AOAC INTERNATIONAL

Official Methods Board

2016 Expert Review Panel (ERP) of the Year Candidates
(2013-2015 Expert Review Panel Profiles)

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2016 AOAC OFFICIAL METHODS BOARD AWARDS

CANDIDATES FOR

EXPERT REVIEW PANEL OF THE YEAR

2013 – 2015 AOAC EXPERT REVIEW PANEL PROFILES

Expert Review Panel of the Year Award Information

The minimum criteria for selection are:

a. The expert review panel must have completed a significant milestone (e.g. First Action Method, Final Action Method, method modification) within the last three years.
b. Generally, some unique or particularly noteworthy aspect of the ERP's work is highlighted as making the ERP worthy of the award, such as innovative technology or application, breadth of applicability, critical need, difficult analysis, or timeliness.
c. The panel report demonstrates significant merit as to the scope of the project, the involvement of a diverse and/or international group of recognized experts or an innovative approach to difficult analytical challenge.

Selection Process:

a. AOAC staff lists all eligible panels for consideration and forwards that list along with the ERP report to the Chair of the Official Methods Board (OMB).
b. The OMB Chair forwards the list along with any supporting information to the OMB.
c. The OMB selects the Expert Review Panel of the Year. Winner is selected by a 2/3 vote. If necessary, the OMB chair may cast tie-breaking vote.

Award

An appropriate letter of appreciation and thanks will be sent to the members of the winning Expert Review Panel. The winning panel will be announced at the appropriate session of the AOAC INTERNATIONAL annual meeting, with presentation of an award. All panelists participating in the winning panel will be acknowledged at the annual meeting, receive an award and a letter of appreciation. The name of the winning ERP, with supporting story, will be carried in the announcement in the *ILM*.

2013 – 2015 AOAC EXPERT REVIEW PANEL PROFILES

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ERP PROFILE SUMMARIES

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPIFAN NUTRIENT METHODS

ERP Name	AOAC Expert Review Panel for SPIFAN Nutrient Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2011	**Number of Methods Adopted**	38 as First Action status	**Number of Methods Recommended**	8 OMAs Final Action; 6 OMAs for repeal
Scope:	Review and adopt methods resulting from SMPRs developed by SPIFAN. Recommend one method per nutrient for Final Action.				

Roster

1.	Darryl Sullivan	Covance (Chair)
2.	John Austad	Covance
3.	Sean Austin	Nestlé ***(FOS/GOS Only)***
4.	Sneh Bhandari ***(OMB Liaison)***	Mérieux NutriSciences
5.	Esther Campos-Gimenez/Adrienne McMahon	Nestlé/Wyeth
6.	Scott Christiansen	Perrigo/PBM Nutritionals
7.	Jon DeVries	Independent Consultant
8.	Sarwar Gilani	Independent Consultant
9.	Brendon Gill/Harvey Indyk	Fonterra
10.	Don Gilliland ***(OMB Liaison)***/Karen Schimpf	Abbott Nutrition
11.	Min Huang	Frontage Labs
12.	Estela Kneeteman	INTI
13.	Bill Mindak	FDA ***(Minerals Only)***
14.	Maria Ofitserova	Pickering Lab
15.	Shay Phillips	Mead Johnson
16.	Guenther Raffler	CLF-Eurofins
17.	Kate Rimmer/Melissa Phillips	NIST ***(Non-Voting)***
18.	Wil van Loon/Hans Cruijsen	FrieslandCampina
19.	David Woollard	Eurofins (NZ) ***(B vitamins only)***
20.	Jinchuan Yang	Waters Corp.

Technical Documents created/used

1. SMPRs for amino acids, biotin, carnitine, carotenoids, chloride, choline, fatty acids, fluoride, folate, fructans (FOS), GOS, inositol, iodine, minerals and trace elements, nucleotides, pantothenic acid, ultra trace minerals, vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, and vitamin K
2. OMA Appendix L
3. OMA Appendix D

Methods Adopted

First Action and Final Action status

AOAC 2016.xx, 2016.xx (biotin);
AOAC 2011.14, 2015.06 (MTE),
AOAC 2012.17 (carnitine)
AOAC 2015.10 , 2014.04 (carnitine/choline)
AOAC 2015.07, 2015.08 (chloride)
AOAC 2012.18, 2012.19, 2012.20 (choline)
AOAC 2011.19 (Cr, Se, Mo)
AOAC 2012.13 (Fatty Acids)
AOAC **2011.05 (repealed),** 2011.06, 2013.13 (folate)
AOAC 2016.xx (fructans)
AOAC 2012.14, 2012.15 (iodine)
AOAC 2011.18**,** 2012.12 (myo-inositol)

Red indicates Final Action OMA status

AOAC 2011.20**,** 2011.21 (nucleotides)
AOAC 2012.16 (pantothenic acid)
AOAC 2011.07, 2011.15,
AOAC 2012.09, 2012.10 (vitamin A /vitamin E)
AOAC 2015.14 (vitamin B1/vitamin B2/vitamin B6)
AOAC **2011.08 (repealed),** 2011.09, 2011.10**,** 2011.16, 2014.02 (vitamin B12)
AOAC 2012.21, 2012.22 (vitamin C)
AOAC 2016.xx, 2011.11, **2011.12, 2011.13,** 2012.11 (vitamin D)
AOAC 2015.09 (vitamin K)
AOAC 2012.07, 2012.08 (Whey Protein:Casein)

Red indicates Final Action OMA status

Final Action Methods Recommended	2012.22 (vitamin C)
Additional Input	
Awards/Recognitions	AOAC OMB ERP of the Year (2012, 2015)

ERP PROFILE SUMMARIES

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPIFAN PESTICIDE CONTAMINANT METHODS

ERP Name	AOAC Expert Review Panel for SPIFAN Pesticide Contaminant Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2015	**Number of Methods Adopted**	3 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPIFAN.				
Roster	1. Darryl Sullivan, *Covance (Chair)* 2. Martin Alewijn, *RIKILT* 3. John Austad, *Covance Labs* 4. Joe Boison, *CFIA* 5. Scott Christiansen, *Perrigo/PBM Nutritionals* 6. Jo Marie Cook, *FL. Dept. of Agriculture* 7. Jon DeVries, *Medallion Labs/General Mills* 8. Harvey Indyk, *Fonterra Cooperative* 9. George Joseph, *AsureQuality* 10. Erik Konings, *Nestlé/ISO* 11. Alex Krynitsky, *FDA-CFSAN* 12. Tom Phillips, *MD. Dept. of Agriculture* 13. Bert Popping, *Mérieux NutriSciences* 14. Murali Reddy, *Abbott Nutrition* 15. Jon Wong, *FDA-CFSAN*				
Technical Documents created/used	1. SMPR for Sodium monofluoroacetate 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2015.02 – Sodium Fluoroacetate in Dairy Powders AOAC 2015.03 – Sodium Fluoroacetate in Infant Formula AOAC 2015.04 - Monofluoroacetate in Powdered Nutritional Products				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPDS – CHONDROITIN, PDE5 INHIBITORS, & ANTHOCYANIN METHODS

ERP Name	AOAC Expert Review Panel for Dietary Supplements – Chondroitin, PDE5 Inhibitors, and Anthocyanin Methods			**Chair(s)**	Brian Schaneberg (Starbucks)
ERP Formed:	2015	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary supplements				
Roster	Chondroitin: 1. Brian Schaneberg, Starbucks (Chair) 2. Jana Hildreth, Synutra Pure 3. Martha Jennens, Covance 4. Phillip Koerner,Phenomenex; 5. Tom Phillips, State of MD; 6. Curtis Phinney, Consultant; 7. Kelly Reins, Consultant; 8. John Spzylka, Merieux NutriSciences; Aniko Solyom, GAAS Corporation; 9. Darryl Sullivan, Covance 10. Liton Roy, Sancillo and Company; 11. Nour Edine ES-SAFI, Mohammad V University Rabat	PDE5 Inhibitors: 1. Brian Schaneberg, Starbucks (Chair); 2. Phil Koerner, Phenomenex; 3. Katerina Mastovska, Covance; 4. Tom Phillips, State of MD; 5. Fenhong Song, FDA; 6. John Spzylka, Mérieux NutriSciences; 7. Darryl Sullivan, Covance. 8. Teresa Cain, FDA; 9. Liton Roy, Sancilio; 10. Jerry Zweigenbaum, Agilent.	Anthocyanins: 1. Brian Schaneberg, Starbucks (Chair); 2. Phil Koerner, Phenomenex; 3. Jungmin Lee, USDA; 4. Melissa Phillips, NIST; 5. Martha Jennens, Covance; 6. Tom Phillips, State of MD; 7. Aniko Solyom, GAAS Analytics; 8. John Spzylka, Mérieux NutriSciences; 9. Darryl Sullivan, Covance 10. Nour Eddine ES-SAFI, Mohammed V University; 11. Liton Roy, Sancilio and Company; 12. Jerry Zweigenbaum, Agilent	
Technical Documents created/used	SMPRs for Chondroitin, PDE5 Inhibitors, and Anthocyanins OMA Appendix K				
Methods Adopted *First Action and Final Action status*	AOAC 2015.11 – Chondroitin AOAC 2015.12 - PDE5 inhibitors				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

ERP PROFILE SUMMARIES

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPDS – ASHWAGANDHA, FOLIN C, AND KRATOM METHODS

ERP Name	AOAC Expert Review Panel for Dietary Supplements – Ashwagandha, Folin C, and Kratom Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2015	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary supplements				
Roster	Ashwagandha: 1. Darryl Sullivan, Covance (Chair) 2. Anton Bzhelyansky, USP 3. Nour-Eddine Es-Safi, Mohammad V University of Rabat 4. Tom Phillips, State of MD 5. Casey Sayre, Roseman University of Health Sciences 6. Catherine Rimmer, NIST; 7. Aniko Solyom, GAAS Analytical; 8. Kurt Young, GNC/Nutra Manufacturing, 9. Prashant Ingle, Herbalife; 10. Yanjun Zhang, Herbalife		Folin C: 1. Darryl Sullivan, Covance (Chair) 2. Nour Eddine Es-Safi, Mohammad V University in Rabat 3. Martha Jennens, Covance 4. Dana Krueger, Krueger Food Laboratories 5. Tom Phillips, State of MD 6. Catherine Rimmer, NIST 7. Aniko Solyom, GAAS Analytical John Spzylka, Mérieux Nutrisciences 8. Joseph Zhou, Sunshineville Health Products 9. John Finley, LSU (Retired) 10. Prashant Ingle, Herbalife 11. Jungmin Lee, USDA		Kratom: 1. Darryl Sullivan, Covance (Chair) 2. Joseph Betz, NIH 3. Nour Eddine Es-Safi, Mohammed V University in Rabat 4. Charles Metcalfe, Custom Analytics 5. Tom Phillips, State of MD 6. John Spzylka, Mérieux Nutrisciences 7. Yan-Hong Wang, University of Mississippi 8. Christine Casey, FDA 9. Catherine Rimmer, NIST
Technical Documents created/used	SMPRs for Ashwagandha, Folin C, and Kratom OMA Appendix K				
Methods Adopted *First Action and Final Action status*	AOAC 2015.17 - Estimation of Withanolides (Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastromonolide, Withanolide A, Withanolide B) in Withania somnifera				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

ERP PROFILE SUMMARIES

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPSFAM HEAVY METAL METHODS

ERP Name	AOAC Expert Review Panel for SPSFAM Heavy Metal Methods			**Chair(s)**	Rick Reba (Nestle)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPSFAM.				
Roster	Rick Reba, Nestle (Chair) Sneh Bhandari, Merieux NutriSciences Michele Briscoe, Brooks Applied Labs Min Huang, Aegis Sciences Corporation Ferry Maniei, The Coca-Cola Company Bill Mindak, US FDA Cory Murphy, CFIA Jenny Nelson, Agilent Technologies Jenny Scifres, USDA Li Sheng, EPL Bioanalytical Christopher Smith, The Coca-Cola Company Darryl Sullivan, Covance				
Technical Documents created/used	1. SMPR for Total Heavy Metals 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2015.01 – Heavy Metals in Food				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPSFAM ST. JOHN'S WORT

ERP Name	AOAC Expert Review Panel for SPSFAM St. John's wort Methods			**Chair(s)**	Shauna Roman (RB)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPSFAM.				
Roster	Shauna Roman, Reckitt Benckiser (Chair) Paula Brown, British Columbia Institute of Technology Nour Eddine Es-Safi, Mohammed V-Agdal University Ikhlas Khan, University of Mississippi Elizabeth Mudge, British Columbia Institute of Technology Klaus Reif, PhytoLab GmbH & Co. KG Brian Schaneberg, Starbucks Maged Sharaf, US Pharmacopeia Darryl Sullivan, Covance Roy Upton, American Herbal Pharmacopeia				
Technical Documents created/used	1. SMPR for St. John's wort				
Methods Adopted *First Action and Final Action status*	AOAC 2013.15 – Hypericin and Pseudohypericin in St. John's wort				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUE METHODS

ERP Name	AOAC Expert Review Panel for Veterinary Drug Residue Methods			**Chair(s)**	Joe Boison (Canadian Food Inspection Agency)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	1 method
Scope:	Review and adopt methods resulting from SMPRs developed by SPMFF.				
Roster	1. Joe Boison, Canadian Food Inspection Agency (Chair) 2. Haejung An, U.S. FDA 3. Martin Danaher, TEAGASC 4. Doug Hite, State of Tennessee - Retired 5. Brian Kinsella, UCT, Inc. 6. Perry Martos, University of Guelph 7. Katerina Mastovska, Covance Laboratories 8. Cory Murphy, Canadian Food Inspection Agency 9. Sherry Turnipseed, U.S. FDA 10. Jon Reuther, Eurofins				
Technical Documents created/used	1. SMPR for Drug Residues in Fish and Seafood 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2012.25 – Residues of Three Triphenylmethane Dyes and Their Metabolites (Malachite Green, Leucomalachite Green, Crystal Violet, Leucocrystal Violet, and Brilliant Green) in Aquaculture Products				
Final Action Methods Recommended	Method listed above.				
Additional Input	Chemical Contaminants Community Veterinary Drug Residues Subgroup				
Awards/Recognitions	ERP of the Year in 2013				

PROFILE OF AOAC EXPERT REVIEW PANEL FOR MICROBIOLOGY METHODS FOR FOODS AND ENVIRONMENTAL SURFACES

ERP Name	AOAC Expert Review Panel for Microbiology Methods for Foods and Environmental Surfaces			**Chair(s)**	Michael Brodsky (Brodsky Consultants) and Wendy McMahon (Mérieux NutriSciences)
ERP Formed:	2012	**Number of Methods Adopted**	10 as First Action status	**Number of Methods Recommended**	7
Scope:	Review and adopt methods resulting from sole source submission of methods for pathogenic and nonpathogenic microbial detection or determination in foods and on environmental surfaces				
Roster	1. Michael Brodsky, Brodsky Consulting (Co-Chair) 2. Wendy McMahon, Silliker Laboratories (Co-Chair) 3. Maya Achen, Abbott Nutrition 4. Patrice Arbault, BioAdvantage 5. Mark Carter, MC2E 6. Yi Chen, U.S. Food & Drug Administration (FDA) 7. Peyman Fatemi, The Acheson Group LLC 8. Maria Christina Fernandez, University of Buenos Aires 9. Tom Hammack, U.S. Food & Drug Administration (FDA) 10. Tony Hitchins, U.S. Food & Drug Administration/CFSAN (Retired) 11. Yvonne Salfinger				
Technical Documents created/used	OMA Appendix J				
Methods Adopted *First Action and Final Action status*	AOAC 2012.02 - Gram-Positive Bacteria Identification AOAC 2013.01 – *Salmonella* in Variety of Foods AOAC 2013.02 – *Salmonella* Species in a Variety of Foods and Environmental Surfaces AOAC 2013.09 – *Salmonella* in Selected Foods AOAC 2013.10 – *Listeria* species in a Variety of Foods and Environmental Surfaces AOAC 2013.11 – *Listeria monocytogenes* in a Variety of Foods AOAC 2013.14 – Identification of *Salmonella* spp from Colony Picks *Red indicates Final Action OMA status*		AOAC 2014.01 – *Salmonella* in Selected Foods AOAC 2014.05 – Enumeration of Yeast of Mold in Food AOAC 2014.06 – *Listeria* species in Selected Foods and Environmental Surfaces AOAC 2014.07 – *Listeria monocytogenes* in Selected Foods and Environmental Surfaces AOAC 2015.13 – Enumeration of Aerobic Bacteria in Food		
Final Action Methods Recommended					
Additional Input	ISPAM				
Awards/Recognitions	Seven methods have Final Action status; ERP of the Year in 2014; AOAC 2013.10 /2013.11 -joint Multi-Laboratory of the Year in 2014; AOAC 2013.14 – Award in Technical and Scientific Excellence in 2014.				

