown environmental samples (Part 1); and the environmental panel of identified environmental organisms (Part 2). This panel will test for potential cross-reactive amplification and/or PCR inhibitors.

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

- The aerosol environmental matrix pools should be used to confirm that there is no detection with the method used i.e. there is no cross reactivity of the target assay with unknown environmental organisms.
- The aerosol environmental matrix pools should also be tested with the target fragment at the AMDL to confirm the filter pool does not interfere with detection by the method used.

· Method developers should obtain environmental matrix samples that are representative and consistent with the collection method that is anticipated to be utilized in generating the sample being analyzed. 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 will test the environmental matrix for interference with sufficient samples to achieve 95% probability of detection.
- Cross-reactivity testing will include sufficient samples and replicates to ensure each environmental condition is adequately represented.

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

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

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

- **Other biothreat agents**

  Bacillus anthracis Ames
  Yersinia pestis Colorado-92
  Francisella tularensis subsp. *tularensis* Schu-S4
  Burkholderia pseudomallei
  Burkholderia mallei
  Brucella melitensis
  Ricinus communis – use ricin plant leaves as source of DNA
  Clostridium botulinum Type A

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

Delftia acidovorans
Escherichia coli K12
Fusobacterium nucleatum
Lactobacillus plantarum
Legionella pneumophila
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

- **DNA Viruses**

Adenovirus vaccine
Herpes simplex virus or *Cytomegalovirus* – whichever is available

- **Microbial eukaryotes**

Freshwater amoebae
Acanthamoeba castellanii
Naegleria fowleri

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

- **DNA from higher eukaryotes**

Plants
Zea mays (corn)
Pollen from *Pinus* spp. (pine)
Gossypium hirsutum (Cotton – use leaves from cotton plant as source of DNA)

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

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

- **Biological insecticides** – includes *Bacillus thuringiensis* subspecies that are widely used in agriculture. It is acknowledged that this organism is a near-neighbor of *B. anthracis* and has been included in the BA exclusivity panel. Furthermore, it is not closely related to *Y. pestis* and *F. tularensis*. However, strains of *B. thuringiensis* present in commercially available insecticides have been extensively used in hoaxes and are likely to be harvested in air collectors. For these reasons, it should be used to assess the specificity of these threat assays.

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

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

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

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

AOAC Stakeholder Panel on Agent Detection Assays:
Stakeholder Panel Meeting

Meeting Minutes
Thursday, March 10, 2015; 2:00 p.m. – 3:00 p.m. EST

Attendees

Panel Members *(Present during all or part of the meeting)*:

Eileen Ostlund, USDA (Chair)
Linda Beck, Naval Surface Warfare Center
Ryan Cahall, Censeo Insight
Joan Gebhardt, NMRC
Pejman Naraghi-Arani, LLNL
Ann Powers, CDC
Darci Smith, SRI

AOAC Staff
***(Present during all or part of the meeting)*:**

Scott Coates
Christopher Dent
Krystyna McIver

Meeting Minutes

I. Welcome and Introductions

All participants were welcomed and roll call was taken.

II. Review of Last Meeting and Fitness for Purpose Statement

Coates reviewed the Fitness for Purpose statement and explained that it had led him to draft SMPRs for VEE and EEV. EEV SMPR is for identification of EEV, WEE and VEE. The VEE-only SMPR is for confirmation methods. For identification methods Probability of Identification (POI) is used instead of Probability of Detection (POD). It would challenge the method with a number of viruses to determine when VEE is there and when it is not.

Coates then asked the group for ideas on the best approach for proceeding with SMPRs for VEE/EEV. Group members said that it would be a great deal of time and effort to take a combined approach. DoD representatives advised that they would be satisfied with one just for VE E – even that alone is complex. Ostlund agreed and stated that the group will focus on an SMPR for VEE only, with the potential to look at the others once VEE is complete.

The group then discussed development of SMPRs for identification, detection, or both. The primary need is to know which strain of the virus is present, so the initial focus will be on identification of VEE. Cahall advised that non pathogenic strains are of little interest.

III. SMPR Development

The group proceeded to modify a draft SMPR. Since the scope is limited to VEE, WEE and EEE were removed.

IV. Adjourn

The meeting adjourned at approximately 3:00 p.m. EST.

MARCH 27, 2015 SPADA VEE MEETING: ACTION ITEMS	
Action	**Owner**
Consider method performance criteria	All
Planning for Inclusivity / Exclusivity Panel discussion	All
Schedule VEE WG Meeting for April 7 at 2:00 p.m.	AOAC

AOAC Stakeholder Panel on Agent Detection Assays:
VEE Working Group Teleconference

Meeting Minutes
Thursday, April 7, 2015; 10:00 a.m. – 11:00 a.m. EST

Attendees

Panel Members *(Present during all or part of the meeting)*:

Eileen Ostlund, USDA (Chair)
Jeff Ballin, ECBC
Linda Beck, Naval Surface Warfare Center
Ryan Cahall, Censeo Insight
Joan Gebhardt, Naval Medical Research Center
Tom Phillips, MD Department of Agriculture
Ann Powers, CDC
Jon Rayner, SRI
Scott Weaver, U. Texas Medical Branch

AOAC Staff
***(Present during all or part of the meeting)*:**

Scott Coates
Christopher Dent
Krystyna McIver
Deborah McKenzie

Meeting Minutes

I. Welcome and Introductions

- Roll call was taken and all were introduced.

II. SMPR Development

- Ostlund explained that although the SMPR has been limited to Venezuelan Equine Encephalitis the next question is which VEEs? Ostlund distributed a spreadsheet[1] containing more information on the various types of VEE. The table was reviewed and there were no objections to including VEE-IAB, VEE-IC, and VEE-IE.
- The group then discussed the inclusion of Mucambo. Beck stated that Mucambo may be so unique that it would be a separate test altogether. Should Mucambo be included in this SMPR? Cahall said that he will take this question back to the DUSA-TE team, who is funding this project, to get clarification on if this is something DoD wants included; however the group came to consensus that for this SMPR Mucambo would not be included.
- Coates recommended the other strains on the table be included as part of the Exclusivity Panel, with the expectation that this SMPr will be specific to VEE-IAB, VEE-IC, and VEE-IE. The group agreed.
- The group also agreed on setting the maximum time to result at four hours.
- Returning to the table in Section 7, the group agreed to an AMIL of 50,000 genome copies per mL. The POI remained at 0.95.
- Environmental testing will be done from buffer.
- For exclusivity, the question remains whether or not to include environmental panel organisms. DNA will be pooled up to 10 at a time for environmental organisms.

[1] Attachment 1: VEE Spreadsheet

- Ostlund asked the group to take time between this and the April 24 in-person working group meeting to consider the environmental list and potentially add to it.
- Coates clarified that there is no requirement for the environmental list to remain consistent for each SPADA SMPR.
- Bioinformatic analysis was used for the Variola SMPR. Coates asked if this is something that could be used for VEE? The group agreed that it could make sense and was asked to consider this as well in preparation for the April 24 meeting.

III. Adjourn

- The meeting adjourned at approximately 11:00 a.m. EST.

FEBRUARY 26, 2015 SPSFAM AP MEETING: ACTION ITEMS	
Action	**Owner**
Seek clarification on need for SMPR for Macambp	Cahall
Reorganize SMPR with exclusivity and inclusivity.	Coates
Consider environmental panel as well as the potential to use bioinformatic analysis.	All

Attachments:

Attachment 1: VEE Spreadsheet
Attachment 2: VEE SMPR v3

AOAC SMPR 2015.XXX; Version 4, April 22, 2015

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

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

1. **Intended Use**: Laboratory or field use by trained operators.

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

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

4. **Definitions**:

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

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

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

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

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

Venezuelan Equine Encephalitis/Encephalomyelitis Virus

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

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

7. **Method Performance Requirements**:

Parameter	**Minimum Performance Requirement**
AMIL	**50,000 genome copies / mL**
POI at AMIL within sample collection buffer	≥ 0.95
POI at ~~AMDL~~ AMIL in an aerosol environmental matrix	≥ 0.95 (Table IV; part 1)
System False-Negative Rate using spiked aerosol environmental matrix	≤ 5% (Table IV; Part 1)
System False-Positive Rate using aerosol environmental matrix	≤ 5% (Table IV; Part 1)

Inclusivity panel purified DNA	All inclusivity strains (Table II) must be correctly identified at 2x the AMIL[†]
Exclusivity panel purified DNA	All exclusivity strains (Table III and Annex IV; part 2) must test negative at 10x the AMIL[†]
Notes: † 100% correct analyses are expected. All aberrations are to be re-tested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures[1]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.	

8. Maximum Time-to-Results: Four hours.

Approval Date:
Final version date:

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

52 **Table I: Controls**

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

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Inhibition Control	This control is designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect the target organism.	Single use per sample run

Table II: Inclusivity Panel

VIRUS	Serotype / Variant	Representative Strain (s)	Human Illness?	Notes
VEEV	VEE-IAB	Trinidad Donkey	Yes	Dnky in Trinidad
		MF-8	Yes	Hu in Honduras
	VEE-IC	ICVE93, ICVE95	Yes	Hu in Venezuela
	VEE-IE	IEMX63, IEPA62	Yes	Hu in Panama, Hu (& eq) inf in Mex (Adams PLOS 2012) Hu in Bolivia, Ecuador (Aguilar *Future Virol* 2011

Table III: Exclusivity Panel (near-neighbor)

VIRUS	Serotype / Variant	Representative Strain (s)	Human Illness?	Notes
VEEV	VEE-ID	1DPA61, 1DPE98, IDPE06	Yes	Hu in Panama, Peru
Mosso das Pedras	VEE-IF	78V 3531	None recognized	Mosq in Brazil
Everglades	VEE-II	Fe-3-7c	Yes	Hu in FL
Mucambo	VEE-IIIA	A	Yes	Monkey in Brazil, Hu in So Am, Trinidad
	VEE-IIIC	C (strain 71D-1252)	Unknown	Mosq in Peru
	VEE-IIID	D	Yes	Human virulent in Peru
Tonate	VEE-IIIB	Tonate	Yes	Bird in Fr. Guiana, Hu in Brazil (Bijou Bridge virus in Cliff swallows of Colorado)
Pixuna	VEE-IV	Pixuna	Yes	Hu in in Brazil
Cabassou	VEE-V	Cabassou	None recognized	Mosq in Fr. Guiana
Rio Negro	VEE-VI	AG 80-663	Yes	Hu in Argentina

Table IV: Environmental Factors Panel For Validating PCR Detectors For Biothreat Agents

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

The Environmental Factors Panel is intended to supplement the biothreat agent near- neighbor exclusivity testing panel, and it should be applicable to all PCR biothreat agent detection assays. The panel criteria are divided into two main groups – the matrix panel of unknown environmental samples (Part 1); and the environmental panel of identified environmental organisms (Part 2). This panel will test for potential cross-reactive amplification and/or PCR inhibitors.

Part 1:

Environmental matrix samples - Aerosol Environmental matrices –

- The aerosol environmental matrix pools should be used to confirm that there is no detection with the method used i.e. there is no cross reactivity of the target assay with unknown environmental organisms.
- The aerosol environmental matrix pools should also be tested with the target fragment at the AMDL to confirm the filter pool does not interfere with detection by the method used.

- Method developers should obtain environmental matrix samples that are representative and consistent with the collection method that is anticipated to be utilized in generating the sample being analyzed. 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 will test the environmental matrix for interference with sufficient samples to achieve 95% probability of detection.
  - Cross-reactivity testing will include sufficient samples and replicates to ensure each environmental condition is adequately represented.

