AOAC INTERNATIONAL

Stakeholder Panel on Strategic Food Analytical Methods

Stakeholder Panel Meeting - March 14, 2016

Salon C/D
Gaithersburg Marriott Washingtonian Center
Gaithersburg, Maryland, 20878
USA

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spsfam@aoac.org

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SPSFAM

2016 AOAC MIDYEAR MEETING, MARCH 14, 2016
STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

RESOURCES

Key Staff Contacts:

Name	Role	Email	Telephone
Scott Coates	AOAC Chief Scientific Officer	scoates@aoac.org	301.924.7077 x 137
Christopher Dent	Standards Development Coordinator	cdent@aoac.org	301.924.7077 x 119
Dawn Frazier	Executive, Scientific Business Development	dfrazier@aoac.org	301.924.7077 x 117
Deborah McKenzie	Sr. Director, Standards Development and Method Approval Processes	dmckenzie@aoac.org	301.924.7077 x 157

Useful Web Links:

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

SPSFAM Microsite: http://bit.ly/1GkSJ07

Stakeholder Panel on Strategic Food Analytical Methods - Chair Biography

Co-Chair, SPSFAM

Erik Konings

Nestle Research Center

Erik Konings has been an active member of AOAC since 1997. He is currently serving as a director on the Board of Directors and a member of the Advisory Panel membership on the Advisory Panel for the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN). 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.

Speaker Bios

Hannah Crum
Kombucha Brewers International
Co-Chair, SPSFAM Kombucha Working Group

Co-founder and President of Kombucha Brewers International, the non-profit trade association for the Kombucha industry, Hannah Crum is a longtime educator and Kombucha advocate. Taking KBI's mission to promote and protect the Kombucha industry worldwide to heart, she has been a featured speaker at conferences, festivals and on television as the leading expert in Kombucha. Founder of the popular educational site, Kombucha Kamp (http://www.kombuchakamp.com/), is the most visited website in the world for Kombucha information, recipes and advice. Along with her partner, KBI co-founder and Chairman of the Board, Alex LaGory, they have directly mentored and consulted Kombucha brewers from start-up to scale-ups since 2007 and have co-written the authoritative tome, The Big Book of Kombucha, due out March 2016.

Rick Reba
Nestlé
Chair, SPSFAM Heavy Metals Expert Review Panel

Rick Reba is a Technical Expert in Food Chemistry for Juice Authenticity, Proximate Analyses, Minerals and Trace Elements in Food. He is also a quality specialist auditor ISO 17025 and ISO 9001 systems. Rick holds a BS Degree in Chemistry from the Ohio State University.

Mr. Vincent Paez

Sr. Director, Food, Environmental & Forensics Testing

SCIEX

Biography

Mr. Vincent Paez is a U.S.A. citizen, who speaks fluent Spanish with good French and German. He is an analytical chemist. In 1984, he received a B.S. degree in chemistry from Stony Brook University in New York. He worked in various analytical chemistry labs with techniques such as atomic absorption, thermal analysis, infrared spectroscopy, gas chromatography, liquid chromatography, mass spectrometry and x-ray diffraction.

In 1990, he received an M.B.A. degree from the Anderson Graduate School of Management at U.C.L.A.

Vincent then joined the Hewlett Packard Company (now called Agilent Technologies). At HP/Agilent, Vincent held several sales and marketing positions, including: Product Manager for Gas Chromatography, Sales Development Manager, European Business Development Manager, and Asia Programs Manager.

In 2004, Vincent joined Thermo Electron Corporation (now called Thermo Fisher Scientific) as Vice President of Americas Sales, responsible for sales in Canada, U.S.A., and Latin America for the Scientific Instruments Division. In 2008, Vincent began his role as Director of Food Safety at Thermo Fisher Scientific. He was responsible for building the global food safety business.

In March of 2012, Vincent joined AB SCIEX (now SCIEX), an operating company of the Danaher Corporation, as Senior Director of Food, Environmental & Forensics Testing, responsible for bringing new mass spectrometry based solutions to these markets. SCIEX is the world's leader in mass spectrometry for food testing. Mass spectrometry is the most reliable form of chemical testing in food. Vincent's team is responsible for helping labs all over the world, especially in China, use mass spectrometry in food testing.

AOAC Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM)
Stakeholder Panel Meeting

Meeting Minutes
Sunday, September 27, 2015; 8:30 a.m. – 12:00 p.m. PT

Attendees:

Erik Konings, Nestle Research Center (SPSFAM Chair)	Josh Messerly, Eurofins Nutrition Analysis Center
Stan Bacler, CFIA/Health Canada	William Mindak, FDA/CFSAN
Brad Barrett, GERSTEL, Inc.	Armen Mirzoian, Alcohol And Tobacco Tax And Trade Bureau
Christopher Blake, Nestec Ltd. - Nestle Research Center	Allen Misa, Phenomenex, Inc.
David Boaz, Caravan Ingredients	Deepali Mohindra, Thermo Fisher Scientific
Michelle Briscoe, Brooks Rand Labs	Jeffrey Moore, US Pharmacopeia (USP)
Michael Brodsky, Brodsky Consultants	Maria Ofitserova, Pickering Laboratories, Inc.
Esther Campos-Gimenez, Nestle Research Center	Lawrence Pacquette, Abbott Nutrition
Pei Chen, USDA - ARS, BHNRC, FCMDL	Shang-Jing Pan, Abbott Nutrition
France Cho, Maxxam Analytics Inc	Melissa Phillips, NIST
Robert Clifford, Shimadzu Scientific Instruments, Inc.	Curtis Phinney, Curtis S. Phinney, CNS
Jo Marie Cook, Florida Department Of Agriculture And Consumer Services	Eric Poitevin, Nestle Research Center
Erin Crowley, Q Laboratories, Inc.	Bert Popping, Mérieux NutriSciences
Xiaojun Deng, Shanghai Exit And Entry Inspection & Quarantine Bureau	Robert Rankin, Infant Nutrition Council of America (INCA)
Jonathan Devries, Retired	Rick Reba, Nestle USA, Inc.
Vanessa Dew, Health-Ade	Murali Reddy, Abbott Nutrition
Linda Dodd, PB Gelatins/PB Leiner	Kunal Rehani, Sigma-Aldrich
Robert Donofrio, NSF International	Klaus Reif, PhytoLab GmbH & Co., KG
Wayne Ellefson, Covance Laboratories	Lars Reimann, Eurofins Scientific, Inc.
Jodie Fung, Kombucha Brewers International	Kyle Rhoden, DuPont Nutrition & Health
Russell Gerads, Brooks Rand Labs, LLC	Catherine Rimmer, NIST
Brendon Gill, Fonterra Co-Operative Group	Shauna Roman, RB (Reckitt Benckiser)
Donald Gilliland, Abbott Nutrition	Joe Romano, Waters Corporation
Jasmine Hagan, ELISA Technologies, Inc.	Brian Schaneberg, Starbucks Coffee Company
Cathy Halverson, TTB	Jenny Scifres, USDA FSIS OPHS LQAD ALP
Norma Hill, US Treasury (Retired)	Li Sheng, EPL Bio Analytical Services
Steve Holroyd, Fonterra Cooperative Group Ltd.	Olga Shimelis, SUPELCO/Sigma-Aldrich
Gregory Hostetler, Perrigo / PBM Nutritionals	Christopher Smith, The Coca-Cola Company
Min Huang, Frontage Laboratories, Inc.	Cheryl Stephenson, Eurofins Central Analytical Laboratory
Prashant Ingle, Herbalife	Linda Stephenson, Sigma Aldrich
Greg Jaudzems, Nestle USA, Inc	Darryl Sullivan, Covance Laboratories
George Joseph, AsureQuality, New Zealand	John Szpylka, Mérieux NutriSciences
David Kennedy, Phenomenex	Nancy Thiex, Thiex Laboratory Solutions LLC
Barbro Kollander, National Food Agency	Daina Trout, Health-Ade LLC
Alex Lagory, Kombucha Brewers International	Wayne Wargo, Abbott Nutrition
Kristie Laurvick, US Pharmacopeia (USP)	Guy Weerasekera, Mead Johnson Nutrition
John Lee, Agilent Technologies, Inc.	Laura Wood, NIST
Farzaneh Maniei, The Coca-Cola Company	Jason Wubben, Archer Daniels Midland Company
Elaine Marley, R-Biopharm Rhone Ltd	Sudhakar Yadlapalli, First Source Laboratory Solutions
Katerina Mastovska, Covance Laboratories	Jinchuan Yang, Waters Corporation
Mary Mcbride, Agilent Technologies, Inc.	Joseph Zhou, Sunshineville Health Products, Inc
	Yang Zhou, Eurofins Scientific Inc.

