March 17, 2015
INTERNATIONAL STAKEHOLDER PANEL ON ALTERNATIVE METHODS (ISPAM)

STAKEHOLDER PANEL MEETING BOOK

Salon D/E
Hilton Washington D.C. North
Gaithersburg, Maryland, 20877

contacts: kmciver@aoac.org or cdent@aoac.org

2015 AOAC MID YEAR MEETING
MARCH 17, 2015
INTERNATIONAL STAKEHOLDER PANEL ON ALTERNATIVE METHODS–
LIST OF REGISTERED ATTENDEES

Name	Affiliation	Country
PATRICE ARBAULT	Nexidia	France
BRAD BARRETT	ABSCIEX	USA
DEANN BENESH	3M Food Safety	USA
JAMES BLACK	The Kroger Company	USA
PETER BODNARUK	Tyson Foods	USA
JOE BOISON	Canadian Food Inspection Agency	Canada
MICHAEL BRODSKY	Brodsky Consultants	Canada
EVAN CHANEY		USA
YI CHEN	FDA - CFSAN	USA
MIKE CLARK	Bio-Rad Laboratories	USA
JO MARIE COOK	Florida Department Of Agriculture And Consumer Services	USA
ERIN CROWLEY	Q Laboratories, Inc.	USA
CHRISTOPHER DENT	AOAC INTERNATIONAL	USA
GREGORY DIACHENKO	FDA - CFSAN	USA
ROBERT DONOFRIO	NSF International	USA
ERIN DREYLING	Roka Bioscience	USA
PHILIP FELDSINE	BioControl Systems, Inc.	USA
IMOLA FERRO	MicroVal	Netherlands
ARLENE FOX	AOAC INTERNATIONAL	USA
VIRENDRA GOHIL	Maxxam Analytics	Canada
QIAN GRAVES	FDA - CFSAN	USA
THOMAS HAMMACK	FDA - CFSAN	USA
ANTHONY HITCHINS	FDA - CFSAN (Retired)	USA
IRENE IUGOVAZ	Health Canada	Canada
ROBERT JECHOREK	3M Food Safety	USA
RONALD JOHNSON	BioMérieux, Inc.	USA

NAME	AFFILIATION	COUNTRY
GEORGE JOSEPH	AsureQuality, New Zealand	New Zealand
DAVID KENNEDY	Phenomenex	USA
ESTELA KNEETEMAN	Instituto Nacional De Tecnologia Industrial Centro De Cereales Y Oleaginosas	Argentina
ANTHONY LUPO	Neogen Corporation	USA
PAUL MILNE	Keurig Green Mountain, Inc.	USA
DEEPALI MOHINDRA	Thermo Fisher Scientific	USA
JEFFREY MOORE	US Pharmacopeia (USP)	USA
MARIA OFITSEROVA	Pickering Laboratories, Inc.	USA
LAWRENCE PACQUETTE	Abbott Nutrition	USA
EFSTATHIA PAPAFRAGKOU	FDA/CSFAN	USA
TOM PHILLIPS	MD Department Of Agriculture	USA
LARS REIMANN	Eurofins Scientific, Inc.	USA
KYLE RHODEN	DuPont Nutrition & Health	USA
LEILA SALDANHA	Office of Dietary Supplements, NIH	
YVONNE SALFINGER	Association Of Public Health Laboratories	USA
BROOKE SCHWARTZ	Brooke Schwartz Consulting	USA
SUPAT SIRIVICHA	Eurofins	USA
JOHN SZPYLKA	Silliker Laboratories	USA
ROBYN WOODBURY	ATCC	USA
JINCHUAN YANG	Waters Corporation	USA
JUPITER YEUNG	Nestle Nutrition	USA
LINGSU ZHANG	USDA-AMS	
JOSEPH ZHOU	Sunshineville Health Products, Inc	USA
JOYCE ZHU	Jamieson Laboratories	Canada
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IMOLA FERRO	MicroVal	Netherlands

ERIN CROWLEY, Q LABORATORIES, INC.

Chair, AOAC INTERNATIONAL STAKEHOLDER PANEL ON ALTERNATIVE METHODS

Erin Crowley has been the Microbiology Research and Development Supervisor at Q Laboratories, Inc. in Cincinnati, Ohio since 2006. For the past 8 years, Erin and her R&D team have served as an independent third-party laboratory with a primary focus on providing high quality method validation for microbiological rapid detection methods. These validations include Independent laboratory evaluations for pathogen detection, qualitative methods and confirmatory biochemical assays for AOAC Official Methods of Analysis, AOAC Research Institute Performance Tested Methods Program and MicroVal. In addition to being an active member of the International Association of Food Protection (IAFP) and AOAC, Erin currently serves as Vice-Chair of the AOAC Official Methods Board and ISPAM Chair and Fresh Produce Initiative Chair of the SMPR Working Group on Salmonella in Leafy Greens. Erin earned a B.S. from the University of Cincinnati in Cincinnati, Ohio and an M.A. from Tufts University in Medford, MA.

BROOKE SCHWARTZ, BROOKE SCHWARTZ CONSULTING

Chair, AOAC ISPAM FRESH PRODUCE INITIATIVE

As Principal of Brooke Schwartz Consulting, Ms. Schwartz consults to life science companies that are expanding through organic growth, acquisition and alliances. Ms. Schwartz deploys small teams with a breadth of business and technical expertise in applied Life Science markets to assist clients with development and commercialization of new technologies. Engagements include the development of market entry strategies and establishment of collaborations with private sector customers, government stakeholders and strategic partners. She currently serves as a Chair-Elect of the AOAC Research Institute; Co-Chair of the AOAC ISPAM Fresh Produce Initiative; and member of the Executive Committee of the AOAC Pacific Southwest Section. Ms. Schwartz previously held business management and corporate development roles at Applied Biosystems / Life Technologies. As Business Segment Leader for Food and Environmental Testing, she led the re-launch of the global food and environmental testing business, including introduction of a validated assay portfolio and development of next generation technologies. As Senior Director for Corporate Strategy and Merger Integration at Applied Biosystems, she led merger integration for acquisitions including Ambion Inc. and Agencourt Personal Genomics. Prior to Life Technologies, she led strategy, innovation and merger integration engagements for Deloitte Consulting's health care and life science practice, and previously served as Director of Biotechnology Alliances and Acquisitions for Monsanto Company. Ms. Schwartz earned an M.B.A from the Harvard Business School, an M.S. in Food and Resource Economics from the University of Florida, and a B.A. in Latin American Studies from the University of California, Los Angeles.

PRESENTER BIOS

TOM HAMMACK, FDA
ISPAM SALMONELLA HARMONIZATION WORKING GROUP

Mr. Hammack has been research microbiologist with the Food and Drug Administration since 1990 and has served as Chief of the Microbial Methods Development Branch of CFSAN's Division of Microbiology since 2009. He is a co-author of FDA's *Bacteriological Analytical Manual's* (BAM) *Salmonella* and *Food Sampling and Preparation of Sample Homogenate* chapters. In addition to his role as a BAM Chapter author, he serves as the Chair of the BAM Council. His research has been concentrated on the development and validation of cultural methods for the detection and isolation of *Salmonella* from foods. Over the last 10 years, the emphasis of his research has been the detection and isolation of *Salmonella* from fresh produce. In addition to his work in the lab, Mr. Hammack has an interest in food microbiology methods validation. Since 2004, he has served as a General Referee (now Process Expert) for food microbiology for AOAC International. In that capacity, he has overseen the validation of numerous microbiological methods for bacterial pathogens, such as *Salmonella*, *Listeria*, and *E. coli* O157:H7 through AOAC International's two methods validation programs: the Official Methods of Analysis and Research Institute Performance Tested Methods Programs. AOAC validated methods are used by FDA and commercial laboratories for the detection of pathogens in foods. Mr. Hammack also serves as Chair of the US Technical Advisory Group to ISO TC 34/SC 9. ISO TC 34/ SC9 is the committee from which all ISO food microbiological methods arise. He received his BS and MS degrees from the University of Maryland at College Park.

ERIK KONINGS, NESTLE
CHAIR, AOAC STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

Erik Konings has been an active member of AOAC since 1997. He is currently serving as a director on the Board of Directors and President of AOAC INTERNATIONAL. Previous AOAC volunteer roles have included chairmanship of the SPIFAN Working Group on Folic Acid, membership on the AOAC Methods Committee on Food Nutrition, and service as a General Referee for Water Soluble Vitamins. Erik Konings started his professional career at the then called Food Inspection Service in Maastricht, the Netherlands. Konings was involved with the development of analytical methods for the analysis of vitamins in food and food products. In 1996 he started his PhD study "Dietary folates in human nutrition" in collaboration with the departments of Human Biology and Epidemiology of Maastricht University. During this study, which he completed in 2001, he obtained a MSc-degree in epidemiology. Konings has worked as Senior Scientific Staff Officer in the department of Research & Development of the Food and Consumer Product Safety Authority (VWA) in the in the Netherlands, as Scientific Officer at the Data Collection and Exposure Unit for the European Food Safety Authority (EFSA) in Parma, Italy, and since June 2009, in a position in the Quality and Safety Department of the Nestlé Research Center in Lausanne, Switzerland. Konings is convenor of a working group on vitamins & carotenoids of the European Committee for Standardization (CEN), a member of the International Dairy Federation (IDF), Standing Committee Analytical Methods for Additives and Contaminants, and participates in Codex Committee for Methods of Analysis and Sampling (CCMAS). In 2012 he was appointed convenor for ISO TC 34 Working Group 14 on Vitamins, carotenoids, and other nutrients. He has (co)authored more than 30 scientific publications.

EFSTATHIA PAPAFRAGKOU, FDA
PRESENTER: Challenges to Testing for Food-bourne Viruses in Food Samples

Dr. Efstathia (Efi) Papafragkou earned her Ph.D in 2007 in Food Microbiology from North Carolina State University. Her thesis focused on the persistence, transfer and detection of human enteric viruses in foods. Dr. Papafragkou was awarded a post-doctoral fellowship from the American Society of Microbiology and joined the National Calicivirus Laboratory at the Centers for Disease Control and Prevention. As a post-doctoral research associate at CDC, she completed her training and specialized on the application of cell culture techniques for cultivation of foodborne viruses. In 2010 she became a member of the Molecular Virology Team at the Center for Food Safety and Applied Nutrition at the Food and Drug Administration. Her research interests include method development for sample preparation, molecular detection, characterization and cell culture for quantifying foodborne pathogenic viruses from food, clinical and environmental samples. She is also involved in method validation studies, and teaching/training courses. Since joining FDA she continues to communicate her research through publications, participation in professional conferences, and presentation in scientific meetings and workshops.