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

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

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

- **Other biothreat agents**

  Bacillus anthracis Ames
  Yersinia pestis Colorado-92
  Francisella tularensis subsp. *tularensis* Schu-S4
  Burkholderia pseudomallei
  Burkholderia mallei
  Coxiella burnetii
  Brucella melitensis
  Ricinus communis – use ricin plant leaves as source of DNA
  Clostridium botulinum Type A

- **Cultivatable bacteria identified as being present in air and soil**
  Acinetobacter lwoffii
  Agrobacterium tumefaciens
  Bacillus amyloliquefaciens
  Bacillus cohnii
  Bacillus psychrosaccharolyticus
  Bacillus benzoevorans

Bacillus megaterium
Bacillus horikoshii
Bacillus macroides
Bacteroides fragilis
Burkholderia cepacia
Burkholderia gladoli
Burkholderia stabilis
Burkholderia plantarii
Chryseobacterium indologenes
Clostridium sardiniense
Clostridium perfringens
Deinococcus radiodurans
Delftia acidovorans
Escherichia coli K12
Fusobacterium nucleatum
Lactobacillus plantarum
Legionella pneumophila
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

- **DNA Viruses**

  Adenovirus vaccine
  Herpes simplex virus or *Cytomegalovirus* – whichever is available

- **Microbial eukaryotes**

  Freshwater amoebae
  Acanthamoeba castellanii
  Naegleria fowleri

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

- **DNA from higher eukaryotes**

  Plants
  Zea mays (corn)
  Pollen from *Pinus* spp. (pine)
  Gossypium hirsutum (Cotton – use leaves from cotton plant as source of DNA)

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

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

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

- **Biological insecticides** – includes *Bacillus thuringiensis* subspecies that are widely used in agriculture. It is acknowledged that this organism is a near-neighbor of *B*. *anthracis* and has been included in the BA exclusivity panel. Furthermore, it is not closely related to *Y. pestis* and *F. tularensis*. However, strains of *B. thuringiensis* present in commercially available insecticides have been extensively used in hoaxes and are likely to be harvested in air collectors. For these reasons, it should be used to assess the specificity of these threat assays.

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

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

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

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

Annex 1: Bioinformatics Analyses of Signature Sequences Underlying Venezuelan Equine Encephalitis Virus Assays

In silico screening will be performed on signature sequences (e.g., oligo primers/probes) to predict specificity to *Variola virus* and inclusivity across all sequenced *Variola virus* strains.

In silico results are suggestive of potential performance issues, so will guide necessary additions to the wet screening panels. *In silico* identification of potential cross-reactions (false positives) or non-verifications (false negatives) would identify the relevant strains to 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, using public Genbank nucleotide [http://www.ncbi.nlm.nih.gov/genbank/]. The selected tool should be able to identify predicted hybridization events based on platform annealing temperatures, thus ensuring an accurate degree of allowed mismatch is incorporated in predictions. The program should detect possible amplicons from any selected database of sequence.

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

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

The method developer submission should include:

- Description of sequence databases used in the *in silico* analysis
- Description of tool used for bioinformatics evaluation
  - Data demonstrating the selected tool successfully predicts specificity that has been confirmed by wet-lab testing on designated isolates
    - This data can be generated retrospectively using published assays
- List of additional strains to be added to the inclusivity or exclusivity panels based on the bioinformatics evaluation

AOAC Stakeholder Panel on Agent Detection Assays

Tuesday, February 3, 2015: SPADA Meeting
Wednesday, February 4, 2015: Working Groups

Meeting Minutes

Attendees *(Present during all or part of the meeting)*:

Matt Davenport, JHU/APL (SPADA Chair)
George Anderson, Naval Research Laboratory
Doug Abbott, USDA (Ret.)
Jessica Appler, DUSA-TE
Jeff Ballin, ECBC
Maureen Beanan, NIH
Linda Beck, DoD NSWC Dahlgren
Brian Bennett, Dugway Proving Ground
Larry Blyn, Ibis
Donna Boston, HHS/BARDA
Ryan Cahall, CTR Support to DUSA-TE
J. Clay McGuyer, NGB
Doug Abbott, USDA (Ret.)
Bruce Goodwin, DoD
Ted Hadfield, Hadeco, LLC
Martha Hale, USAMRIID
Anthony Hitchins, FDA (Ret)
Paul Jackson, LLNL (Ret)
Malcolm Johns, DHS/OHA
Cecilia Kato, CDC
Alex Kayatani, PFPA
Liz Kerrigan, ATCC
Katalin Kiss, ATCC
John Lednicky, University of Florida
Matt Lesho, Luminex
Nancy Lin, NIST
Tim Minogue, USAMRIID
Stephen Morse, CDC (Ret)
Pejman Naraghi-Arani, LLNL
Kate Ong, JPM, NBCCA
Eileen Ostlund, USDA
Roberto Rebeil, ECBC
Kris Roth, FDA
James Samuel, Texas A&M
Mark Scheckelhoff, DHS/OHA
Deborah Shuping, DUSA-TE
Darci Smith, SRI
Shanmuga Sozhamannan, CRP/DOD
Sandra Tallent, FDA
Scott Weaver, University of Texas
Christian Whitchurch, DTRA

AOAC Staff *(Present during all or part of the meeting)*:

Jim Bradford, Scott Coates, Christopher Dent, Dawn Frazier, Krystyna McIver, Alicia Meiklejohn, Anita Mishra, Robert Rathbone

Meeting Minutes

I. Welcome and Introductions

Bradford opened at the meeting 9:00 a.m. and all participants were introduced. Bradford explained AOAC's consensus-based standards development model and that voting members are chosen prior to each meeting to provide a balance of perspectives.

II. Overview of SPADA Project

Davenport delivered a presentation[1] to review SPADA's past work, as well as the scope and timeline of the current initiative, which is to develop analytical method performance requirements for: 1) *Coxiella burnetti;* 2) Venezuelan Equine Encephalitis (VEE) with potential for EEE and WEE; and 3) Staphyloccocus enterotoxin B *(*SEB).

III. Overview of AOAC Standards / Concepts and Terminology

Coates directed the attention of the group to the working group sign up forms found in the SPADA Meeting Book[2] and asked members to sign up for the working groups they wish to participate in.

Coates gave a presentation[3] regarding AOAC standards development, in particular AOAC *Standard Method Performance Requirements*® (SMPRs). Coates advised that Appendix F[4] in the *Official Methods of Analysis of AOAC INTERNATIONAL* book provides a detailed overview on SMPRs.

Several stakeholders enquired as to the intended use of the SMPRs to be developed. Appler responded that the primary purpose for DoD is for environmental testing, however, if other uses can be added on without too much trouble then that would be acceptable. Coates added that, in many cases, more than one SMPR has been developed for a single analyte to reflect the broader needs of the community.

Coates then delivered a second presentation[5] explaining AOAC /SPADA acronyms, terms, and concepts. Coates explained the purpose of stakeholder panels, and that Stakeholder panels include subject matter experts from various perspectives. Working groups, subsets of the stakeholder panel, are formed to address specific topics, for example, detection of *Bacillus anthracis* from aerosol collection devices.

A SPADA member stated that previous SPADA projects have avoided clinical samples and asked if that will be the case for this project as well. Appler replied that the primary goal of the project is the development of the standards for environmental detection. However, clinical samples could be considered if the standard fits well with environmental.

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[1] Attachment 1: Davenport Presentation
[2] February, 2015 SPADA Meeting eBook: http://griegler-aoac-org.cld.bz/AOAC-SPADA-February-2015-Meeting-Book
[3] Attachment 2: Coates SMPR Presentation
[4] AOAC Official Methods of Analysis, Appendix F: http://www.eoma.aoac.org/app_f.pdf
[5] Attachment 3: Coates "Terms and References" Presentation

IV. DoD TECMIPT Presentation

Bennett, who serves as co-chair for DoD's Test and Evaluation Capability and Methodology Integrated Process Team (TECMIPT)[6], provided a presentation on TECMIPT. TECMIPT's priority is to standardize procedures for the evaluation of threat agent detection devices; and to ensure that threat agent detection devices work correctly, are reliable, and support the decision makers, and ultimately, the war fighters.

US Army Dugway Proving Ground (DPG) has been developing a new BSL3 facility which includes a large environmental chamber that can be used to evaluate aerosol collection devices. Bennett advised that the facility is near completion, and explained how it will be used to evaluate threat agent detection devices and personal protective equipment.

V. Discussion on Scope

Coates stated that in the past SPADA has always focused only on the analytical portion of threat agent detection systems, but this project may be slightly different due to the combat application. Coates explained that discussing the scope now would clarify what methods the SMPRs should be developed for, and will help the working group chairs in the next step.

Shuping and Appler explained that these methods will be used to support the war fighters in the field, and it is expected that the detection devices will be in the field or near the front line, and used to test the environment, principally testing the air.

Hadfield said that there are two main components of threat detection systems: 1) collection devices, and 2) analytical devices. Davenport reiterated Hadfield's point and said that historically, the system has been split into two parts: 1) collection and 2) analysis. Previous SPADA projects have focused on testing after collection and treated aerosol collection as a separate function because there were so many uncontrollable variables with testing when using aerosols.

Blyn reminded the group that costs of testing must also be considered, because companies that develop these assays will be expected to use these SMPRs as part of their process for qualifying a detection system, and if the cost of testing is too high, then companies will not develop detection systems.

After some discussion, the group agreed to develop SMPRs for analytical devices starting with dry filters and/or aqueous collection solutions.

VI. Working Group Launch: Venezuelan Equine Encephalitis

[6] Attachment 4 – Bennett TECMIPT Presentation

Ostlund delivered a presentation[7] to launch the SPADA Venezuelan Equine Encephalitis (VEE) project. She reviewed the history of the virus, its geographic prevalence and the closely related Western Equine Encephalitis (WEE) and Eastern Equine Encephalitis (EEE). VEE has been experimented with as a biological weapon as an incapacitating agent. It is a select agent and there are a limited number of laboratories that are permitted to work with it. Not many methods have been published for PCR detection of VEE, WEE or EEE. The goal of the working group is to develop SMPRs for detection of VEE by PCR methods, with the possibility of developing a single SMPR for a combination of VEE, WEE and EEE.

After further discussion the group agreed to the following fitness for purpose statement to help guide the working group in their proceedings:

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

VII. Q-Fever

Samuels delivered a presentation[8] describing the background, impact, regulatory guidance and current detection technologies for *Coxiella burnetti*, the causative agent of Q-Fever. The organism is found in goats and other domestic mammals. Hundreds of cases of Q-Fever were documented during the Iraq war, and it is prevalent on Dutch goat farms. The typical route for transmission is aerosolization of contaminated soils. There is a currently a vaccine approved for use in Australia for people at the highest risk, such as goat farmers. There are two common targets in existing PCR assays, IS1111 and Com01. 95% of work currently being conducted is with the Nine Mile RSA439 clone.

After a lengthy discussion on potential requirements and geographic challenges, the group agreed to the following fitness for purpose statement:

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

VIII. Staphyloccocus enterotoxin B (SEB)

Tallent delivered a presentation[9] on the history, background, and current technologies for thedetection of SEB. There are 23 homologous distinct staphylococcus enterotoxins identified and all of them are superantigenic. They are considered biological threat agents because they can be collected and disseminated quickly, and potentially cause widespread illness. The general analytical need would be to detect low levels of SEA, SEB and SEC. Tallent led the group in a discussion on the proposed fitness for purpose for the SEB Working Group and the following statement was agreed upon:

[7] Attachment 5 – Ostlund VEE Presentation
[8] Attachment 6 – Samuel Q-Fever Presentation
[9] Attachment 7 – Tallent SEB Presentation

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

IX. SPADA Executive Advisory Panel Discussion

Davenport took the floor and explained that the United States Department of Homeland Security (DHS) has provided infrastructure funding to SPADA to encourage discussions on future priorities, especially the needs of first responders for standards and rapid assays that have been tested to those standards. The discussion that ensued was part of DHS infrastructure support.

Davenport said that he has spoken to David Ladd (IAB / Massachusetts Department of Fire Services) who is working on a position paper for DHS to move further and faster in the development of handheld technologies for first responders that have been tested and proven to perform to a known level of standard. Often first responders must make important decisions (closing down a facility, for example) based on the hand-held assay results, but do not know how well those assays perform. Lin advised that there are multiple layers that need to be developed to meet first responder needs. Reference materials need to be updated and developed to meet today's standards and be readily available for assay manufacturers. The assays themselves need to be developed and tested to established performance standards. And training for the first responder in the use of those assays in the field is critical.

Johns suggested the development of tactical standards for threats that are currently unknown, and asked if a system could be developed to rapidly develop standards for these unknowns.

Another SPADA member suggested revisiting the older SPADA standards. There is a tremendous amount of genotypic availability around the world and it could be worthwhile to revisit some of the older strain panels to ensure they are diverse enough. A DoD representative volunteered to bring this concept to the attention of the DTRA Program Manager.

X. Adjourn

The SPADA Meeting adjourned at approximately 4:00 p.m. EDT. McIver announced that the working groups would be held consecutively on the following day beginning at 9:00 a.m. EDT.