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PROPRIETARY VITAMIN METHODS

ERP Name	AOAC Expert Review Panel for Proprietary Vitamin Methods			**Chair(s)**	Shang-Jing (Jean) Pan (Abbott Nutrition)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	1 method
Scope:	Review and adopt methods resulting from sole source method developer				
Roster	1. Shang-Jing (Jean) Pan, Abbott Nutrition (Chair) 2. John Austad, Covance 3. Sneh Bhandari, Merieux NutriSciences 4. Johanna Camera, NIST 5. Sarwar Gilani, Health Canada (retired) 6. Erik Konings, Nestle 7. John Szpylka, Merieux NutriSciences 8. Dave Woollard, Eurofins				
Technical Documents created/used	1. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	*Method not published as ERP requested revisions made by method developer prior to publication and revisions were not completed. No OMA number assigned.*				
Final Action Methods Recommended	Method listed above.				
Additional Input					
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR FOOD ALLERGEN METHODS - GLUTEN

ERP Name	AOAC Expert Review Panel for Food Allergens - Gluten Methods			**Chair(s)**	Terry Koerner (Health Canada) and Shang-Jing (Jean) Pan (Abbott Nutrition)
ERP Formed:	2014	**Number of Methods Adopted**	3 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the detection or determination of food allergen compounds in food products				
Roster	1. Terry Koerner, Health Canada 2. Shang-Jing Pan, Abbott Nutrition 3. Joe Boison, Canadian Food Inspection Agency 4. Clyde Don, Foodphysica 5. Bert Popping, Mérieux NutriSciences 6. Girdhari Sharma, US FDA 7. Paul Wehling, Medallion Labs / General Mills 8. Jupiter Yeung, Nestle Nutrition 9. Sneh Bhandari, Silliker 10. Eric Garber, US FDA				
Technical Documents created/used	OMA Appendix L OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.03 - Gluten in Rice Flour and Rice-Based Food Products AOAC 2015.05 – Partially Hydrolyzed Gluten in Fermented Cereal-Based Products AOAC 2015.16 – Gluten in Processed and Nonprocessed Corn Products				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

ERP PROFILE SUMMARIES

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PAH METHODS

ERP Name	AOAC Expert Review Panel for PAH Methods			**Chair(s)**	Tom Phillips (Maryland Department of Agriculture)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of PAHs in seafood				
Roster	1. Tom Phillips, Maryland Department of Agriculture (Chair) 2. Mark Crosswhite, Florida Department of Agriculture 3. Julie Kowalski, RESTEK 4. Cheryl Lassitter, DOC, NOAA, NMFS, NSIL 5. Kai Liu, Eurofins 6. Jian Wang, Canadian Food Inspection Agency (CFIA) 7. Xiaoyan Wang, United Chemical Technologies, Inc. (UCT) 8. Stephen A. Wise, National Institute of Standards and Technology (NIST) 9. Tracy Collier - (Alternate), NOAA (retired) 10. Lowri de Jager, U.S. Food and Drug Administration				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.08 - Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions	Method of the Year in 2014				

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PESTICIDE RESIDUE METHODS

ERP Name	AOAC Expert Review Panel for Pesticide Residue Methods			**Chair(s)**	Joe Boison (Canadian Food Inspection Agency)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of pesticide residues in teas				
Roster	1. Joe Boison, Canadian Food Inspection Agency (CFIA) 2. Amy Brown, Florida Department of Agriculture 3. Jo Marie Cook (Alternate), Florida Department of Agriculture 4. Julie Kowalski, Restek Corporation 5. John Reuther, Eurofins 6. Marina Torres, LATU 7. Jian Wang, Canadian Food Inspection Agency (CFIA) 8. Xiaoyan Wang, United Chemical Technologies, Inc. (UCT)				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.09 - Residues of 653 Multiclass Pesticides and Chemical Pollutants in Tea				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions	ERP of the Year in 2015; Method of the Year in 2014				

PROFILE OF AOAC EXPERT REVIEW PANEL FOR DIETARY STARCH METHODS

ERP Name	AOAC Expert Review Panel for Dietary Starch Methods			**Chair(s)**	Lars Reimann (Eurofins)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary starch determination in animal feed and pet food.				
Roster	1. Lars Reimann, Eurofins 2. Sean Austin, Nestle Research Centre 3. Sneh Bhandari, Silliker, Inc. 4. Kommer Brunt, Rotating Disc BV 5. Jon DeVries, Medallion Laboratories 6. Kai Liu, Eurofins 7. Barry McCleary, Megazyme International Ireland 8. Tom Phillips, MD Department of Agriculture 9. John Szpylka, Silliker, Inc. (Alternate)				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.10 - Dietary Starch in Animal Feed and Pet Food				
Final Action Methods Recommended					
Additional Input	AOAC Community on Agricultural Materials				
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR FERTILIZER METHODS

ERP Name	AOAC Expert Review Panel for Fertilizer Methods			**Chair(s)**	Bill Hall (Mosaic)
ERP Formed:	2014	**Number of Methods Adopted**	2 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of fertilizers				
Roster	*Slow and Controlled Release Fertilizers* 1. Bartos, James 2. Hall, William 3. Hartshorn, Jon 4. Hojjatie, Michael 5. Nacharaju, Krishnamurthy (Murthy) 6. Nagarajan, Rajamani *7. Parisi, Salvatore* *8. Provance-Bowley, Mary*	*P and K Inorganic Fertilizers* 1. Bartos, James 2. Hall, William 3. James, Barbara 4. Kariuki, Solomon 5. Parisi, Salvatore 6. Phillips, Heidi 7. Shelite, Kristopher 8. Tan, Rechel 9. Tsourides, Dion	*Total Sulfur in Fertilizers* 1. Bartos, James 2. Hall, William 3. Kariuki, Solomon 4. Parisi, Salvatore 5. Phillips, Heidi *6. Provance-Bowley, Mary* 7. Wegner, Keith	*Ar, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se, Zn in Fertilizers* 1. Bartos, James 2. Hall, William 3. Kariuki, Solomon 4. Parisi, Salvatore 5. Phillips, Heidi 6. Provance-Bowley, Mary 7. Reba, Rick 8. Shelite, Kristopher 9. Shoemaker, Dirk D 10. Tan, Rechel 11. Tsourides, Dion 12. Wegner, Keith	
Technical Documents created/used	Fertilizer Forum Documents OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2015.15 - Nitrogen, Phosphorus, and Potassium Release of Slow- and Controlled Release Fertilizers AOAC 2015.18 - Phosphorus and Potassium in Commercial Inorganic Fertilizers				
Final Action Methods Recommended					
Additional Input	AOAC Community on Agricultural Materials				
Awards/Recognitions					

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPIFAN NUTRIENT METHODS

ERP Name	AOAC Expert Review Panel for SPIFAN Nutrient Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2011	**Number of Methods Adopted**	38 as First Action status	**Number of Methods Recommended**	8 OMAs Final Action; 6 OMAs for repeal
Scope:	Review and adopt methods resulting from SMPRs developed by SPIFAN. Recommend one method per nutrient for Final Action.				

Roster

1.	Darryl Sullivan	Covance (Chair)
2.	John Austad	Covance
3.	Sean Austin	Nestlé ***(FOS/GOS Only)***
4.	Sneh Bhandari ***(OMB Liaison)***	Mérieux NutriSciences
5.	Esther Campos-Gimenez/Adrienne McMahon	Nestlé/Wyeth
6.	Scott Christiansen	Perrigo/PBM Nutritionals
7.	Jon DeVries	Independent Consultant
8.	Sarwar Gilani	Independent Consultant
9.	Brendon Gill/Harvey Indyk	Fonterra
10.	Don Gilliland ***(OMB Liaison)***/Karen Schimpf	Abbott Nutrition
11.	Min Huang	Frontage Labs
12.	Estela Kneeteman	INTI
13.	Bill Mindak	FDA ***(Minerals Only)***
14.	Maria Ofitserova	Pickering Lab
15.	Shay Phillips	Mead Johnson
16.	Guenther Raffler	CLF-Eurofins
17.	Kate Rimmer/Melissa Phillips	NIST ***(Non-Voting)***
18.	Wil van Loon/Hans Cruijsen	FrieslandCampina
19.	David Woollard	Eurofins (NZ) ***(B vitamins only)***
20.	Jinchuan Yang	Waters Corp.

Technical Documents created/used

1. SMPRs for amino acids, biotin, carnitine, carotenoids, chloride, choline, fatty acids, fluoride, folate, fructans (FOS), GOS, inositol, iodine, minerals and trace elements, nucleotides, pantothenic acid, ultra trace minerals, vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, and vitamin K
2. OMA Appendix L
3. OMA Appendix D

Methods Adopted

First Action and Final Action status

AOAC 2016.xx, 2016.xx (biotin);
AOAC 2011.14, 2015.06 (MTE),
AOAC 2012.17 (carnitine)
AOAC 2015.10 , 2014.04 (carnitine/choline)
AOAC 2015.07, 2015.08 (chloride)
AOAC 2012.18, 2012.19, 2012.20 (choline)
AOAC 2011.19 (Cr, Se, Mo)
AOAC 2012.13 (Fatty Acids)
AOAC **2011.05 (repealed),** 2011.06, 2013.13 (folate)
AOAC 2016.xx (fructans)
AOAC 2012.14, 2012.15 (iodine)
AOAC 2011.18**,** 2012.12 (myo-inositol)

Red indicates Final Action OMA status

AOAC 2011.20**,** 2011.21 (nucleotides)
AOAC 2012.16 (pantothenic acid)
AOAC 2011.07, 2011.15,
AOAC 2012.09, 2012.10 (vitamin A /vitamin E)
AOAC 2015.14 (vitamin B1/vitamin B2/vitamin B6)
AOAC **2011.08 (repealed),** 2011.09, 2011.10**,** 2011.16, 2014.02 (vitamin B12)
AOAC 2012.21, 2012.22 (vitamin C)
AOAC 2016.xx, 2011.11, **2011.12, 2011.13,** 2012.11 (vitamin D)
AOAC 2015.09 (vitamin K)
AOAC 2012.07, 2012.08 (Whey Protein:Casein)

Red indicates Final Action OMA status

Final Action Methods Recommended	2012.22 (vitamin C)
Additional Input	
Awards/Recognitions	AOAC OMB ERP of the Year (2012, 2015)

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

MEETING HELD AT

Washington DC/Rockville Hotel & Executive Meeting Center
Rockville, MD

Tuesday, March 12, 2013

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Member Attendees:

Darryl Sullivan, Chair, *Covance Laboratories*
John Austad, *Covance Laboratories*
Sneh Bhandari, *Silliker Laboratories*
Scott Christiansen, *Perrigo Nutritionals*
Jonathan DeVries, *Medallion Labs/Gen. Mills*
Brendon Gill, *Fonterra*
Don Gilliland, *Abbott Nutrition*
Min Huang, *Aegis Corp.*
Erik Konings, *Nestlé*
Adrienne McMahon, *Pfizer Nutrition*
Shay Phillips, *Mead-Johnson Nutritional*
Kate Rimmer, *NIST*
Jeanne Rader, *FDA*
Jinchuan Yang, *Waters Corp.*

AOAC Staff including:

Delia Boyd
E. James Bradford
Scott Coates
Arlene Fox
Nora Marshall
Alicia Meiklejohn
Anita Mishra
Robert Rathbone
Gar Riegler

I. WELCOME/INTRODUCTIONS

Jim Bradford (AOAC)/Darryl Sullivan (Covance), Chair of the Stakeholder Panel on Infant Formula and Adult Nutrition, introduced and welcomed the participants to Expert Review Panel (ERP) meeting of the SPIFAN project. The ERP members in attendance were introduced.

II. OVERVIEW OF DOWN SELECTION PROCESS & EVALUATION FORM

Erik Konings (Nestlé) provided an overview of the down selection process, rationale and other criteria for completing the Method Evaluation form.

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP)

The Expert Review Panel (ERP) discussed the methods and selected a single method for multi-lab testing (MLT) through the SPIFAN process. The following is the result of the discussions and vote.

1) Vitamin A/E

a. OMA# 2012.09 (VitA-16/VitE-18) & OMA# 2012.10 (VitA-17/VitE-19)
b. **Score**: 469 points (OMA# 2012.09) / 501 points (OMA# 2012.10)

2) Vitamin B_{12}

a. OMA# 2011.08 (B_{12}-03) & OMA# 2011.10 (B_{12}-12)
b. **Score**: 418 points (OMA# 2011.08) / 454 points (OMA# 2011.10)

3) Vitamin D

a. OMA# 2011.11 (VitD-01) & OMA#2012.11 (VitD-16)
b. **Score**: 428 points (OMA# 2011.11) / 410 points (OMA# 2012.11)

4) Inositol

a. OMA# 2011.18 (INOS-34) & OMA# 2012.12 (INOS-39)
b. **Score**: 493 points (OMA# 2011.18) / 460 points (OMA# 2012.12

5) Nucleotides

a. OMA# 2011.20 (Nuc-01) & OMA# 2011.21 (Nuc-02)
b. **Score**: 493 points (OMA# 2011.20) / 452 points (OMA# 2011.21)

Motion: To advance the selected method for reproducibility study

Method	Method Title	Reviewer(s)	Score	Vote	Recommendations
VitA-16 **VitE-18**	*2012.09 - Simultaneous Determination of 13-Cis and All-Trans Vitamin A Palmitate, Vitamin A Acetate, Alpha Vitamin E Acetate, and Alpha Tocopherol by HPLC and Column Switching. Submitted by Abbott Nutrition.* *Vitamin A & E Single Laboratory Validation Submitted by Abbott Nutrition.*	Scott Christiansen Jinchuan Yang	**469**		
VitA-17 **VitE-19**	*2012.10 - Simultaneous Determination of Vitamins A, E and Beta Carotene/Mixed Carotenoids in Infant Formula by Normal Phase HPLC. Submitted by Pfizer Nutrition.*	Erik Konings Kathy Sharpless/Kate Rimmer	**501**	**Motion:** Sneh Bhandari Scott Christiansen **Vote:** Yes- 9/ No-0 /Abstain-5	◊ Study director to continue with accuracy for each of the matrices. ◊ Need to change in SMPR back from .7 mcg to 7 mcg on the ERP score sheet
$VitB_{12}$-03	*2011.08 - Determination of Vitamin B12 in Infant Formula and Adult/Pediatric Nutritional Formula by Optical Biosensor Protein-Binding Assay. Submitted by Nestlé.*	Scott Christiansen Estela Kneeteman	**418**		◊ Question about Proprietary immune affinity column information
$VitB_{12}$-12	*2011.10 - Determination of Vitamin B12 in Infant Formula and Adult/Pediatric Nutritional Formula by HPLC. Submitted by Abbott Nutrition.*	Jon DeVries Shay Phillips	**454**	**Motion:** Scott Christiansen John Austad **Vote:** Yes- 9/ No-0 /Abstain-4	◊ More spike recoveries
VitD-01	*2011.11 - AOAC SPIFAN Single Laboratory Validation for Vitamin D Analysis in Infant Formula and Adult Nutritionals and Addendum No. 1a. Submitted by Covance Laboratories*	Brendon Gill Sneh Bhandari	**428**	**Motion:** Sneh Bhandari Erik Konings **Vote:** Yes- 9/ No-0 /Abstain-4	◊ Indicate the sample size ◊ Update method with C30 column. Data collected on modified method. ◊ No issue on D2
VitD-16	*2012.11 - Simultaneous Determination of Vitamin D3 and D2 by ESI LC-MS/MS. Submitted by Abbott Nutrition*	Jeanne Rader Guenther Raffler	**410**		