AOAC Staff:

Jim Bradford, Delia Boyd, Scott Coates, Christopher Dent, Dawn Frazier, Deborah McKenzie, Tien Milor, LaKia Phillips, Bob Rathbone

Meeting Minutes

I. Welcome and Introductions

Roll call was taken and Konings opened the meeting at approximately 8:30 a.m. PT.

II. Policies, Procedures and Past Meeting Minutes

Konings directed all participants to the AOAC Policies and Procedures located in their meeting books.[1] Konings also asked the group for a motion to approve the March 16, 2015 SPSFAM Meeting Minutes.

MOTION to Approve the March 16, 2015 SPSFAM Meeting Minutes (Sullivan/Barrett)
17 in favor, 0 opposed, 2 abstain. The motion passed.

III. Stakeholder Panel Updates

Konings introduced Crowley, Chair of the AOAC International Stakeholder Panel on Alternative Methods (ISPAM). Crowley took the floor with a presentation[2] on the activities of the ISPAM panel, including their work on a produce sampling plan and harmonization of *Salmonella* methods. Currently, there is no support from potential working group members for the development of rapid microbiological methods as a collaborative effort between ISPAM and SPSFAM. Konings then provided a verbal update on the activities of the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) who are discussing potential future activities to establish *Standard Method Performance Requirements*® (SMPRs) for nutrients and contaminants in dairy ingredients

IV. Ingredient Quality / Glyphosates Soft Launch

Konings advised that SPSFAM is now operating under the new working group model, which allows organizations to fund a working group under an existing panel rather than an entire stakeholder panel. Because Glyphosates is not yet a supported initiative, AOAC is offering a soft launch on this topic to gauge interest. Konings then introduced Popping to provide a presentation on Glyphosates, which some authorities are beginning to label as carcinogenic. Popping explained that for SPSFAM to take on this project, proper expertise as well as funding will be required. A small group will be meeting later during the Annual Meeting to discuss next steps, additional interest, and to identify potential companies to support the working group.

V. Working Group Launch: Allergens

Konings explained that while the last presentation was from a working group that needs funding, SPSFAM does now have several new working groups that are funded and ready to launch. The Allergens Working Group will be Chaired by Vincent Paez of SCIEX.

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[1] http://griegler-aoac-org.cld.bz/September-27-2015-SPSFAM-Meeting-Meeting-Book
[2] Attachment 1: Crowley Presentation

Paez took the floor with a presentation[3] on the background, significance, analytical needs, regulatory guidance, existing methods, and challenges facing the topic of allergens. He followed this with a proposed fitness for purpose statement for a new SPSFAM Allergens Working Group. After some discussion, the fitness for purpose was revised to read:

Mass spectrometry based method or methods able to detect and/or quantify peanut, tree nuts, soy, egg, gluten, shellfish, fish and milk allergens in selected finished products and ingredients. Each allergen should be uniquely identified.

Coates highlighted that this fitness for purpose statement will likely result in two SMPRs, one for quantitation and one for detection.

MOTION to accept the proposed fitness for purpose statement and launch the SPSFAM Allergens Working Group (Paez/Gilliland).

20 in favor, 0 opposed, 0 abstain. The motion passed.

VI. Working Group Launch: Kombucha

Konings introduced Crum and Trout, co-presenters for the Kombucha Working Group launch presentation. Crum explained that she is from Kombucha Brewers International, a trade association to protect Kombucha worldwide. Trout leads the legislative outreach committee for Kombucha. They provided a presentation[4] on the background, current methodology and regulations, and challenges in measuring alcohol content in Kombucha. After some discussion, the following fitness for purpose statement was proposed:

Methods need to accurately and precisely measure the ethanol concentrations to comply with alcohol and non-alcohol declarations in Kombucha in-process and finished products.

MOTION to accept the proposed fitness for purpose statement for the SPSFAM Kombucha Working Group (M. Phillips/Zweigenbaum).

20 in favor, 0 opposed, 0 abstain. The motion passed.

VII. Other Business and Adjourn

Konings thanked the group for a successful SPSFAM Meeting. He advised that the Arsenic Call for Methods has been extended until January 1, 2016 and that interested parties should submit their methods before that date. He also advised that a small discussion to gauge interest in the potential glyphosate working group will be held later during the annual meeting, and participants will be informed. SPSFAM participants were invited to sign up for a working group on Allergens and Kombucha respectively to develop SMPR's for discussion and agreement during the Mid-year meeting in March 2016. The meeting adjourned at approximately 12:00 pm PT.

[3] Attachment 2: Paez Presentation
[4] Attachment 3: Crum/Trout Presentation

Attachments:

Attachment 1: Crowley ISPAM Presentation

Attachment 2: Paez Allergens Presentation

Attachment 3: Crum/Trout Kombucha Presentation

The Scientific Association Dedicated to Analytical Excellence®

Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM)

March 14, 2016 | 1:00PM – 5:30PM ET
Registration Opens at 12:00 p.m.

Gaithersburg Marriott Washingtonian Center | 9751 Washingtonian Blvd | Gaithersburg, MD, USA
Conference Room: Salon CD

A G E N D A

I. Welcome and Introductions (1:00 p.m. – 1:15 p.m.)
Erik Konings, Nestlé, SPSFAM Chair
   a. Approval of September 27, 2015 Minutes

II. Policies and Procedures (1:15 p.m. – 1:20 p.m.)
Erik Konings, Nestlé, SPSFAM Chair

III. Heavy Metals ERP Update (1:20 p.m. – 1:50 p.m.)
Rick Reba, Nestlé, Heavy Metals ERP Chair

IV. Glyphosate Update (1:50 p.m. – 2:00 p.m.)
Erik Konings, Nestlé, SPSFAM Chair

V. SMPR Presentation: Kombucha* (2:00 p.m.- 3:30 p.m.)
Hannah Crum, Kombucha Brewers International

VI. SMPR Presentation: Allergens* (3:45 p.m. – 5:15 p.m.)
Vincent Paez, SCIEX

VII. Other Business and Next Steps (5:15p.m. – 5:30 p.m.)
Erik Konings, Nestlé, SPSFAM Chair

VIII. Adjourn

**Item requires a vote*

AOAC INTERNATIONAL STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS (SPSFAM)

Hannah Crum, Kombucha Brewers International
Kombucha Working Group
March 14, 2016

Gaithersburg, Maryland

Fitness for Purpose

Methods need to accurately and precisely measure the ethanol concentrations to comply with alcohol and non-alcohol declarations in kombucha in-process and finished products.

Kombucha Working Group Members

Hannah Crum, KBI (Chair)
Sam LaBonia, Cornerstone Labs (Co-Chair)
Michael Beshore, Humm Kombucha
Tim Beshore, Humm Kombucha
GT Dave, Millennium Products, Inc.
Vanessa Dew, Health-Ade
Blake Ebersol. NaturPro Scientific
John Edwards, Process NMR Associates
Cathy Halverson, Alcohol And Tobacco Tax And Trade Bureau
Eldon Hurley, Enology Analytical Services Laboratory
Rachel Kanaan, Unity Vibration Living Kombucha
Erik Konings, Nestle
Alex LaGory, Kombucha Brewers International
Armen Mirzoan, Alcohol And Tobacco Tax And Trade Bureau
Deepali Mohindra, Thermo Scientific
James Neal-Kababick, Flora Research Labs
Theresa Pham, Holy Kombucka
Melissa Phillips, NIST
Catherine Rimmer, NIST
Maged Sharaf, APHA
Gary Spedding, BDAS
Katherine Stenerson, Sigma Aldrich
Rachel Stryffeler, Coca Cola
Darryl Sullivan, Covance
John Szpylka, Mérieux NutriSciences
Daina Trout, Health Ade
Sudhakar Yadlapalli, First Source Laboratory Solutions LLP
Jinchaun Yang, Waters
Chen Zhang, Coca Cola

Kombucha Working Group Work to Date

- 3 teleconferences (November 2015 – December 2015)
- 1 SMPR Drafted
- Public comment period (January 8, 2016 – February 5, 2016)
- SMPRs made ready for SPDS review and approval

Background

WHAT IS KOMBUCHA?