AOAC INTERNATIONAL Stakeholder Panel on Agent Detection Assays
Working Group Sessions - February 4, 2015 (Day 2)

I. Welcome and Introductions

Davenport opened the second day of SPADA proceedings and explained that since all were present from the previous day's SPADA meeting, all were welcome to participate in the working groups. To sign up for specific working groups and stay engaged with them in the future, participants were encouraged to sign up through AOAC's working group sign up form[10].

II. VEE Working Group

Ostlund led the group in a discussion on the SMPR for VEE. The group agreed that the purpose of this SMPR will be the detection of agents if there is an aerosol release. There was a discussion on whether or not to develop one SMPR or two, one for confirmation and one for screening. The group agreed to limit the scope of the working group to one SMPR applicable to the detection of known human pathogenic strains of VEE, and possibly EEV and WEEV, in dry filters and or liquid samples from aerosol collectors, with the preferential method being a field-deployable assay that can detect specific viruses.

A draft version of the *Variola* SMPR was used as a starting point and the group continued to progress through that template, adjusting it to fit VEE as needed. Definitions were reviewed and Ostlund took the action to provide an adequate definition of *Venezuelan Equine Encephalitis/Encephalomyelitis Virus.* AOAC took the action to incorporate the validation guidance and introduction paragraphs into the VEE SMPR. The group concluded its revisions with the first page of the template and agreed to continue progress by teleconference (attachment 1).

III. SEB Working Group

Tallent led the group in a discussion on the SMPR for SEB. Applicability was agreed as specific detection of SEA, SEB, and SEC dry filters and/or liquid samples from aerosol collectors, with the preference of a field deployable assay. The first page of the template SMPR was reviewed and Tallent took the action to provide a definition of SEA-C for the definitions section. A lengthy discussion ensued regarding the Acceptable Minimum Detection Level (AMDL) for SEB. The group tentatively agreed to an AMDL of 25 ng / mL., but also agreed to revisit this issue once more data is available. Johns took the action to research this and bring it back to the next meeting of the SEB Working Group.

IV. Q-Fever Working Group

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[10] AOAC SPADA Working Group Sign Up: https://adobeformscentral.com/?f=Wwp88CLBMHw7QQ4Cr02L4g

Samuel led a discussion on Q-Fever. The group discussed the number of strains that should be used to evaluate candidate methods. Samuel indicated he already has information on which strains are most appropriate. Validation guidance was modified to state that strains must be confirmed and documented.

This led to a discussion on how, and by whom, strains and species should be confirmed and documented. Davenport noted that this has been an issue with SPADA for years and that the assumption has been that you are testing with qualified materials. Coates proposed a sub-group to discuss this further because this issue runs across all of the working groups. Blyn, Hadfield, Kiss, Lednicky, Naraghi-Arani, and Weaver all volunteered to participate on this group. Davenport suggested contacting Goodwin and Wolcott (USAMRIID) as well.

The working group reviewed an SMPR developed in 2007-2010 for detection of *Bacillus anthracis* by PCR. The group tentatively agreed to use the AMDL specified in the SMPR: 20,000 genome equivalents on dry filter and/or 2,000 per mL genome equivalents in collection buffer. Blyn, Hadfield, and Davenport discussed the rationale for the AMDL. Coates volunteered to search through the archives to determine the rationale for these AMDLs.

The working group agreed to an applicability statement as the specific detection of *Coxiella burnetii* in dry filters and/or collection buffer from aerosol collection devices. The preferential method would be a field-deployable assay. The group agreed to continue to work on this at the next teleconference meeting, which is still to be determined.

FEBRUARY 3-4, 2015 SPADA MEETING: ACTION ITEMS	
Action	**Owner**
Provide definition for *Venezuelan Equine Encephalitis/Encephalomyelitis Virus* for use in SMPR at the next working group meeting.	Ostlund
Incorporate the validation guidance and introduction paragraph into the VEE SMPR.	Coates / Dent
Provide a definition of SEA-C for the definitions section of the SEB SMPR.	Tallent
Research appropriate AMDL for SEA-C.	Johns
Contact Goodwin and Wolcott for strain characterization sub-group.	McIver / Dent
Search AOAC archive for rationale of 2000 genome equivalents per mL., and 20,000 genome equivalent / dry filter.	Coates / Dent
Organize follow up working group calls.	Coates / Dent

Stakeholder Panel on Agent Detection Assays

SPADA MEETING 15

03 FEB 2015

Matthew G. Davenport, Ph.D.
SPADA Chair
The Johns Hopkins University Applied Physics Laboratory

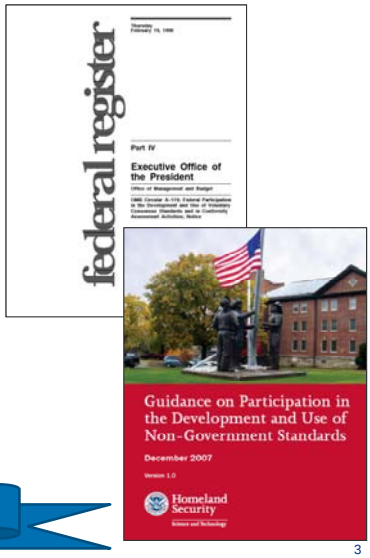
James Bradford
Executive Director
AOAC INTERNATIONAL

SPADA Objectives & History

- **Original Objectives in 2007**
  - Establish standards to validate Polymerase Chain Reaction (PCR)-based technologies that detect aerosolized *Bacillus anthracis*, *Yersinia Pestis*, or *Francisella tularensis*
  - Pilot the validation process with an assay that detects *B. anthracis*
- **2009**
  - Develop standards to validate immunoassay-based Hand-Held Assays (HHAs) that detect *B. anthracis* or Ricin in suspicious powders
  - Test commercially-available HHAs
- **2010**
  - Develop standards to validate PCR-based technologies that detect aerosolized *Burkholderia psuedomallei* and *Burkholderia mallei*
  - Develop standards to validate PCR-based technologies that detect *B. anthracis* in suspicious powders
- **2011**
  - Develop recommendations on controls needed for field-based assays
- **2013**
  - Develop standards to validate PCR-based technologies that detect aerosolized Variola
  - Establish First Responder Working Group
  - Maintain a SPADA Executive Steering Committee
- **2014**
  - Establish standards to validate technologies that detect Venezuelan Equine Encephalitis Virus, Staphylococcus Entertoxin B, and *Coxiella burnetti* (Q-fever)

Federal development and use of standards is guided by NTTAA and OMB A-119*

- OMB Circular A-119; Federal Participation in the Development and Use of Voluntary Consensus Standards and in Conformity Assessment Activities
  - Directs Federal agencies to develop and use voluntary consensus standards in lieu of government-unique standards
    - When practicable
    - Use in whole or in part
- The Homeland Security Act of 2002 (Public Law 107-296) directs DHS to conduct all standards activities in accordance with the NTTAA and OMB A-119

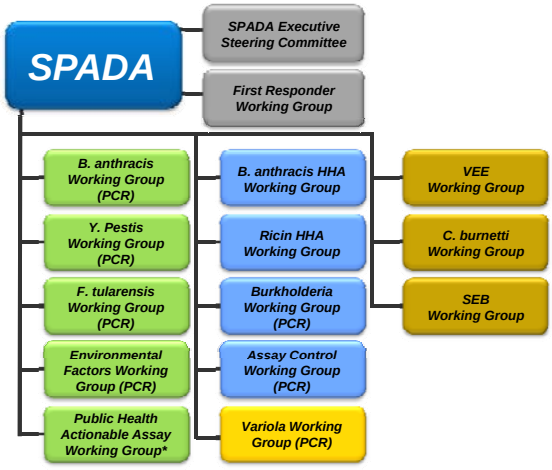


GOAL: Produce open consensus standards that serve the community

*National Technology Transfer and Advancement Act of 1995 (Public Law 104-113)

SPADA Sets Standards

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



❖ ***All SPADA members volunteer their time and expertise***

*The SPADA PHAAWG did not develop strain panels and method performance requirements; rather, the WG discussed necessary elements of an actionable assay (e.g., performance standards, user training, ConOps)

SPADA Working Group Chairs

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

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

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

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

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

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

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

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

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

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

Venezuelan Equine Encephalitis Working Group
James Samuel (U of Texas, A&M)

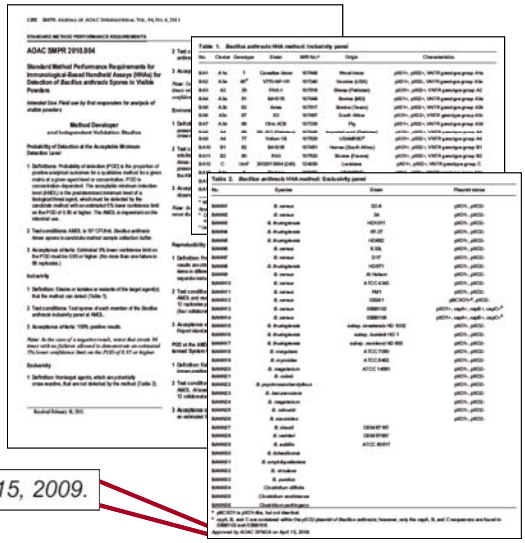
***C. burnetti* Working Group**
Eileen Ostlund (USDA)

SEB Working Group
Sandra Tallent (FDA)

Standard Method Performance Requirements

SPADA approved strain panels and method performance requirements are consolidated into a Standard Method Performance Requirements (SMPR) document for publication in the *Journal of AOAC INTERNATIONAL*

Example:
AOAC SMPR 2010.004 Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of *Bacillus anthracis* Spores in Visible Powders


AOAC SMPR 2010.004

Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of *Bacillus anthracis* Spores in Visible Powders

Method Developer

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

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

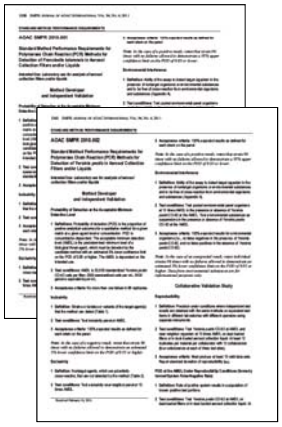
Approved by AOAC SPADA on April 15, 2009.

SPADA Standards Are Published

Each will be published as a Standard Method Performance Requirements (SMPR) document in the *Journal of AOAC INTERNATIONAL*

Five SMPRs and two additional publications are published:

- Development of Standard Method Performance Requirements for Biological Threat Agent Detection Methods (SMPR-SPADA Overview)
- AOAC Biological Threat Agent Method Validation Guideline (BTAM Guideline)
- **AOAC SMPR 2010.001** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Francisella tularensis* in Aerosol Collection Filters and/or Liquids
- **AOAC SMPR 2010.002** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Yersinia pestis* in Aerosol Collection Filters and/or Liquids
- **AOAC SMPR 2010.003** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* in Aerosol Collection Filters and/or Liquids
- **AOAC SMPR 2010.004** Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of *Bacillus anthracis* Spores in Visible Powders
- **AOAC SMPR 2010.005** Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of Ricin in Visible Powders
- **AOAC SMPR 2014.006** Detection and Identification of Variola Virus



Additional SPADA Standards

More SMPRs to be published:

- **AOAC SMPR 2011.XXX** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Burkholderia psuedomallei* in Aerosol Collection Filters and/or Liquids
- **AOAC SMPR 2011.XXX** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Burkholderia mallei* in Aerosol Collection Filters and/or Liquids
- **AOAC SMPR 2011.XXX** Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* Spores in Visible Powders

Uses of SMPRs

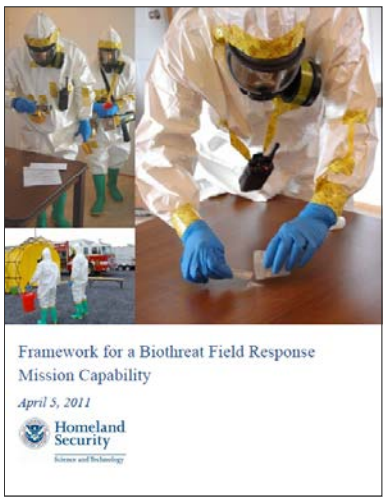
Development of validation protocols

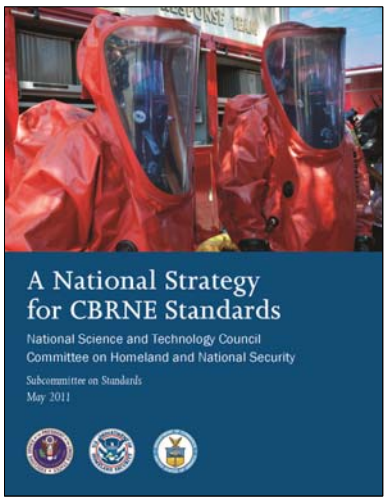
- Development of three validation protocols within the AOAC *Performance Tested* ^{SM} *Methods* and *Official Methods of Analysis*^{SM} program