Inos-34	*2011.18 - Determination of Myo-inositol in Infant Formula and Adult/Pediatric Nutritional Formula by HPLC Column Switching and Pulsed Amperometry. Submitted by Abbott Nutrition*	Brendon Gill Harvey Indyk	**493**	**Motion:** Sneh Bhandari Jon DeVries **Vote:** Yes- 10/ No-0 /Abstain-3	
Inos-39	*2012.12 - Analysis of Free and Total myo-Inositol in Foods, Feeds, and Infant Formula by HPAEC-PAD Including a Novel Total Extraction Using Microwave-Assisted Acid Hydrolysis and Enzymatic Treatment. Submitted by Covance Lab*	Brendon Gill Karen Schimpf	**460**		
Nuc-01	*2011.20 - Routine Analysis of 5'-Mononucleotides in Infant Formula and Adult/Pediatric Nutritional Formula by Liquid Chromatography. Submitted by Fonterra*	Sneh Bhandari Estela Kneeteman	**493**	**Motion:** Sneh Bhandari Don Gilliland **Vote:** Yes- 9/ No-0 /Abstain-4	◊ Modify to incorporate starch based products ◊ Need to see additional recovery
Nuc-02	*2011.21. Submitted by Kinjo Gakin Univ. (Nestlé)*	Adrienne McMahon Min Huang	**452**		

***Include starch with all other methods going forward.**

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

MEETING HELD AT

Palmer House Hilton
Chicago, IL

Tuesday, August 27, 2013

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Member Attendees:

Darryl Sullivan, Chair, *Covance Laboratories*
John Austad, *Covance Laboratories*
Sneh Bhandari, *Silliker Laboratories*
Esther Campos-Giménez/Adrienne McMahon, *Nestlé/Wyeth*
Scott Christiansen, *Perrigo Nutritionals*
Jonathan DeVries, *Medallion Labs/Gen. Mills*
Don Gilliland/Karen Schimpf, *Abbott Nutrition*
Min Huang, *Aegis Corp.*
Harvey Indyk/Brendon Gill, *Fonterra*
Estela Kneeteman, *INTI*
Bill Mindak, *FDA*
Shay Phillips, *Mead-Johnson Nutritional*
Melissa Phillips/Kate Rimmer, *NIST*
Jinchuan Yang, *Waters Corp.*

AOAC Staff including:

Delia Boyd
E. James Bradford
Scott Coates
Dawn Frazier
Deborah McKenzie
Alicia Meiklejohn
Anita Mishra

Expert Review Panel Members Not in Attendance:

Jeanne Rader, *FDA*
Günther Raffler, *Danone*

I. WELCOME/INTRODUCTIONS

Darryl Sullivan (Covance), Chair of the Stakeholder Panel on Infant Formula and Adult Nutrition, introduced and welcomed the participants to Expert Review Panel (ERP) meeting of the SPIFAN project. The ERP members in attendance and participants were introduced.

II. DOWN SELECTION PROCESS & REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP)

The Expert Review Panel (ERP) discussed the methods and selected a single method for multi-lab testing (MLT) through the SPIFAN process. The following is the result of the discussions and vote.

1) Vitamin C

a. OMA# 2012.21 (VitC-02) & OMA# 2012.22 (VitC-03)
b. **Score**: 757 points (OMA# 2012.21) / 826 points (OMA# 2012.22)

2) Choline

a. OMA# 2012.18 (Chol-03) & OMA# 2012.20 (Chol-07)
b. **Score**: 852 points (OMA# 2012.18) / 869 points (OMA# 2012.20)

3) Iodine

a. OMA# 2012.14 (Iod-01) & OMA#2012.15 (Iod-02)
b. **Score**: 838 points (OMA# 2012.14) / 877 points (OMA# 2012.15)

4) Pantothenic Acid

a. OMA# 2012.16 (Panto-01) - **Moved to MLT**

5) Carnitine

a. OMA# 2012.17 (Carn-01) - **Method Withdrawn**

1. Move to advance the selected method for reproducibility study

Method	Method Title	Reviewer(s)	Score	Vote	Comments
VitC-02	*2012.21 - Determination of Vitamin C by HPLC with UV Detection*	Brendon Gill John Austad	757		➢ No measurable bias against the SRM ➢ Met all SMPR requirements ➢ SRM results; average was higher ➢ Which option was used for data collection?
VitC-03	*2012.22 - HPLC-UV Determination of Total Vitamin C in a Wide Range of Fortified Food Products*	Jon DeVries Harvey Indyk	826	Yes- 11/ No-0 /Abstain-2	
Chol-03	*2012.18 - Simultaneous Determination of Free Carnitine and Total Choline by Liquid Chromatography/Mass Spectrometry in Infant Formula and Health-Care Products: Single-Laboratory Validation*	John Austad Sneh Bhandari	852		➢ Within RSD requirements ➢ Meets SMPR
Chol-06	*2012.19 - Method Development for Determination of Total and Free Choline in Nutritional Products by LC–MS/MS*	Method withdrawn			
Chol-07	*2012.20 - Determination of Choline in Powdered Infant Formula*	Scott Christiansen Shay Phillips	869	Yes- 12/ No-0 /Abstain-2	➢ Repeatability o Pretty rugged ➢ Meets SMPR ➢ Instruction o When predicted life time ➢ Use of guard column should be part of system suitability
Iod-01	*2012.14 - Determination of Total Iodine in Infant Formula and Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single Laboratory Validation*	Bill Mindak Min Huang	838		➢ Well written method ➢ Difference in the sample prep ➢ digestion
Iod-02	*2012.15 - Method of Analysis for the Determination of Total Iodine in Foods and Dietary Supplements Using Inductively Coupled Plasma-Mass Spectrometry*	Esther Campos-Giménez Min Huang	877	Yes- 12/ No-0 /Abstain-2	➢ May require additional detail ➢ Decide on one (1) digestion technique only ➢ See data on both through MLT
Panto-01	*2012.16 - Pantothenic Acid (Vitamin B5) in Fortified Foods: Comparison of a Novel Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry Method and a Microbiological Assay (AOAC*	John Austad Don Gilliland		Yes- 13/ No-0 /Abstain-1	➢ Units were wrong o Units in grams should be milligrams ➢ Address high fat issue ➢ Use free pantothenic acid according to NIST ➢ Define what the prep looks

	Official MethodSM 992.07)				like o No option (one or the other)
Carn-01	*2012.17 - Single-Laboratory Validation of a Liquid Chromatographic/Tandem Mass Spectrometric Method for the Determination of Free and Total Carnitine in Infant Formula and Raw Ingredients*	Method withdrawn			

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS

The Expert Review Panel (ERP) members Primary and Secondary Reviewers provided updates on their assigned method(s) and rendered a decision on First Action *Official Method* status. The ERP collectively discussed the method(s) and selected a single method to move forward through the SPIFAN process.

1. **Folate**
   a. **Fol-20**
   b. **Fol-21**

2. **<u>Move to advance the selected method for First Action *Official Method*^{SM} Status</u>**

Method	Method Title	Reviewer(s)	ERP Vote	Reviewer's Comments
Fol-20	*A validated method to determine folic acid and 5-methyl THF in adult/infant nutritional formulae using ultra performance liquid chromatography-tandem mass spectrometry*	Adrienne McMahon Melissa Phillips/Kate Rimmer	Yes- 3/ No-5/Abstain-2	➢ Recovery didn't meet the SMPR ➢ Extraction o Difference in the totals ➢ Values in the SRM is high
<u>ERP RECOMMENDATIONS FOR FOL-20:</u> ➢ More data on SPIFAN suite o Choose between heat treatment or o Tri-enzyme ➢ Check calculations *Will not move to MLT at this time **Study director proposes to have additional data within one month				
Fol-21	*Single-Laboratory Validation - Free Folates in Infant Formula and Adult/ Pediatric Nutritional Formula by UHPLC-UV*	Min Huang Shay Phillips	Yes- 10/ No-1/Abstain-1 **Second vote:** Yes- 10/ No-0 /Abstain-2	➢ Meets SMPR ➢ Sample prep ➢ Standard purity
<u>ERP RECOMMENDATIONS FOR FOL-21:</u> ➢ Check methodology (concentration of calibration standard) ➢ Use spectro-photometric combined with HPLC (recommend both) ➢ MS/MS not UV ➢ Tri-enzyme Caveat ➢ Measurement of poly-glutamate				

IV. UPDATE ON MULTI-LABORATORY TESTING SCHEDULE

Robert Rankin (International Formula Council) provided an update on the Multi-Laboratory Testing schedule.

V. UPDATE ON VITAMIN A & E

Adrienne McMahon of Wyeth Nutrition (formerly Pfizer) provided an update on the Vitamin A & E method slated to move to multi-lab testing.

Method	Method Title	Original Reviewer(s)	Vote	Recommendation
VitA-17 **VitE-19**	*2012.10 - Simultaneous Determination of Vitamins A, E and Beta Carotene/Mixed Carotenoids in Infant Formula by Normal Phase HPLC. Submitted by Pfizer Nutrition.*	Erik Konings Kathy Sharpless/ Kate Rimmer	Yes- 12/ No-0 /Abstain-1	➢ Move to MLT

VI. REVIEW ACTIONS FROM PREVIOUS MEETINGS

Darryl Sullivan & Anita Mishra reviewed previous actions/items from past meetings.

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Meeting held at
Hilton Washington DC North/Gaithersburg, MD
Wednesday, March 19, 2014 - 3:00pm (Eastern US)

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Members (in attendance):

Darryl Sullivan	***Covance Labs*** *(Chair)*
John Austad	Covance Labs
Sneh Bhandari	Mérieux NutriSciences & OMB
Esther Campos-Gimenez/Adrienne McMahon	Nestlé
Brendon Gill	Fonterra
Don Gilliland/Karen Schimpf	Abbott Nutrition
Estela Kneeteman	INTI
Bill Mindak	FDA
Shay Phillips	Mead Johnson
Guenther Raffler	CLF-Eurofins
Kate Rimmer/Melissa Phillips	NIST
Matt Sliva (for Scott Christiansen)	Perrigo/PBM Nutritionals
Jinchuan Yang	Waters Corp.

Expert Review Panel Members (unable to attend):

Scott Christiansen	Perrigo/PBM Nutritionals
Jon DeVries	General Mills/Medallion Labs
Sarwar Gilani	Consultant
Min Huang	Frontage Labs
Maria Ofitserova	Pickering Labs
Jeanne Rader	FDA (CFSAN)

AOAC Staff Includes:
Delia Boyd
E. James Bradford
Scott Coates
Dawn Frazier
Deborah McKenzie
Alicia Meiklejohn
Tien Milor
Anita Mishra
Bob Rathbone

I. WELCOME AND INTRODUCTIONS

Darryl Sullivan welcomed all participants to the ERP meeting and introduced the ERP members.

II. REVIEW ACTIONS FROM PREVIOUS MEETING

Darryl Sullivan reviewed previous actions/items from past meetings.

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS

For each method, the ERP/Working Group Co-Chairs discussed methods submitted.

Carnitine – *Co-Chairs:* John Austad (Covance) & Guenther Raffler (CLF-Eurofins)
Choline – *Co-Chair:* Sneh Bhandari (Silliker) & Nick Cellar (Abbott)

To study directors, methods should be able to detect both carnitine and choline, but evaluate the methods independently.

IV. REVIEW OF MODIFICATION FOR OMA# 2011.08 (Vitamin B_{12})

V. The Expert Review Panel (ERP) reviewed the modification to OMA method 2011.08. The primary (Christiansen/Sliva) and secondary (Kneeteman) reviewers provided their evaluation along with other members of the ERP.

- This is a new method, it contains significant changes. Should check if it should be major or minor modification
- For AOAC First Action, the method must have SPIFAN SLV data
- For publication, reference the previous method
- Vote the current modified method as AOAC First Action
- HPLC/ULC – have data reflect both HPLC & ULC
- Have a dispute resolution method, but keep as a back-up
- Caveat – back up method if study director doesn't want to continue
- Re-introduce as a new method

VI. REPEAL OF FIRST ACTION METHODS

The Expert Review Panel (ERP) members collectively discussed the next steps on the remaining First Action *Official Methods*^{SM} currently in the SPIFAN system.

Method	Method Title	Reviewer(s)	Vote	Comments
Folate	*2011.05 - Folate in Infant Formula and Adult/Pediatric Nutritional Formula Optical Biosensor Assay*	Sneh Bhandari Melissa Phillips	*Motion to repeal Yes- 11/ No-0 /Abstain-1	◊ Method is being used, but no SLV data to be produced
	2011.06 - Total Folates in Infant Formula and Adult Nutritionals by Trienzyme Extraction and UPLC-MS/MS Quantitation: First Action 2011.06	Adrienne McMahon Matt Sliva for Scott Christiansen	*Motion to extend method for one (1) year Yes- 10/ No-0 /Abstain-1	◊ No further work will be done ◊ Extend method for one(1) year ◊ If method is kept, someone should take it on ◊ 1st Action status should have meaning ○ Proprietary method (single source)
Nucleotides	*2011.21 - Development and Application of an HILIC-MS/MS Method for the Quantitation of Nucleotides in Infant Formula*	Estela Kneeteman Min Huang	*Motion to keep Yes- 11/ No-0 /Abstain-1	◊ Keep as a back-up method

Vitamin A	*2011.07 - Vitamin A in Infant Formula and Adult Nutritionals UPLC-UV*	Don Gilliland Jinchuan Yang	*Motion to keep method Yes- 11/ No-0 /Abstain-1	◊ Keep method ◊ Desire for combo method ◊ Has SLV data, but not published
	2011.15 - Vitamin A (Retinol) in Infant Formula and Adult Nutritionals Liquid Chromatography	Sneh Bhandari Adrienne McMahon	*Motion to repeal Yes- 11/ No-0 /Abstain-1	
Vitamin D	*2011.12 - Vitamins D_2 and D_3 in Infant Formula and Adult Nutritionals Ultra Pressure Liquid Chromatography with Mass Spectrometry Detection (UPLC-MS/MS)*	Brendon Gill Adrienne McMahon	*Motion to repeal Yes- 10/ No-0 /Abstain-1	
	2011.13 - Vitamins D_2 and D_3 in Infant Formula and Adult Nutritionals LC-MS/MS	Matt Sliva for Scott Christiansen Shay Phillips	*Motion to repeal Yes- 9/ No-0 /Abstain-1	◊ Original method was withdrawn and resubmitted
Vitamin B12	*2011.08 - Improved AOAC First Action 2011.08 for the analysis of vitamin B_{12} in Infant Formula and Adult/Pediatric Formulas. Single Laboratory Validation* ***(Modification)***	Bill Mindak Matt Sliva for Scott Christiansen Brendon Gill Shay Phillips	*Motion to give the method a new number (TBD) Yes- 11/ No-0 /Abstain-1 *Motion to repeal old number (2011.08) Yes- 10/ No-0 /Abstain-1	◊ Keep or give it a new number? ◊ Reference back to the old number from the AOAC Journal ◊ ***See section 4 for additional comments***
	2011.09 - Vitamin B_{12} in Infant Formula and Adult Nutritionals HPLC After Purification on an Immunoafinity Column	Matt Sliva for Scott Christiansen Estela Kneeteman	*Motion to keep Yes- 11/ No-0 /Abstain-1	◊ Keep the method
	2011.16 - Vitamin B_{12} in Infant Formula and Adult Nutritionals Surface Plasmon Resonance	Shay Phillips John Austad	*Motion to repeal Yes- 10/ No-0 /Abstain-1	

VII. DISCUSS REQUIREMENTS/EXPECTATIONS RELATED TO MULTI-LABORATORY TESTING REPORTS, AN UPDATE ON MULTI-LABORATORY TESTING AND SCHEDULE

Robert Rankin (International Formula Council) discussed the template to be used for Multi-Laboratory Testing (MLT) reports; the reports will be in a standardized format. The draft template and will be available soon, it's currently in review with the Methods Committee on Statistics.