•Fermented tea: Naturally rich in probiotics and healthy acids
•4 ingredients: Tea, Water, Sugar, and SCOBY
- SCOBY = Symbiotic Culture of Bacteria & Yeast
- Most brands brew "raw", **contain some level of alcohol**

•Enjoyed for thousands of years to promote HEALTH!
- 150 years of research, mostly international
- Improved digestion, immunity, energy, metabolism
- "Makes me feel good"

•Naturally low in calories and sugar, effervescent and DELICIOUS!
•Kombucha has a unique composition:

- Acetic acid ferment (like vinegar)
- Plethora of organic & amino acids
- Low pH (typically 2.9-3.5)
- Residual sugars
- Complex sediments
- Natural carbonation

Background

HISTORY OF COMMERCIAL GROWTH

•1995: First to market, GT's Kombucha
•2000's: Steady growth with new brands
•2010: Voluntary withdrawal of kombucha
- Alcohol thought to be >0.5% ABV
- Reformulated brands came back

•Since 2010: HUGE GROWTH
- +30% growth in natural channel
- +50% + growth in grocery channel
- ~$600M in 2015
- Growth BEYOND kombucha

•2014: KBI formed
- 40 founding companies, now 120 breweries
- 90% of commercial bottles on shelves
- WE NEED AN APPROPRIATE & FAIR STANDARD!

KOMBUCHA BREWERS INTERNATIONAL

SMPR Key Points

- Repeatability - Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SDr); or % repeatability relative standard deviation (%RSDr).
- Reproducibility - The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SDR); or % reproducibility relative standard deviation (% RSDR).
- Recovery Factor - The fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

4. **Method Performance Requirements**:

Analytical range (% ABV)	0.1 to 2.8
Limit of Quantitation (LOQ) (% ABV)	≤ 0.05
Accuracy (% of mean spiked recovery over the range of the assay)	97 to 102
Repeatability (RSD_r) %	≤ 4
Reproducibility (RSD_R)%	≤ 6

ABV= alcohol by volume

Comments Submitted (if any)

Kombucha SMPR: Comment 1

SMPR Comments for:	Kombucha
Submitter"s Name	Armen Mirzoian
Submitter"s Email Address	armen.mirzoian@ttb.gov
Organization	TTB
Types of Comments	Technical
Line Numbers (If Applicable)	66-67
Comments (Justification of Change(s))	Sigma-Aldrich 200 Proof Ethanol cannot be used as a reference material because it does not arrive with certificate of analysis that indicates exact concentration of ethanol. It can be used in some cases for preparation of standards only after accurate concentration of ethanol is determined by means of specific gravity.
Proposed Change(s)	Remove the mention of Sigma Aldrich 200 proof ethanol as a reference material

Chair / CSO Response:

This is what we are having to do since the only available reference standard we can find is a 1.0%. We are using the 200 Proof from Aldrich and standardizing it using the Certified 1% standard. Sam

Kombucha SMPR: Comment 2

SMPR Comments for:	Kombucha
Submitter"s Name	Kathy Stenerson
Submitter"s Email Address	katherine.stenerson@sial.com
Organization	MilliporeSigma
Types of Comments	Technical
Line Numbers (If Applicable)	51
Comments (Justification of Change(s))	The analytical range is too wide, that is if this refers to a working range. It will be difficult for most analytical techniques to be linear in this wide of a range. Since the limit to be considered nonalcoholic is 0.5% ABV, going up to 2.8% really should not be necessary.
Proposed Change(s)	Reduce this range to .1% to 1 % ABV

Chair / CSO Response:

This was discussed by the working group and a majority of the working group members agreed to leave the Sigma-Aldrich material on the list because it is a very high concentration (>99.5%) material that can be diluted to lower concentrations at which any minor deviations in the claimed concentration do not matter. Perhaps a parenthetical statement to the effect that "this product does not come with a certificate of analysis" can be added to the SMPR. This issue can be re-examined when the Stakeholder Panel meets.

Discussion?

- What methods currently exist that might fill this need?
- Are there newer technologies that may be more accurate?
- Are there low-cost, in-house solutions also available?

DRAFT AOAC SMPR 2016.XXX; Version 4; December 9, 2015.

Standard Method Performance Requirements for Determination of Ethanol in Kombucha

Intended Use: Use by trained technicians in a laboratory for routine quality assurance testing.

1. **Applicability**:
   Determination of low levels of ethanol as expressed as alcohol by volume (ABV) in kombucha.

2. **Analytical Technique**:
   Any analytical technique that meets the following method performance requirements is acceptable.

3. **Definitions**:

Alcohol by volume (%ABV)
A standard measure of how much alcohol (ethanol) is contained in a given volume of an alcoholic beverage (expressed as a volume percent).

Ethanol
The 2-carbon alcohol with a molecular formula of CH_3CH_2OH. CAS Registry Number: 64-17-5

Kombucha
Kombucha is a fermented, effervescent tea beverage made by adding a symbiotic culture of bacteria and yeast (SCOBY) to a solution of tea and sugar, and may include other ingredients.

Limit of Quantitation (LOQ)
The minimum concentration which quantitative results may be obtained with 95% confidence.

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

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

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

4. Method Performance Requirements:

Analytical range (% ABV)	0.1 to 2.8
Limit of Quantitation (LOQ) (% ABV)	≤ 0.05
Accuracy (% of mean spiked recovery over the range of the assay)	97 to 102
Repeatability (RSD_r) %	≤ 4
Reproducibility (RSD_R)%	≤ 6

ABV= alcohol by volume

5. System suitability tests and/or analytical quality control:

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

6. Reference Material(s):

NIST Standard Reference Material®

2893 Ethanol-water solution (nom. 0.08 %)
2894 Ethanol-water solution (nom. 0.1 %)
2895 Ethanol-water solution (nom. 0.2 %)
2896 Ethanol-water solution (nom. 0.3 %)
2897 Ethanol-water solution (nom. 2 %)

Sigma Aldrich

459836 200 proof, anhydrous, ≥99.5% (Sigma-Aldrich)

Cerilliant CRMs

E-037 Ethanol-80 (5 ampoule multi-pack), 80 mg/dL
E-038 Ethanol-100 (5 ampoule multi-pack), 100 mg/dL
E-039 Ethanol-200 (5 ampoule multi-pack), 200 mg/dL
E-041 Ethanol-150 (10 ampoule multi-pack), 150 mg/dL
E-044 Ethanol-400 (5 ampoule multi-pack), 400 mg/dL

LGC Standards

BCR-651, beer at 0.505 % (v/v) ethanol.
BCR-652, beer at 0.051 % (v/v) ethanol .

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

7. **Validation Guidance**:

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available on line at: http://www.eoma.aoac.org/app_k.pdf

8. **Maximum Time-To-Result:** None

Allergen Working Group Topics for Discussion

1. Nature of Analyte

The precise nature of the analyte has not been resolved. Working Group members have discussed peptides, proteins, and commodities.

Allergens are regulated by the FDA and EU as the whole allergen (i.e., peanuts) and products thereof.

The draft SMPR specifies mass spectrometry as the analytical technique, and presumably methods will detect/measure the peaks associated with certain peptides. However, the exact peptide is left up to the method developer. Methods may differ as to the peptide and fragmentation methods. Therefore the exact ratio of peptide to whole commodity may differ. It should be left up to the method develop to: 1) decide which peptide and fragmentation method; and 2) determine the appropriate conversion factor. The AOAC Expert Review Panel will review the method developer's proposed conversion factors as part of the method review.

RECOMMENDATION: PPM OF ALLERGEN PER COMMODITY.

WORKING GROUP DECISION: **AGREED.**

2. Commutability

It was suggested that reference materials should be commutable. The term "commutability" was first used to describe the ability of a reference or control material to have interassay properties comparable to the properties demonstrated by authentic clinical samples when measured by more than one analytical method.

Commutability is not an AOAC requirement for evaluation of methods.

While commutability would seem ideal, it may not be practical. A commutable allergen reference material would require that a reference material provider demonstrate that equivalent results are obtained using a variety of techniques, or example ELISA. Lateral flow, LC-MS, MALDI-TOF-MS, and PCR. It would seem unlikely that many, if any, reference materials would be characterized by multiple techniques, and just as unlikely that equivalent results would be demonstrated.

RECOMMENDATION: DO NOT REQUIRE COMMUTABILITY OF REFERENCE MATERIALS.

WORKING GROUP DECISION: **AGREED.**

ADDITIONAL: COATES TO REACH OUT TO DAIRY COMMUNITY TO IDENTIFY ADDITIONAL REFERENCE MATERIALS USED BY THE INDUSTRY.