FABIENNE LOISY-HARMON, ceeram
PRESENTER: ISO Technical Specifications of Viruses: How are they Relevant to Service Laboratories and Assay Manufacturers?

Fabienne Loisy-Hamon has a PhD in microbiology with a specialty in molecular virology. She has been studying human enteric viruses in environment and food samples since 12 years, developing molecular methods for the detection of these viruses and studying their persistence in different types of environment. From 2005 to 2014, she was, with Benoît Lebeau, the co-founder and CSO of ceeram, a company specialized in molecular identification of microbial agents. She is now bioMérieux food business- R&D virology manager. She is an expert member for the European Committee of Normalization, for Afnor and also Afssa in working groups concerning food borne viruses and molecular detection of food and feed pathogens. She has been publishing several papers and giving several oral communication in international conferences concerning viruses subject. Her expertise is so worldwide recognized. In July 2013, ceeram was the recipient of the “Food Safety Innovation Award” for its expertise in food borne viruses.

The Scientific Association Dedicated to Analytical Excellence®

Meeting of the International Stakeholder Panel on Alternative Methods (ISPAM)

March 17, 2015
10:30AM – 5:00PM EDT

Hilton Washington DC North | Perry Parkway | Gaithersburg, MD, USA

DRAFT MEETING AGENDA

I. Welcome and Introductions (10:30 a.m. – 10:45 a.m.)
Erin Crowley, Q Laboratories, Inc., Chair, ISPAM

II. Standards Development Overview (10:50 a.m. – 11:10 a.m.)
Deborah McKenzie, AOAC INTERNATIONAL

III. Update: ISPAM Fresh Produce Initiative (11:10 a.m. – 11:30 a.m.)
Brooke Schwartz, Brooke Schwartz Consulting, Chair, ISPAM Fresh Produce

IV. Stakeholder Panel on Strategic Food Analytical Methods Update (11:30 a.m. – 12:00 p.m.)
In conjunction with the SPSFAM Chair Erik Konings, Erin Crowley will lead a discussion on areas of potential overlap between the two panels.

V. Working Group Launch: Harmonization of Salmonella Methods (1:00 p.m. – 2:30 p.m.)
Tom Hammack, FDA, CFSAN

a. Presentation of WG objectives and goal, *Tom Hammack, FDA, CFSAN & Chair, WG*
b. Discussion and Vote on Working Group objectives and goal – *ISPAM**

----------Lunch 12:00 p.m. – 1:00 p.m. On Your Own----------

VI. Overview of Standards for the Detection of Viruses (2:30 p.m. – 4:30 p.m.)
Patrice Arbault, BioAdvantage Consulting;

a. Challenges to Testing for Foodborne Viruses in Food samples: Current Standard Methods and Future Directions – *Efi Papafragkou, FDA, CFSAN*
b. ISO Technical Specifications for Viruses: How are they Relevant to Service Laboratories and Assay Manufacturers – *Fabienne Loisy, CEERAM (European Centre for Expertise and Research on Microbial Agents);*
c. SPADA and the Development of Standard Method Performance Requirements (SMPR) for Smallpox – *Scott Coates, AOAC Chief Scientific Officer*

VII. Next Steps (4:30 p.m. – 5:00 p.m.)
Erin Crowley, Q Laboratories, Inc., Chair, ISPAM

VIII. Adjourn

Meeting of the International Stakeholder Panel on Alternative Methods (ISPAM)

March 17, 2015
10:30AM – 5:00PM EDT

Erin Crowley
Chair, ISPAM
Microbiology R&D Supervisor, Q Laboratories, Inc.

Agenda

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c. SPADA and the Development of Standard Method Performance Requirements (SMPR) for Smallpox – ***Scott Coates****, AOAC Chief Scientific Officer*

VI. Next Steps (4:30 p.m. – 5:00 p.m.)

Erin Crowley, Q Laboratories, Inc., Chair

Update on Initiatives

Annual Meeting 2014- Boca Raton

- Brainstormed Ideas on Future Initiatives
  1. Approved **WG development of Harmonization of BAM and ISO Salmonella methods**
     - *Chaired by Tom Hammack- FDA-CFSAN*
     - *15 member group as of 1/20*
  2. Viruses
     - SMPRs
     - Certified Reference Material
  3. Review of current Validation Guidelines for Identification Methods (SO/WD 16140-6)

Next Steps- Fresh Produce

- First method validated?
- Identify next product for development of SMPR and expansion of Sampling Plan
  - Tomatoes?
  - Fresh herbs?
  - Peppers?
- Engage Key Opinion Leaders in FP Industry to expand on ideas and collaborations

ISPAM Fresh Produce Initiative Update

Presentation to International Stakeholder Panel on Alternative Methods (ISPAM)
March 17, 2015

Brooke Schwartz
Principal, Brooke Schwartz Consulting
Co-Chair, ISPAM Fresh Produce Initiative

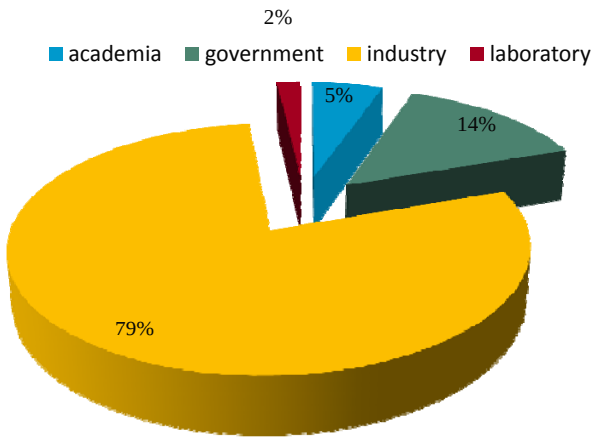
Fresh Produce Project Overview

- The produce industry was identified as a community that is underserved by AOAC
- Produce industry input on key issues – sampling was highest priority.
- Project adopted by ISPAM in 2013 and funded by AOAC Research Institute
- Initial focus: *Salmonella* in leafy greens
- Initial goals:
  - Develop best practices for sampling *Salmonella* in leafy greens fields
  - Develop an SMPR for *Salmonella* detection methods for leafy greens
  - Integrate SMPR and sampling best practices

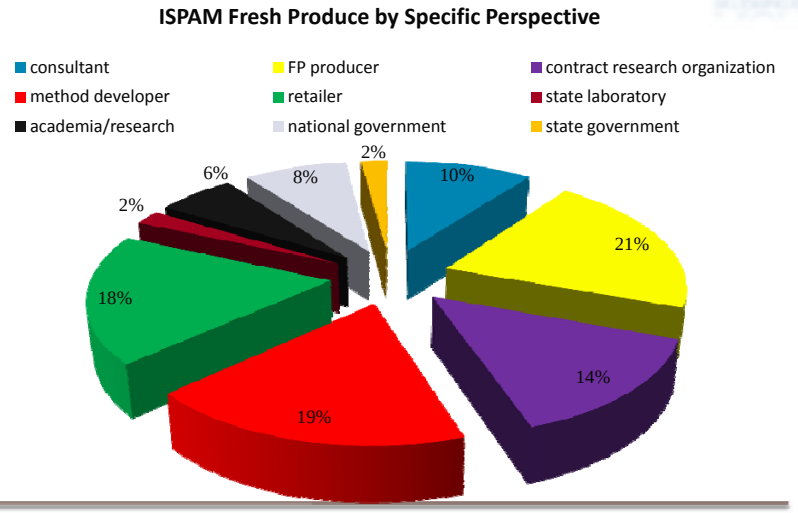
Stakeholder Participation

Chair, Fresh Produce Stakeholder Panel	David Acheson *The Acheson Group LLC*
Co-Chair, Fresh Produce Stakeholder Panel	Brooke Schwartz *Brooke Schwartz Consulting*
Chair, Working Group on Sampling Plan	David Gombas *United Fresh Produce Association*
Chair, Working Group on SMPR for *Salmonella*	Erin Crowley *Q Laboratories*

Stakeholder Participation



Stakeholder Participation



Visits to Growers and Processors

- April 2014 - Participants Fresh Produce Working Groups toured Salinas Valley produce fields and processing facilities
- Team observed in-field sampling and harvesting activities, and processing / packaging of fresh and bagged products
- Products included leafy greens and strawberries
- Tour organized by David Gombas, United Fresh, and hosted by
  - Church Brothers
  - Naturipe Berry Growers
  - Earthbound Farm
  - Dole Fresh Vegetables

Visits to Growers and Processors – Salinas Valley

SMPR Working Group

Chair – Erin Crowley, Q Laboratories

Salmonella SMPR Working Group Work to Date

- First Meeting on November 14th, 2013
- Telecons every 2 weeks. 1 x month post- Mid-Year Meeting
- 2 face-to-face meetings
- SMPR Document Drafted
- 30 day public comment period (June 25, 2014 – July 25, 2014)
- SMPRs approved by ISPAM/FP at Annual Meeting September 2014

Salmonella SMPR Working Group

- Drafted SMPR Document: *Detection of Salmonella species in romaine lettuce and baby spinach*
- Submitted for public comment
- Reviewed and addressed comments
- Reviewed and approved by ISPAM/FP

SMPR Key elements:

- Applicability
- Definitions
- Method performance criteria
- Inclusivity / Exclusivity

SMPR Key Points

- ***Applicability***
  - Pre-Harvest Commodities
- ***Definitions***
  - Align with current validation guidelines
  - AOAC Appendix J
  - ISO 16140 (2003) Standard
  - ISO/FDIS 16140-1

SMPR Key Points cont'd

- ***Method Performance Criteria***
  - SLV, MLV, Verification
  - Statistical Considerations
  - Maximum Time to Determination
- ***Inclusivity/Exclusivity***
  - Common set of genera and species for Inclusivity and Exclusivity
  - Inclusivity- strains implicated in the past 5 years, produce specific
  - Exclusivity- Critical cross-reacting genera should be represented