VIII. UPCOMING METHOD AUTHOR ORIENTATION

Deborah McKenzie, AOAC provided an overview of the upcoming method author orientation including requirements for submission of Multi-Laboratory Testing (MLT) reports.

IX. NEXT STEPS/FEEDBACK FROM ERP

Darryl Sullivan provided next steps including deadline dates. The study directors will need to complete the evaluation sheets and the ERP may have seven (7) MLT reports for review with a mid-July deadline, while providing the ERP with four (4) weeks to complete a thorough review. AOAC will need to be informed if ERP members will be unable to attend the AOAC Annual Meeting in Boca Raton, FL. No feedback/comments were received from the ERP pertaining to the meeting.

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Meeting held at

Boca Raton Resort & Club

Tuesday, September 9, 2014 - 8:30am (Eastern US)

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Members (in attendance):

Darryl Sullivan	***Covance Labs*** *(Chair)*
John Austad	Covance Labs
Sneh Bhandari	Mérieux NutriSciences & OMB
Esther Campos-Gimenez/Adrienne McMahon	Nestlé/Wyeth Nutrition (formerly Pfizer)
Scott Christiansen	Perrigo/PBM Nutritionals
Jon DeVries	General Mills/Medallion Labs
Brendon Gill/Harvey Indyk	Fonterra
Sarwar Gilani	Consultant
Min Huang	Frontage Labs, Inc.
Estela Kneeteman	INTI
Maria Ofitserova	Pickering Labs, Inc.
Melissa Phillips/Kate Rimmer	NIST
Shay Phillips	Mead Johnson Nutrition
Guenther Raffler	CLF-Eurofins
Karen Schimpf	Abbott Nutrition
Scott Christiansen	Perrigo/PBM Nutritionals
Jinchuan Yang	Waters Corp.

Expert Review Panel Members (unable to attend):

Don Gilliland	Abbott Nutrition
William Mindak	FDA
Jeanne Rader	FDA (CFSAN) - Retired

AOAC Staff Includes:

Delia Boyd
E. James Bradford
Scott Coates
Deborah McKenzie
Tien Milor
Anita Mishra
Bob Rathbone

ERP PROFILE SUMMARIES

Observers:

Martin Alewijn, *RIKILT*
Sean Austin, *Nestlé Research Center*
Brad Barrett, *ABSciex*
Christopher J. Blake, *Nestlé Research Center*
Martin Bucknall, *UNSW*
Marti Cenky, *Abbott Nutrition*
France Cho, *Maxxam Analytics Inc.*
Mark Collison, *Archer Daniels Midland Co.*
Hans Cruijsen, *FrieslandCampina Domo*
Brian De Borba, *Thermo Fisher Scientific*
Jean-Luc Deborde, *SCL Laboratoire de Strasburg*
Rachel de Guzman, *Mead Johnson Nutrition*
Marieke de Laat, *Mead Johnson Nutrition*
XiaoJun Deng, *SHCIQ*
Marcel deVreeze, *NEW/ISO*
Aurélie Dubois-Lozier, *IDF*
Wayne C. Ellefson, *Covance Laboratories*
Jaap Evers, *ISO Rep. (Fonterra Co-op.)*
Ping Feng, *Consultant*
Jennifer Fruth, *Mead Johnson Nutrition*
Pierre-Alain Golay, *Nestlé Research Center*
Phillip Haselberger, *Abbott Nutrition*
Melissa Holskey, *Abbott Nutrition*
Steve Holroyd, *IDF Rep. (Fonterra Co-op.)*
Gregory Hostetler, *Perrigo/PBM Nutritionals*
Wesley Jacobs, *Abbott Nutrition*
Greg Jaudzems, *Nestlé*
Elaine Jobgen, *Eurofins*
George Joseph, *AsureQuality*
Bert Klarenbeek, *FrieslandCampina Domo*
Khammawan Kohler, *Covance Laboratories*
Erik J. M. Konings, *Nestlé Research Center*
Li Xian Liang, *China ChongQing CIQ*
Stephen Lock, *ABSciex*
E. Marley, *R-Biopharm Rhome Ltd. Scotland*
Frederic Martin, *Nestlé Research Center*
Josh Messerly, *Eurofins*
Deepali Mohindra, *Thermo Fisher Scientific*
Nancy Montgomery, *Abbott Nutrition*
Mardi Mountford, *IFC*
Norriel Nipales, *Wyeth Nutrition (formerly Pfizer)*
Lawrence Pacquette, *Abbott Nutrition*
Shang-Jing Pan, *Abbott Nutrition*
Eric Poitevin, *Nestlé Research Center*
Al Poland, *AOAC Consultant*
Robert Rankin, *IFC*
Lars Reimann, *Eurofins*
Joe Romano, *Waters Corporation*
Steve Royce, *Agilent Technologies, Inc.*
Louis Salvati, *Abbott Nutrition*
Dan Schmitz, *Abbott Nutrition*
Matthew Sliva, *Perrigo/PBM Nutritionals*
Angela Song, *Abbott Nutrition*
Karla Steele, *Mead Johnson Nutrition*
John Szpylka, *Merieux NutriSciences*
Joseph J. Thompson, *Abbott Nutrition*
Linda Thompson, *Abbott Nutrition*
Melissa Thompson, *Covance Laboratories*
Laszlo Torma, *Pickering Laboratories, Inc.*
Marina Torres Rodriguez, *LATU*
Martijn Vermeulen, *TNO*
Wayne Wargo, *Abbott Nutrition*
Guy Weerasekera, *Mead Johnson Nutrition*
Laura Wood, *NIST*
David Woollard, *Hill Laboratories*
Wayne Wolf, *USDA (Retired)*
Jinchuan Yang, *Waters Corporation*
Linda Zhao, *Abbott Nutrition*
Yang Zhou, *Eurofins*

I. WELCOME AND INTRODUCTIONS

Darryl Sullivan welcomed all participants to the ERP meeting and introduced the ERP members.

II. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS

For each method, the ERP/Working Group Co-Chairs discussed methods submitted.

- **Folate – *Chair:*** Erik Konings (Nestlé)

  Fol-20: Method was withdrawn for additional information

- **Carnitine – *Co-Chairs:*** John Austad (Covance) & Günther Raffler (CLF-Eurofins)
  Choline – *Co-Chair:* Sneh Bhandari (Silliker) & Nick Cellar (Abbott)
  - Instructions to study directors to collect choline data as well as carnitine

Method	Method Title	Reviewer(s)	N Vote	Comments
Folate	*Fol-20 - Analysis of Folic acid and 5-Methyltetrahydrofolate in Infant and Adult Nutritional formula using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry*	Adrienne McMahon Min Huang	Method withdrawn from consideration	◊ SPIFAN matrices did not produce internal peaks (repeat work) ◊ Concerned with interferences ◊ Confirmation ion is required ◊ Did not meet LOQ
Carnitine	*Carn-05 - Single Laboratory Validation of CARN-05: Determination of Free and Total Carnitine and Choline in Infant Formulas and Adult Nutritional Products*	John Austad Sneh Bhandari	John Austad moved & Sneh Bhandari second *Motion: move method to First Action *Official Method*^{SM} status **Yes- 14/ No-0 /Abstain-1**	◊ Collect choline data as well as carnitine ◊ Will not be considered dispute resolution method for choline ◊ Investigate choline adult powder milk
	Carn-06 - Simultaneous Determination of Carnitine and choline in Infant Formula, Adult/Pediatric Nutritional Formula, food and feed ESI LC-MS/MS	Günther Raffler John Austad	Method not recommended at this time	◊ Collect choline data as well as carnitine ◊ Recoveries needed at different levels ◊ Must use SPIFAN matrices ◊ Need SPIFAN data ◊ Resubmit for March 2015

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FINAL ACTION *OFFICIAL METHOD*^{SM} STATUS

The Expert Review Panel (ERP) reviewed seven (7) methods for Final Action *Official Methods*^{SM} status. Six (6) were recommended for Final Action to the Official Methods Board (OMB).

Method	Method Title	Reviewer(s)	Vote	Comments
Vitamin B_{12}	*2011.10 - Vitamin B_{12}: - Determination of Vitamin B12 in Infant Formula and Adult Nutritionals by HPLC*	John DeVries Shay Phillips	John DeVries moved & Scott Christiansen second *Motion: move to Final Action Yes- 11/ No-1 /Abstain-3 ——————— *Second vote: Yes- 11/ No-1 /Abstain-3	◊ Question about SPE overload – How to qualify ◊ Include safety for cyanide ◊ Chromatography resolution
Vitamin D	*2011.11 - Vitamin D - Determination of Vitamin D2 and D3 in Infant and Adult/Pediatric Nutritionals and Utilizing Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC-MS/MS)*	Sneh Bhandari Brendon Gill/ Harvey Indyk	Sneh Bhandari moved & Sarwar Gilani second *Motion: move to Final Action ——————— ~Motion: withdrawn	◊ Editorial changes ◊ Method not a significant improvement over existing method(s) ◊ 50% of samples do not apply to SMPR ◊ Qualifier removed ◊ Single platform – Blinded
Inositol	*2011.18 - Inositol - Determination of Myo-Inositol (Free and Bound as Phosphatidylinositol) in Infant Formula and Adult Nutritionals by Liquid Chromatography/Pulsed Amperometry with Column Switching*	Brendon Gill/ Harvey Indyk	Esther Campos-Gimenez moved & Melissa Phillips second *Motion: move to Final Action Yes- 12/ No-1 /Abstain-2 ——————— *Second vote: Yes- 12/ No-1 /Abstain-2	◊ Remove phosphatidylinositol (data does not support) ◊ Method does not match SMPR ◊ Sum of free myo-inositol (change total to free in method) ◊ Remove "phosphorylated forms" from applicability section ◊ Sum and Free and phosphydal Inositol; c**apture in applicability statement of SMPR.**
UTM	*2011.19 - Ultra Trace Minerals - Simultaneous Determination of Chromium, Selenium, and Molybdenum in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma/Mass Spectrometry*	Min Huang Sneh Bhandari	Min Huang moved & Sneh Bhandari second *Motion: move to Final Action Yes- 13/ No-0 /Abstain-1	
Nucleotides	*2011.20 - Nucleotides - Nucleotides in Infant Formula by HPLC-UV*	Sneh Bhandari Estela Kneeteman	Sneh Bhandari moved & Estela Kneeteman second *Motion: move to Final Action Yes- 13/ No-0 /Abstain-1	

Vitamin A/E	*2012.10 - Vitamin A/E - Simultaneous Determination of 13-Cis and all-trans Vitamin A Palmitate (retinyl palmitate), Vitamin A Acetate (retinyl acetate), and Total Vitamin E (α-Tocopherol and DL-α-Tocopherol Acetate) in Infant Formula and Adult Nutritionals by Normal Phase HPLC*	Scott Christiansen Jinchuan Yang	Scott Christiansen moved & Brendon Gill second *Motion: move to Final Action Yes- 13/ No-0 /Abstain-1	◊ Require use of primary standards ◊ Simplify calibration curve (all trans)
Fatty Acids	*2012.13 - Fatty Acids - Determination of Fatty Acids, including LCPUFAs, in Infant and Adult/Pediatric Nutritional Formula*	Jon DeVries Karen Schimpf	Jon DeVries moved & Karen Schimpf second *Motion: move to Final Action Yes- 13/ No-0 /Abstain-1	◊ Reference materials with higher RSD ◊ Double check chromatography for clarity (peaks)

IV. NEXT STEPS/FEEDBACK FROM ERP

Darryl Sullivan provided next steps including deadline dates. The ERP provided feedback on lessons learned from reviews.

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Meeting held at

Hilton Washington DC North/Gaithersburg

Wednesday, March 18, 2015 - 8:30am (Eastern US)

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Members (in attendance):

Darryl Sullivan	***Covance Labs*** *(Chair)*
John Austad	Covance Labs
Esther Campos-Gimenez/Adrienne McMahon	Nestlé/Wyeth Nutrition (formerly Pfizer)
Scott Christiansen	Perrigo/PBM Nutritionals
Jon DeVries	General Mills/Medallion Labs
Harvey Indyk	Fonterra
Estela Kneeteman	INTI
Maria Ofitserova	Pickering Labs, Inc.
Melissa Phillips/Kate Rimmer	NIST
Shay Phillips	Mead Johnson Nutrition
Günther Raffler	CLF-Eurofins
Karen Schimpf	Abbott Nutrition
Jinchuan Yang	Waters Corp.

Expert Review Panel Members (unable to attend):

Sneh Bhandari	Mérieux NutriSciences & OMB
Sarwar Gilani	Consultant
Brendon Gill	Fonterra
Don Gilliland	Abbott Nutrition
Min Huang	Frontage Labs, Inc.