3. VITAL reference doses

3.1 Voluntary Incidental Trace Allergen Labeling (VITAL) is a project of the Allergen Bureau based in Australia/New Zealand. There is also an EU-VITAL. VITAL is a voluntary consensus project to establish maximum levels of allergens for safe consumption by individuals with food allergies.

The maximum allergen concentrations are calculated using the specification provided by the commenter for a 200 g serving size of food.

	serving size (g)	converted to kg	mg/kg
hazel nut	200	0.2	3.2
milk	200	0.2	15.2
peanut	200	0.2	4
whole egg	200	0.2	1.2

As the commenter noted, the maximum permissible concentration of allergens are all below the proposed LOQ in the SMPR.

While the VITAL concentrations are not binding, they are based on scientific studies.

RECOMMENDATION: LOQ AND RANGES CONSISTENT WITH THE VITAL WOULD SEEM TO BE A BENEFIT FOR INTERNATIONAL TRADE, AND THEREFORE A GOOD REASON TO REVISE THE SMPR.

WORKING GROUP DECISION: VITAL IS NOT AN INTERNATIONALLY RECOGNIZED STANDARD. **AGREED NOT REVISE THE SMPR.**

3.2 VITAL values are based on amount of protein per service size. Therefore, the definition of the food allergens as “food commodities” without mentioning the protein content will establish a non-comparability between results obtained by an LC-MS/MS method and VITAL values.

Method developers can provide conversion factors in their methods for peptide to protein to “whole” allergen.

RECOMMENDATION: NO CHANGE RECOMMENDED.

WORKING GROUP DECISION: AGREED WITH RECIMMENDATION. **NO CHANGE RECOMMENDED.**

AOAC SPSFAM ALLERGENS DRAFT SMPR - COMMENTS on ALLERGENS SMPR FINAL

Item	Line Numbers (If Applicable)	Comment	Proposed Change(s)	Response
1	139 (table 2)	Chocolate is an important matrix for peanut, hazelnut and milk.	Chocolate should be included into the list of priority allergens. If chocolate is a known problem than the applicability should clearly state that chocolate is not possible to measure using the validated method.	**No change.** Chocolate is an optional matrix to be tested for candidate method that claim to work in chocolate.
2	56-65	Should the precision data obtained over the whole analytical range? Number of levels?	Describe the validation of precision in a more precise way e.g. include number of levels and replicates	**Additional reference** to Appendix D and F are added
3	116 (table 1)	By definition the analytical range can only start with an LoQ. MDL only gives a yes or no.		**No change recommended.** The comment is true but there is not any prohibition against the LOQ = MDL.
4		After validation, LC-MS/MS methods will be used for comparison with ELISA results. An commercial ELISA is (often) calibrated to the whole allergenic food while LC-MS/MS is calibrated to peptides. Is comparability established via reference materials? (again: traceability of LC-MS/MS to these RMs is mandatory!)	Discuss traceability and comparability to ELISA results (note: this SMPR discuss a possible reference method for cGMP compliance!)	**No change.** The working group did not agree to tie LC-MS/MS results to ELISA results.
5	96	NIST SRM 2387 is not pure peanut but a mixture of roasted peanut, sugar, partially hydrogenated vegetables oils and salt. See NIST certificate: protein content is given but not peanut content.	Discuss suitability of this SRM in the working group and give conversion factor	**No change.** That's will be left up to the methode developer.
6	92	NIST SRM 1549 is superseded by NIST 1549a	Delete NIST SRM 1549	**Agree. Replace NIST SRM 1549 wuth 1549a.**
7	85	NIST 8445 is a whole egg powder with a given protein content. How should a method developer trace it to whole egg without conversion factor?	Discuss traceability in the working group and discuss a conversion factor	**Working Group agreed that all results to be "reported as ppm of the target allergen in food commodity".**
8	67	Recovery: What kind of samples is required? Spiked or incurred? For ELISA incurred is preferred.	We should follow the guideline for ELISA which prefer incurred	**Add a reference to Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods.** Appendix M does mandate the use of incurred samples. AOAC policy allows for both kinds of samples. Method developer discretion.
9	67	Recovery: How should a method developer determine this parameter? By spiking with reference materials or peptides or a different material. One should remember that it is not allowed to use a reference material for calibration AND spiking! If peptides are used for calibration, how was traceability established?	Discuss in the working group and remember to solve the traceability problem	**No change recommended.** Method development issue not SMPR issue.
10	62	Since reproducibility determination is only possible by a collaborative study, an intra-laboratory reproducibility should be defined to ease single-lab validations at the beginning	Inlcude a new clause after repeatability and describe the validation to be done	**No change.** All previous SMPRs used RSDR and RSDr.

11	50	MDL: How should a method developer estimate this parameter? By using blank matrices or blank matrices spiked with reference materials/peptides? How many replicates? We have very clear guidelines for allergen determination by ELISA-why not for "Reference methods for cGMP compliance"?	Discuss in the working group maybe follow ELISA guidelines	**Reference to Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods added to SMPR.** SMPR will also refer to FDA and/or EPA definition for MDL.
12	46	LoQ: How should a method developer determine or even estimate this parameter? By using reference materials or peptide solutions or blank matrices or blank matrices spiked with reference materials/peptides? How many replicates? We have very clear guidelines for allergen determination by ELISA-why not for "Reference methods for cGMP compliance"?	Discuss in the working group maybe follow ELISA guidelines	**Reference to Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods added to SMPR.**
13	46-69	LoQ, MDL, recovery and precision data need to be determined for every claimed matrix	include a sentence for each parameter that explains the parameter-specific validation	**Line 108 of version revised** to recommend "LOQ, MDL, recovery and precision" data for every claimed matrix.
14	116 (table 1)	By taking the latest published VITAL reference doses C18(Food Chem. Toxicol. 63: 9-17, 2014) it is obvious that the MDLs/LoQs in table 1 are not sufficient when a food is analyzed that is consumed in a service size of more than 50 g. Lower MDL/LoQ appropriately to the following table. Note: C19 Hazelnut: Reference dose as protein: 0.1 mg; Reference dose as allergenic food: 0.64 mg; Minimum concentration to be quantified when consuming 50 g food: 12.8 mg/kg and for 200 g 3.2 mg/kg. Milk: Reference dose as protein: 0.1 mg; Reference dose as allergenic food: 3.03 mg; Minimum concentration to be quantified when consuming 50 g food: 60.6 mg/kg and for 200 g 15.2 mg/kg. Peanut: Reference dose as protein: 0.2 mg; Reference dose as allergenic food: 0.8 mg; Minimum concentration to be quantified when consuming 50 g food: 16 mg/kg and for 200 g 4 mg/kg. Whole egg: Reference dose as protein: 0.03 mg; Reference dose as allergenic food: 0.25 mg; Minimum concentration to be quantified when consuming 50 g food: 4.8 mg/kg and for 200 g 1.2 mg/kg.	Change MDLs/LoQ in table 1 according to the VITAL values and calculations given under comments. Discuss in the working group	**No change.** Working Group discussed on 3/3/2016. There are multiple VITALs with different maximum permissiable concentrations. The Working Group consensus is that none of the VITALs are international concensusn standards, and declined to reset the LOQs or MDLs based on VITALmaximum permissiable concentrations.
15		How should a method developer prove that the selected peptides are not "too" specific e.g. a sequence is used that is not present in every commercially available peanut or hazelnut variety. On the opposite, if the selected peptides are not specific enough, near botanical relatives are detected which are maybe not allergenic or regulated (see prunus mahaleb example).	At minimum a chapter describing the known specificities/selectivities should be provided. (Note: Unknown occurrence of peptides that are not from a allergenic source will always occur in the future, see also prunus mahaleb)	**No change.** The working group did not agree.
16		What are the minimum performance criteria for peptide selection?	Include criteria for peptide selection or give reference	**No change.** The working group did not agree.
17		VITAL values are based on amount of protein per service size. Therefore, the definition of the food allergens as "food commodities" without mentioning the protein content will establish a non-comparability between results obtained by an LC-MS/MS method and VITAL values.	Include some guidance for the user or let the method developer describe his way of establishing traceability to VITAL values	**No change.** Working Group discussed on 3/3/2016. There are multiple VITALs with different maximum permissiable concentrations. The Working Group consensus is that none of the VITALs are international concensusn standards, and declined to reset the LOQs or MDLs based on VITALmaximum permissiable concentrations. AND E25
18	9	collaborative test: It should be critically checked if Appendix D is sufficient in the case where LESS than 8 participants (and/or LC-MS/MS machines) are available. Is this still collaborative or forbidden at all?	discuss in the working group	**No change.** AOAC policy not a working group decision.
19	3	The title is unclear	change to "…selected food allergens"	**Change title to "…selected food allergens."**
20	9	This means a method comparison between the original method (checked by an ERP) and this method transferred to another lab. Are there any guidelines for this case? What is the minimum required number of measurements to be sure that both methods are comparable?	Include minimum requirements for verification	**No change.** Method comparision is not a verification requirement.