Comments Received

- 66 comments received and addressed by WG
- General comments regarding footnotes, typos and clarification
- Revised definition of Baby Spinach and Romaine Lettuce
- Eliminated Annex I: Controls (positive, negative, inhibition control)
- Specified Maximum Time to Determination as ≤ 24 hours.
- Content-specific
  - Inclusivity- specify "must-test" and minimum representation of subspecies (*salamae, houtenae, bongori, arizonae, diarizonae*)
- Follow-up question needed to be addressed by ISPAM
  - RLOD

Comments Received

1. Method Performance Requirements

Parameter	Parameter Requirements	Target Test Concentration*	Minimum Acceptable Results
Acceptable Minimum Detection Level (AMDL)	SLV: Minimum of 20 replicates per food type, artificially inoculated as outlined in internationally accepted method validation guidelines.	1 to 5 cfu / test portion	25 to 75% positive rate; and dPOD ≥ 0, LCL < 0, UCL > 0 **
High concentration	SLV: Minimum of 5 replicates per food type artificially inoculated as outlined in internationally accepted method validation guidelines at 10x the AMDL concentration.	10 to 50 cfu / test portion	100% correct analyses are expected per food type‡
Zero concentration	SLV: Minimum of 5 replicates per food type that have tested negative with the reference method in the validation study and have not been artificially inoculated.	0 cfu / test portion	
LPOD	Multi-laboratory study.	1 – 10 cfu / test portion 10 to 50 cfu / test portion	0.15 ≥ $LPOD_c$ ≥ 0.85 dLPOD† = LPOD§ ≥ 0.95 dLPOD† =
$LPOD_{(0)}$	Multi-laboratory study.	0 cfu / Test portion	LPOD‡‡ ≤ 0.05
RLOD	Single laboratory study	Combined levels	RLOD =TBD
	Multi-laboratory study		RLOD = TBD

Motion Approved

- Motion to accept the SMPRs for *Detection of* Salmonella *species in romaine lettuce and baby spinach* as presented.
- Unanimous approval on 9/6/14

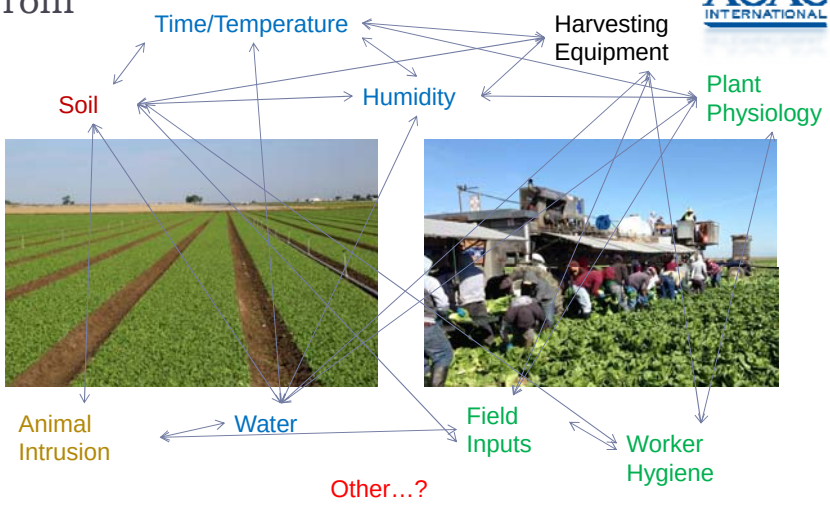
Next Steps

- Identify next product for development of SMPR and expansion of Sampling Plan
  - Tomatoes?
  - Fresh herbs?
  - Peppers?
- Engage Key Opinion Leaders in FP Industry to expand on ideas and collaborations

Sampling Plan Working Group

Chair – David Gombas, United Fresh

Where Does Contamination come From



Sampling Plan Update

Current Situation:

- Statistically valid sampling plans (e.g., ICMSF) were developed for processed foods, where assumption of "contamination uniformly distributed" is likely to be valid
- Published studies and industry testing has demonstrated that field contamination, when it occurs, is most likely to be sporadic, not uniformly distributed, so assumption is invalid
- Most sporadic detections in field are inexplicable and non-repeatable

Sampling Plan Update

Current Situation:

- Some operations using "Z-pattern", some using multiple Z-patterns, some using serpentine, some using directional sampling, some test upon receipt at the processing facility
  - None are developed to be statistically valid

- Currently no statistically valid field sampling protocol
  - A single "positive" condemns the whole field – no depth of analysis to indicate the degree of field contamination.
  - Negative test results are meaningless – a future "positive" invalidates the field test results

Sampling Plan Update

Current Situation:

- Fields will not be sterile
  - Industry data: leafy greens field operating under GAPs will still have about 0.2% frequency of detectable pathogens in field
  - Sampling to prove "pathogen-free" is impractical

Sampling Plan Update

Objective:

- Evaluate existing sampling protocols (industry, FDA "site-specific risk-based approach")
- Identify/recommend/develop a field sampling protocol, e.g.,
  - For routine sampling (e.g., to meet a customer requirement)
  - For cause or investigative sampling (e.g., if a potential food safety issue is identified)
    - Directional/gradient sampling (e.g., from least likely to most likely areas)
  - Define a sampling lot, while considering field assessments and historical data
- Determine what level of statistical confidence can be achieved

Sampling Plan Update

Objective:

- Use "testing of a Romaine lettuce field for Salmonella" as the model
  - Develop a field sampling program
  - Training program for samplers, including a test and hold, taking in to account normally occurring events (weather events, field activity, employee limitations) that impact implementation of program
  - Evaluate whether the sampling protocol can be extended to other commodities (e.g., spinach or strawberries for EHEC) and target analytes
- Highlight the need for rapid and fit for purpose methods that ensure that data collected are reliable and repeatable and the method is implementable in other labs.

Next Steps

- Finalize Sampling Plan
- Reengage produce industry / expand participation to determine interest in next set of crops / matrices / targets

AOAC® Standards Development and Official Methods of Analysis Overview

Deborah McKenzie, רב
AOAC INTERNATIONAL,
Sr. Director, Standards Development &
AOAC Research Institute

March 2015

AOAC INTERNATIONAL HEADQUARTERS
2275 Research Boulevard, Suite 300
Rockville, Maryland 20840, USA

About AOAC INTERNATIONAL

AOAC is a scientific standards development association dedicated to analytical excellence.

- ~ 3000 members worldwide including **organizational affiliate members**
  - 1/3 of members overseas
- Established a wholly owned subsidiary – AOAC Research Institute
  - administers AOAC conformity assessment programs
- Maintains 16 active international sections representing over 90 countries
- Develops voluntary consensus standard method performance requirements (SMPRs)
- Publishes the *Official Methods of Analysis of AOAC INTERNATIONAL*
- Maintains an accredited Laboratory Proficiency Testing Program
- Governed by a membership-elected volunteer Board of Directors

AOAC® INTERNATIONAL (AOAC) is an independent third-party international standards developing organization and AOAC has no vested interest in the development of standards or in the evaluation of methods of analysis.

About AOAC INTERNATIONAL

AOAC leverages its networks to gather stakeholders and experts to:

- Develop international voluntary consensus standards method performance requirements
- Discuss & adopt methods that are published in the *Official Methods of Analysis of AOAC INTERNATIONAL* using judgment of the world's leading experts.

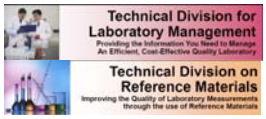
Providing fit for purpose methods through standards development

● *General Locations of AOAC stakeholder panel participants* ● *General Locations of the 16 AOAC INTERNATIONAL current Sections*

About AOAC INTERNATIONAL

- AOAC offers a number of resources through its goods and services; however, AOAC does not:
  - Regulate products
  - Buy or sell food, beverage products, or proprietary technologies
  - Promote specific food and beverage products
  - Set tolerance levels
  - Own a laboratory or provide laboratory services

About AOAC INTERNATIONAL - Resources



Analytical Communities

AOAC's Proficiency Testing Program

SMPR℠

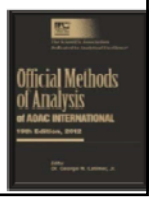
AOAC Mid-Year Meeting

AOAC Annual Meeting & Exposition

EDUCATE NETWORK COLLABORATE

AOAC RESEARCH INSTITUTE

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 000000

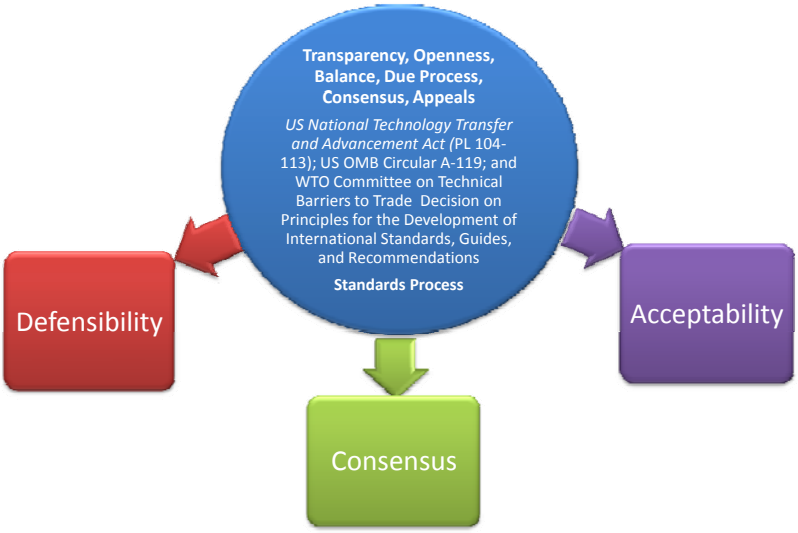


About AOAC INTERNATIONAL - Power of Many

As a scientific association, AOAC *brings scientists together to do a job together that they should not do alone.*

- AOAC leverages its global networks and the value of its independent third party status to provide opportunities for scientific stakeholder groups to *talk about methods* driven by the need for reliable, scientifically valid, fit for purpose methodology.
- Reliable, scientifically valid, fit for purpose methodology are attained by beginning with the development of voluntary consensus standards.
- Methods deemed that meet the voluntary consensus standard are considered fit for purpose and are adopted and published in the *Official Methods of Analysis of AOAC INTERNATIONAL.*

AOAC Creates International Standards



AOAC INTERNATIONAL

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

Transparency

Openness

Balance of Interests

Due Process

Consensus

Appeals

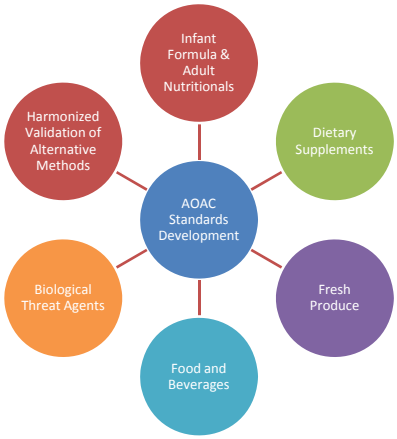
Accomplishments

77	**The number of new fit for purpose First Action methods adopted and published in the *Official Methods of Analysis of AOAC INTERNATIONAL* since 2011**
11	The number of First Action OMA adopted through the AOAC Research Institute since 2013
47	**The number of AOAC voluntary consensus standards developed since 2010**
65	The number of analytes covered by AOAC voluntary consensus standards since 2010
35	The number of analytes for which AOAC voluntary consensus standards are currently in development
12	The number of working groups in process for drafting AOAC voluntary consensus standards
7	The number of working groups being launched in 2015
>230	The number of methods processed and reviewed by AOAC ERPs

ISO and AOAC Sign Cooperation Agreement for Joint Development and Approval of Common Standards
(for milk and milk products)

How does AOAC do this?