AOAC Staff Includes:

Delia Boyd
E. James Bradford
Scott Coates
Arlene Fox
Deborah McKenzie
Alicia Meiklejohn
Tien Milor
Bob Rathbone
Gar Riegler

Observers:

Martin Alewijn, *RIKILT*
Sean Austin, *Nestlé Research Center*
Brad Barrett, *Sciex*
Anne Bienvenue, *U.S. Dairy Export Council*
Christopher Blake, *Nestlé Research Center*
Bob Clifford, *Shimadzu*
Hans Cruijsen, *FrieslandCampina Domo*
Marcel deVreeze, *NEN/ISO*
Jaap Evers, *IDF Rep. (Fonterra Co-op.)*
Jennifer Fruth, *Mead Johnson Nutrition*
Christophe Fuerer, *Nestlé Research Center*
Jim Griffiths, *CRN*
Jim Harnly, *USDA*
Phillip Haselberger, *Abbott Nutrition*
Steve Holroyd, *IDF Rep. (Fonterra Co-op.)*
Gregory Hostetler, *Perrigo/PBM Nutritionals*
Harvey Indyk, *Fonterra Co-op.*
Wesley Jacobs, *Abbott Nutrition*
Greg Jaudzems, *Nestlé*
George Joseph, *AsureQuality*
Bert Klarenbeek, *FrieslandCampina Domo*
Erik Konings, *Nestlé Research Center*
Frederic Martin, *Nestlé Research Center*
Josh Messerly, *Eurofins*
Paul Milne, *Keurig Green Mountain Inc.*
Deepali Mohindra, *Thermo Fisher Scientific*
Matt Noestheder, *Sciex*
Mike Nygaard, *U.S. Dairy Export Council*
Lawrence Pacquette, *Abbott Nutrition*
Bert Popping, *Mérieux NutriSciences*
Robert Ragan, *Abbott Nutrition*
Robert Rankin, *INCA*
Rick Reba, *Nestlé USA, Inc.*
Murali Reddy, *Abbott Nutrition*
Lars Reimann, *Eurofins*
Kate Rimmer, *NIST*
Shauna Roman, *Reckitt Benckiser (RB)*
Joe Romano, *Waters Corporation*
Steve Royce, *Agilent Technologies, Inc.*
Louis Salvati, *Abbott Nutrition*
Dan Schmitz, *Abbott Nutrition*
Olga Shimelis, *SUPELCO/Sigma-Aldrich*
Brian Shira, *Mead Johnson Nutrition*
Matthew Sliva, *Perrigo/PBM Nutritionals*
Karla Steele, *Mead Johnson Nutrition*
John Szpylka, *Merieux NutriSciences*
Joseph J. Thompson, *Abbott Nutrition*
Linda Thompson, *Abbott Nutrition*
Melissa Thompson, *Covance Laboratories*
Marina Torres Rodriguez, *LATU*
Wil Van Loon, *FrieslandCampina*
Martijn Vermeulen, *TNO*
Wayne Wargo, *Abbott Nutrition*
Laura Wood, *NIST*
David Woollard, *Hill Laboratories*
Wayne Wolf, *USDA (Retired)*
Jason Wubben, *Archer Daniels Midland Co.*
Jinchuan Yang, *Waters Corporation*
Joyce Zhu, *Jamieson Lab*
Richard Zywicki, *Covance Laboratories*

I. WELCOME AND INTRODUCTIONS

Darryl Sullivan welcomed all participants to the ERP meeting and introduced the ERP members.

II. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS – AOAC SPIFAN I

For each method, the ERP/Working Group Co-Chairs discussed methods submitted.

- **Folate – *Chair:*** Erik Konings (Nestlé)

- **Carnitine – *Co-Chairs:*** John Austad (Covance) & Günther Raffler (CLF-Eurofins)

  Choline – *Co-Chairs:* Sneh Bhandari (Silliker) & Nick Cellar (Abbott)

Method	Method Title	Reviewer(s)	Vote	Comments
Folate	*AOAC Official Method 2011.06 (Fol-22) - Validation of A LC-MS/MS Method for Folate Analysis in Infant Formula and Adult Nutritional Samples*	Adrienne McMahon	Adrienne McMahon recommended method proceed in AOAC SPIFAN process (DRM) *Method retains First Action *Official Method*^{SM} status	▪ Method meets SMPR ▪ Detects the poly glutamate ▪ Process is long ▪ SLV data is available ▪ Folate reported as total folate ▪ Needs % of folate ▪ Standard corrective for water; 8% ▪ Accuracy that doesn't have methyl; non folic ▪ 5 methyl being different
Carnitine	*Carn-07 - Analysis of Free and Total Carnitine and Choline in Infant Formula and Adult Nutritionals*	Günther Raffler Sneh Bhandari (by evaluation form)	Günther Raffler moved & Melissa Phillips second *Motion: recommended method to First Action *Official Method*^{SM} status & proceed in AOAC SPIFAN process (DRM) **Yes- 11/ No-0 /Abstain-1**	▪ Recovery rates are good ▪ SLV report complete ▪ Meets requirements

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS – AOAC SPIFAN II

For each method, the ERP/Working Group Co-Chairs discussed methods submitted. Five (5) methods received First Action *Official Method*^{SM} status with two (2) retaining the original status for a total of seven (7).

Method	Method Title	Reviewer(s)	Vote	Comments
Amino Acid	*Amino-01 - Determination of amino acids in infant and adult/pediatric nutritional formula by UHPLC/UV*	Maria Ofitserova Shay Phillips	Method not recommended for First Action at this time	▪ Method is well written; sound methodology ▪ Study author should use all AOAC SPIFAN samples and provided the information ▪ Analytical range; LOQ ▪ Lysteine has limited range ▪ Title change – total amino acid ▪ Does not capture tryptophan ▪ Proprietary technique ▪ Recovery over 110% ▪ Method has potential; needs optimization
Biotin	*Bio-01 - An inter-laboratory study to extend the scope of the CEN biotin method by HPLC with post-column derivatization and fluorimetric detection*	Estela Kneeteman Scott Christiansen	Method not recommended for First Action at this time	▪ Five (5) different samples used ○ Infant milk powder ▪ Simple method ▪ No LOQ data or recovery ▪ Need more information on the method
	Bio-02 - Determination of Biotin by High Performance Liquid Chromatography coupled with EASI-EXTRACT Biotin Immunoaffinity column cleanup extraction	Scott Christiansen Karen Schimpf	Method not recommended for First Action at this time	▪ Semi proprietary method ▪ No chromatography ▪ Two (2) samples used ▪ Free or total? ▪ Use NIST 1849a as reference ▪ Not enough SLV data ▪ Columns from two (2) different manufacturers
	Boi-03 - Simultaneous Determination of Seven Water Soluble Vitamins in Products by LC-MS/MS	Adrienne McMahon Estela Kneeteman	Method not recommended for First Action at this time	▪ Lower LOQ ▪ Needs full recovery ▪ Needs more AOAC SPIFAN samples ▪ Bound forms need to be captured
Chloride	*Chlor-01 - AOAC Official Method 986.26 Chloride in Milk-Based Infant Formula. Final action 1988.*	Brendon Gill Karen Schimpf Bill Mindak	Method not recommended for First Action at this time	▪ Applicable to milk based infant formula ▪ May help to have figures; can't distinguish ▪ No AOAC SPIFAN matrices ▪ For medical nutritionals please provide information
	#Chlor-02 - Infant Formula and Adult Nutritionals Chloride by Potentiometry	Günther Raffler Shay Phillips Bill Mindak	Scott Christiansen moved Jon DeVries second *Motion: move to First Action Yes- 10/ No-2 /Abstain-1 ——————— Second vote Yes- 10/ No-2 /Abstain-1	▪ Hydrolyzed method ▪ No SLV data included ▪ Simple method ▪ Address issue with high protein & fat in adult nutritionals ▪ Recovery/repeatability meets the SMPR
	Chlor-03 - Determination of Chloride in Infant Formula	Shay Phillips Günther Raffler Bill Mindak	Method not recommended for First Action at this time	▪ Different approach ▪ Needs AOAC SPIFAN SLV ▪ Newer technology ▪ Needs reagents ▪ Performance based method is needed not equipment

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

	#Chlor-04 - Chlorine in Infant Formula and Adult/Pediatric Nutritional Formula by Potentiometric Titration	Adrienne McMahon Bill Mindak	Moved second *Motion: move to First Action Yes- 12/ No-0 /Abstain-0	▪ No recovery completed
Fluoride	*Fluor-01 - Determination of fluoride in dietetic food products by ISE*	John Austad Melissa Phillips Bill Mindak	Method not recommended for First Action at this time	▪ No SLV data ▪ May not meet the analytical range ▪ 5-200mg 100% ▪ 2.5 = 200/250 high end
	Fluor-02 - Determination of Fluoride in Infant Food	John Austad Melissa Phillips Bill Mindak	Method not recommended for First Action at this time	▪ Not a full set of SLV data available ▪ Promising method, but needs more information ▪ Specific to the Dionex ▪ Ion chromatography
Fructans	*Fos-01 - AOAC Official Method 997.08 Fructans in Food Products - Ion Exchange Chromatographic Method*	Sean Austin Estela Kneeteman	Method not recommended for First Action at this time	▪ Method looks promising; awaiting additional data ▪ High content of free sugars ▪ SLV data not complete ▪ No LOQ or recovery information
	Fos-02 - Determination of Fructans in Foods	Sean Austin Brendon Gill	Method not recommended for First Action at this time	▪ Basic principle-hydrolyzed sucrose ▪ Method should realize more saccharides ▪ Significant lose in fructose ▪ Need raw materials ▪ Reference to the Nestlé method not cited ▪ May meet SMPR ▪ looks promising; awaiting additional data
	Fos-03 - Determination of Fructans in Infant Formula and Adult Nutritionals (High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection)	Jon DeVries Karen Schimpf	Method not recommended for First Action at this time	
	Fos-04 - Determination of Fructans in Infant and Adult/Pediatric Nutritional Formulas as well as ingredient commodities	Jon DeVries Sean Austin	Method not recommended for First Action at this time	
GOS	*Gos-01 - Single Lab Validation Report for GOS in Infant Formula and Adult Nutritionals*	Estela Kneeteman Maria Ofitserova	Estela Kneeteman moved Maria Ofitserova second *Motion: move to First Action Yes- 1/ No-8 /Abstain-2 Method not recommended for First Action at this time	▪ HPLC method ▪ No data on LOQ ▪ Significant gaps that need to be addressed ▪ Direct quantification of the Gos ▪ Current AOAC SPIFAN matrices may not be the best to use
	Gos-02 - Determination of trans-Galacto-oligosaccharides (TGOS) in Infant Milk Formula (Ion-Exchange Chromatography)	Sean Austin Maria Ofitserova	Method not recommended for First Action at this time	▪ Specific for Gos ▪ Sample prep not written clearly ▪ Lengthy sample prep, but improves the existing method ▪ Send SLV data to ERP ▪ Lactose from Gos is confusing ▪ With the clean up, how specific is the removal of lactose or other di-saccharides? ▪ Need additional data

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

Minerals & Trace Elements	*MTE-01 - 2013 AOAC INTERNATIONAL AOAC Official Method 2011.14 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products*	Bill Mindak Jinchuan Yang	*Method retains First Action *Official Method*[SM] status	▪ Good method; great update ▪ SLV used pertinent samples ▪ Method meets most of the SMPR ▪ LOQ meets SMPR ▪ Spiked recovery meeting SMPR (90-110%) ▪ Analytical range recovered higher ▪ Did not use AOAC SPIFAN samples
	MTE-02 - Determination of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo by ICP-MS	Bill Mindak Jinchuan Yang	Moved second *Motion: move to First Action Yes- 9/ No-2 /Abstain-1 _____ Second vote Yes- 10/ No-2 /Abstain-2	▪ Too much instrument details ▪ Internal standard added before ▪ Meets most of the SMPR ▪ Carbon buffer ▪ Used AOAC SPIFAN samples ▪ No scope applicability ▪ SRM too high ▪ Written for only one model ▪ Potassium has high RSD ▪ Need to see modified write up/data
	MTE-03 - Milk and milk products Determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc contents - Inductive coupled plasma atomic emission spectrometric method (ICP-AES)	Bill Mindak Jinchuan Yang	Bill Mindak moved Jinchuan Yang second Method withdrawn/tabled until data can be reviewed by ERP	▪ No internal standard ▪ Copper did not meet the low range ▪ Remove the "dry ash" from the method ▪ Microwave digestion with internal standard
Vitamin K	*VitK-01 - Validation of A LC-MS/MS Method for Vitamin K Analysis in Infant Formula and Adult Nutritional Samples*	Esther Campos-Gimenez Scott Christiansen	Method not recommended for First Action at this time	▪ Peak in the chromatograms are not labeled correctly ▪ Extraction procedure; separation of the cis/trans could be compromised ▪ Method seems promising; has potential ▪ LOQ/accuracy meets the SMPR ▪ No primary stock standard used ▪ Variability & precision is a concern ▪ No purity check on the standard
	VitK-02 - Determination of Trans Vitamin K1 by HPLC and Fluorescence Detection	Scott Christiansen Esther Campos-Gimenez	Scott Christiansen moved Esther Campos-Gimenez second *Motion: move to First Action Yes- 10/ No-0 /Abstain-1	

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

IV. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FINAL ACTION *OFFICIAL METHOD*^{SM} STATUS

The Expert Review Panel (ERP) reviewed four (4) methods for Final Action *Official Methods*^{SM} status. Two (2) were recommended to the Official Methods Board (OMB) for Final Action *Official Method*^{SM} consideration.

Method	Method Title	Reviewer(s)	Vote	Comments
Iodine	*2012.15 - Determination of Total Iodine in Infant Formula and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)*	Esther Campos-Gimenez	Esther Campos-Gimenez moved Jon DeVries second *Motion: move to Final Action Yes-11/ No-0 /Abstain-1	▪ Fourteen (14) labs participated; only thirteen (13) submitted data ▪ Used red dye #3 (strawberry drink mix) ▪ Six (6) matrices/one (1) SRM ▪ Used three (3) different suppliers ▪ Three (3) labs used microwave digestion ▪ Complete method ▪ May need maintenance ▪ Add precautions ○ Lens stack ⮝ Replace or have a dedicated set for iodine only
Pantothenic Acid	*2012.16 - AOAC Official Method 2012.16 Pantothenic Acid (Vitamin B5) in Infant Formula and Adult/Pediatric Nutritional Formula*	John Austad Don Gilliland/ Karen Schimpf	John Austad moved Karen Schimpf second *Motion: move to Final Action Yes- 12/ No-0 /Abstain-1	▪ Sixteen (16) labs participated ○ One (1) lab dropped out ○ One (1) lab did not qualify ○ Fourteen (14) labs provided data ⮝ Two (2) labs provided data as one (1) ▪ Consider a way to dry ○ Need moisture content addressed
Vitamin C	*2012.22 - Vitamin C (ascorbic acid) in Infant Formula and Adult/Pediatric Nutritional Formula by UHPLC-UV*	Jon DeVries Brendon Gill/ Harvey Indyk	Jon DeVries moved Scott Christiansen second Motion: move to Final Action Yes- 4/ No-4 /Abstain-4 *Method retains First Action *Official Method*^{SM} status	▪ Twenty-six (26) labs originally participated ○ Two (2) labs failed ○ Twenty- four (24) labs provided data ⮝ Fourteen (14) labs provided data using UPLC ⮝ Nine (9) labs provided data using HPLC ⮝ Due to customs restrictions, one (1) lab used known samples ○ Two (2) labs slightly passed ▪ Method needs more guidance ○ Stability ○ Timing ▪ May be oxidized ▪ Samples should be in sealed containers with the same lot number ▪ System suitability included ▪ Precision data from the two (2) sample types ▪ Add a control

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

Vitamin D	*2011.11 - Vitamin D - Determination of Vitamin D2 and D3 in Infant and Adult/Pediatric Nutritionals and Utilizing Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC-MS/MS)*	Sneh Bhandari (via review form) Brendon Gill/ Harvey Indyk	Method not recommended for Final Action *Method retains First Action *Official Method*^{SM} status	▪ Method author needs to publish two (2) sets of data ○ Second set of data should stand alone ▪ Clearly identify which data was previously used and which in new ⅄ Do not exclude data ⅄ Need outliers in the report ▪ Clarify labs that participated in the study Remediation ○ Clean up data ⅄ UPLC ⅄ HPLC

V. NEXT STEPS/FEEDBACK FROM ERP

Darryl Sullivan provided next steps.

#The ERP has requested combining the two (2) methods (Chlor-02 & Chlor-04)

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Meeting held at

Westin Bonaventure Hotel
404 South Figueroa Street
Los Angeles, California 90071

Tuesday, September 29, 2015 - 8:30am (Pacific US)

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

Expert Review Panel Members (in attendance):

Darryl Sullivan	***Covance Labs*** *(Chair)*
John Austad	Covance Labs
Sean Austin	Nestlé Research Centre
Sneh Bhandari	Mérieux NutriSciences & OMB
Esther Campos-Giménez/Adrienne McMahon	Nestlé/Wyeth Nutrition
Scott Christiansen	Perrigo Nutritionals
Hans Cruijsen/Wil van Loon	FrieslandCampina Domo
Jon DeVries	Independent Consultant
Sarwar Gilani	Consultant
Brendon Gill	Fonterra Cooperative
Don Gilliland/Karen Schimpf	Abbott Nutrition
Estela Kneeteman	INTI
Bill Mindak	FDA CFSAN
Maria Ofitserova	Pickering Labs, Inc.
Melissa Phillips/Kate Rimmer	NIST
Shay Phillips	Mead Johnson Nutrition
Günther Raffler	Eurofins/CLF
Jinchuan Yang	Waters Corp.