21		Hazelnuts and peanut are not defined in sense of their variety while milk and egg are not defined in sense of their origin	A method developer should validate the differences between different varieties of hazelnuts and peanuts in sense of traceability and measurement uncertainty. A method developer should validate different species that deliver milk (cow, goat, sheeo etc.); same for egg. Maybe an in-silico analysis of peptide sequences is sufficient.	**Species names were added to the SMPR. The working group group did not agree to requireing different varieties.**
22		The term "allergen" is not defined	The SMPR should contain a clarifying chapter or clause that explains that the term “allergen” is used in an analytical and not immunological way. “Allergen” could also mean a specific protein from hazelnut that differs between regions and varieties.	**No change recomended.** Although the term "allergen" itself is not defined, the identification of food allergens types in the SMPR provides all of the needed information for method developers. No definition were found that weren't circular. i.e., an allergen is a molecule causes an allergic reaction.
23		A general problem of this SMPR is traceability of results. In detail, an LC-MS/MS user prepared peptide solutions on a weight by weight basis and uses reference materials also on a weight by weight basis. Are these reference materials of a higher order in the calibration hierarchy or are the peptides of higher level? Furthermore, how should we re-calculate to the “analyte” which is for example “milk”. There is no conversion factor to re-calculate from “weight whole milk powder from NIST” to “milk”. Even more problematic is the recalculation from “peak area of a specific milk peptide” to “milk”.	At a minimum, each validation report should contain a chapter that clearly describes how the validation manager solved this fundamental problem for each allergen or describes the limitations of quantitative results.	Working Group agreed that all results to be "reported as ppm of the target allergen in food commodity".
24		I have developed a sign system for the 14 allergens which, according to the European Union, must be indicated in food packagings. It has being the work of 7 months for my final grade in Graphic Design.	Implement your standard regulation by applying the sign system for the allergens (eggs, milk, nuts, peanuts, celery, sesame, mustard, sulphites, fish, crustaceans, mollusks, gluten, lupin and soy). It would help to the interpretation of the ingredients independently from the country where people live or the language that they speak. Thank you	**No change.** Irrelevant

DRAFT AOAC Allergen SMPR Version 5; March 10, 2016.

Detection and Quantitation of Selected Food Allergens

Intended Use: Reference method for cGMP compliance.

1. **Purpose:** AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.

2. **Applicability**:
Detection and quantitation of egg, milk, peanut, and hazelnut food allergens in finished food products and ingredients. Method(s) shall uniquely identify each allergen.

3. **Analytical Technique**:
Mass spectrometry based methods.

4. **Definitions**:

Food Allergens

Hazelnut

Any of the nuts deriving from species of the genus *Corylus*, especially the nuts of the species *Corylus avellana* (the common hazel tree).

Milk

For the purposes of this SMPR: "milk" refers to pasteurized whole cow's (*Bos Taurus)*. milk, and shall contain not less than 8 1/4 percent milk solids not fat and not less than 31/4 percent milkfat.[1]

Peanut

The seed of the *Arachis hypogaea* plant. For the purposes of this SMPR, includes both raw and roasted peanuts.

[1] Code of Federal Regulations; Title 21 - Food and Drugs, § 131.110. Other internationally recognized definition may be applied.

Whole Egg

A combination of pasteurized chicken (*Gallus gallus domesticus*) egg whites and egg yolks from the same production batch blended together in their entirety, in natural proportions.[2]

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result. LOQ = average (blank) + 10 * s0 (blank).*

Method detection limit (MDL)

Method detection limit (MDL) is the minimum concentration of a substance than can be measured and reported with 99% confidence that the analyte concentration is greater than zero. It is determined from analysis of a sample in a given matrix containing the analyte. [3]

The minimum concentration of a substance that can be measured (detected) and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte using at least two ion MS/MS transitions. See 4 (a) of 40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision.[4]

Repeatability

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

Reproducibility

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

Recovery

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

5. Method Performance Requirements:

See table 1.

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[2] Introduction to Egg Products, USDA Food Safety and Inspection Service, website: http://www.fsis.usda.gov/wps/wcm/connect/c5c85914-5055-4f09-8098-1a179a1c6e14/EPT_Introduction.pdf?MOD=AJPERES, accessed 12/15/2015.

* See Table A3 in Appendix F: *Guidelines for Standard Method Performance Requirements* for additional guidance.

** See Spiking method in Appendix M in the Official Methods of Analysis.

[3] Volume II - Methods, Method Verification and Validation ORA-LAB.5.4.5; DOCUMENT NO.: IV-02; VERSION NO.:1.7; Section 2 – Microbiology; EFFECTIVE DATE: 10-01-03; REVISED: 08-25-14; WEBSITE: http://www.fda.gov/ScienceResearch/FieldScience/ucm171877.htm, ACCESSED: Feb. 22, 16.

[4] 40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision (link)

6. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. **Potential Reference Material(s):**

Whole Egg
- NIST 8445
- LGC SAL-RSM-5 (Check for characterization level)

Nonfat Milk Powder
-
- NIST SRM 1549a (whole milk powder)

Peanut
- NIST SRM 2387 (peanut butter)
- LGC FAL-RFM1017-XXX

Hazelnut
- LGC FAL-RFM1015-50 or FAL-RFM1015-50Â or FAL-RFM1015-5

Additional materials can be found at the LGC and FAPAS websites
LGC = http://www.lgcstandards.com/HK/en/search/?q=allergen:relevance:category:CEFP_77243
FAPAS = http://fapas.com/quality-control-materials/Available-quality-control-materials.cfm

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

NIST = National Institute for Standards and Technology.
LGC = formerly the UK *Laboratory of the Government Chemist*, now simply "LGC Standards".
FAPAS = formerly the *Food Analysis Performance Assessment Scheme* in the UK, now simply "FAPAS".

8. **Validation Guidance**:

Method developers shall submit LOQ, MDL, recovery and precision data for the matrices in Table 2.

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

9. **Maximum Time-To-Result:** None

Table 1: Method performance requirements.

Parameter	Target allergen			
	whole egg	**milk**	**peanut**	**hazelnut**
Analytical Range (ppm)	10-1000	10-1000	10-1000	10-1000
LOQ (ppm*)	5	10	10	10
MDL (ppm*)	1.65	3	3	3
Recovery (%)	60-120%	60-120%	60-120%	60-120%
% RSD_r	≤20 %	≤20 %	≤20 %	≤20 %
% RSD_R	≤ 30%	≤ 30%	≤ 30%	≤ 30%

* Reported as ppm of the target allergen in food commodity, i.e., 25 ppm of "whole egg" in cookies.

Definitions for LOQ and MDL provided in section 4.

Table 2: Priority Allergen/Matrix Combinations

whole egg	cookies bread dough salad dressing wine
milk	cookies, baked goods infant formula wine dark chocolate (optional matrix for methods that claim a chocolate matrix)
peanut	cookies ice cream breakfast cereal milk chocolate (optional matrix for methods that claim a chocolate matrix)
hazelnut	cookies ice cream breakfast cereal milk chocolate (optional matrix for methods that claim a chocolate matrix)

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.

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40 CFR Part 136, Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision

analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

(http://ecfr.gpoaccess.gov/graphics/ec15no91.208.gif)

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

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 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.