Minimum acceptance criteria for federal acquisition programs

Provides guidance for development of new environmental detection capabilities

SPADA has impacted national policy





Timeline of current project

- February 3 – 4, 2015
  - Project Launch – SPADA and WG meetings
- February – April 2015
  - WG teleconferences to develop Draft SMPRs
- April 2015
  - Face-to-face WG meetings, one for each agent, to discuss/revise Draft SMPRs
- May – June 2015
  - WG teleconferences to continue to draft SMPRs
- June 2015
  - AOAC outreach and post SMPRs on AOAC website for public comment

Timeline of current project

- July – August 2015
  - Preparation for final draft SMPR for each agent by WG Chair and AOAC Chief Scientific Officer
    - Collect and compile comments
    - Address comments
    - Revise SMPRs as appropriate
- August – September 2015
  - WG Teleconferences
    - Resolve issues
    - Agree on final draft SMPR to be submitted for SPADA approval
- September 2015
  - SPADA face-to face meeting to discuss/approve SMPRs

Summary

- SPADA develops open, documented consensus standards, consistent with NTTAA and OMB A-119, that support the biothreat detection community
- SPADA has developed and published Standard Method Performance Requirements for a number of biothreat agents
- SPADA has developed a testing and certification process for biological threat detectors
- Tools have been tested to SPADA Standards
- SPADA standards have supported government programs
  - BioWatch Gen3 T&E
  - DoD Critical Reagents Program
  - Guided sequencing programs of DHS S&T and DTRA-CB
- SPADA currently supports the standards needs of the DHS BioWatch Program and the First Responder Community

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AOAC INTERNATIONAL

Standard Method Performance Requirements (SMPRs)

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- Introduction
- Background
- Format
- Process
- Guideline for Development of SMPRs
- Performance parameters

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Standard Methods Performance Requirements

- Commonly referred to as:
  - SMPRs
  - “Smipper”s


Standard Method Performance Requirements | AOAC INTERNATIONAL (2011)

AOAC SMPR 2011.008

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

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SMPRs

- documents a community’s analytical method needs.
- very detailed description of the analytical requirements.
- includes method acceptance requirements.
- published as a standard.

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Uses of SMPRs

- Basis for method acceptance and approval.
- Guidance to method developers for the development of new methods.
- Advance the state-of-the-art in a particular direction.
- Address specific analytical needs.
- Allow AOAC to reach a broader community of method developers and users.

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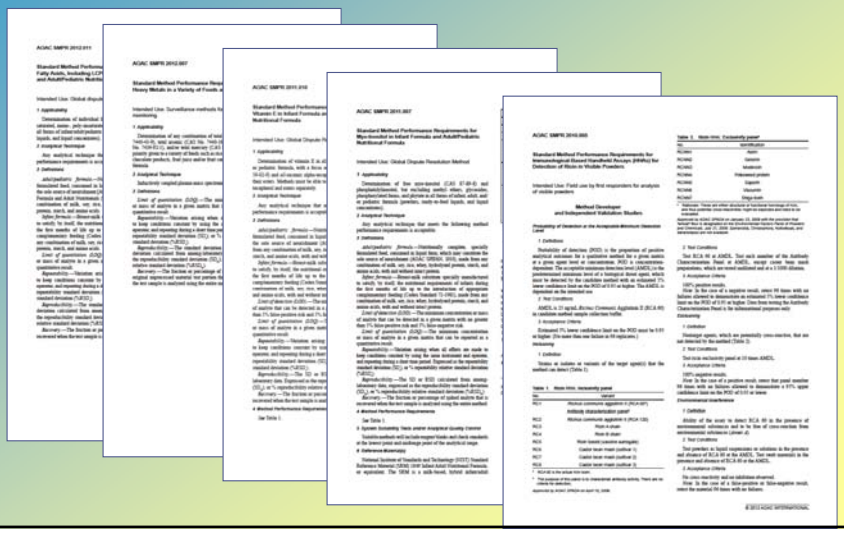
AOAC has adopted 50+ SMPRs



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SMPR Format

- Intended use
- Applicability
- Analytical technique
- Definitions

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SMPR Format

- System suitability
- Reference materials
- Validation guidance
- Maximum time-to-determination
- Method performance requirements table

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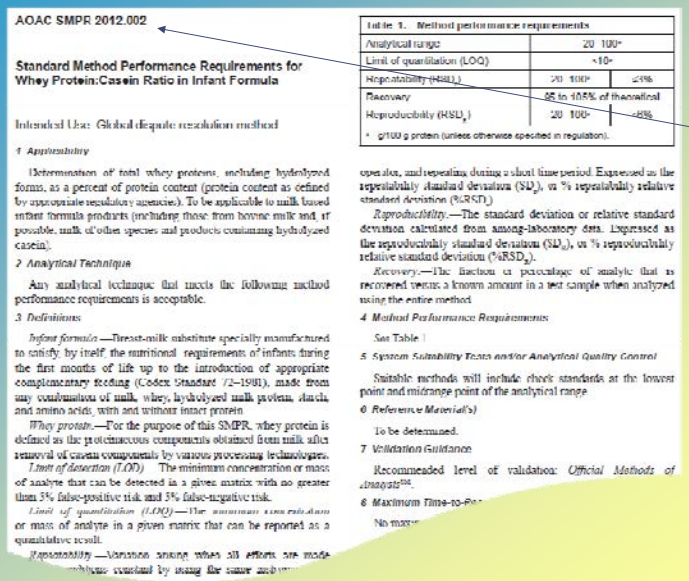

AOAC SMPR 2012.002

Standard Method Performance Requirements for Whey Protein:Casein Ratio in Infant Formula

Intended Use: Global dispute resolution method

1 Applicability

Determination of total whey proteins, including hydrolyzed forms, as a percent of protein content (protein content as defined by appropriate regulatory agencies). To be applicable to milk based infant formula products (including those from bovine milk and, if possible, milk of other species and products containing hydrolyzed casein).

2 Analytical Technique

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

3 Definitions

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, whey, hydrolyzed milk protein, starch, and amino acids, with and without intact protein

Whey protein.—For the purpose of this SMPR, whey protein is defined as the proteinaceous components obtained from milk after removal of casein components by various processing technologies.

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 analyst

Table 1. Method performance requirements		
Analytical range	20–100*	
Limit of quantitation (LOQ)	<10*	
Repeatability (RSD_r)	20–100*	≤4%
Recovery	95 to 105% of theoretical	
Reproducibility (RSD_R)	20–100*	≤8%

* g/100 g protein (unless otherwise specified in regulation).

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 standard deviation (SD_R), or % reproducibility relative standard deviation (%RSD_R).

Recovery.—The fraction or percentage of analyte that is recovered versus a known amount in a test sample when analyzed using the entire method

4 Method Performance Requirements

See Table 1

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include check standards at the lowest point and midrange point of the analytical range

6 Reference Material(s)

To be determined.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*℠.

8 Maximum Time-to-Re

No maxi

SMPRs are published in the OMA.

SMPR ID numbers use the year and 3 numerals.

OMA ID numbers use the year and 2 numerals.

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SMPRs can be developed for all types of methods:

Quantitative methods

- Trace components: arsenic in food.
- Main components: nutrients in infant formula.

Qualitative methods

- Trace components: *Listeria* in cheese.
- Main components: chondroitin sulfate.

Identification methods: PDE5-Inhibitors in supplements.

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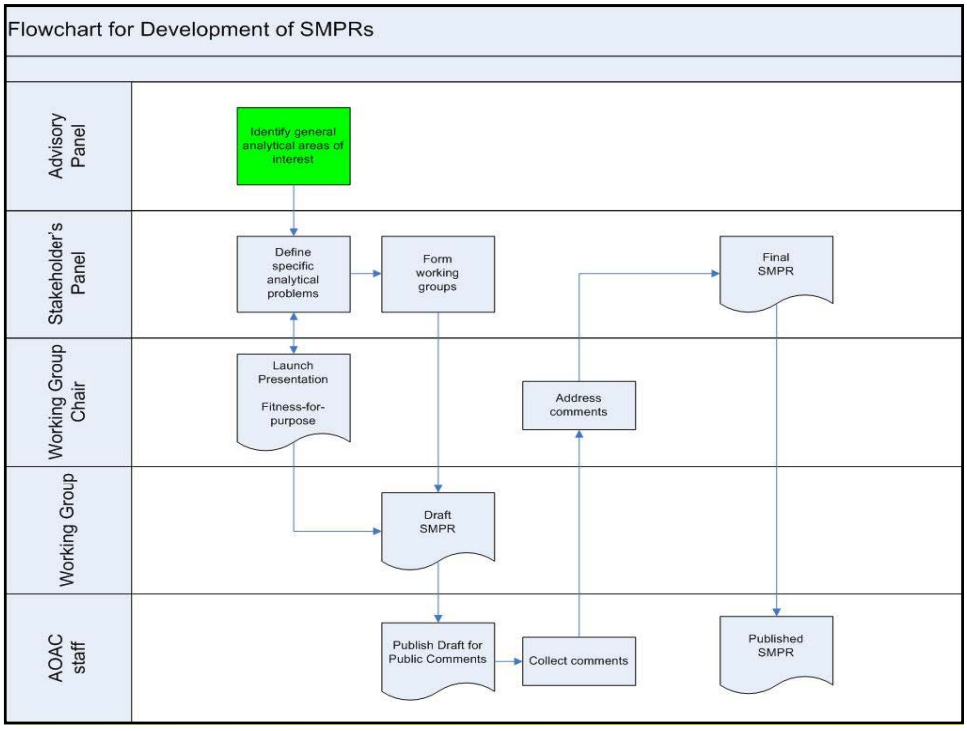
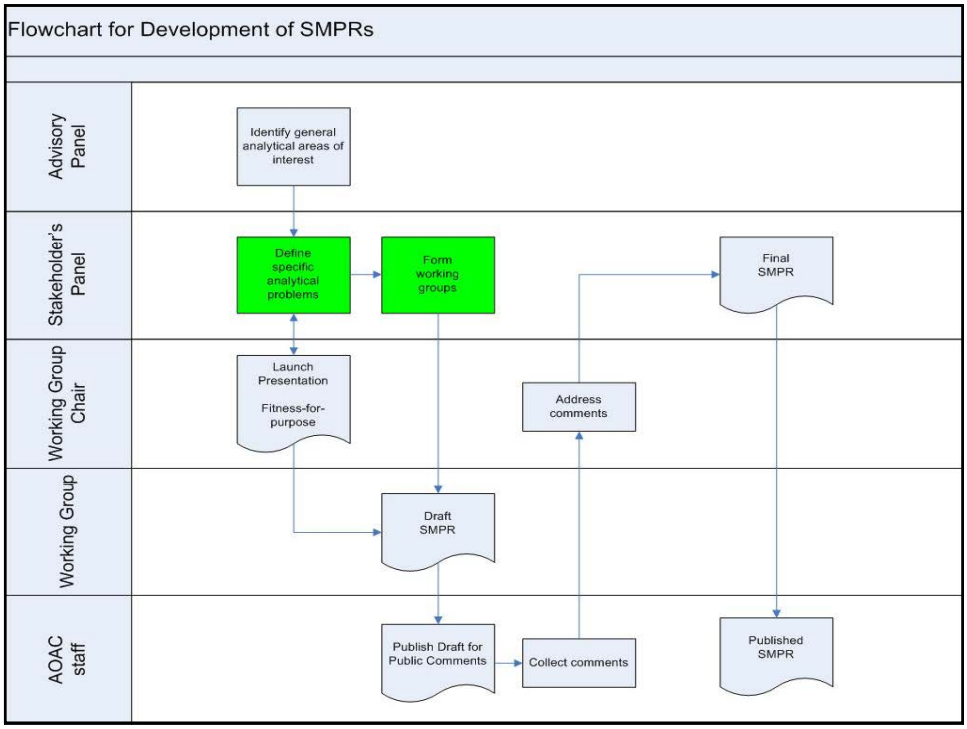
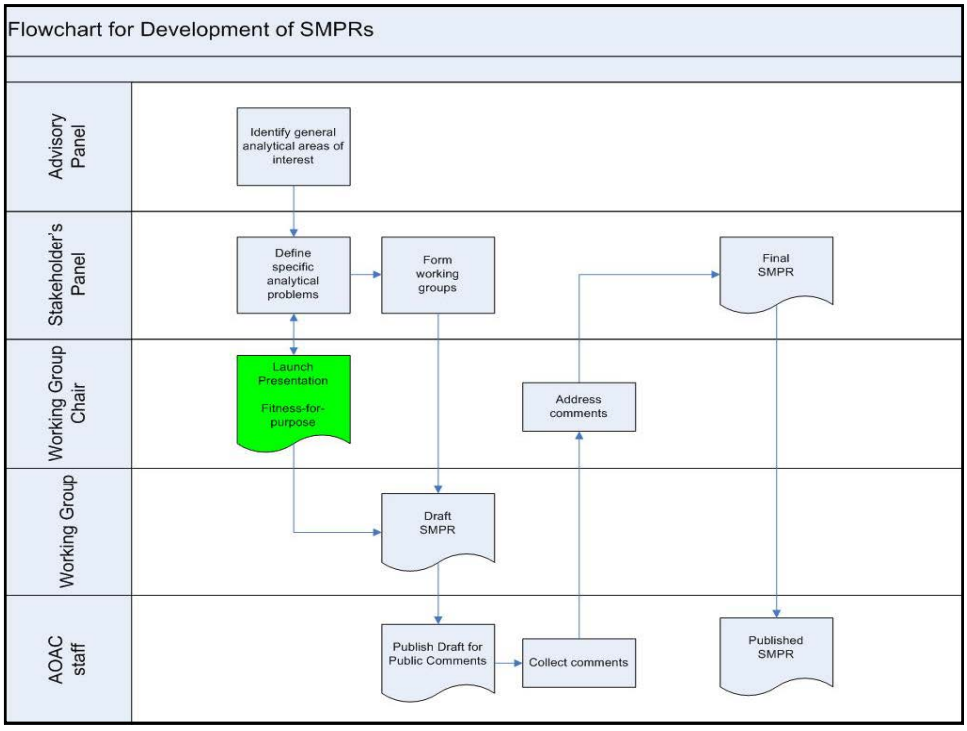
SMPR Process