- Active AOAC stakeholder panels cover a range of topics including



Advisory Panel*
Stakeholder Panel*
Working Group*
Expert Review Panel*
AOAC Official Methods Board
AOAC Board of Directors

**Managed by AOAC Staff*

AOAC Advisory Panels

Works with staff to:

- Identify key stakeholders
- Identify subject matter experts
- Frame issues & set priorities for standards development
- Facilitate financial support
- Stakeholder Panel Chair moderates panel discussions

Working Group (WG) Initiative

- December 2014, AOAC Board of Directors initiates WG Initiative
  - as an a mechanism for AOAC Organizational Affiliate members to initiate relevant standard development projects using existing AOAC stakeholder panels
    - Expressed a need for a consensus standards and scientifically valid fit for purpose consensus methodology
    - WG supported through AOAC Organizational Affiliates funded and formed through AOAC staff
    - AOAC works with Organizational Affiliates to find additional Organizational Affiliates with the same need for scientifically valid fit for purpose methodology
  - WG will develop SMPR to present to an existing stakeholder panels for review

Why the new WG Initiative?

- Offers companies the opportunities to solve challenges without waiting on priorities of existing stakeholder panels
  - Advisory Panel participation and discussion
- WG's funded by current OA's and new companies interested in addressing immediate needs
  - for analytical standards/standard method performance requirements; and
  - scientifically valid fit for purpose methodology.

Stakeholder Panel Composition

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

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

AOAC Stakeholder Panels

- To deliberate on priorities that result in reaching consensus on AOAC voluntary consensus standards
  - Chair of Stakeholder Panel vetted by the AOAC Official Methods Board and appointed by the President of AOAC.
  - Representative Stakeholder Voting Panel members vetted by AOAC Official Methods Board to ensure balance of perspectives represented in determining consensus.
  - Anyone with a material interest can participate in stakeholder panel deliberations.
- Stakeholder Panel form working groups and uses working groups to develop draft standards.
- Working group chair presents standard to stakeholders.

Stakeholder Panels – Voting Panel

- To demonstrate consensus of the stakeholder panel
- Organizations do not have permanent seats or appointments on any given stakeholder panel
  - Balance of Perspectives driven
- Voting panel is determined for each meeting of a stakeholder panel using those registered for a stakeholder panel meeting
  - Vetting through AOAC Official Methods Board

Working Groups

- Chair approved/appointed by Stakeholder Panel chair
- Engage in the detailed discussions and work of the stakeholders
- Develop draft fitness for purpose and standard method performance requirements (SMPRs) or other draft standard as proposed by stakeholder panel
- Recommend draft standards to the stakeholder panel
- Managed by staff

Standard Methods Performance Requirements (SMPRs)

AOAC SMPR 2011.006

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

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

After SMPRs are Approved

AOAC *Official Methods of Analysis*℠

- AOAC issues a Call for Methods
  - Using the stakeholder voluntary consensus SMPR
- AOAC issues a Call for Experts
  - Establish an AOAC Expert Review Panel to review methods for AOAC Official

After SMPRs are Approved

- AOAC *Performance Tested Methods*℠
- AOAC *Official Methods of Analysis*℠
  - Commercial/Proprietary Method Developers can submit their methods to AOAC Research Institute for either or both (harmonized) AOAC programs
  - Will follow normal processes for each program.

AOAC Expert Review Panel (ERP)

- All candidates are vetted by AOAC Official Methods Board (OMB)
- Approved members are appointed by President of AOAC
- ERP member must go through ERP Orientation
- ERP Review methods for AOAC First Action *Official Methods* status
- Adopt methods as AOAC First Action *Official Methods* status
- Tracks First Action methods for 2 years after adoption

Final Action *Official Methods*

- During the Tracking Period:
  - ERP reviews any information on reproducibility, user feedback, etc.. using guidance by AOAC OMB (OMA, Appendix G)
- When ERP has sufficient information it can:
  - Make a recommendation for Final Action Official Method status
  - Make a recommendation to repeal the Official Method
- Official Methods Board
  - Reviews ERP recommendations and renders decisions on Final Action status or repeal

Documentation and Communication

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

Questions?

Thank you.

Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM)

Erik J.M. Konings
Nestlé Research Center, Nestec Ltd.
Lausanne, Switzerland

AOAC SPSFAM History

- AOAC initiated this panel to address issues of Organizational Affiliate (OA) members - specifically the multi-national food and beverage companies
- SPSFAM focuses on the OA issues and builds consensus within the community related to food or strategic growth of the food industry

SPSFAM Participants and Agenda

- AOAC INTERNATIONAL Organizational Affiliates
- Multinational Food Companies
- All give direction on the analytical needs for the food industry

SPSFAM Inaugural Meeting

- SPSFAM Inaugural Meeting held on June 30, 2011
- SPSFAM Meeting held twice a year
- Initial areas decided by the Advisory Panel include antioxidants, contaminants, flavanols, and ingredients
- Working groups initiated and *Standard Method Performance Requirements* (SMPRs) developed in each area

AOAC Organizational Affiliate Members

- 3M Food Safety
- Abbott Nutrition
- AB SCIEX
- Agilent Technologies, Inc.
- American Proficiency Institute
- Archer Daniels Midland Company
- Bio-Rad Laboratories
- BioControl Systems, Inc.
- bioMérieux, Inc.
- Bruker Daltonics
- Canadian Food Inspection Agency
- CEM Corporation
- Coca-Cola Company
- DuPont Qualicon
- Elanco/Eli Lilly & Co.
- Fertilizer Institute
- Fonterra Cooperative Group Ltd.
- Health Canada
- Herbalife
- Hershey Center for Health And Nutrition
- Kellogg's Company
- Kraft Foods, Inc.
- Mars
- Mead Johnson Nutrition
- Medallion Labs
- Merck KGaA – EMD Millipore
- Mérieux NutriSciences
- Microbac Laboratories, Inc.
- Microbiologics, Inc.
- MPI Research
- Neogen Corporation
- Nestlé
- NSF International
- NSI Solutions
- Pepsi-Cola Company
- Q Laboratories, Inc.
- QIAGEN
- R-Biopharm, Inc.
- ROMER Labs Division Holding GmbH
- Shimadzu International
- Starbucks Coffee Company
- Synutra Internatiopnal
- Thermo Fisher Scientific
- Waters Corporation

SPSFAM Advisory Panel

- Chaired by Erik Konings, Nestle
- Advisory Panel Companies
  - Abbott Nutrition
  - Archer Daniels Midland
  - The Coca-Cola Company
  - General Mills, Inc.
  - Hershey Center for Health And Nutrition
  - Kellogg Company
  - Kraft Foods, Inc.
  - Mars Chocolate
  - Mead Johnson
  - Nestle Research Center
  - PepsiCo
  - Starbucks Coffee Company

Achievements to date: SMPRs

Analyte	Matrices	SMPR
Antioxidants	Foods, Beverages, Beverage Materials, Dietary Supplements	2011.11
Flavenols	Foods, Beverages and Beverage Materials, Fruit Juice, wines, Fruit & Fruit products, Cocoa Powder Chocolate, Spices and Condiments	2012.01
Heavy Metals	Foods, Beverages and Beverage Materials, Chocolate, Chocolate products, Fruit Juices, Infant formula	2012.07
St. John's Wort	Dietary Supplements	2013.01
Vitamin A	Foods	2012.03
Vitamin D	Foods	2012.04
Vitamin E	Foods	2012.05
Vitamin K	Foods	2012.06

Achievements to date: OMs First Action

AOAC Official Method First Action	Title
2012.04	Method for the Determination of Antioxidant Activity in Foods and Beverages by Reaction with 2, 2'-diphenyl-1-picrylhydrazyl (DPPH): Collaborative Study
2012.03	Analytical Parameters of the Microplate-Based ORAC-Pyrogallol Red Assay
2012.23	Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe
2013.04	Method for the Determination of Catechin and Epicatechin Enantiomers in Cocoa-Based Ingredients and Products by High Performance Liquid Chromatography: Single-Laboratory Validation
2012.24	Determination of Flavanol and Procyandin (by Degree of Polymerization 1-10) Content of Chocolate, Cocoa Liquors, Powder(s), and Cocoa Flavanol Extracts by Normal Phse High-Performance Liquid Chromotography: Collaborative Study
2013.03	Analysis of Cocoa Flavanols and Procyanidins (DP 1-10) in Cocoa-Containing Ingredients and Products by Rapid Resolution Liquid Chromatography

Outcome SPSFAM Meeting September 2014

- Launch of Heavy metal speciation working group, approved fitness for purpose
- Prioritization future SPSFAM area
  - Food Safety Panel (D. Acheson. B. Brackett, S. Godefroy) discussion
  - GFSI (P. Wissenburg) Industry response on Food Fraud
  - Proposal working group for meat authenticity (T. Delatour)

Priorities identified by Stakeholder Panel

- Meat/Fish species
- Validation guidelines for non-targeted analysis
- Fast methods for pathogens
- Fast methods for quatification (micro)
- Guidelines for laboratory sample preparation

Working Group (WG) Initiative

- AOAC Board of Directors initiates WG Initiative on December 9, 2014
- Individual or entity who expresses a need for a method
- WG may be funded and formed with assistance of AOAC
- WG will develop SMPR to present to an existing stakeholder panels for review

Why the new WG Initiative?