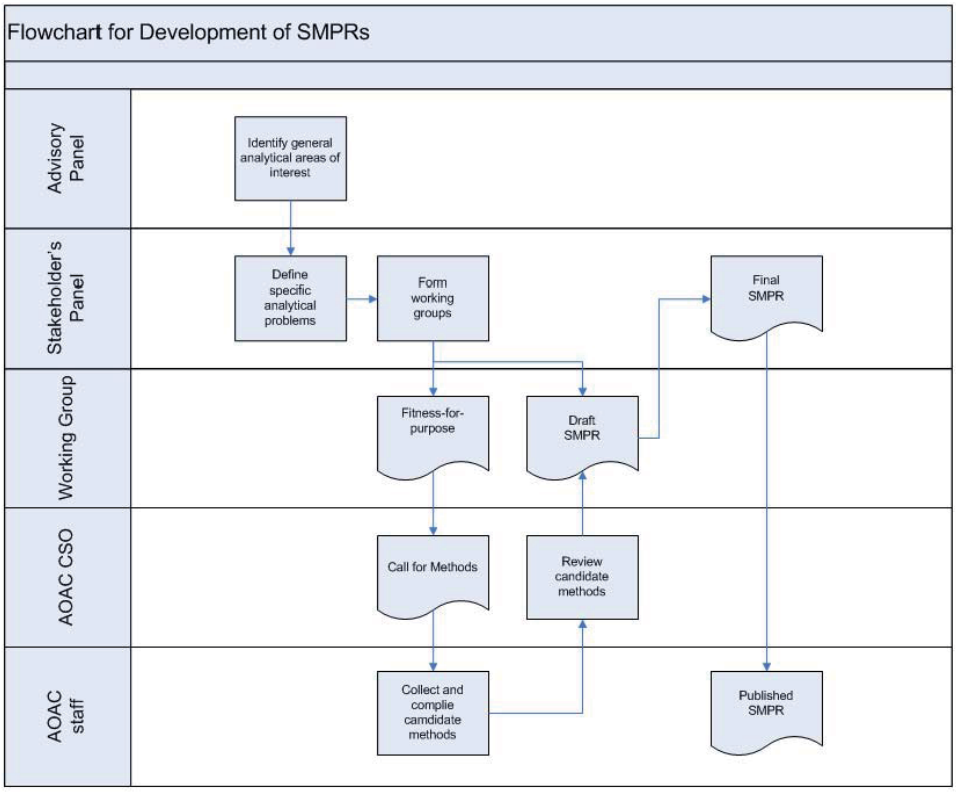

Figure 1. Schematic flowchart diagram of the SMPR development process.

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.

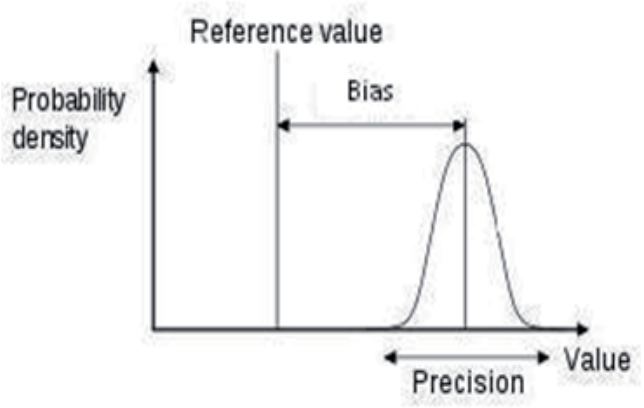

Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

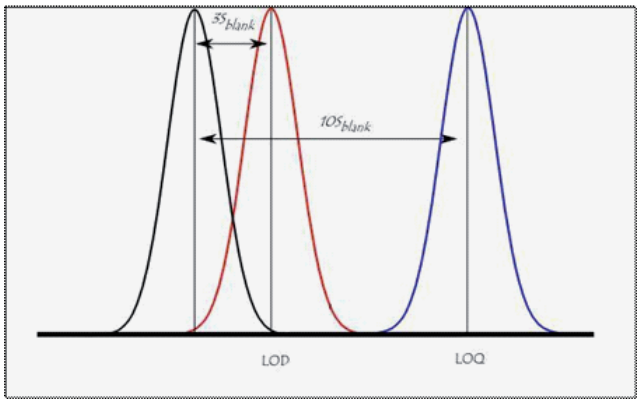

Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

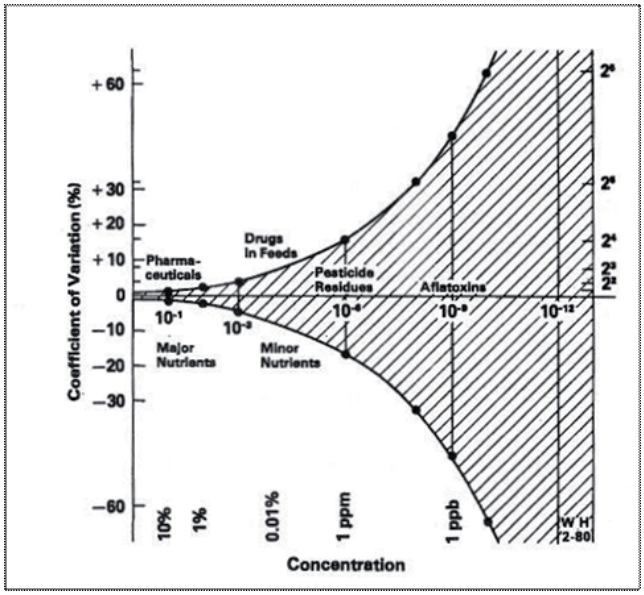

Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

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.

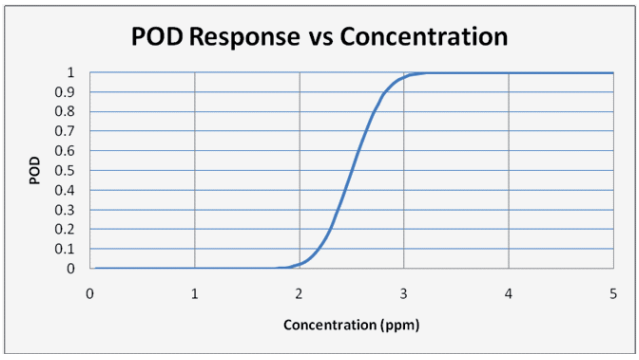

Figure C1. Theoretical POD curve for a qualitative detection method.

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

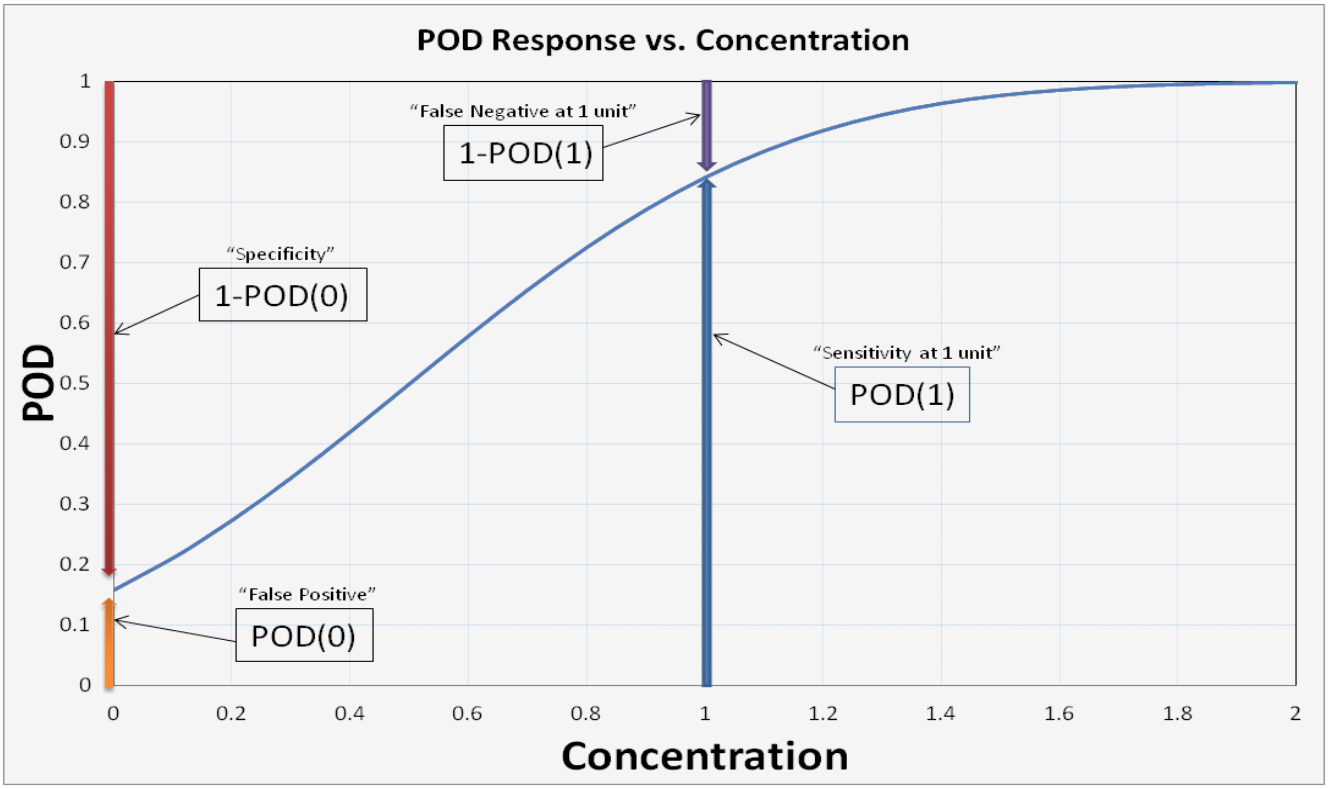

Figure C2. Comparison of POD model terminology to other obsolete terms.