Flowchart for Development of SMPRs
Advisory Panel
Stakeholder's Panel
Working Group Chair
Working Group
AOAC staff
Identify general analytical areas of interest
Define specific analytical problems
Form working groups
Final SMPR
Launch Presentation
Fitness-for-purpose
Address comments
Draft SMPR
Publish Draft for Public Comments
Collect comments
Published SMPR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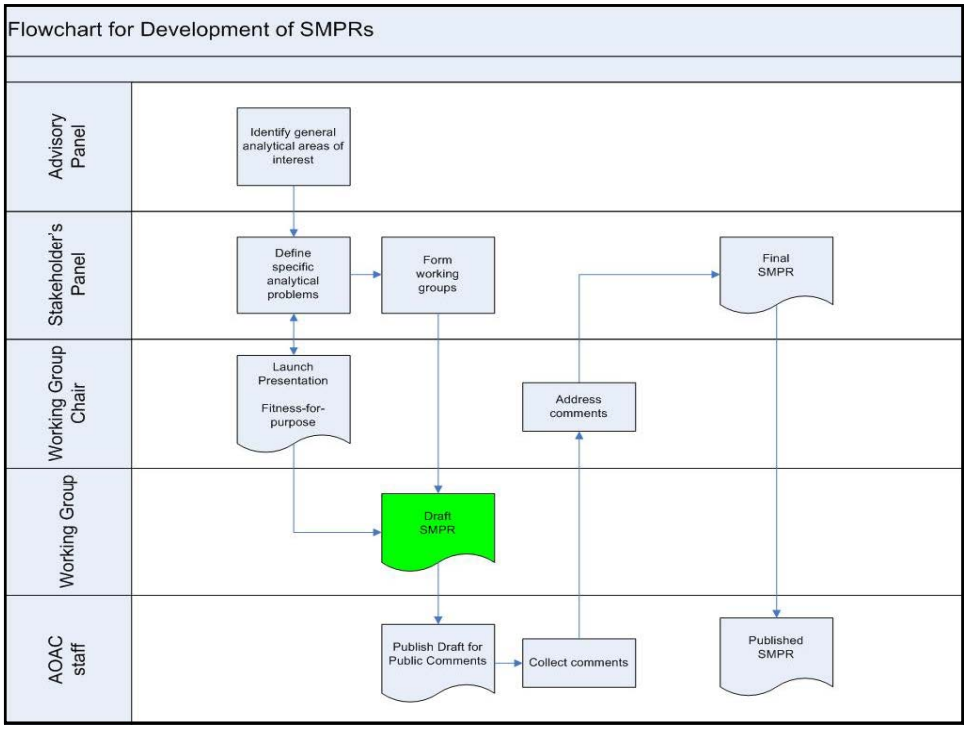
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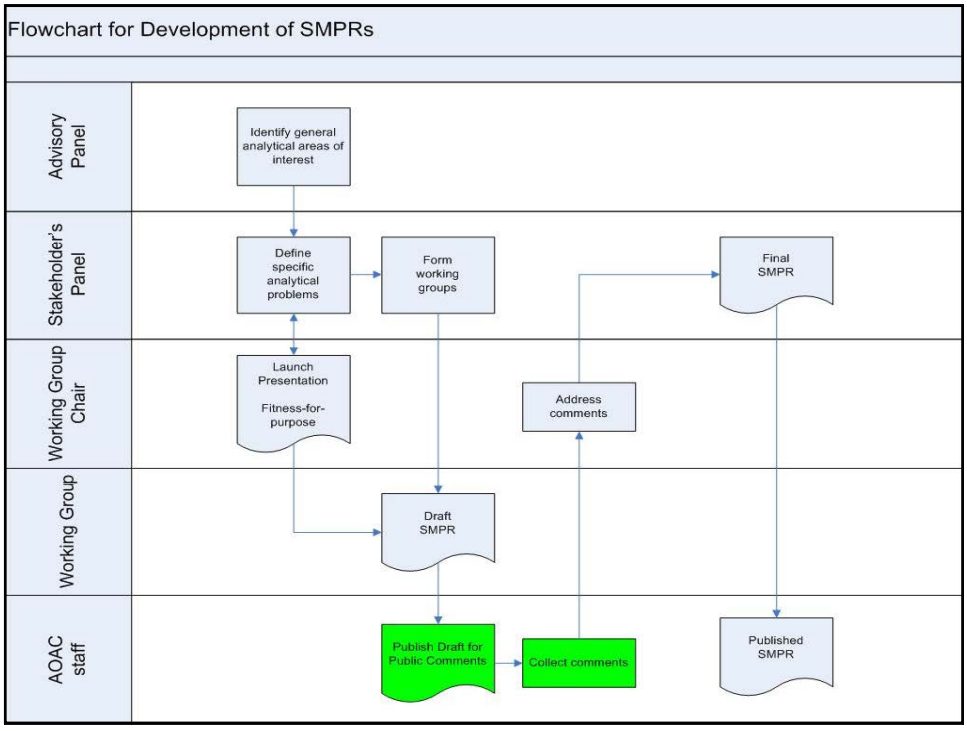
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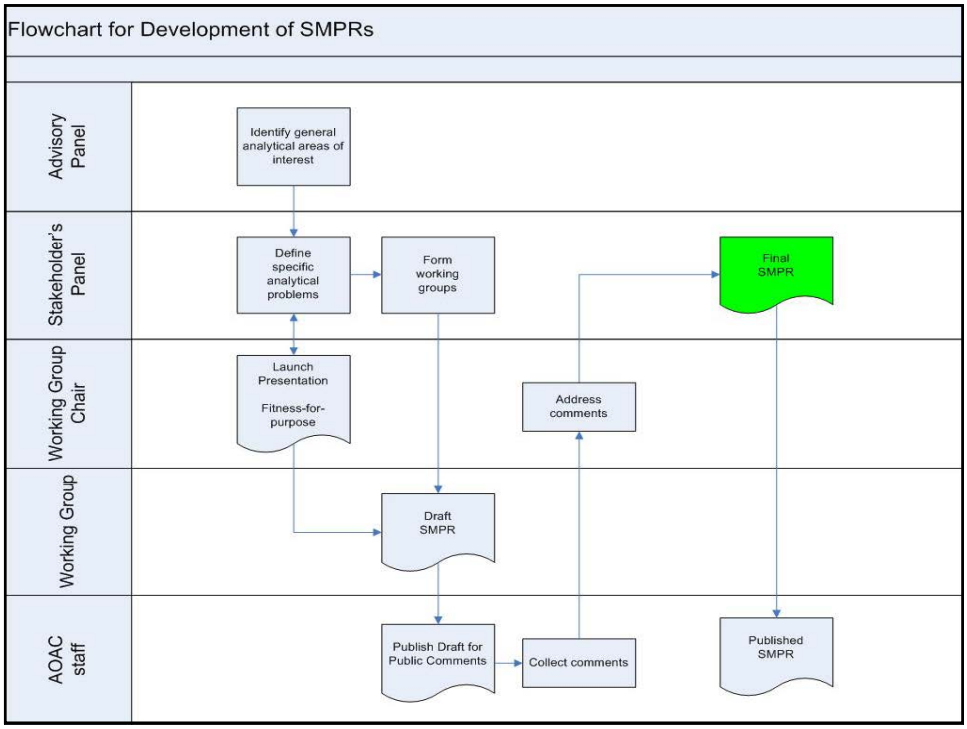
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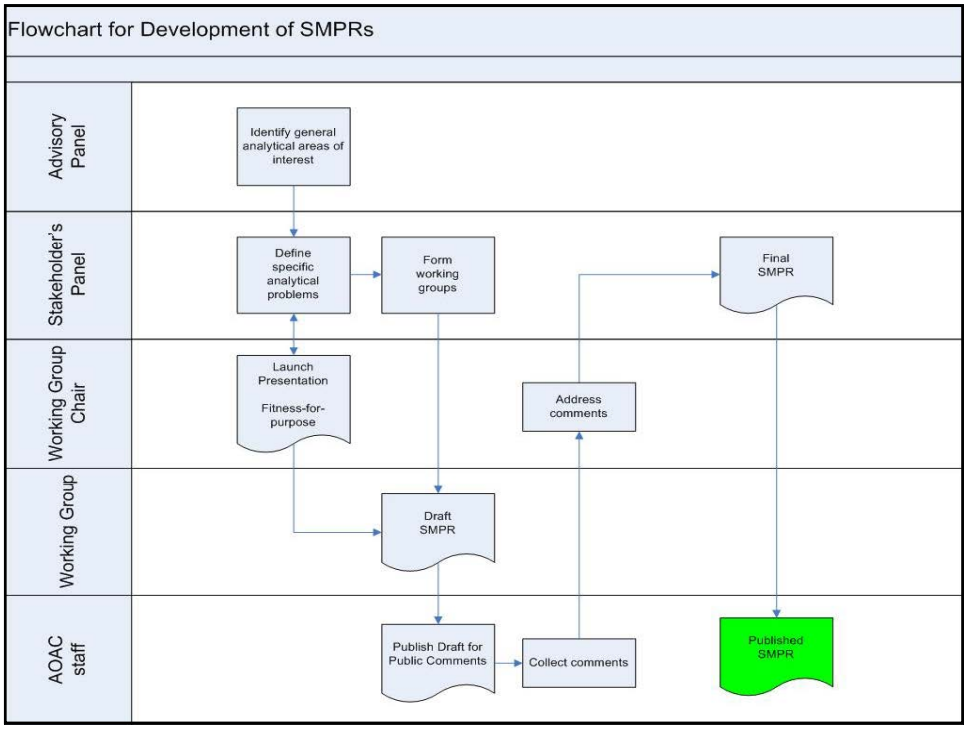
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Fitness-for-Purpose

- Very early in process
- General statement of method performance
- No or few acceptance criteria
- 1 or 2 paragraphs
- No formal format
- Not a standard

SMPR

- A deliverable
- Very detailed specification of method performance requirements
- Acceptance criteria
- 2 to 3 pages
- Standard format
- Formal AOAC standard
- Published in the OMA

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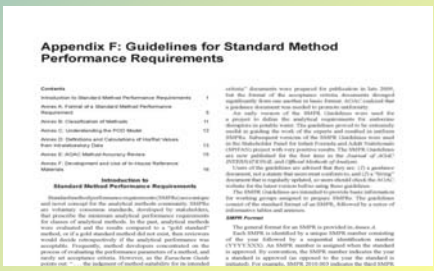
Appendix F: Guideline to SMPRs

- **Complete** guidance describing SMPRs and general validation requirements.
- 19th ed. of OMA
- On-line at: http://www.eoma.aoac.org/app_f.pdf


Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

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Performance parameters

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Quantitative methods

- Analytical range
- Limit of detection
- Limit of Quantitation
- Repeatability
- Recovery
- Reproducibility

4. Method Performance Requirements

Analytical range	0.01–5.0[a]	
Limit of detection (LOD)	≤0.004[a]	
Limit of quantitation (LOQ)	≤0.01[a]	
Repeatability (RSD_r)	0.01[a]	≤15%
	0.2[a]	≤7%
	0.5[a]	
	5.0[a]	
Recovery	0.01[a]	90–110%
	0.2[a]	
	0.5[a]	
	5.0[a]	
Reproducibility (RSD_R)	0.3	≤11%
	0.6	
	1.0	
	2.5	
	5.0	
Concentrations apply to (*1*) "ready-to-feed" liquids "as is"; (*2*) reconstituted powders (25 g into 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight.		
[a] µg/100 g expressed as cyanocobalamin in reconstituted final product.		