- Offers companies the opportunities to solve challenges without waiting on priorities of existing stakeholder panels
- WG's funded by current OA's and new companies interested in solving problems

Questions?

ISPAM *Salmonella* Methods Harmonization Working Group

Thomas Hammack

Chief
Microbial Methods Development Branch
Division of Microbiology
Office of Regulatory Science
Center for Food Safety and Applied Nutrition

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Background

- **ISPAM *Salmonella* Methods Harmonization Working Group formed in January 2015**
  - **Formed to determine how and if the US and ISO reference methods for *Salmonella* can be harmonized**
- **3 Teleconferences**
- **Accomplishment to date**
  - **Drafting committee has developed a charge for the working group**

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Salmonella Methods Harmonization Working Group Members

Thomas Hammack (Chair)	FDA - CFSAN
Patrice Arbault	Nexidia
Marcia Armstrong	QIAGEN Gmbh
Mike Clark	Bio-Rad Laboratories
Erin Crowley	Q Laboratories, Inc.
Leanne DeWinter	Health Canada
Philip Feldsine	BioControl Systems, Inc.
Paul In't Veld	Netherlands Food and Consumer Product Safety Authority
Irene Iugovaz	Health Canada
Balamurugan Jagadeesan	Nestec S.A
Ron Johnson	bioMerieux
Adrianne Klijn	Nestle Quality Assurance Laboratory
Wendy Lauer	Bio-Rad Laboratories, Inc.
Wendy McMahon	Silliker Inc.
Sam Mohajer	Canadian Food Inspection Agency
Kirsten Mooijman	Coordinator EURL-Salmonella
Mark Mozola	Neogen Corporation
Brooke Schwartz	Brooke Schwartz Consulting
Meredith Sutzko	Romer Labs
Morgan Wallace	DuPont Nutrition & Health

Office of Regulatory Science

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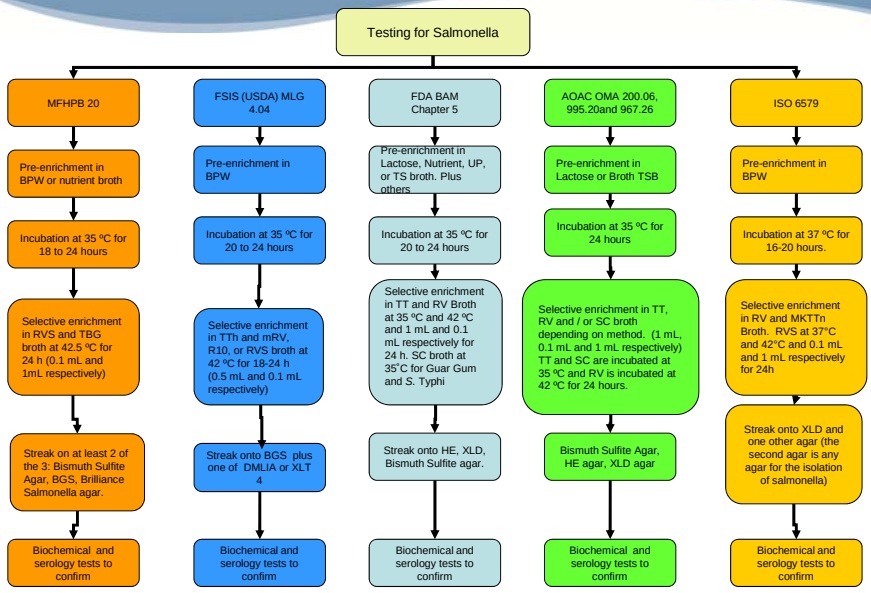

Slide Courtesy Donna Douey, Canadian Food Inspection Agency

Proposed Charge

"The charge of the working group is to provide recommendations for the process of harmonizing the US (BAM/MLG) and ISO Salmonella reference culture methods. The first step of this process is to determine if there are matrices for which the US and ISO Salmonella methods are statistically equivalent using ISO16140 Part 2. This will be done through the analysis of available existing data and through side-by-side comparisons of the methods with selected matrices. This comparative data will be the basis for determining which steps should be taken to harmonize the US and ISO Salmonella methods."

Existing Data

- **AOAC Official Method 2002.10**
  - **Salmonella Detection in Fresh Cheese, Dried Egg Products, and Fresh Chilled and Frozen Poultry**
- **DuPont/Campden data**
  - **Chilled ready meal (vegetable bake), Hard Cheese, Soft Cheese, RTE Salad, Milk Chocolate, Prawns, Black Pepper, Dry Pet Food, Raw Pizza Dough, Egg and Cress Sandwiches, and Custard**

AOAC Official Method 2002.10

Matrix	Concentration MPN[a]/25g	N	ISO Reference			AOAC Reference			$dPOD_C$	95% CI
			X	POD_C	95% CI	x	POD_R	95% CI		
Cheese	0.00	75	0	0.00	0.00, 0.05[h]	0	0.00	0.00, 0.05	0.00	(-0.05), 0.05
	0.70	75	57	0.76	0.65, 0.84	65	0.87	0.77, 0.93	-0.11	(-0.23), 0.02
	37.25	75	66	0.88	0.79, 0.94	70	0.93	0.85, 0.97	-0.05	(-0.15), 0.04
Egg Powder 1	0.00	74	0	0.00	0.00, 0.05	0	0.00	0.00, 0.05	0.00	(-0.05), 0.05
	9.63	75	73	0.98	0.91, 0.99	75	1.00	0.95, 1.00	-0.03	(-0.09), 0.03
	115.5	74	73	0.99	0.93, 0.99	74	1.00	0.95, 1.00	-0.01	(-0.07), 0.04
Egg Powder 2	0.00	40	0	0.00	0.00, 0.09	0	0.00	0.00, 0.09	0.00	(-0.09), 0.09
	0.70	40	13	0.33	0.20, 0.48	19	0.48	0.33, 0.63	-0.15	(-0.34), 0.06
Poultry 1	0.00	75	0	0.00	0.00, 0.05	0	0.00	0.00, 0.05	0.00	(-0.05), 0.05
	3.68	74	72	0.97	0.91, 0.99	39	0.53	0.41, 0.64	0.45	(0.32), 0.56
	5.78	75	75	1.00	0.95, 1.00	70	0.93	0.85, 0.97	0.07	(0.005), 0.15
Poultry 2	0.23	78	15	0.19	0.12, 0.29	14	0.18	0.11, 0.28	0.01	(-0.11), 0.14
	1.05	78	14	0.18	0.11, 0.28	24	0.31	0.22, 0.42	-0.13	(-0.26), 0.01
Poultry 3	0.58	24	13	0.54	0.35, 0.72	16	0.67	0.48, 0.82	-0.13	(-0.37), 0.14
	1.05	24	17	0.71	0.51, 0.85	20	0.83	0.64, 0.93	-0.13	(-0.35), 0.11

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DuPont/Campden Data

Matrix	Concentration MPN/25g	N	FDA-BAM Reference			ISO Reference			$dPOD_C$	95% CI
			X	POD_C	95% CI	x	POD_R	95% CI		
Chilled ready meal (vegetable bake)	0.00	5	0	0.00	0.00, 0.46[h]	0	0.00	0.00, 0.46[h]	0.00	-0.43, 0.43
	6	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
	23.25	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
Hard Cheese	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	6	20	15	0.75	0.53, 0.89	19	0.95	0.76, 0.99	-0.20	-0.42, 0.03
	27.5	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
Soft Cheese	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.4	20	18	0.90	0.70, 0.97	5	0.25	0.11, 0.47	0.65	0.35, 0.81
	5.25	20	20	1.00	0.84, 1.00	14	0.70	0.48, 0.85	0.30	0.077, 0.52
RTE Salad	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.23	20	4	0.20	0.081, 0.42	14	0.70	0.48, 0.85	-0.50	-0.70, -0.19
	10.75	20	14	0.70	0.48, 0.85	20	1.00	0.84, 1.00	-0.30	-0.52, 0.077
Milk Chocolate	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	2.33	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
	37.5	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
Milk Chocolate	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.575	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28

DuPont/Campden Data

Matrix	Concentration	N	FDA-BAM Reference			ISO Reference			$dPOD_C$	95% CI
	MPN/25g		X	POD_C	95% CI	x	POD_R	95% CI		
Seafood (prawns)	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.375	20	5	0.25	0.11, 0.47	2	0.10	0.028, 0.30	0.15	-0.09, 0.38
	1.075	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
Spice (black pepper)	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	6	20	19	0.95	0.76, 0.99	20	1.00	0.84, 1.00	-0.05	-0.24, 0.12
	10.75	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
Dry Pet Food	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.575	20	13	0.65	0.43, 0.82	19	0.95	0.76, 0.99	-0.30	-0.52, -0.049
	2.325	20	20	1.00	0.84, 1.00	19	0.95	0.76, 0.99	0.05	-0.12, 0.24
Raw Pizza Dough	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	0.95	20	11	0.55	0.34, 0.74	18	0.90	0.70, 0.97	-0.35	-0.57, -0.072
	23.25	20	18	0.90	0.70, 0.97	20	1.00	0.84, 1.00	-0.10	-0.30, 0.077
Egg and Cress Sandwiches	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	<0.075	20	20	1.00	0.84, 1.00	13	0.65	0.43, 0.82	0.35	0.11, 0.57
	9.5	20	16	0.80	0.58, 0.92	20	1.00	0.84, 1.00	-0.20	-0.42, 0.0005
Chilled Dairy Desert (custard)	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	18.75	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
	60.0	20	20	1.00	0.84, 1.00	20	1.00	0.84, 1.00	0.00	-0.16, 0.16
Chilled Dairy Desert (custard)	0.00	5	0	0.00	0.00, 0.46	0	0.00	0.00, 0.46	0.00	-0.43, 0.43
	6.0	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26

Annex A

"Classification of sample types and suggested target combinations for validation studies"