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

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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.

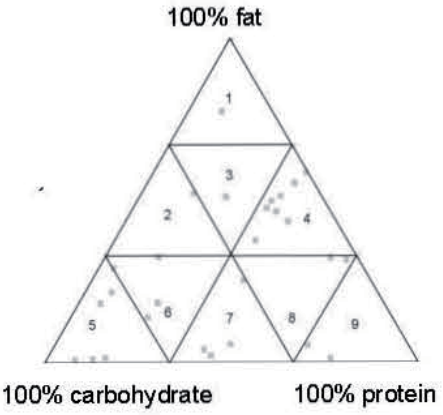
Why Use an In-House Reference Material?

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.

Stakeholder Panel on Strategic Food Analytical Methods
As of: March 10, 2016

Erik J. M. Konings, Chair
Nestle Research Center
Quality And Safety Dept, PO Box 44
Vers-chez-les-Blanc
Lausanne 26, CH-1000
Switzerland
Tel. +41 21 785 8232 (O)
Fax. +41 21 785 8553
Email: erik.konings@rdls.nestle.com
Term: February 5, 2014 - February 5, 2017

Michael Beshore, Member
Humm Kombucha LLC
1125 NE 2nd St
Bend, OR 97701
USA
Tel. +1(541) 306-6329 (O)
Term: September 14, 2015 - September 14, 2018

Tim Beshore, Member
Chemours
,
Email: timbeshore@gmail.com
Term: September 14, 2015 - September 14, 2018

Jeannie Buscher, Member
Buchi Kombucha
,
Email: jeannine@drinkbuchi.com
Term: November 23, 2015 - November 23, 2018

Jim Cali, Member
Promega Corporation
2800 Woods Hollow Rd
Madison, WI 53711
USA
Tel. +1(608) 443-3503 (O)
Email: jim.cali@promega.com
Term: January 6, 2016 - January 6, 2019

Esther Campos-Gimenez, Member
Nestle Research Center
PO Box 44, Vers-chez - 65 - Blanc
Lausanne 26, Vaud, 1000-CH
Switzerland
Tel. ++41 21 785 8250 (O)
Fax. ++41 21 785 8553
Email: esther.campos-gimenez@rdls.nestle.com
Term: September 4, 2015 - September 4, 2018

Mr. Niladri Sekhar Chatterjee, Ph.D, Member
Indian Agricultural Research Institute
ROOM NO.84, VASANT HOSTEL
IARI (PUSA CAMPUS)
NEW DELHI, 110012
India
Tel. +(996) 837-0481 (O)
Email: niladri.sekhar.chatterjee@gmail.com
Term: June 1, 2014 - June 1, 2017

Geoffrey Cottenet, Member
Nestle Research Center
Route Du Jorat 57
Vers-Chez-Les-Blanc
Lausanne, 1000
Switzerland
Tel. +0041 21 785 8085 (O)
Email: geoffrey.cottenet@rdls.nestle.com
Term: January 20, 2015 - January 20, 2018

Hannah Crum, Member
Kombucha Brewers International
8950 W Olympic Blvd Ste 494
Beverly Hills, CA 90211
USA
Email: info@kombuchabrewers.org
Term: August 27, 2014 - August 28, 2018

GT Dave, Member
Millennium Products, Inc.
4646 Hampton St
Vernon, CA 90058
USA
Tel. +1(310) 709-9955 (O)
Email: gt@drinkgts.com
Term: September 14, 2015 - September 14, 2018

Carmen Diaz-Amigo, Member
2 Chemin Des Muilles
Villa 6
Grézieu-la-Varenne, 69290
France
Email: carmen.diaz.amigo@gmail.com
Term: November 20, 2015 - November 20, 2018

Robert Donofrio, Member
NSF International
789 N Dixboro Rd
Ann Arbor, MI 48105
USA
Tel. +1(734) 827-6894 (O)
Fax. +1(734) 827-7191
Email: donofrio@nsf.org
Term: September 4, 2015 - September 4, 2018

Blake E. Ebersole, Member
Verdure Sciences
,
Email: blake@npscientific.com
Term: November 24, 2015 - November 24, 2018

Katherine Fiedler, Member
U.S. FDA
HFS-707/BE-018
5100 Paint Branch Pkwy
College Park, MD 20740
USA
Tel. +1(240) 402-5055 (O)
Email: katherine.l.fiedler@fda.hhs.gov
Term: October 30, 2015 - October 30, 2018

Jodie Fung, Member
Kombucha Brewers International
3347 Motor Ave #200
Los Angeles, CA 90034
USA
Tel. +1(949) 648-1928 (O)
Email: lgo@kombuchabrewers.org
Term: September 4, 2015 - September 4, 2018

Russell Gerads, Member
Brooks Rand Labs, LLC
3958 6th Ave NW
Seattle, WA 98107
USA
Tel. +1(425) 483-3300 (O)
Email: russ@brooksrand.com
Term: September 4, 2015 - September 4, 2018

Brendon D. Gill, Member
Fonterra Co-operative Group Ltd.
Cnr. No.1 Road & S.H. 26
PO Box 7
Waitoa, 3341
New Zealand
Tel. ++64-7-8893989 (O)
Fax. +(647) 887-1501
Email: brendon.gill@fonterra.com
Term: September 4, 2015 - September 4, 2018

Jasmine Hagan, B.S., Member
ELISA Technologies, Inc.
2501 NW 66th Ct
Gainesville, FL 32653
USA
Tel. +1(352) 337-3929 (O)
Email: jasmine@elisa-tek.com
Term: September 4, 2015 - September 4, 2018

Kevin Hegarty, Member
Effendorf
,
Email: hegarty.k@eppendorf.com
Term: July 1, 2014 - July 1, 2017

Norma R. Hill, Member
US Treasury (Retired)
1382 Deerfield Ct
Concord, CA 94521
USA
Tel. +1(925) 681-8672 (O)
Email: normarhill@gmail.com
Term: September 4, 2015 - September 4, 2018

Prashant Ingle, Member
2811 W 235th St Apt 9
Torrance, CA 90505
USA
Tel. +1(510) 378-7213 (O)
Email: prashant.ingle@hotmail.com
Term: September 4, 2015 - September 4, 2018

Brian P. Jackson, Member
Dartmouth College
Earth Sciences
HB 6105 Fairchild Bldg
Hanover, NH 03755
USA
Tel. +1(603) 646-1272 (O)
Fax. +1(603) 646-3922
Email: brian.p.jackson@dartmouth.edu
Term: June 1, 2014 - June 1, 2017

Rasu Jayabalan, Member
National Institute Of Technology Rourkela
,
Email: jayabalanr@nitrkl.ac.in
Term: September 14, 2015 - September 14, 2018

David C. Kennedy, Ph.D, Member
Phenomenex
1583 Redford Dr Unit A
Palm Springs, CA 92264
USA
Tel. +1(402) 304-5022 (O)
Fax. +1NA
Email: davidk@phenomenex.com
Term: September 4, 2015 - September 4, 2018

Barbro Inga Kristina Kollander, Ph.D, Member
National Food Agency
Livsmedelsverket, 75162
Box 622
Uppsala,
Sweden
Tel. ++46(0)18175360 (O)
Fax. ++46(0)18105848
Email: barbro.kollander@slv.se
Term: September 4, 2015 - September 4, 2018

Alex LaGory, Member
Kombucha Brewers International
8950 W. Olympic Blvd. Suite 494
Beverly Hills, CA 90211
USA
Tel. +1(424) 245-5867 (O)
Email: admin@kombuchakamp.com
Term: September 4, 2015 - September 4, 2018