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Qualitative methods

- Probability of Detection (POD)
- Acceptable Limit of Detection (AMDL)
- Inclusivity
- Exclusivity

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Summary

- SMPRs provide a logical way to define what we need in a method.
- SMPRs provide a way to standardize inclusivity/exclusivity panels.
- The process allows a community to agree on and set the minimum performance requirements for a class of methods.

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Summary

- SMPRs provide an objective standard to judge candidate methods.
- SMPRs are unique in the analytical community.
- AOAC and its volunteers have produced 50+ SMPRs in 4 years, even for the toughest analytes.

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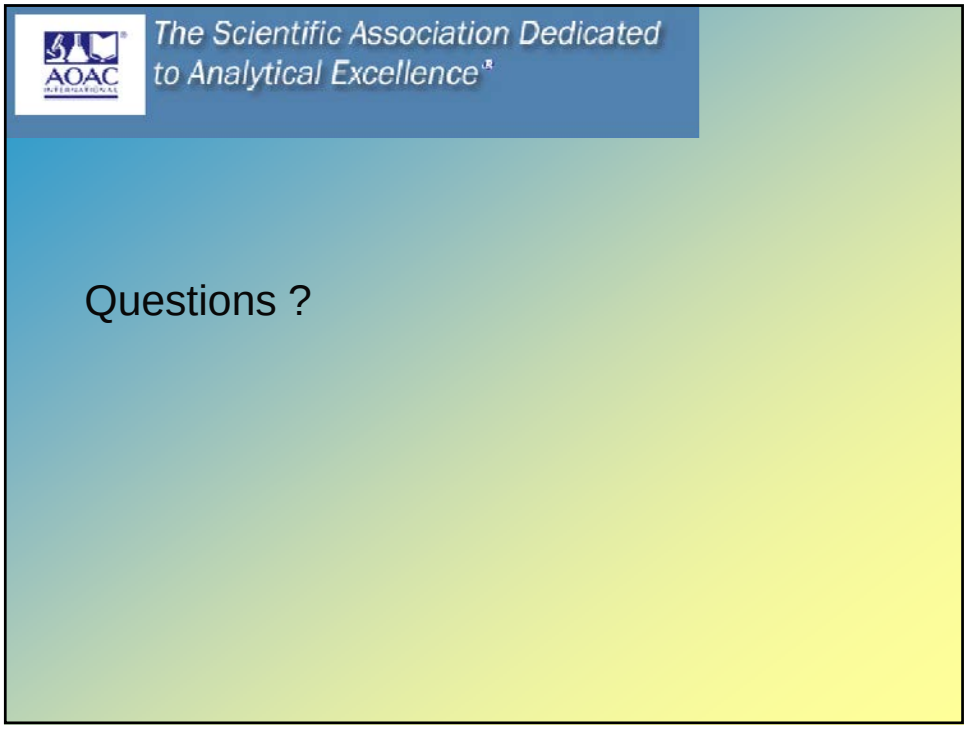
Don’t worry -

- It’s a great process.
- We’ll be there at your side every step of the way.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Questions ?

AOAC Concepts and Terms

SPADA
February 2015
AOAC Headquarters
Rockville, Maryland

AOAC Groups

Stakeholder Panel

1. Comprised of subject matter experts
    a. Voting members vetted by AOAC Official Methods Board
2. Identifies specific analytical topics within the general analytical problem described by the advisory panel
3. Forms working groups to address the specific analytical topics
4. Identifies additional subject matter experts needed for the working groups
5. Provides oversight of the SMPR development
6. Formally adopts SMPRs drafted by working groups

AOAC Groups

Working Group

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.

Official Methods Board (OMB)

A thirteen-member Board, appointed by the President of AOAC, that sets AOAC scientific policy, oversees the activities and composition stakeholder panels, and reviews recommendations for Final Action *Official Methods of Analysis*^{SM}

General Concepts

Verification Study

The evaluation of whether or not a product, service, or system complies with a regulation, requirement, specification, or imposed condition. Often an **internal** process.

Single Laboratory Validation (SLV)

The assurance that a product, service, or system meets the needs of the customer and other identified stakeholders. Often involves acceptance and suitability with external customers.

General Concepts

Test method

Specified technical procedure for detection of an analyte (synonymous with assay).

Screening

Tests of high diagnostic sensitivity suitable for large-scale application.

Confirmation

Test methods of high diagnostic specificity that are used to confirm results, usually positive results, derived from other test methods.

General Concepts

Collaborative Study Validation

Validation study carried out at multiple sites using the same method and equipment on common samples. Primarily used to determine reproducibility (inter-laboratory variability).

General Concepts

Assay

Synonymous with test or test method, e.g. enzyme immunoassay, complement fixation test or polymerase chain reaction tests. AOAC defines an assay as the total of all of the steps from sample collection to final result.

Analyte

Constituent that is of interest in an analytical procedure.

Matrix

Totality of components of a material system except the analyte. Ex.: soil, water, air, etc.

General Concepts

Qualitative method

Analytical method that results in a binary result: present or absent. Frequently, the method has a design cut-off point at which the method is designed to be positive at all concentrations about the cut-off point.

Quantitative method

Analytical method that determines the absolute or relative abundance (often expressed as a concentration) of one, several, or all particular substance(s) present in a sample. [1]

Qualitative Method Specific Terms

Acceptable minimum detection level (AMDL)

Predetermined minimum level of an analyte which must be detected by a candidate method at a specified probability of detection (POD).

Probability of detection (POD)

Proportion of positive analytical outcomes for a qualitative method for a given matrix at a specified analyte level or concentration with a ≥0.95 confidence interval .

Qualitative Method Specific Terms

Inclusivity

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

Exclusivity

Study involving pure nontarget strains, which are potentially cross-reactive, that shall not be detected or enumerated by the tested method. Also known as "Near-Neighbor."

Environmental Factors Study

Supplements the exclusivity testing panel and tests for potential cross-reactivity and/or inhibition. DUSA uses term "Interferents."

Qualitative Method Specific Terms

Laboratory probability of detection (LPOD)

POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration.

False-negative rate

Proportion of test results that are negative, contained within a population of known positives.

False-positive rate

Proportion of test results that are positive, contained within a population of known negatives.

Qualitative Method Specific Terms

Predictive value (negative)

The probability that an animal is free from exposure or infection given that it tests negative; predictive values are a function of the DSe (diagnostic sensitivity) and DSp (diagnostic specificity) of the diagnostic assay and the prevalence of infection.

Predictive value (negative)

The probability that an animal has been exposed or infected given that it tests positive; predictive values are a function of the DSe and DSp of the diagnostic assay and the prevalence of infection.

Sensitivity (analytical)

Synonymous with “Limit of Detection“, smallest detectable amount of analyte that can be measured with a defined certainty; analyte may include antibodies, antigens, nucleic acids or live organisms.

Guidelines

Appendix I: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures

http://www.eoma.aoac.org/app_i.pdf

Appendix F: Guidelines for Standard Method Performance Requirements

http://www.eoma.aoac.org/app_f.pdf

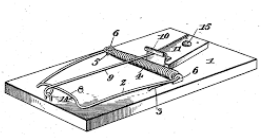
Test and Evaluation Capability and Methodology Integrated Process Team

(TECMIPT)

Reset, Sustain, Prepare, and Modernize

Brian Bennett

Jan, 21, 2015



TECMIPT

Mission

To provide technical expertise, reviews, and recommendations to the CBRND T&E Executive in the identification of T&E capabilities gaps and the development of T&E capability standards.

Chemical and Biological Contamination Survivability

Smartman Mask Testing

TECMIPT

Test and Evaluation Capability and Methodology Integrated Process Team

Mr. James Cooke
Assistant Deputy Under Secretary of the Army T&E

Ms. Deborah Shuping
Chief, CBRND Defense Division, DUSA-TE

8 Commodity Groups + SMEs

- Biological
- Contamination Avoidance
- Advanced Threat
- Radiological/Nuclear Defense
- Modeling & Simulation
- Individual Protection
- Collective Protection
- Deconn

TECMIPT Mission

Established by the Chemical and Biological Defense Program Test & Evaluation (T&E) Executive in 2005

Ensure testing resources are in place, maintained, and sufficiently contemporary to support incoming T&E program test requirements.

I. Adequate fidelity and scale

II. Test results that are scientifically reproducible, legally defendable

Continual Task For TECMIPT Members

1. Screen incoming test requirements and identify gaps
2. Provide POM input to man, equip, train, and sustain ARMY/CBDP T&E Infrastructure
3. Review Validation plans, Test Reports, and TOPS

Test to fully understand how well systems work and what limitations exist under threat conditions

- Support the War Fighter -

Two Principle Documents

#1 Shortfalls, Gaps, and Modernization

- Low level of detail
- Not necessarily program driven

#2 Test and Evaluation Capability Needs

- High Level of detail
- Driven by test program performance and technical requirements
- Feeds POM

Examples:

Can chamber reach test conditions?

Are referees of adequate sensitivity?

CAPAT SME Responsibilities

Screen future programs for technical requirements

a. Early involvement (TEMP, SEP, ORD) is paramount.

b. Are range of test conditions threat representative , operationally relevant, and of sufficient rigor?

c. Does technology exist to adequately test?

d. Work with government S&T organizations to develop weak areas.

Understand current test infrastructure capabilities.

a. Test fixture: capacity, fidelity, range of performance

b. Referee system compared to incoming needs

c. Ensure data set has maximum forward compatibility. Legacy data should be comparable to modern data sets whenever possible.

-Identify existing gaps and publish in SG&M and TECN-

Bio CAPAT Overview

- Point Detectors

- Sample Aerosol Directly
- Reliability, Time to Detect, Distinguish from Background
- Particle Size: Threat Based (1 - 10 micron)

- Identifiers

-qPCR, ELISA, UV Emission, AA

- Standoff Detectors (AOF not funded)

- Back Scatter + Emission: Good Sizing, Concentration
- Poor ID

- Decon and Equipment Survivability

- Particulate in nature (surface) , deactivation, filtration efficacy. Nuclear decon often related

A Few Biological TECNs

- Standardized Interferent Recipes.
- Standard Unit of Measure for Bio Detection
  ACPLA, TALA*p* BUALA.
  Infectivity, Virulance and Viablity Based

More Short Term

- Background vs Challenge: UV Vis Particle Sizer.
- Threat realistic Aerosolizer for chamber tests.
  Crude method (threat based), Wide PSD
  Flux must be suitable for the low levels
  associated with chamber work
  Current Sonotek method is very refined

DUGWAY PROVING GROUND

Questions?