- **18 categories**
  - **Raw milk and dairy products (4)**
  - **Heat processed milk and dairy products**
  - **Raw meat and ready-to-cook meat products (except poultry)**
  - **Ready-to-eat, ready-to-reheat meat products**
  - **Raw poultry and ready-to-cook poultry products (1)**
  - **Ready-to-eat, ready-to-reheat meat poultry products**
  - **Eggs and derivatives (1)**
  - **Raw and ready-to-cook fish and seafood (unprocessed) (1)**
  - **Ready-to-eat, ready-to-reheat fishery products**

Annex A
continued

- **Categories (continued)**
  - **Fresh produces and fruits**
  - **Processed fruits and vegetables**
  - **Infant formula and infant cereals**
  - **Dried cereals, fruits, nuts, seeds and vegetables (2)**
  - **Chocolate, bakery products and confectionary (1)**
  - **Multi-component foods or meal Components (2)**
  - **Pet food and animal feed (1)**
  - **Environmental samples (food or feed production)**
  - **Primary production samples (PPS)**

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Next Steps

- **Analyze existing data with RLOD statistics**
- **Decide what more needs to be done**
  - **Ask for input from various stakeholders, including regulatory bodies**
- **Analysis of additional matrices**
- **How do we move forward?**

Thank you

ISO TECHNICAL SPECIFICATIONS FOR VIRUSES: HOW ARE THEY RELEVANT TO SERVICE LABORATORIES AND ASSAY MANUFACTURERS

PIONEERING DIAGNOSTICS

ceeram
ANTICIPATE TO BETTER PREVENT

BIOMÉRIEUX
INDUSTRY

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INDUSTRY

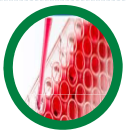
1. ISO/TS 15216-1 and 2
2. service laboratories point of view
3. assay manufacturer perception

Virus : detection method?

Electronic Microscope

\+ For all viruses

\- Sensitivity Issue



Antigene Detection, Immuno Tests

\+ ELISA : RV, AsV, AdV

\- Sensitivity, Specificity

Cell Culture

\+ Virucidal Effects of Process

\- In the early stage of culture (2014 publication)

Viral Genome Detection

\+ ALL Viruses

\- PCR or RT-PCR

Virus : detection method?

CEN/TC275/ WG6/TAG4

ISO/TS 15216-1 et 2

Horizontal Method

TARGETED VIRUS

NOROVIRUS GI & GII

HEPATITIS A VIRUS

SALADS & HERBS

SOFT FRUITS

BIVALVE MOLLUSKS

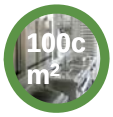
BOTTLED WATER

HARD SURFACES

Virus : detection method?

PROTOCOL	• Elution • Concentration	CEN/TC275/WG6/TAG4 ISO/TS 15216 PART 1 & 2
QUANTITY	 2g  25g  1L  100 cm²	
PURIFIED VIRAL RNA	From a know volume of concentrate/extraction by standard method	
ANALYSIS	Real-time (TaqMan) RT-PCR	
2 PARTS	Determination	
	Quantitative	**Qualitative**

Virus : detection method?

PRETREATMENT & EXTRACTION OF NUCLEIC ACIDS	CEN/TC275/WG6/TAG4 ISO/TS 15216 Part 1 & 2
 100c m²	**SWABBING FOLLOWED BY ELUTION**
 25g	**ELUTION WITH AGITATION & PRECIPITATION WITH PEG / NaCl**
 2g	**DIGESTIVE TISSUE PRETREATMENT BY CRUSHING AND PROTEINASE K**
 1L	**ADSORPTION & ELUTION ON LOADED MEMBRANES & CONCENTRATION / ULTRAFILTRATION**

Virus : detection method?

CONTROLS

CEN/TC275/WG6/TAG4
ISO/TS 15216
Part 1 & 2

ANALYSIS	Reproducible & Repeatable
COMPLEX METHODS	Several levels of controls
EXTRACTION EFFICACY	Use of a tracer (mengo virus vMC0)
RT-PCR EFFICACY	Internal Control integration + (IPC)
QUANTIFICATION	Done with Plasmides
RT-PCR	Control + (Virus deactivated or plasmides)
NEGATIVE CONTROLS	for pre-treatment, extraction & RT-PCR

Virus : detection method?

Shelf Life : 12 Months

CEN/TC275/WG6/TAG4
ISO/TS 15216
Part 1 & 2

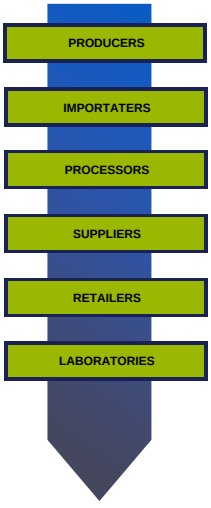
Comply to ISO/TS

Virus : detection method?

Virus : detection method?

Validation on a large panel of matrices : Shellfish (oysters, mussels, cockles, clams, scallops…) Fruits (berries, seeds,...) Vegetables (salads, tomatoes, carots, …) Ready-to-eat, complex foods Herbs and spices Surfaces Water, sewage water, process water, ...

Fresh, frozen, dried, lyophilized, coulis, purée, powder ...



Virus : detection method?

OPTIMIZED FOR ROUTINE ANALYSIS

ceeramTOOLS

- FAST
- SENSITIVE
- 95 % AMPLIFICATION EFFICACY
- SA STANDARDIZED AMPLIFICATION
- M MULTIPLEXING

CEN/TC275/WG6/TAG 4
ISO/TS 15216

- ROBUST
- SPECIFIC
- MULTI-PLATEFORMS
- MULTI-MATRICES
- EXTERNAL CONTROL MENGOVIRUS

Virus : detection method?

VALIDATED PROCEDURE DIAGRAM

CEN/TC275/WG6/TAG4
ISO/TS 15216

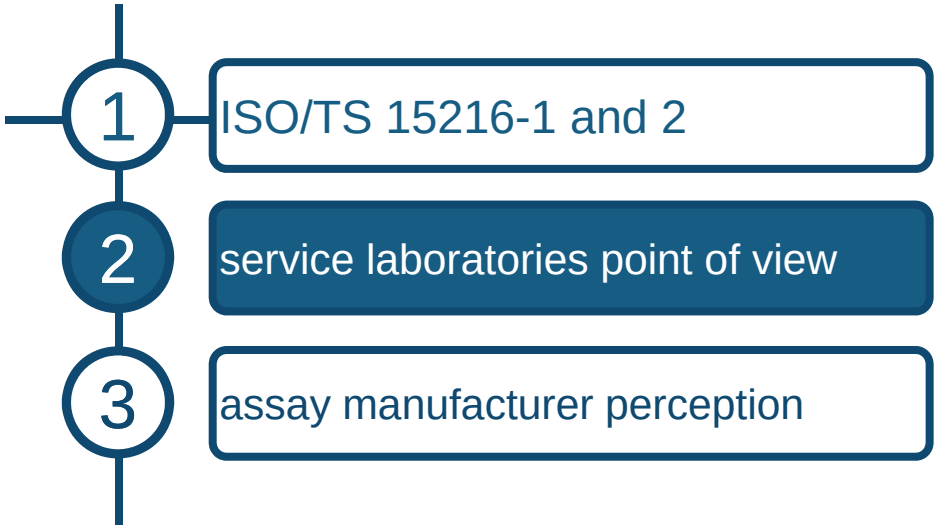
MENGOVIRUS ADDED		VIRUS PRETREATMENT	EXTRACTION RNA	DETECTION
HARD SURFACES	100 cm²	SWABBING FOLLOWED BY ELUTION	Nucleic acids extraction on 1mL concentrated virus, lysis with guanidine isothiocyanate & silica binding. Elution RNA in 100 µl (= MiniMag equipment + Nuclisens reagents bioMérieux)	ceeramTOOLS Mengovirus DETECTION & targets
SALADS VEGGIES FRUITS HERBS SPICES	25g	GLYCINE BUFFER ELUTION PRECIPITATION PEG / NaCl CLARIFICATION CHLOROFORM BUTANOL		
BIVALVE MOLLUSKS	2g	DIGESTIVE TISSUES ELUTION PROTEINASE K INCUBATION 37° THEN 60° CENTRIFUGATION		
LIQUID SAMPLES	1L	CONCENTRATION / FILTRATION TANGENTIAL CASSETTES, PRECIPITATION PEG CLARIFICATION CHLOROFORM BUTANOL		RESULTS INTERPRETATION

Virus : detection method?

ISO/TS 15216: what have been done on 2014?

- international interlaboratory study:
18 laboratories, 7 matrices (surface, lettuce, green onion, raspberry, oyster, mussel, bottled water), 10 labs/ tested matrices, 3 virus (NoV GI, NoV GII, HAV), 3 levels of contamination (low, medium, high)/ tested virus.
Determination of repeatability and reproducibility limits

- redaction of a new draft including slight modifications on protocols and data from interlaboratory study :
submitted to CEN secretariat for voting procedure on Feb 4th 2015



Service laboratories point of view

Advantages

- ISO standard
-Standardized protocols for pre-treatment, extraction, results interpretation
- Main matrices included
- Ease to set-up methods (protocol, list of reagents and equipment needed)
-Intercomparability, Ring trial efficacy
- facilitate accreditation

Service laboratories point of view

Drawbacks

- Need a class 2 lab and people skilled in molecular biology
- Methods very different from bacteria testing (several labs asked for practical training + technical support)
- High cost to implement standardized virus methods
- Potential equipment change, leading to higher budget
- Numbers of controls (normative part)
- Long time to result
- Expensive analyses
- A limited number of matrices within the scope, hence difficulties for complex ones (requests from service labs customers)
- Few manufacturers offering validated solutions
- ISO 17025 accreditation: difficult (depending on countries), cost effective (several matrices, several viruses), ISO 16140 difficult to apply for viruses

Assay manufacturers point of view

Advantages

- If you follow the standard, this is a great opportunity to have a chance to sell more, at higher price, and gain competitive advantage
-More labs will be able to start virus testing. More food companies will trust the advantages of testing. – possibility to make comparison of on performance criteria available commercial assays in comparison with the ISO standard method

Assay manufacturers point of view

Drawbacks

- Difficult to provide a simple solution all in one including all the required controls
- Methods very different from bacteria testing (several labs asked for practical training + technical support): you need skilled people in foodborne virus issue for technical support
- No available referential to follow for certification (ISO 16140 non applicable)
- Absence of valuable reference materials (ex: no cell culture for NoV = human stool samples only)
- Numbers of details in informative annexes that labs considered as normative
- Several normative points related to IP issue
- Several matrices, several viruses : cost of validation
- Absence of reference laboratories except for viruses in shellfish (NRL, CRL in Europe), difficulties to ask for validation or interlaboratory studies
- A limited number of matrices within the scope, hence difficulties for complex ones (requests from service labs customers)
-

Relevance of ISO/TS 15216

As a whole pretty much relevant to bring confidence in the method of virus testing for all parties.