Expert Review Panel Members (unable to attend):

Min Huang	Frontage Labs, Inc.
Harvey Indyk	Fonterra Cooperative

AOAC Staff Includes:

Delia Boyd
E. James Bradford
Scott Coates
Arlene Fox
Deborah McKenzie
Tien Milor

Working Group Chairs:

Amino Acids:	Ping Feng (Wyeth) & Wes Jacobs (Abbott)
B Vitamins:	Louis Salvati (Abbott)
Carnitine:	John Austad (Covance) & Günther Raffler (CLF-Eurofins)
Chloride:	Christopher Blake (Nestlé)
Choline:	Sneh Bhandari (Silliker) & Nick Cellar (Abbott)
Folate:	Erik Koinings (Nestlé)
Fructans (FOS) & GOS:	Sean Austin (Nestlé)
Minerals & Trace Elements:	Eric Poitevin (Nestlé)
Vitamin D:	Don Gilliland (Abbott)
Vitamin K:	Sneh Bhandari (Mérieux NutriSciences)

Observers:

Simm Bevis, *R-Biopharm Rhone*
Chris Blake, *Nestlé Research Center*
Kommer Brunt, *Rotating Disc*
Nick Cellar, *Abbott Nutrition*
Susie Dai, *Office of the Texas State Chemist*
Raquel de Guzman, *Mead Johnson*
Marcel de Vreeze, *NEN/ISO*
Jean-Luc Deborde, *SCL*
Xiaojun Deng, *CIQ-Shanghai*
Jon DeVries, *Consultant*
Aurélie Debois, *IDF*
Jaap Evers, *Fonterra Cooperative/IDF*
Ping Feng, *Wyeth Nutrition*
Bill Hammonds, *Mead Johnson*
Philip Haselberger, *Abbott Nutrition*
Steve Holroyd, *Fonterra Cooperative/IDF*
Greg Hostetler, *Perrigo Nutritionals*
Wes Jacobs, *Abbott Nutrition*
Greg Jaudzems, *Nestlé USA, Inc.*
George Joseph, *AsureQuality*
Erik Konings, *Nestlé/ISO*
Sookwang Lee, *FDA*
Qi Lin, *Abbott Nutrition*
Elaine Marley, *R-Biopharm Rhone*
Josh Messerly, *Eurofins*
Deepali Mohindra, *Thermo Fisher Scientific*
Mardi Mountford, *INCA*
Norriel Nipales, *Mead Johnson*
Maria Ofitserova, *Pickering Labs*
Uwe Oppermann, *Shimadzu Europa*
Lawrence Pacquette, *Abbott Nutrition*
Quangson Pham, *Abbott Nutrition*
Shang-Jing Pan, *Abbott Nutrition*
Yuefen Peng, *CAIQ*
Melissa Phillips, *NIST*
Shay Phillips, *Mead Johnson*
Eric Poitevin, *Nestlé*
Robert Rankin, *INCA*
Lars Reimann, *Eurofins*
Maurice Seegers, *Mead Johnson*
Emma Shi, *CIQ-Shanghai*
Angela Song, *Abbott Nutrition*
Saovaros Srichimuttayomphol, *Mead Johnson*
Monique Steegmans, *Tienen Miher/Beneo Orafti*
Karla Steele, *Mead Johnson*
Joe Thompson, *Abbott Nutrition*
Leala Thomas, *Thermo Fisher Scientific*
Marina Torres-Rodriguez, *LATU*
Harrie van den Bijgaart, *Qlip/ISO/IDF*
Martijn Vermeulen, *TNO Triskelion*
Mark Wade, *Mead Johnson*
Wayne Wargo, *Abbott Nutrition*
Laura Wood, *NIST*
Chunyan Zhang, *Abbott Nutrition*
Linda Zhao, *Abbott Nutrition*
Joseph Zhou, Sunshineville Health Products, Inc.
Yang Zhou, *Eurofins*

I. WELCOME AND INTRODUCTIONS

Darryl Sullivan welcomed all participants to the ERP meeting and introduced the ERP members.

II. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS – AOAC SPIFAN I

For each method, the ERP/Working Group Chairs including (Co-Chairs) discussed methods submitted.

Method	Method Title	Reviewer(s)	Vote	Comments
Carnitine/ Choline	*Carn-06 - Carnitine Quantitated by liquid chromatography and isotope dilution and Choline quantitated by liquid chromatography and isotope dilution mass spectroscopy*	Sneh Bhandari John Austad	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Method can analyze for free and total Carnitine ▪ Validation data includes different units ▪ Spiked recovery in the data but not in the evaluation form ▪ 1849a used to establish free Carnitine ▪ The method is well designed and employs internal standard for precision purposes ▪ The method is MS based and thus quite specific to the analyte it measures. ▪ Method Author to provide ERP with information on retention time

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS – AOAC SPIFAN II

For each method, the ERP/Working Group Co-Chairs discussed methods submitted. Nine (9) methods were received and reviewed and one (1) method received First Action *Official Method*^{SM} status.

Method	Method Title	Reviewer(s)	Vote	Comments
Amino Acid	*Amino-02 - HPLC Determination of Total Tryptophan in Infant Formula and Adult/Pediatric Nutritional Formula Following Enzymatic Hydrolysis*	Maria Ofitserova Shay Phillips	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ The amino acid profile only measures tryptophan ▪ For chosen samples the method showed good precision and accuracy. ▪ Data for NIST SRM 1849a is presented for 2 laboratories and accuracy for this matrix meets SMPR. ▪ The sample prep takes less time ▪ Sample size is too small/precision ▪ No SPIFAN matrices were used for validation. Limited data for 3 matrices are presented – NIST SRM 1849a, soy formula and Hypoallergenic formula. o Method needs more data on SPIFAN materials ▪ Method uses 3-point calibration instead of recommended 6-point and no information on the range is given. ▪ Actual levels of Tryptophan in studied matrices (except for NIST SRM 1849a) and spike levels used for accuracy studies are not listed. ▪ Background Tryptophan from self-digest of enzymes in the absence of the sample is a concern since this can affect accuracy of analysis at low levels. ▪ One weakness is the limit of quantitation range ▪ Provide additional information on the following: o Analytical range o wide calibration range o system suitability
Carotenoids	*Carot-01 - Determination of Carotenoids in Infant Formula and Adult/Pediatric Nutritional Formula using High-Performance Liquid Chromatography with Photo Diode Array Detection.*	Jon DeVries Adrienne McMahon	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Method uses best of the technology available for Carotenoids ▪ Use of internal standards & PKB columns could improve ▪ Need safety comments for the isopropyl ether/ peroxides ▪ determination of the beta carotene stock solution needs a second step ▪ Mobil phase D has to be stirred constantly ▪ A working standard injection is specified, but nothing on how to prepare or any chromatograms o Information provided on six (6) samples and one (1) lab o Awaiting additional data ▪ Good method, but needs additional work and clarification on standards (include control sample) and purity checks ▪ Need to see a standard chromatogram (including reference) ▪ Method needs enough samples of Carotenoids to do an SLV ▪ Temperature is very high (85˚)

Chloride	*Chlor-03 -Single Laboratory Validation for Chloride Analysis in Infant Formula and Adult Nutritionals: AOAC SMPR 2014.015*	Bill Mindak Günther Raffler Karen Schimpf	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Dilution factor ▪ general ion chromatography to do chloride ▪ method is sophisticated ▪ Contains high bias on the reference material ▪ Method does not meet some of the SMPR ▪ Lower linear limit did not meet SMPR ▪ Write method more neutral/general ▪ Need report/data
Fluoride	*Fluor-02 - Single Laboratory Validation for Fluoride Analysis in Infant Formula and Adult Nutritionals: AOAC SMPR 2014.016*	John Austad Melissa Phillips Bill Mindak	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Failed to meet SMPR ▪ Needs additional work done ▪ Method did not meet the SRM certified reference range ▪ Need additional information on true value w/NIST
Fructans (FOS)	*Fos-04 - Determination of Fructans in Infant and Adult/Pediatric Nutritional Formulas as well as ingredient commodities*	Jon DeVries Sean Austin Hans Cruijsen	Jon DeVries (moved) Brendon Gill (second) Yes-4/ No-10/Abstain-1 _____ Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Data will meet SMPR ▪ Spiked recovery o Part 1 - oligosaccharide (need to see data) ▪ The units used in the report are not clearly described
GOS	*Gos-02 - Determination of trans-Galacto-oligosaccharides (TGOS) in Infant Milk Formula (Ion-Exchange Chromatography)*	Sean Austin Maria Ofitserova Estela Kneeteman	Maria Ofitserova (moved) Estela Kneeteman (second) Yes-2/ No-13/Abstain-1 _____ Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Method not easy to run (part 1 & 2) ▪ No blank samples (analyze all SPIFAN samples) ▪ Method does not meet SMPR ▪ Four (4) spiked levels w/ two (2) below ▪ Higher GOS levels got worst ▪ Meets repeatability requirement ▪ Need more data/reference ▪ Method doesn't capture DP2 ▪ Uses commonly available equipment ▪ Removes Lactose to improve accuracy of GOS analysis ▪ SPIFAN matrices were used for Single Laboratory Validation Study
Minerals & Trace Elements	*MTE-03 - ISO/CD 15151/IDF 229 Milk and milk products-Determination of calcium, copper, iron , magnesium, manganese, phosphorus, potassium, sodium and zinc contents. Inductive coupled plasma atomic emission spectrometric method (ICP-AES)*	Bill Mindak Jinchuan Yang	Jinchuan Yang (moved) Scott Christiansen (second) Yes-6/ No-7/Abstain-1 _____ Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Method has a simple microwave digestion ▪ No ionization buffer is required o should be required & stated o do background correction ▪ Specify the wavelength ▪ LOQ for three (3) elements o copper o iron o manganese ▪ No data on SPIFAN matrices
B Vitamins (1, 2, 3, 6)	*BVit-01 - Simultaneous Determination of Thiamine HCl, Riboflavin, Niacin (Nicotinic Acid and Nicotinamide), Pantothenic acid, Vitamin B6 (Pyridoxine, Pyridoxal and Pyridoxamine) and Biotin in Infant Formula and Adult Nutritionals*	Esther Campos-Gimenez Brendon Gill Scott Christiansen	Method not adopted/ moved to First Action *Official Method*^{SM} status	▪ Method is easy to follow ▪ Method is not clearly written ▪ Recovery rates were combined ▪ Free forms are in range ▪ The scope of the method does not meet the SMPR ▪ Applicability does not meet SMPR ▪ Does not capture phosphate
	BVit-02 - Simultaneous Determination of Total Vitamin B6, B2, B3 and B1 in Infant Formula Products by LC-MS/MS Using Enzymatic Digestion	Esther Campos-Gimenez Scott Christiansen Estela Kneeteman	Scott Christainsen (moved) Sarwar Gilani (second) Yes-6/ No-9/Abstain-1 _____ Melissa Phillips (moved) Esther Campos-Giminez (second) Yes-11/ No-2/Abstain-1 Yes-12/ No-2/Abstain-1 **Method adopted/ moved to First Action *Official Method*^{SM} status for **$B_{1, 2, 6}$ only**	▪ FAD/FAB o FAB is missing ▪ Use of 1849a is not needed and should not compare ▪ ERP wants to see blanks ▪ Niacin in most is lower

IV. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FINAL ACTION *OFFICIAL METHOD*^{SM} STATUS

The Expert Review Panel (ERP) reviewed Two (2) methods for Final Action *Official Methods*^{SM} status. Zero (0) methods were recommended to the Official Methods Board (OMB) for Final Action *Official Method*^{SM} consideration.

Method	Method Title	Reviewer(s)	Vote	Comments
Minerals & Trace Elements	***OMA# 2011.14*** *(MTE-01) - 2013 AOAC INTERNATIONAL AOAC Official Method 2011.14 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products*	Sneh Bhandari Brendon Gill/ Harvey Indyk Don Gilliland	Bill Mindak (Moved) Brendon Gill (Second) Motion: withdrawn _____ Method not recommended for Final Action status *Method retains Final Action *Official Method*^{SM} status with a change in the applicability for infant formula	▪ Copper did not meet SMPR for LOQ ▪ Clarification on LOQ ▪ Need diversity of infant formula ▪ Need justification from ERP on SMPR
Vitamin D	***OMA# 2011.11*** *(VitD-01) - Vitamin D - Determination of Vitamin D2 and D3 in Infant and Adult/Pediatric Nutritionals and Utilizing Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC-MS/MS)*	Sneh Bhandari Brendon Gill/ Harvey Indyk	Jon DeVries (Moved) Sarwar Gilani (Second) Yes-2/ No-11/Abstain-2 _____ Method not recommended for Final Action status *Method retains First Action *Official Method*^{SM} status	▪ Only one (1) sample was used for D_2 ▪ Exclude HPLC ▪ 8/16 had performance issues ▪ Next steps o Open call for methods o Discuss with stakeholders

IV. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR REPRODUCIBILITY TESTING

The ERP will collectively discuss the methods and select a single method to move forward through the AOAC SPIFAN process for reproducibility testing.

Method Evaluation Forms - Methods processed via the numbering system:

Method	Method Title	Number Voting	Comments
Carnitine/ Choline	***OMA# 2014.04 (****Car n-05) - Single Laboratory Validation of CARN-05: Determination of Free and Total Carnitine and Choline in Infant Formulas and Adult Nutritional Products*	**1204**	
	OMA# 2015.10 (*Car n-07) - Analysis of Free and Total Carnitine and Choline in Infant Formula and Adult Nutritionals*	~Move to reproducibility testing **1207**	
Chloride	***OMA# 2015.08*** *(Chlor-02) - Infant Formula and Adult Nutritionals Chloride by Potentiometry*	~Move to reproducibility testing **1413**	▪ CAIQ & Nestle to work together to get one method
	OMA# 2015.07 *(Chlor-04) Chlorine in Infant Formula and Adult/Pediatric Nutritional Formula by Potentiometric Titration*	**1411**	
Folate	***OMA# 2013.13*** *(Fol-21) - Single-Laboratory Validation - Free Folates in Infant Formula and Adult/ Pediatric Nutritional Formula by UHPLC-UV*	**No Vote**	▪ Method doesn't do bound folate ▪ Nestle & Silliker to work together and write up a document explaining - should harmonize. ▪ Exact match label compound ▪ define "total" ▪ If you leave in "use internal standard" remove the compound ▪ Defer to March 2016

Method Evaluation Forms - Methods processed via the voting system:

Method	Method Title	Reviewer(s)	Vote	Comments
B Vitamin	*BVit-02 - Simultaneous Determination of Total Vitamin B6, B2, B3 and B1 in Infant Formula Products by LC-MS/MS Using Enzymatic Digestion* ***(**First Action granted during this meeting)***	Melissa Phillips (Moved) Bill Mindak (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	▪ Complete accuracy study in single lab
Minerals & Trace Elements	***OMA# 2011.14*** *(MTE-01) - 2013 AOAC INTERNATIONAL AOAC Official Method 2011.14 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products*	Shay Phillips (Moved) Hans Cruijsen (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	▪ Complete MLT data on SPIFAN matrices
	OMA# 2015.06 *(MTE-02) - Determination of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo by ICP-MS*	Shay Phillips (Moved) Hans Cruijsen (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	
Vitamin K	***OMA# 2015.09*** *(VitK-02) - Determination of Trans Vitamin K1 by HPLC and Fluorescence Detection*	Brendon Gill (Moved) John Austad (Second)	~Motion: move to reproducibility testing Yes-14/ No-0/Abstain-1	▪ Need to see the published method

V. NEXT STEPS/FEEDBACK FROM EXPERT REVIEW PANEL

Darryl Sullivan provided next steps including suggestions from the Expert Review Panel requesting that some of the SMPRs® be revisited and have the stakeholder panel weigh in. Calls for methods in the nutrients that haven't yielded/generated sufficient response was also proposed.