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STAKEHOLDER PANEL ON AGENT DETECTION ASSAYS

Background & Fitness for Purpose

Venezuelan Equine Encephalomyelitis Virus

Eileen N. Ostlund, DVM, PhD
Diagnostic Virology Laboratory,
National Veterinary Services Laboratories, USDA

AOAC, International Rockville, MD
3 February 2015

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VEEV Historical Background

- VEEV was first isolated in 1936 from a horse brain during an outbreak of fatal equine encephalomyelitis in the Guajira region of Venezuela
  - The isolate was serologically distinct from eastern and western equine encephalomyelitis viruses (EEEV, WEEV)

- Prior outbreaks in horses, mules, donkeys identified retrospectively (1920's and 1930's, included Colombia)

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VEEV Historical Background (continued)

- Outbreaks in 11 of 26 years from 1935-1961
  - Colombia, Venezuela, Trinidad, Peru, etc.
- Outbreaks nearly every year from 1962-1973

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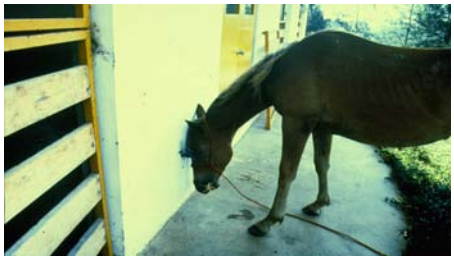
VEEV Clinical Signs in Horses



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VEEV History (continued)

- 1943-1963 – VEE isolated from locations in South America, Central America, Caribbean
  - First reported human VEEV infections were in laboratory workers
  - Mild human infections during 1944 Trinidad outbreak
  - Severe human infections during subsequent outbreaks
- Outbreak from 1969 to 1972 eventually reached the USA (Texas) and was stamped out by vaccination and movement control of horses
  - May have originated from incomplete formalin inactivation of vaccine strains

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VEEV History (continued)

- 1995 –outbreak in Venezuela and Colombia
  - 75,000-100,000 people affected
- Little epizootic VEE activity in nature in recent years
- Locations of activity not well reported
- Locations of activity may not be safe for investigators
- Inter-epizootic maintenance of VEEV not well understood

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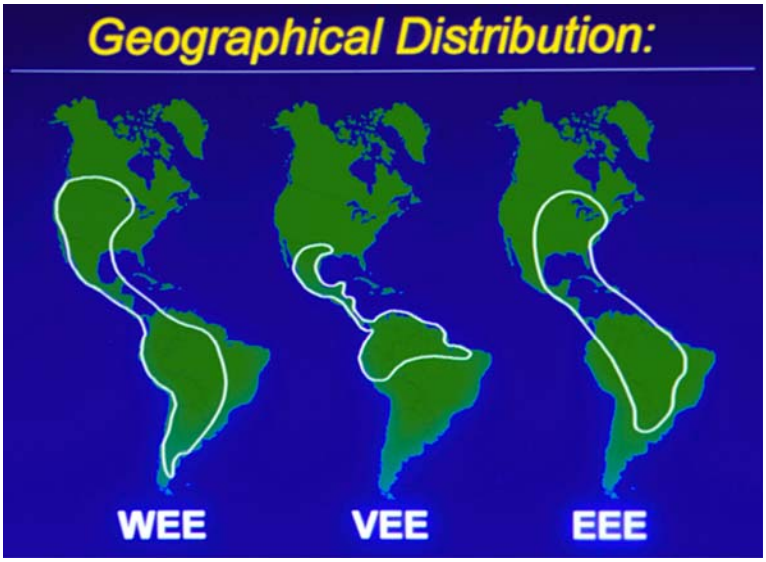
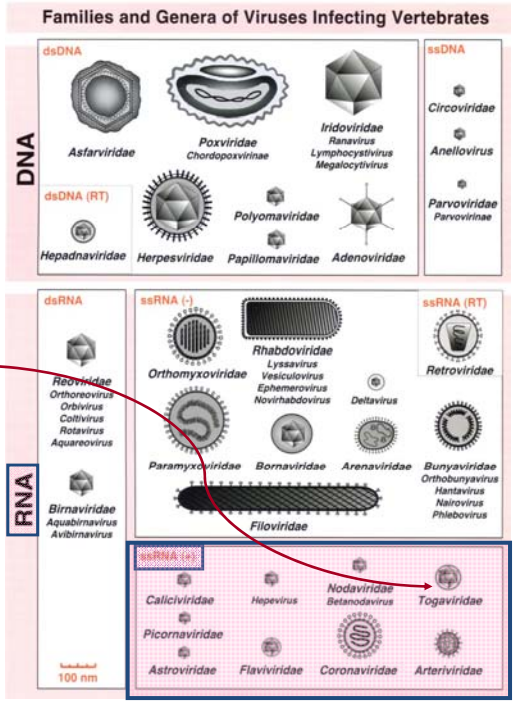


Virus Classification

Family: Togaviridae
Genus: Alphavirus

Arboviruses
ss +RNA (infectious)

Antigen complexes include:
- EEE complex
- WEE complex
- **VEE complex**



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VEE

Subtype	Species	Serotype	Transmission Pattern
I	VEE virus	AB	Epizootic
	VEE virus	C	
	VEE virus	D	Enzootic
	VEE virus	E	
	Mosso das Pedras virus	F	
II	Everglades virus		
III	Mucambo Virus	A	
	Tonate virus	B	
	Mucambo virus	C	
	Mucambo virus	D	
IV	Pixuna virus		
V	Cabassou virus		
VI	Rio Negro virus		

Enzootic VEE Transmission

Primary Vector
Culex (Melanoconion) species

Vertebrate Host: Rodents

Dead end hosts: Humans (sporadic clinical and subclinical cases)

Epizootic VEE Transmission

Primary Vector
multiple
mosquito species

Other species
naturally infected
but not amplifiers

Dead-end hosts:
Humans –epidemics of 'flu
like illness, encephalitis

Vertebrate Host:
Horses

VEE - high viremia in horses – movement control is appropriate

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Epizootic VEEV infections

- Equine pathogen
  - In natural outbreaks, equine cases precede human
    - Equids are primary amplifier for human infections
    - Expect simultaneous impact in bioterrorism event

- Human pathogen
  - Adults experience influenza-like illness
    - Attack rate nearly 100%
    - 1-5 day incubation, illness 1-2 weeks
    - Malaise, spiking fever, rigors, severe headache, photophobia, myalgia in legs and lumbosacral area
    - Nausea, vomiting, cough, sore throat, diarrhea

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Epizootic VEEV infections (continued)

- Human pathogen (continued)
  - Neurologic manifestations in small percent of elderly and children
    - Neck stiffness, convulsions, coma, paralysis.
    - Most VEE fatalities occur in children (up to 20%)
- Transmissible by aerosol
  - Human-human transmission in natural outbreak is negligible
  - Virus is stable in aerosol form

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Case Fatality Rates

	Equine	Human
WEE	20-40%	~10%
EEE	~ 90%	~33%
VEE	38-90%	<1 – 20%*

*most VEE fatalities occur in children

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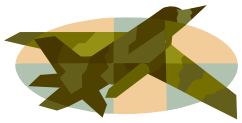

VEE as Bioweapon

- FDR authorized development of VEE as a biological warfare agent with offensive and defensive objectives
  - Incapacitating agent
  - Led to development of attenuated TC-83 vaccine strain and inactivated C-84 vaccine strain
  - Aerosol, solid, liquid forms possible
- R. Nixon cancelled biological weapons program
- Other countries were/are suspected of VEE as BT

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VEEV – Existing PCR assays

- **Purpose: Detect VEEV RNA in clinical samples from horses**
  - Brain is preferred sample
  - Horse is an amplifying host
- **Target: E2 membrane glycoprotein**
  - Associated with virus attachment to cells
  - Antibody to E2 neutralizes VEEV
  - Subtype differentiation by primer selection and sequencing of amplicon
- **Nested PCR method**
  - Enhances sensitivity
  - Cross contamination risks

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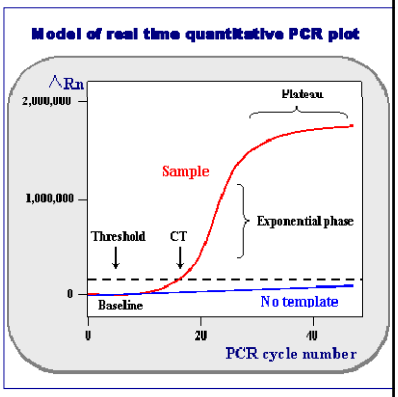
General Analytical Needs

- Infectious dose for humans 10-100 organisms
- Diagnostic testing via PCR
  - To date, no real time PCR for VEE published
- Need to include Subtypes IAB and IC
- Ideal to exclude other Subtypes and serotypes (ID, IE, IF, II, III, IV, V)
- Need to distinguish EEE, WEE, VEE



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VEEV is an Overlap Select Agent USDA/HHS Regulations

- **Includes VEEV Subtypes IAB and IC only**
- Any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC are excluded from Select Agent status, provided that the individual or entity can verify that the agent is within the exclusion category.
  - TC-83 vaccine strain and vaccine candidate strain V3526 are excluded from Select Agent Regulations

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Regulatory Guidance (continued)

- VEE is a **BSL3 Agent**
- TC-83 is <u>not</u> a select agent, can be manipulated at BSL2
- If aerosols of TC-83 are generated, it **reverts to BSL3** due to infectious nature for humans
- Human vaccination is limited to administration by the military as part of the Special Immunization Program / Investigational New Drug Authority
  - Military personnel, laboratory workers

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Standard Method Performance Requirements - Goal

Development of Standard Method Performance Requirements (SMPR) for Venezuelan Equine Encephalitis virus by PCR method, with the possibility of developing a single SMPR for a combination of Venezuelan Equine Encephalitis virus, Western Equine Encephalitis virus, and Eastern Equine Encephalitis virus

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Fitness for Purpose (proposal)

Identification of VEEV in environmental samples

The method must be able to identify VEEV RNA resulting from aerosol, solid or liquid samples. Ideally, the method should include only epizootic VEEV (Subtypes 1AB, 1C) and should exclude all other VEEV. The limit of detection must be lower than one infectious human dose of VEE.

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Fitness for Purpose (proposal)

Identification of VEEV in environmental samples

The useable matrices for the PCR test (aerosol collections, environmental swabs, air filters, etc.) are defined with respect to their complexity, presence of organic material and inorganic material, and the expected duration of detectable alphavirus RNA by PCR.

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Fitness for Purpose (proposal - continued)

Identification of VEEV in environmental samples

The preferential method would be a field-deployable real time PCR. Quantitation of the analyte based on reference standards is desired.

Expansion of an acceptable PCR test to a combination of assays or a multiplex format that would identify and distinguish VEEV, EEEV, and WEEV is desirable.

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QUESTIONS?

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STAKEHOLDER PANEL ON
Agent detection assay:PCR

Background & Fitness for Purpose

Coxiella burnetii

James E. Samuel, PhD
Professor and Chair, TAMHSC
February 3, 2015

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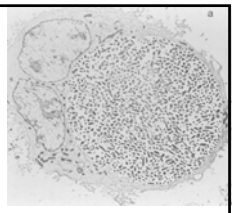

Baca *et al.* 1984

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

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*Seshadri *et al.* PNAS 2003

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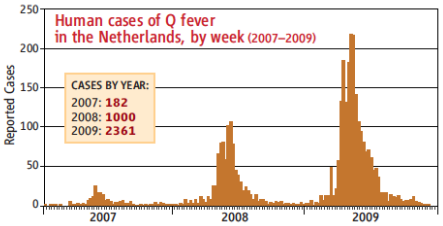
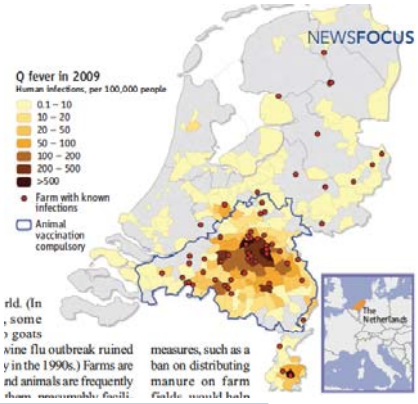


Rising tide. The number of human Q-fever cases exploded in the past 3 years, and the disease, originally concentrated in the south, spread north and east.



rld. (In
, some
› goats
wine flu outbreak ruined measures, such as a
y in the 1990s.) Farms are ban on distributing
nd animals are frequently manure on farm

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

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*15 JANUARY 2010 VOL 327 SCIENCE

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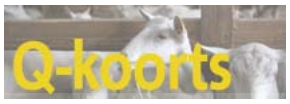