It is a must to be in accordance with ISO/TS 15216-1 or 2 on normative AND informative parts

THANK YOU FOR YOUR ATTENTION


BIOMÉRIEUX
INDUSTRY
PIONEERING DIAGNOSTICS

AOAC SMPR 2014.XXX; Version 7.5, August 14, 2014

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

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

1. **Intended Use**: Laboratory use by trained technicians.

2. **Applicability**: Detection and identification of *Variola virus* DNA in aerosol collection filters and/or liquids.

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

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

4. **Definitions**:

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

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

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

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

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

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.

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

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

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

7. **Other Requirements:** Method developer must present the positive predictive value in their submission since Smallpox is an eradicated disease. The positive predictive value must be based on data generated within the environmental matrix.

8. Method Performance Requirements:

Parameter	Minimum Performance Requirement
Acceptable Minimal Detection Level (AMDL)	50,000 copies/ml of *Variola virus* target DNA in the candidate method sample collection buffer. Copies/ml refers to number of viral genomes or equivalent plasmid copies containing target viral gene or gene fragment.
Probability of Detection at AMDL within sample collection buffer	≥ 0.95
Probability of Detection at AMDL in an aerosol environmental matrix	≥ 0.95 (Annex IV; part 1)
Inclusivity panel purified DNA	All inclusivity strains (Annex II and Annex V) must test positive at 2x the AMDL†
Exclusivity panel purified DNA	All exclusivity strains (Annex III and Annex IV; part 2 and Annex V) must test negative at 10x the AMDL†
System False-Negative Rate using spiked aerosol environmental matrix	≤ 5% (Annex IV; Part 1)
System False-Positive Rate using aerosol environmental matrix	≤ 5% (Annex IV; Part 1)
Maximum Time to Assay Result	24 hours
Notes: † 100% correct analyses are expected. All aberrations are to be re-tested following the AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures[5]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.	

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

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

Annex II: Inclusivity Panel

The inclusivity panel shall include:

- Sequences from at least two representative strains from each major clade of *Variola virus*
- Any other strain with differences in the assay primer and/or probe target sequences based on bioinformatic analysis (Annex V).

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

More details can be found at:

http://www.who.int/csr/disease/smallpox/SummaryrecommendationsMay08.pdf

Annex III: Exclusivity Panel (near-neighbor)

The exclusivity panel shall include:

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

CORE EXCLUSIVITY PANEL

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

Annex 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 working group determined that some of the environmental factors listed in the 2010 panel are not applicable to *Variola* virus detection assays, and so have been removed. Other various clarifications have been included. September 2014.]

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 V: Bioinformatics Analyses of Signature Sequences Underlying *Variola 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

2015 AOAC MID YEAR MEETING MARCH 17, 2015
INTERNATIONAL STAKEHOLDER PANEL ON ALTERNATIVE METHODS

RESOURCES

ISPAM
Key Staff Contacts:

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

ISPAM Microsite: http://bit.ly/1lhEULh

AOAC Acronyms and Abbreviations

AMDL	acceptable minimum detection level
AOAC	AOAC INTERNATIONAL (AOAC formerly stood for *Association of Official Analytical Chemists*, but long-name no longer used)
CSO	chief scientific officer
ERP	expert review panel
ISPAM	International Stakeholder Panel on Alternative Methods
ISO	International Organization for Standardization
LOD	limit of detection
LPOD	laboratory probability of detection
NGO	non-governmental organization
OMA	*Official Methods of Analysis*, frequently pronounced like “o maa”
OMB	Official Methods Board
POD	probability of detection
PTM	*Performance Tested Methods*
RI	AOAC Research Institute
RSDR	Relative Standard Deviation of Reproducibililty, sometimes referred to as “RSD big R”. The variation between laboratories.
RSDr	Relative Standard Deviation of Repeatability, sometimes referred to as “RSD little R”. The within laboratory variation, also called precision.
SMPR	*Standard Method Performance Requirement*, frequently pronounced as in “smipper”.

AOAC Stakeholder Panel Voting Members

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

Due Process and Balance

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

Composition

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

Approval Process

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

Roles and Responsibilities

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

AOAC INTERNATIONAL

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

VOLUNTEER ROLE DESCRIPTION

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

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

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

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

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

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

AOAC RESOURCES:

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

STAFF LIASON:

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

TERMS OF REVIEW:

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

DATES REVISED:

Stakeholder Panel

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

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

Expert Review Panel

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

Working Group

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

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

Helpful Definitions & Terminology

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

Appendix W

POLICY AND PROCEDURES ON VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

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

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

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

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

Illustrations of Conflicts of Interest

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

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

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

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

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

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

Do's and Don't's

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

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

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

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

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

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

Procedures

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

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

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

* * * * * *

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

Appendix U

ANTITRUST POLICY STATEMENT AND GUIDELINES

Introduction

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

Responsibility for Antitrust Compliance

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

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

Antitrust Guidelines

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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

Appendix V

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

Introduction

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

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

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

The Scientific Association Dedicated to Analytical Excellence®

Policy

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

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

the purposes for which permission has been specifically granted.

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

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

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

Instructions

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

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

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

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

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

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

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

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

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

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

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

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

Sanctions

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

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

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

* * * * * *

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

Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

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

International Voluntary Consensus Standards

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

Guidance for Standard Method Performance Requirements

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

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

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

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

SMPR Format

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

where C is expressed as a mass fraction.

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

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

Initiation of an SMPR

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

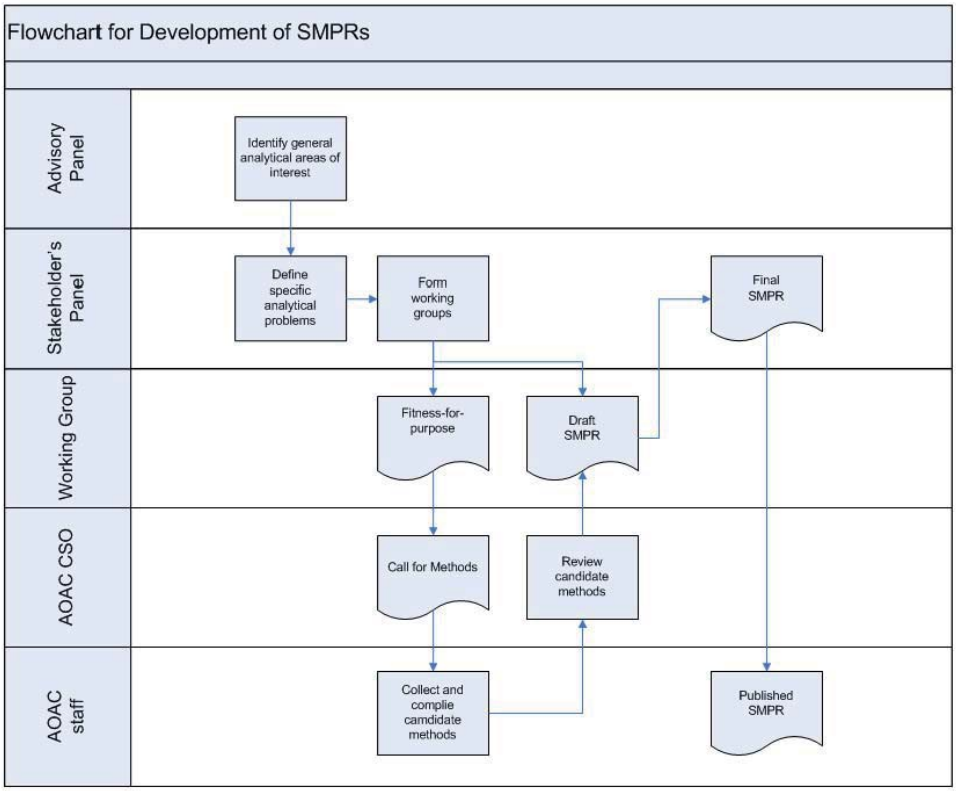


Figure 1. Schematic flowchart diagram of the SMPR development process.

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

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

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

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

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

Fitness-for-Purpose Statement and Call for Methods

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

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

Creating an SMPR

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

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

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

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

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

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

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

Table 2. Example of method performance table for multiple analytes

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

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

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

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

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

Open Comment Period

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

Submission of Draft SMPRs to the Stakeholder Panel

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

Publication

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

Conclusion

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

References

(1) Eurachem, *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, *Validation*, http://www.eurachem.org/guides/pdf/valid.pdf, posted December 1998, accessed March 2012
(2) *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis*, *Appendix D*, AOAC INTERNATIONAL, Gaithersburg, MD
(3) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix I, Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*, AOAC INTERNATIONAL, Gaithersburg, MD
(4) *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD
(5) Codex Alimentarius Codex Procedure Manual
(6) International Organization for Standardization, Geneva, Switzlerland

ANNEX A
Format of a
Standard Method Performance Requirement

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

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

Approved By: Name of stakeholder panel or expert review panel

Final Version Date: Date

Effective Date: Date

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

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

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

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

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

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

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

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

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

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

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

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

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

Table A1. Performance requirements

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

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

[b] ≥100 g/kg.

[c] <100 g/kg.

[d] If a reference material is available.

[e] At a critical level.

[f] AMDL = Acceptable minimum detection level.

[g] LPOD = CPOD.

Table A2. Recommended definitions

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

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

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

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

[d] ISO 5725-1-1994.

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

Table A3. Recommendations for evaluation

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

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

[b] Codex Alimentarius Codex Procedure Manual.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[b]

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

[d] AOQL = Average outgoing quality level.