Alex LaGory, Member
Kombucha Brewers International
8950 W. Olympic Blvd. Suite 494
Beverly Hills, CA 90211
USA
Tel. +1(424) 245-5867 (O)
Email: admin@kombuchakamp.com
Term: September 14, 2015 - September 14, 2018

Kristie Laurvick, MS, Member
US Pharmacopeia (USP)
8116 Valley Run Dr
Clifton, VA 20124
USA
Tel. +1(301) 816-8356 (O)
Fax. +1(301) 816-8373
Email: kxb@usp.org
Term: October 8, 2013 - December 31, 2016

John Lawry, Member
Covance
,
Email: john.lawry@covance.com
Term: October 30, 2015 - October 30, 2018

Mr. John W. Lee, Member
MasterFoods USA
3250 E 44th St
Vernon, CA 90058
USA
Tel. +1(323)586-4837 (O)
Fax. +1(323)586-8386
Email: john.lee@effem.com
or john.lee@effem.com
Term: September 4, 2015 - September 4, 2018

Daniel Lopez-ferrer, Member
Thermo Scientific
,
Email: daniel.lopezferrer@thermofisher.com
Term: October 30, 2015 - October 30, 2018

Eric Marceau, Member
Canadian Food Inspection Agency (CFIA)
1001 Saint-Laurent St W
Longueuil Laboratory
Longueuil, QC J4K 1C7
Canada
Tel. +(450) 928-4367 (O)
Fax. +(450) 928-4249
Email: eric.marceau@inspection.gc.ca
Term: September 25, 2015 - September 25, 2018

Elaine Catherine Marley, Ph.D, Member
R-Biopharm Rhone Ltd
Acre Road
Block 10 Todd Campus WSSP
Glasgow, G200XA
United Kingdom
Tel. +00441419452924 (O)
Fax. +00441419452925
Email: elaine@r-biopharmrhone.com
Term: September 4, 2015 - September 4, 2018

Katerina Mastovska, Ph.D, Member
Covance Laboratories
3138 Spring Mill Rd
Plymouth Meeting, PA 19462
USA
Tel. +1(317) 371-2968 (O)
Email: katerina.mastovska@covance.com
Term: September 4, 2015 - September 4, 2018

Allen Misa, Member
Phenomenex, Inc.
411 Madrid Ave
Torrance, CA 90501
USA
Tel. +1(310) 212-0555 (O)
Email: AllenM@phenomenex.com
Term: September 4, 2015 - September 4, 2018

Lisa Monteroso, Member
3M Food Safety
3M Center Bldg 0260-06-B-01
Food Safety Dept
Saint Paul, MN 55144-1000
USA
Tel. +1(651) 736-2913 (O)
Fax. +1(651) 733-1805
Email: lmonteroso@mmm.com
Term: December 3, 2015 - December 3, 2018

Christine Parker, Member
FDA
,
Email: Christine.Parker@fda.hhs.gov
Term: October 30, 2015 - October 30, 2018

Son Quang Pham, Ph.D, Member
Abbott
20 Biopolis Way #09-01/02
Centros Building
Singapore, 138668
Singapore
Tel. ++65 68016346 (O)
Fax. ++65 6478 9010
Email: quangson.pham@abbott.com
Term: September 25, 2015 - September 25, 2018

Theresa Pham, Member
Holy Kombucha, Inc.
,
Email: theresa@holycombucha.com
Term: September 25, 2015 - September 25, 2018

Tom Phillips, Member
MD Department Of Agriculture
50 Harry S Truman Pkwy
State Chemist Section
Annapolis, MD 21401
USA
Tel. +1(410) 841-2713 (O)
Fax. +1(410) 841-2740
Email: tom.phillips@maryland.gov
Term: July 1, 2014 - July 1, 2017

Eric Poitevin, Member
Nestle Research Center
PO Box 44, Vers-chez - 65 - Blanc
Lausanne 26, Vaud, 1000-CH
Switzerland
Tel. ++41 21 785 9545 (O)
Fax. ++41 21 785 8553
Email: eric.poitevin@rdls.nestle.com
Term: September 4, 2015 - September 4, 2018

Jenny Scifres, Member
USDA FSIS OPHS LQAD ALP
950 College Station Rd
Athens, GA 30605
USA
Tel. +1(706) 546-2337 (O)
Fax. +1(706) 546-4265
Email: jenny.scifres@fsis.usda.gov
Term: September 1, 2015 - September 1, 2018

Cheryl Stephenson, Ph.D., Member
Eurofins Central Analytical Laboratory
2248 Jefferson St
Mandeville, LA 70448
USA
Tel. +1(504) 293-2739 (O)
Email: cherylstephenson@eurofinsus.com
Term: June 1, 2014 - June 1, 2017

Rachel Stryffeler, Member
Coca-Cola Company
TEC 605 , 1 Coca-Cola Plaza NW
Atlanta, GA 30301
USA
Tel. +1(404) 676-5210 (O)
Email: rstryffeler@coca-cola.com
Term: September 25, 2015 - September 25, 2018

Julie Sundgaard, Member
ROMER Labs Inc
2456 133rd Ln NW
Andover, MN 55304
USA
Tel. +1(320) 241-8617 (O)
Fax. +1(320) 253-6250
Email: julie.sundgaard@romerlabs.com
Term: November 23, 2015 - November 23, 2018

Daina Trout, Member
Health-Ade LLC
3347 Motor Ave #200
Los Angeles, CA 90034-3711
USA
Tel. +1(202) 297-0248 (O)
Email: daina@health-ade.com
Term: September 14, 2015 - September 14, 2018

Jolanta Vidugiriene, Member
Promega
,
Email: jolanta.vidugiriene@promega.com
Term: January 6, 2016 - January 6, 2019

Azza M. Waggy, Member
Edgar A. Weber & Co.
549 Palwaukee Dr
Wheeling, IL 60090
USA
Tel. +1(847) 215-1980 x176 (O)
Fax. +1(847) 392-8414
Email: azzaw@weberflavors.com
Term: December 1, 2014 - December 1, 2017

Wayne Wargo, Member
Abbott Nutrition
3300 Stelzer Rd Bldg RP4-2
Columbus, OH 43219
USA
Tel. +1(614) 624-3456 (O)
Fax. +1(614) 727-3456
Email: wayne.wargo@abbott.com
Term: September 1, 2015 - September 1, 2018

Guy Weerasekera, Member
Mead Johnson Nutrition
2400 W Lloyd Expy
Global Quality Assurance
Evansville, IN 47721
USA
Tel. +1(812) 429-5940 (O)
Fax. +1(812) 429-7492
Email: gayanga.weerasekera@mjn.com
Term: September 1, 2015 - September 1, 2018

Jason Lynn Wubben, Member
Archer Daniels Midland Company
1001 N Brush College Rd
James R Randall Research Ctr
Decatur, IL 62521
USA
Tel. +1(217) 451-4740 (O)
Fax. +1(217) 451-2457
Email: jason.wubben@adm.com
Term: October 1, 2014 - October 1, 2017

Chen Zhang, Member
Coca-Cola Company
TEC 6, 1 Coca-Cola Plaza NW
Atlanta, GA 30301
USA
Tel. +1(404) 676-4895 (O)
Email: chezhang@coca-cola.com
Term: September 25, 2015 - September 25, 2018

Scott G. Coates, AOAC Staff
AOAC INTERNATIONAL
2275 Research Blvd Ste 300
Rockville, MD 20850-3250
USA
Tel. +1(301) 924-7077 x137 (O)
Fax. +1(301) 924-7089
Email: scoates@aoac.org
Term: April 1, 2015 - April 1, 2020

Christopher Dent, AOAC Staff
AOAC INTERNATIONAL
2275 Research Blvd Ste 300
Rockville, MD 20850-3250
USA
Tel. +1(301) 924-7077 x119 (O)
Fax. +1(301) 924-7089
Email: cdent@aoac.org
Term: April 1, 2015 - April 1, 2020

Dawn L. Frazier, AOAC Staff
AOAC INTERNATIONAL
2275 Research Blvd Ste 300
Rockville, MD 20850-3250
USA
Tel. +1(301) 924-7077 x117 (O)
Fax. +1(301) 924-7089
Email: dfrazier@aoac.org
Term: April 1, 2015 - April 4, 2020

Deborah McKenzie, AOAC Staff
AOAC INTERNATIONAL
2275 Research Blvd Ste 300
Rockville, MD 20850-3250
USA
Tel. +1(301) 927-7077 x157 (O)
Fax. +1(301) 924-7089
Email: dmckenzie@aoac.org
Term: April 1, 2015 - April 1, 2020