Texas A&M
Health Science Center
Graduate School of Biomedical Sciences

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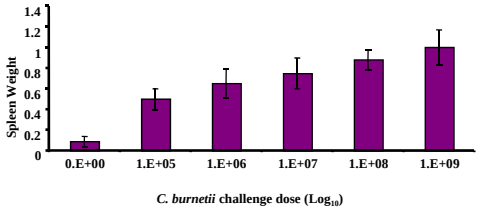
Mouse sublethal challenge model

Infected mice:IP

Wild type mice relatively resistant to clinical disease but readily colonized: splenomegaly correlates with bacterial load

Aerosol and oral challenges models developed for virulence and protection studies



Zhang *et al.* IAI 2004

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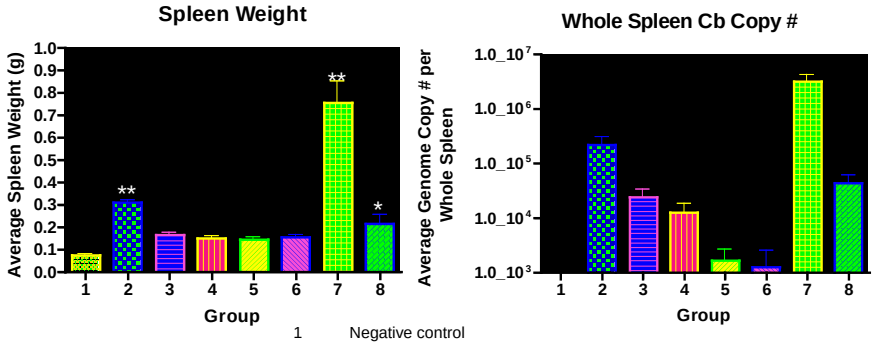
Mouse Infection-derived Immunity: IP Challenge



1	Negative control
2	10^6 Cb inf (3 wk)
3	10^6 Cb inf (3 wk), Doxy (2 wk), rest (1 wk)
4	10^6 Cb inf (6 wk)
5	10^6 Cb inf (3 wk), Doxy (2 wk), rest (1 wk), 10^7 Cb inf (2wk)
6	10^6 Cb inf (6 wk), 10^7 Cb inf (2wk)
7	Non-inf (6 wk), 10^7 Cb inf (2wk)
8	pI vax (6 wk), 10^7 Cb inf (2wk)

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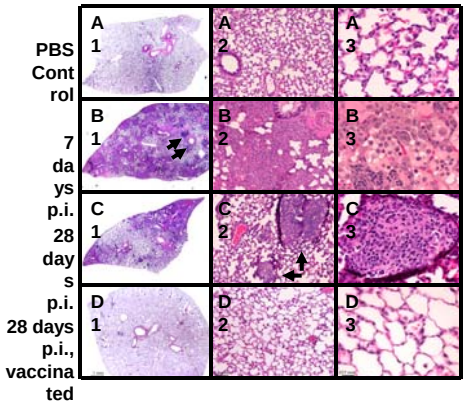
Q Fever Pneumonia: Guinea pig model



C. burnetii infection leads to pneumonia at high doses.
7d pi – panleukocytic bronchointerstitial pneumonia
28d pi – lymphohistiocytic interstitial pneumonia

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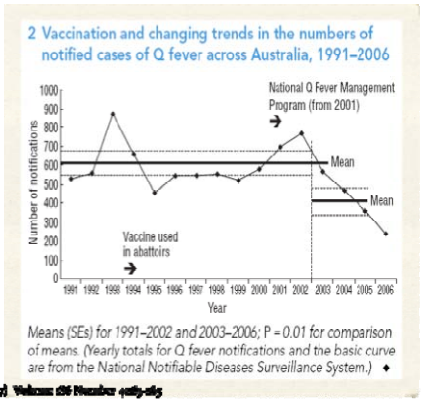
- Formalin inactivated Whole Cell Vaccine (WCV)
  - Q-vax®: Effective Q fever vaccine licensed for used in Australia
  - Long-lived, single dose
  - Not FDA approved: IND material
  - Serious side effects in previously sensitized individuals (local and systemic)
  - Requires pre-screening: Skin test and phase I/phase II serology

- **Next generation vaccines under development**



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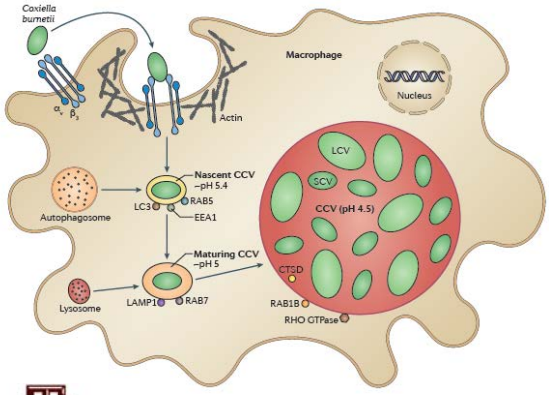
Intracellular lifecycle



Natually obligately intracellular

Recent advances in axeminic media (ACCM2)

Recent genetic and mutant analysis

Primary virulence tools include T4SS and T2SS effectors

van Schaik et al. Nat Rev 2013

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Comparative genomics: Pathotype model

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

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Samuel et al. 1985

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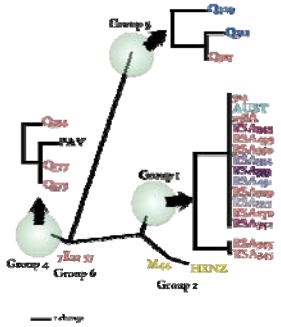
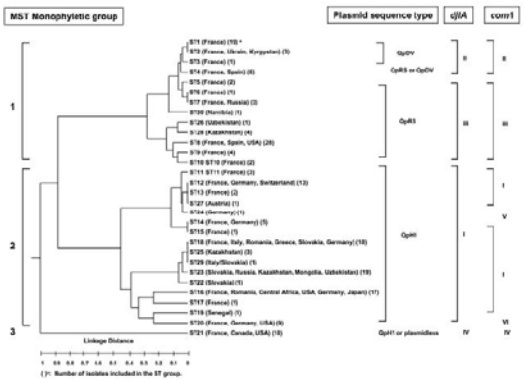
C. burnetii phylogenetic organization



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

Pearson, Keim et al. ASM2005



Glazunova *et al.* EID 2005

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C. burnetii – Existing PCR assays

- **Purpose: Detect *C. burnetii* DNA in clinical and environmental samples**
  - Environmental samples, milk, soil, animal Tissues, air sampling
  - Human blood samples
- **Most common targets:**

✓ **IS1111, mulit-copy IS element**

  - Range in copy number among isolates (~20)

✓ **Com-1: encodes outer membrane (DsbA/C) protein**

  - Highly conserved among isolates
- **qPCR method**
  - Brennan et al (IAI 2003)
  - Sensitivity approaches one genome equivalent

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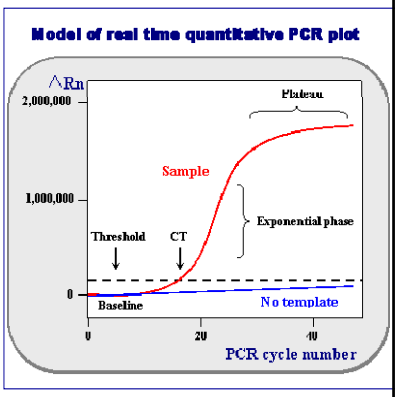
General Analytical Needs

- Infectious dose for humans 10-100 organisms
- Diagnostic testing for Q fever is serologic tittering of IgG and IgM, using phase I and phase II antigens
- To date, no PCR based diagnostic approved for human samples to diagnose acute Q fever, in part, because of transient appearance in serum and whole blood



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Regulatory Guidance (continued)

- Coxiella burnetii is a B list Select Agent requiring **BSL3 containment**
- Nine Mile, RSA439, clone 4, is not a Select Agent, can be manipulated at BSL2 and does **not revert** because of a well characterized, large deletion ((~20 Kbp) which encodes critical O-antigen biosynthetic genes.
- Human vaccination is not available in US and Q-vax, is not licensed in US

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Standard Method Performance Requirements - Goal

Development of Standard Method Performance Requirements (SMPR) for Coxiella burnetii by PCR method, with the possibility of developing a single SMPR for the detection of the diversity of isolate variation

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Fitness for Purpose (proposal)

Identification of *C. burnetii* in environmental samples

The useable matrices for the PCR test (aerosol collections, environmental swabs, air filters, etc.) are defined with respect to their complexity, presence of organic material and inorganic material, and the expected duration of detectable DNA by PCR.

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Fitness for Purpose (proposal - continued)

Identification of C. burnetii in environmental samples

The preferential method would be a field-deployable real time PCR. Quantitation of the analyte based on reference standards is desired.

Expansion of an acceptable PCR test to a combination of assays or a multiplex format is desirable.

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QUESTIONS?

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STAKEHOLDER PANEL ON Agent Detection Assays

Background & Fitness for Purpose

Staphylococcal enterotoxin B

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February 3, 2015

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Background on Staphylococcal enterotoxins

- Pyrogenic exoproteins
- Stable proteins
  - Water soluble
  - Heat resistant
  - Protease resistant
  - Tolerate extreme pH changes
- Twenty-three homologous serologically distinct SEs identified
- SEA-SEE select agent status
- Superantigenic

Human illness associated with SEs

- Staphylococcal food poisoning (SFP)
- Inhalation
- Toxic shock

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Staphylococcal food poisoning

- Ingestion of pre-formed SE
- Nausea, vomiting, abdominal cramping within 2-8 hours of ingestion
- Self-limiting resolving within 24-48 hours

Aerosol exposure

- Based upon accidental laboratory inhalation exposure
- Symptoms noted within 90 minutes-24 hours after exposure
- Symptomatic 3-4 days
  - Fever
  - Headache
  - Muscle aches
  - Pulmonary symptoms
  - GI symptoms

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Superantigenic properties of SEs

- Cross link T-cells with Antigen Presenting Cells
- By-passing antigen processing
- Activates large populations of T-cells
- Release of cytokines
- Toxic shock syndrome symptoms
  - Fever, hypotension vomiting, diarrhea, rash, renal failure
- Associated with SEA, SEB, and SEC

SEs as biothreat agents

- Universal availability
- Ease of production and dissemination
- Potentially could cause widespread illness
- Common in the environment
- Diseases are similar to others
- Initial aerosolization research performed on SEB only
- SEA & SEC shown later to have similar effects

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Category B biothreat agent

- Low mortality/High morbidity
- Easily disseminated in a crowd incapacitating hundreds
- Amount SEB required less than synthesized chemicals
  - ~400 pg/kg body weight incapacitates 50% human population exposed to aerosol attack
    - 175 pound person ~32 ng
  - ~200 ng/kg body weight would be lethal for 50% human population exposed to aerosol attack
    - 175 pound person ~15,800 ng

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Challenges: Ordinary event or act of bioterrorism?

- Accidental food poisoning cases common
  - 241,148 illnesses; 1064 hospitalizations
- Occasional TSS cases reported
- Challenges will be:
  - Monitoring for a common environmental agent
  - Establishing baseline levels

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General analytical needs

- Detection of low levels SEA, SEB, & SEC
- Testing field labs and medical labs
- Variety matrices
  - Food
  - Air filters, water, environmental surfaces
  - Human samples including nasal swabs, sterile body fluids

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Existing Methods - Immunoassays

Commercially available

- ELISA and ELFA
- Lateral flow device
- Surface plasmon resonance

Research only

- Nanopartilce immunosensing
- Electro-Chemiluminescence
- Array Biosensor
- Multiplex suspension array
- Mass spectrometry

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Regulatory Guidance

- Per Food and Cosmetic Act food products with SEA-SEE are violative
- SEA-SEE are on the Select Agent list. Users can possess up to 5mg.

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Fit for purpose: Staphylococcal food poisoning

- Emetic activity linked to SEA-SEE, SEG, SEH, SER, and SES.
- Assay should detect <200 ng/g
- Food matrices
- Time-to-detection 4 hours
- Trained laboratory personnel
- Designed for use as reference method

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Fitness for Purpose: Inhalation

- Toxic shock due to inhalation linked to SEA, SEB and SEC
- Assay should detect <400 pg/kg body weight
- Water, environmental surfaces, nasal swabs, air filters
- Time-to-detection 4 hours
- Trained laboratory personnel
- Designed for use as reference method

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QUESTIONS??

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