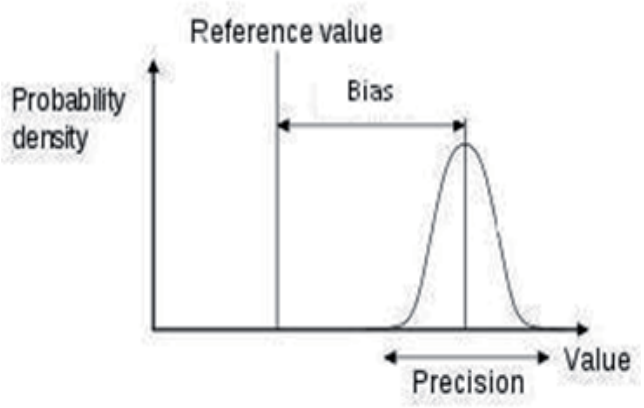


Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

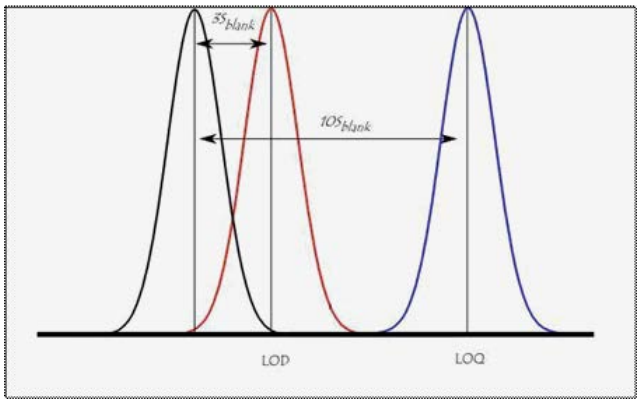


Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

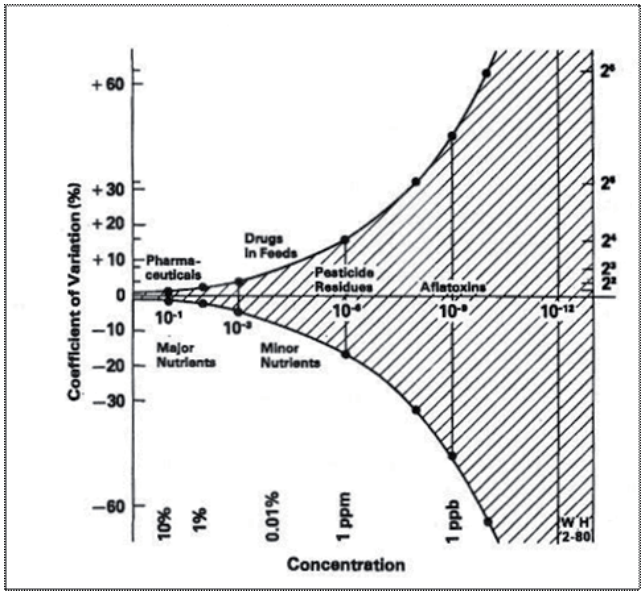


Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

ANNEX B
Classification of Methods

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

Type I: Quantitative Methods

Characteristics: Generates a continuous number as a result.

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

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

Type II: Methods that Report Ranges

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

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

Type III: Methods with Cutoff Values

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

Recommendation: Use performance requirements specified for qualitative methods.

Type IV: Qualitative Methods

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

Recommendation: Use performance requirements specified for qualitative methods.

Type V: Identification Methods

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

Recommendation: Use performance requirements specified for identification methods.

ANNEX C
Understanding the POD Model

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

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

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

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

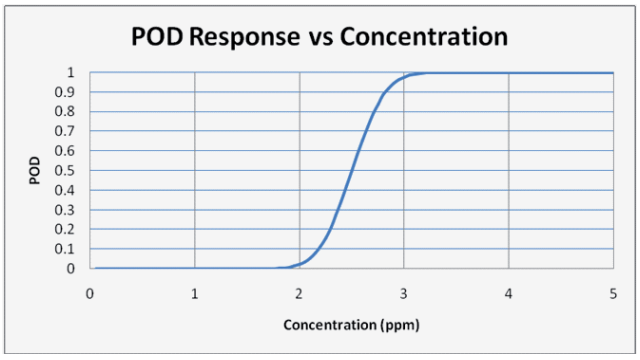


Figure C1. Theoretical POD curve for a qualitative detection method.

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

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

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

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

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

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

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

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

Table C1. Terminology

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

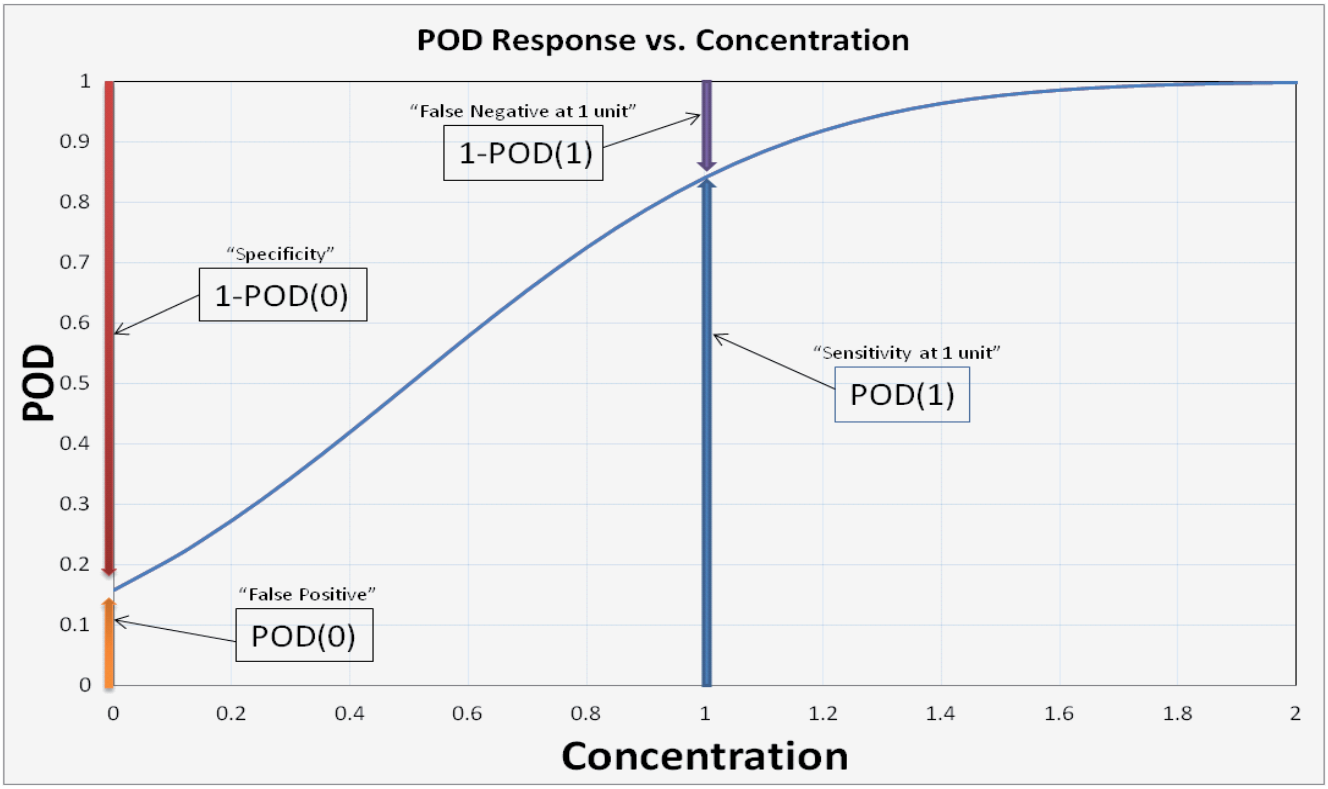


Figure C2. Comparison of POD model terminology to other obsolete terms.

ANNEX D
Definitions and Calculations of HorRat Values from Intralaboratory Data

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

1. Definitions

1.1 Replicate Data

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

1.2 Pooled Data

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

1.3 Average

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

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

1.4 Standard Deviation

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

1.5 Relative Standard Deviation

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

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

The relative standard deviation calculated from within-laboratory data.

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

The relative standard deviation calculated from among-laboratory data.

Table D1. Predicted relative standard deviations

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

1.6 Mass Fraction

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

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

The reproducibility relative standard deviation calculated from the Horwitz formula:

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

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

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

1.8 HorRat Value

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

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

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

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

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

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

2 *Acceptable HorRat Values*

2.1 For Interlaboratory Studies

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

Table D2. Predicted relative standard deviations

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

2.1.1 Limitations

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

2.2 For Intralaboratory Studies

2.2.1 Repeatability

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

2.2.2 HorRat(r)

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

Calculate HorRat(r) from the SLV data:

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

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

2.3 Other Limitations and Extrapolations

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

ANNEX E
AOAC Method Accuracy Review

Accuracy of Method Based on Reference Material

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Demonstration of Method Accuracy when No Reference Material Is Available

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

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

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

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

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

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

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

ANNEX F
Development and Use of In-House Reference Materials

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

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

What Is a Reference Material?

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

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

Why Use Reference Materials?

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

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

What Certified Reference Materials Are Currently Available?

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

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

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

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

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

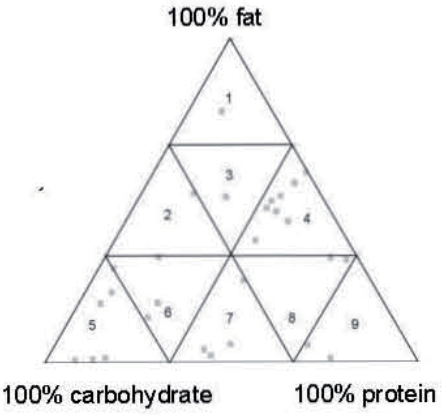
Why Use an In-House Reference Material?

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

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

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

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



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

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

How Do I Create an In-House Reference Material?

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

References

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

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

(2) Wolf, W.R., & Andrews, K.W. (1995) *Fresenius' J. Anal. Chem.* **352**, 73–76
(3) Wolf, W.R. (1993) *Methods of Analysis for Nutrition Labeling*, D.R. Sullivan & D.E. Carpenter (Eds), AOAC INTERNATIONAL, Gaithersburg, MD
(4) European Reference Materials (2005) *Comparison of a Measurement Result with the Certified Value*, Application Note 1
(5) *ISO Guide 34 General Requirements for the Competence of Reference Material Producers* (2009) 2nd, International Organization for Standardization, Geneva, Switzerland
(6) *Guide 35 Certification of Reference Materials—General and Statistical Principles* (2006) International Organization for Standardization, Geneva, Switzerland

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