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPIFAN PESTICIDE CONTAMINANT METHODS

ERP Name	AOAC Expert Review Panel for SPIFAN Pesticide Contaminant Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2015	**Number of Methods Adopted**	3 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPIFAN.				
Roster	1. Darryl Sullivan, *Covance (Chair)* 2. Martin Alewijn, *RIKILT* 3. John Austad, *Covance Labs* 4. Joe Boison, *CFIA* 5. Scott Christiansen, *Perrigo/PBM Nutritionals* 6. Jo Marie Cook, *FL. Dept. of Agriculture* 7. Jon DeVries, *Medallion Labs/General Mills* 8. Harvey Indyk, *Fonterra Cooperative* 9. George Joseph, *AsureQuality* 10. Erik Konings, *Nestlé/ISO* 11. Alex Krynitsky, *FDA-CFSAN* 12. Tom Phillips, *MD. Dept. of Agriculture* 13. Bert Popping, *Mérieux NutriSciences* 14. Murali Reddy, *Abbott Nutrition* 15. Jon Wong, *FDA-CFSAN*				
Technical Documents created/used	1. SMPR for Sodium monofluoroacetate 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2015.02 – Sodium Fluoroacetate in Dairy Powders AOAC 2015.03 – Sodium Fluoroacetate in Infant Formula AOAC 2015.04 - Monofluoroacetate in Powdered Nutritional Products				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

AOAC INTERNATIONAL

Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)

Meeting at Hilton Washington DC North/Gaithersburg

EXPERT REVIEW PANEL (CONTAMINANTS)

REPORT OF THE EXPERT REVIEW PANEL (ERP) PROCEEDINGS

SPIFAN Chair – Darryl Sullivan

(Tuesday, March 17, 2015)

EXPERT REVIEW PANEL MEMBERS

Martin Alewijn, *RIKILT*
John Austad, *Covance Labs*
Joe Boison, *CFIA*
Scott Christiansen, *Perrigo/PBM Nutritionals*
Jo Marie Cook, *FL. Dept. of Agriculture*
Jon DeVries, *Medallion Labs/General Mills*
Harvey Indyk, *Fonterra Cooperative*
George Joseph, *AsureQuality*
Erik Konings, *Nestlé/ISO*
Alex Krynitsky, *FDA-CFSAN*
Tom Phillips, *MD. Dept. of Agriculture*
Bert Popping, *Mérieux NutriSciences*
Murali Reddy, *Abbott Nutrition*
Darryl Sullivan, *Covance Labs*
Jon Wong, *FDA-CFSAN*

MEETING PARTICIPANTS (part or all of the meeting)

Sean Austin, *Nestlé*
Brad Barrett, *Sciex*
Anne Bienvenue, *U.S. Dairy Export Council*
Chris Blake, *Nestlé Research Center*
Esther Campos-Gimenez, *Nestlé*
Erin Crowley, *Q Labs, Inc.*
Hans Cruijsen, *FrieslandCampina*
Marcel de Vreeze, *NEN/ISO*
Clay Detlefsen, *National Milk Producers Federation*
Jaap Evers, *Fonterra Cooperative/IDF*
Jennifer Fruth, *Mead Johnson Nutrition*
Christophe Fuerer, *Nestlé Research Center*
Jim Harnly, *USDA*
Steve Holroyd, *Fonterra Cooperative*
Greg Hostetler, *Perrigo/PBM Nutritionals*
Harvey Indyk, *Fonterra Cooperative*
Wes Jacobs, *Abbott Nutrition*
Greg Jaudzems, *Nestlé USA, Inc.*
Ron Johnson, *Merieux NutriSciences*
Estela Kneeteman, *INTI*
Markus Lipp, *USP*
Frederic Martin, *Nestlé Research Center*
Adrienne McMahon, *Nestlé Research Center*
Josh Messerly, *Eurofins*
Paul Milne, *Keurig Green Mountain Inc.*
Bill Mindak, *FDA-CFSAN*
Deepali Mohindra, *Thermo Fisher Scientific*
Shu Na, *Abbott Nutrition*
Matt Noestheder, *Sciex*
Mike Nygaard, *U.S. Dairy Export Council*
Lawrence Pacquette, *Abbott Nutrition*
Shay Phillips, *Mead Johnson*
Günther Raffler, CLF-Eurofins
Robert Ragan, *Abbott Nutrition*
Robert Rankin, *INCA*
Rick Reba, *Nestlé USA, Inc.*
Lars Reimann, *Eurofins*
Shauna Roman, *Reckitt Benckiser (RB)*
Joe Romano, *Waters Corp.*
Steve Royce, *Agilent Technologies, Inc.*
Louis Salvati, *Abbott Nutrition*
Dan Schmitz, *Abbott Nutrition*
Olga Shimelis, *SUPELCO/Sigma-Aldrich*
Brian Shira, *Mead Johnson*
David Stone, *University of Oregon*
Darryl Sullivan, *Covance*
Fabrizis Suarez, *Abbott Nutrition*
John Szpylka, *Silliker/Merieux NutriSciences*
Joe Thompson, *Abbott Nutrition*
Marina Torres-Rodriguez, *LATU*
Wil Van Loon, *FrieslandCampina*
Wayne Wargo, *Nestlé*
David Woollard, *Hill Labs, Ltd.*
Jinchuan Yang, *Waters Corp.*
Jupiter Yeung, *Nestlé Nutrition*
Joyce Zhu, *Jamieson Labs*

AOAC STAFF

Delia Boyd
E. James Bradford, *Executive Director*
Scott Coates, *CSO*
Arlene Fox
Deborah McKenzie
Nora Marshall
Alicia Meiklejohn
Tien Milor
La'Kia Phillips
Gar Riegler
Bob Rathbone

I. WELCOME AND INTRODUCTIONS

Darryl Sullivan welcomed all participants to the ERP meeting and introduced the ERP members.

II. EXPERT REVIEW PANEL (ERP) ORIENTATION

Deborah McKenzie provided the ERP members with an overview of the process including policies, procedures and logistics.

III. REVIEW OF METHODS BY EXPERT REVIEW PANEL (ERP) FOR FIRST ACTION *OFFICIAL METHOD*^{SM} STATUS

For each method, the ERP/Working Group Chair discussed methods submitted. The ERP also provided comments to the methods authors that were recommended for First Action.

- **Compound 1080 – *Chair:*** Joe Boison (CFIA)

Method Title	Reviewer(s)	Vote	Comments
***MFA-01** - Determination of sodium monofluoroacetate (1080) in dairy powders by Gas Chromatography Tandem Mass Spectography (GC-MS/MS)*	Jo Marie Cook Alex Krynitsky	Method not recommended for First Action	✧ Calibration curve ✧ Method meets criteria in SMPR ✧ Confirmatory method ✧ Sample chromatograms needed ✧ Covered different types of matrices ✧ Two (2) internal standards used ✧ Uses two (2) types of extractions
***MFA-02** - Determination of sodium monofluoroacetate in dairy powders by Liquid Chromatography Tandem Mass Spectography (LC-MS/MS)*	Scott Christiansen Bert Popping	Jon DeVries moved Jo Marie Cook second *Motion: recommend for First Action Yes- 11/ No-3 /Abstain 0 ——————— *Second vote: Yes- 13/ No-0 /Abstain-1	✧ 5 pt. calibration curve ✧ Need to see chromatograms ✧ Consider use of one internal standard a. Suitability b. 10.2.28 needs to be rephrased for clarification ✧ Need completed validation information to demonstrate compliance with SMPR
***MFA-03** - Sodium Fluoroacetate By LC-MS/MS*	Alex Krynitsky John Austad	Jon DeVries moved Jon Wong second *Motion: recommend for First Action Yes- 13/ No-0 /Abstain-1	✧ Ruggedness data ✧ Column performance over extended use ✧ Need completed validation information to demonstrate compliance with SMPR
***MFA-04** - Rapid quantitative and qualitative confirmatory method for the determination of monofluoroacetic acid in foods by liquid chromatography mass spectrometry*	Tom Phillips George Joseph	Method not recommended for First Action	✧ Does not meet LOD requirements ✧ No supporting data ✧ Optimized extraction conditions ✧ No system suitability ✧ Calibration range does not meet SMPR
***MFA-05** - Quantitation of Fluoroacetic Acid and Fluoracetamide with Mass Spectrometric Detection*	Murali Reddy Erik Konings	Method not recommended for First Action	✧ Matrix is drinking water; not shown for infant formula ✧ Does not have sufficient data
***MFA-06** - Method Validation: Determination of Monofluoroacetate in Milk Products by LC-MS/MS*	Jon Wong Jo Marie Cook	Method not recommended for First Action	✧ Uses a 3 point calibration curve (1, 2, 5 pp) ✧ Requires overnight derivitation ✧ Procedure has met the performance criteria
***MFA-07** - Determination of Sodium Monofluoroacetate in Dairy Powder by Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS)*	Jo Marie Cook Alex Krynitsky	Method not recommended for First Action	**Comments are the same as MFA-01**

*MFA-01 & 07 are the same method submitted by 2 different method authors

MFA-08 *- Determination of Monofluoroacetate by LC-MS/MS*	Tom Phillips Martin Alewijn	Tom Phillips moved Martin Alewijn second *Motion: recommend for First Action Yes- 10/ No-3 /Abstain 1 ——————— *Second vote: Yes- 7/ No-5 /Abstain-2 (did not pass)	✧ Non-derivitized method ✧ Samples were separated in UPLC ✧ No clean-up step ✧ Extremely clear & easy to run ✧ Confirmatory method; good recovery ✧ Doesn't meet SMPR ✧ Low manual for high throughput
MFA-09 *- Determination of monofluoroacetate in powdered nutritional products by derivatization with 2-nitrophenylhydrazide and LC-MS/MS*	Jon DeVries Jon Wong	Tom Phillips moved Scott Christiansen second *Motion: recommend for First Action Yes- 11/ No-2 /Abstain-2 ——————— *Second vote: Yes- 11/ No-2 /Abstain-2	✧ Over spiked ✧ Confirmatory method ✧ Can detect at lower levels ✧ 2nd transition ion required ✧ Need completed validation information to demonstrate compliance with SMPR

*MFA-01 & 07 are the same method submitted by 2 different method authors

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPDS – CHONDROITIN, PDE5 INHIBITORS, & ANTHOCYANIN METHODS

ERP Name	AOAC Expert Review Panel for Dietary Supplements – Chondroitin, PDE5 Inhibitors, and Anthocyanin Methods			**Chair(s)**	Brian Schaneberg (Starbucks)
ERP Formed:	2015	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary supplements				
Roster	Chondroitin: 1. Brian Schaneberg, Starbucks (Chair) 2. Jana Hildreth, Synutra Pure 3. Martha Jennens, Covance 4. Phillip Koerner,Phenomenex; 5. Tom Phillips, State of MD; 6. Curtis Phinney, Consultant; 7. Kelly Reins, Consultant; 8. John Spzylka, Merieux NutriSciences; Aniko Solyom, GAAS Corporation; 9. Darryl Sullivan, Covance 10. Liton Roy, Sancillo and Company; 11. Nour Edine ES-SAFI, Mohammad V University Rabat	PDE5 Inhibitors: 1. Brian Schaneberg, Starbucks (Chair); 2. Phil Koerner, Phenomenex; 3. Katerina Mastovska, Covance; 4. Tom Phillips, State of MD; 5. Fenhong Song, FDA; 6. John Spzylka, Mérieux NutriSciences; 7. Darryl Sullivan, Covance. 8. Teresa Cain, FDA; 9. Liton Roy, Sancilio; 10. Jerry Zweigenbaum, Agilent.	Anthocyanins: 1. Brian Schaneberg, Starbucks (Chair); 2. Phil Koerner, Phenomenex; 3. Jungmin Lee, USDA; 4. Melissa Phillips, NIST; 5. Martha Jennens, Covance; 6. Tom Phillips, State of MD; 7. Aniko Solyom, GAAS Analytics; 8. John Spzylka, Mérieux NutriSciences; 9. Darryl Sullivan, Covance 10. Nour Eddine ES-SAFI, Mohammed V University; 11. Liton Roy, Sancilio and Company; 12. Jerry Zweigenbaum, Agilent	
Technical Documents created/used	SMPRs for Chondroitin, PDE5 Inhibitors, and Anthocyanins OMA Appendix K				
Methods Adopted *First Action and Final Action status*	AOAC 2015.11 – Chondroitin AOAC 2015.12 - PDE5 inhibitors				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

EXPERT REVIEW PANEL FOR DIETARTY SUPPLEMENT METHODS - SET 1 INGREDIENTS

(Anthocyanins, Chondroitin, and PDE5 Inhibitors)

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

Brian Schaneberg, Starbuck's, SPDS Set 1 ERP Chair

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the SPDS Set 1 Ingredient (Anthocyanins, Chondroitin, and PDE5 Inhibitors) Expert Review Panel held on August 3-4, 2015.

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON SPDS SET 1 INGREDIENTS (Anthocyanins, Chondroitin, and PDE5 Inhibitors)

Conclusion: The Expert Review Panel reviewed four (4) Chondroitin methods, three (3) Anthocyanins methods, and five (5) PDE5 Inhibitors Methods, which were submitted in response to the Call for Methods. One Chondroitin Method (CHON-004) and one PDE5 Inhibitor Method (PDE5-005) were adopted as First Action *Official Methods*^{SM}.

Methods Reviewed: Each Anthocyanins, Chondroitin, and PDE5 Inhibitors method collected by AOAC for consideration by this ERP was assigned a primary and secondary reviewer. The decisions of the August 3-4, 2015 ERP are shown below.

I. Chondroitin – August 3, 2015

Chondroitin ERP Members Present: Brian Schaneberg, Starbucks (Chair); Jana Hildreth, Synutra Pure; Martha Jennens, Covance; Phillip Koerner, Phenomenex; Tom Phillips, State of MD; Curtis Phinney, Consultant; Kelly Reins, Consultant; John Spzylka, Merieux NutriSciences; Aniko Solyom, GAAS Corporation; Darryl Sullivan, Covance.

Chondroitin ERP Members Absent: Liton Roy, Sancillo and Company; Nour Edine ES-SAFI, Mohammad V University Rabat

AOAC Method #	Manuscript Title and Submitter	ERP Decisions	Consensus	Reviewers
CHON-001	**Title:** "Selected Adulterants in Dietary Ingredients and Dietary Supplements Containing Chondroitin Sulfate" **Submitted by:** Gabriel Giancaspro, USP	**Motion 1:** MOTION to not move CHON-001 to First Action *Official Methods*^{SM} status. **Motion 2:** MOTION that the method author should submit probability of detection (POD) data at the minimum detection level for the compounds listed in Annex I of AOAC SMPR 2014.008.	**Motion 1:** Hildreth/Phinney, 9/0/1 (Abstain: Covance) **Motion 2:** Hildreth/Jennens, 9/0/1 (Abstain: Covance)	Primary: Jana Hildreth Secondary: Curtis Phinney
CHON-002	**Title:** Isotachophoretic Determination	**Motion 1:** MOTION to not move CHON-002 to *First Action*	**Motion 1:** Reins/Hildreth,	Primary: Kelly Reins

	of GA and CS in Dietary Supplements **Submitted by:** Frantisek Kvasnicka, University of Chemistry and Technology, Prague	*Official Methods*[SM] status.	9/0/1 (Abstain: Covance)	Secondary: Curtis Phinney
CHON-003	**Title:** Chondroitin by IR and Dimethylmethylene Blue (DMMB) Spectrophotometry **Submitted by:** Xun Yan, Amway	**Motion 1:** MOTION to not move CHON-003 to *First Action Official Methods*[SM] status. **Motion 2:** MOTION to ask the method author whether they can provide additional data regarding the interfering compounds that are listed on the SMPR008 and if they have additional data for system suitability / all validation data.	**Motion 1:** Solyom/Spzylka 9/0/1 (Abstain: Covance) **Motion 2:** Solyom/Hildreth 9/0/1 (Abstain: Covance)	Primary: Aniko Solyom Secondary: Nour Eddine Es-Safi
CHON-004	**Title:** Determination of Chondroitin Sulfate Content in Raw Materials and Dietary Supplements by High-Performance Liquid Chromatography with Ultraviolet Detection After Enzymatic Hydrolysis **Submitted by:** David Ji, Analytical Laboratories in Anaheim, Inc.	**MOTION 1:** MOTION to move CHON-004 to First Action *Official Methods*[SM] status. **Motion 2:** MOTION to be considered for *Final Action* Official Methods[SM] status, the author(s) should: • Optimize and control the moisture in the chondroitin sulfate including appropriate vessels and glassware. • Investigate alternative LC columns. • Optimize the LC conditions. • Look at lessons learned from USP. • Include a potency evaluation of the enzyme use. • Investigate use of the USP standard that is currently available. • Certified reference material recommended.	**Motion 1:** Sullivan/Koerner, 8/0/2 (Abstain: Covance, Hildreth) **Motion 2:** Sullivan/Reins 8/0/2 (Abstain: Covance, Hildreth)	Primary: Darryl Sullivan Secondary: Phillip Koerner

II. **Anthocyanins - August 3, 2015**

Anthocyanins ERP Members Present: Brian Schaneberg, Starbucks (Chair); Phil Koerner, Phenomenex; Jungmin Lee, USDA; Melissa Phillips, NIST; Martha Jennens, Covance; Tom Phillips, State of MD; Aniko Solyom, GAAS Analytics; John Spzylka, Mérieux NutriSciences; Darryl Sullivan, Covance

Anthocyanins ERP Members Absent: Nour Eddine ES-SAFI, Mohammed V University; Liton Roy, Sancilio and Company; Jerry Zweigenbaum, Agilent

AOAC Method #	Manuscript Title and Submitter	ERP Decisions	Consensus	Reviewers
ANTH-001	**Title:** Determination of Anthocyanins in Brazilian and Floridian Açaí (Euterpe oleraceae Mart) Using LC-MS/MS **Submitter:** Kevin Tran, FDA	**Motion 1:** MOTION to not move ANTH-001 to First Action *Official Methods*^{SM} status with no further action.	**Motion 1:** Lee/Phillips, 7/0/1 (Abstain: Covance)	Primary: Jungmin Lee Secondary: Melissa Phillips
ANTH-002	**Title:** Total Monomeric Anthocyanins By HPLC **Submitter:** Melanie Bush, Artemis International	**Motion 1:** MOTION to not move ANTH-002 to First Action *Official Methods*^{SM} status with no further action.	**Motion 1:** Jennens/Solyom, 7/0/1 (Abstain: Covance)	Primary: Aniko Solyom Secondary: Martha Phillips
ANTH-003	**Title:** Anthocyanin Profiles by HPLC with DAD and MS Detections **Submitter:** Bob Durst, Oregon State Unviersity	**Motion 1:** MOTION to not move ANTH-003 to First Action *Official Methods*^{SM} status with no further action.	**Motion 1:** Spzylka/Solyom, 7/0/1 (Abstain: Covance)	Primary: John Spzylka Secondary: Not Submitted

III. PDE5 Inhibitors– August 4, 2015

PDE5 Inhibitors ERP Members Present: Brian Schaneberg, Starbucks (Chair); Phil Koerner, Phenomenex; Katerina Mastovska, Covance; Tom Phillips, State of MD; Fenhong Song, FDA; John Spzylka, Mérieux NutriSciences; Darryl Sullivan, Covance.

PDE5 Inhibitors ERP Members Absent: Teresa Cain, FDA; Liton Roy, Sancilio; Jerry Zweigenbaum, Agilent.

AOAC Method #	Manuscript Title	ERP Decisions	Consensus	Reviewers
PDE5-001	**Title:** Screening Method for Phosphodiesterase Type 5 (PDER5) Inhibitors in Dietary Ingredients and Supplements using High Resolution Mass Spectrometry **Submitter:** Brian Musselman, IonSense	**Motion 1:** MOTION to not move PDE5-001 to First Action *Official Methods*^{SM} status. **Motion 2:** ERP finds this method intriguing and would like to see a further submission of data demonstrating applicability towards the SMPR 2014.012.	**Motion 1:** Phillips/Sullivan, 6/0/1 (Abstain: Covance) **Motion 2:** Phillips/Sullivan, 6/0/1 (Abstain: Covance)	**Primary:** Tom Phillips **Secondary:** Not Submitted
PDE5-002	**Title:** Screening Method for Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Ingredients and Supplements **Submitter:** Tien Do, Camag	**Motion 1:** MOTION to not move PDE5-002 to First Action *Official Methods*^{SM} status. **Motion 2:** MOTION that the ERP finds this method valuable for screening and would like to see a further submission of data demonstrating applicability towards the SMPR 2014.012.	**Motion 1:** Mastovska/Koerner, 6/0/1 (Abstain: Covance) **Motion 2:** Mastovska/Spzylka, 6/0/1 (Abstain: Covance)	**Primary:** Katerina Mastovska **Secondary:** Not submitted
PDE5-003	**Title:** Adaption of the LC-MS Screen for PDE5 Inhibitors to UHPLC-MS **Submitter:** Elisa Nickum, FDA	**Motion 1:** MOTION to not move PDE5-003 to First Action *Official Methods*^{SM} status. **Motion 2:** MOTION that this method should: 1. Supply single lab validation data related to LOD. **2.** Supply LIB data if available, and any other	**Motion 1:** Koerner/Phillips, 5/0/2 (Abstain: Covance, Song) **Motion 2:** Koerner/Spzylka, 6/0/1 (Abstain:	**Primary:** Phillip Koerner **Secondary:** John Spzylka

		data that exists in support of this method's applicability to the SMPR 2014.012 or 2014.010.	Covance, Song)	
PDE5-004	**Title:** Testing for Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Supplements **Submitter:** Said Goueli, Promega *Note: This method was supplemented with a presentation from the method author.*	**Motion 1:** MOTION to not move PDE5-004 to First Action *Official Methods*^{SM} status. **Motion 2:** MOTION that ERP finds this method valuable for screening and would like to see a further submission of data demonstrating applicability towards the SMPR 2014.012.	**Motion 1:** Phillips/Sullivan, 7/0/1 (Abstain: Covance) **Motion 2:** Phillips/Spzylka, 7/0/1 (Abstain: Covance)	**Primary:** Jerry Zweigenbaum **Secondary:** Teresa Cain
PDE5-005	**Title:** SLV Study of a Method for Screening **Submitter:** Katerina Mastovska, Covance	**Motion 1:** MOTION to move PDE5-005 to First Action *Official Methods*^{SM} status. **Motion 2**: MOTION to be considered for *Final Action* Official Methods^{SM} status, the method author(s) should: 1. Provide reproducibility data 2. Break out AOAC SMPR 2014.011: -Matrix match spike recovery -Recovery options 3. Beak out AOAC SMPR 2014.012 and AOAC SMPR 2014.010. -Feedback from other users and a mechanism to receive that feedback.	**Motion 1:** Phillips/Koerner, 5/0/2 (Mastovska , Sullivan) **Motion 2:** Phillips/Spzylka, 5/0/2 (Mastovska , Sullivan)	**Primary:** Teresa Cain **Secondary:** Tom Phillips

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPDS – ASHWAGANDHA, FOLIN C, AND KRATOM METHODS

ERP Name	AOAC Expert Review Panel for Dietary Supplements – Ashwagandha, Folin C, and Kratom Methods			**Chair(s)**	Darryl Sullivan (Covance)
ERP Formed:	2015	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary supplements				
Roster	Ashwagandha: 1. Darryl Sullivan, Covance (Chair) 2. Anton Bzhelyansky, USP 3. Nour-Eddine Es-Safi, Mohammad V University of Rabat 4. Tom Phillips, State of MD 5. Casey Sayre, Roseman University of Health Sciences 6. Catherine Rimmer, NIST; 7. Aniko Solyom, GAAS Analytical; 8. Kurt Young, GNC/Nutra Manufacturing, 9. Prashant Ingle, Herbalife; 10. Yanjun Zhang, Herbalife	Folin C: 1. Darryl Sullivan, Covance (Chair) 2. Nour Eddine Es-Safi, Mohammad V University in Rabat 3. Martha Jennens, Covance 4. Dana Krueger, Krueger Food Laboratories 5. Tom Phillips, State of MD 6. Catherine Rimmer, NIST 7. Aniko Solyom, GAAS Analytical John Spzylka, Mérieux Nutrisciences 8. Joseph Zhou, Sunshineville Health Products 9. John Finley, LSU (Retired) 10. Prashant Ingle, Herbalife 11. Jungmin Lee, USDA		Kratom: 1. Darryl Sullivan, Covance (Chair) 2. Joseph Betz, NIH 3. Nour Eddine Es-Safi, Mohammed V University in Rabat 4. Charles Metcalfe, Custom Analytics 5. Tom Phillips, State of MD 6. John Spzylka, Mérieux Nutrisciences 7. Yan-Hong Wang, University of Mississippi 8. Christine Casey, FDA 9. Catherine Rimmer, NIST	
Technical Documents created/used	SMPRs for Ashwagandha, Folin C, and Kratom OMA Appendix K				
Methods Adopted *First Action and Final Action status*	AOAC 2015.17 - Estimation of Withanolides (Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastromonolide, Withanolide A, Withanolide B) in Withania somnifera				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

EXPERT REVIEW PANEL ON SPDS SET 2 INGREDIENTS

(Ashwagandha, Folin C and Kratom)

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned Chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the SPDS Set 2 Ingredients (Ashwagandha, Folin C and Kratom) Expert Review Panel held on December 9-10, 2015.

_____

DARRYL SULLIVAN, SPDS SET 2 INGREDIENT EXPERT REVIEW PANEL CHAIRMAN

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

EXPERT REVIEW PANEL ON SPDS SET 2 INGREDIENTS

METHODS FOR CONSIDERATION:

Conclusion: The Expert Review Panel reviewed one (1) Ashwagandha method, three (3) Folin-C methods, and five (5) Kratom Methods, which were submitted in response to the Call for Methods.

Methods Reviewed: Each Ashwagandha, Folin-C and Kratom method collected by AOAC for consideration by this ERP was assigned a primary and secondary reviewer. The decisions of the December 9-10, 2015 ERP are shown below.

I. Ashwagandha

Ashwagandha ERP Members Present: Darryl Sullivan, Covance (Chair); Anton Bzhelyansky, USP; Nour-Eddine Es-Safi, Mohammad V University of Rabat; Tom Phillips, State of MD; Casey Sayre, Roseman University of Health Sciences; Catherine Rimmer, NIST; Aniko Solyom, GAAS Analytical; Kurt Young, GNC/Nutra Manufacturing

Ashwagandha ERP Members Absent: Prashant Ingle, Herbalife; Yanjun Zhang, Herbalife

AOAC Method #	Manuscript Title	ERP Decisions	Consensus	Decision Date
ASH-01	Withanolide Glycosides and Aglycones of Ashwagandha (*W. somnifera*)	**The ERP moved this method to First *Action Official Methods* Status.** **The ERP recommended the method author complete the following actions to be completed prior to Final Action consideration:**	MOTION, Young/Bzhelyansky, to move ASH-01 First Action. **8 in favor, 0 opposed, 0 abstain. The motion passed.**	**December 10, 2015**

		• Investigate a better baseline separation for Peak 1 • pH of phosphate buffer • Column temperature • Heat stability during the extraction • Simplify the preparation of the extract products • Limit number of reference standards to one withanolide and one withanoside with use of response factors • Provide data on finished products • When specifying columns put "or equivalent." • Clarify the discard volume in the filtration step • Provide data on method LOQ		

ACTION ITEMS:

1. **Staff: Send notification to method author, requesting MS Word copy of method/manuscript for publication. Send follow up documentation on requirements and ERP recommendations for Final Action Official Methods status consideration.**

II. Folin C

Folin C ERP Members Present: Darryl Sullivan, Covance (Chair); Nour Eddine Es-Safi, Mohammad V University in Rabat; Martha Jennens, Covance; Dana Krueger, Krueger Food Laboratories; Tom Phillips, State of MD; Catherine Rimmer, NIST; Aniko Solyom, GAAS Analytical; John Spzylka, Mérieux Nutrisciences; Joseph Zhou, Sunshineville Health Products

Folin C ERP Members Absent: John Finley, LSU (Retired), Prashant Ingle, Herbalife; Jungmin Lee, USDA

AOAC Method #	Manuscript Title	ERP Decisions	Consensus	Decision Date
FOL-01	**Folin-Ciocalteau Reagent for**	The ERP agreed not to take action on this	**No motion made.**	**December 10, 2015**

	Polyphenolic Assay	method at this time. The ERP determined that the following actions must be completed before this method can reconsidered for First Action *Official Methods* status: • Clarify benefits of single vs. dual; ask which of the two is being submitted • Clarification on purpose of the cleanup steps • Clarify the major differences in the data between the two methods. • Ask if there is data on sample matrices addressed in SMPR • Need data on reference materials (available from Kate Rimmer, NIST) • Use Gallic Acid for calibrant • Provide raw data		
FOL-02	**Method for the estimation of total phenolic content using the Folin-C assay**	The ERP agreed not to take action on this method at this time. The ERP determined that the following actions must be completed before this method can reconsidered for First Action *Official Methods* status: • Need data on reference materials (available from Catherine Rimmer, NIST) • Ask if there is data on sample matrices addressed in SMPR • Expand analytical range • Data not completely clear • More supporting data for	**No motion made.**	**December 10, 2015**

		recovery and LOQ		
FOL-03	**Modified Folin–Ciocalteu Antioxidant Capacity Assay for Measuring Lipophilic Antioxidants**	The ERP agreed not to take action on this method at this time. The ERP determined that the following actions must be completed before this method can reconsidered for First Action *Official Methods* status: • Need more data to meet SMPR • Need data on reference materials (available from Catherine Rimmer, NIST) • Ask if there is data on sample matrices addressed in SMPR • Express as Gallic Acid equivalent • Comparative data between traditional folin and modified folin between a range of matrices is required.	**No motion made.**	**December 10, 2015**

Note: *The Expert Review Panel for Folin-C has asked for additional clarification of the Standard Method Performance Requirements (SMPRs) , specifically the title, as this SMPR is* ***not*** *for total phenolics.*

ACTION ITEMS:

1. **Staff: Send notification to method authors respectively of the ERP decision and relevant ERP's suggestions for each Folin C method to be considered for First Action Official Methods status.**
2. **Staff: Send notification to ERP member, Catherine Rimmer regarding the availability of reference materials for the Folin C methods.**
3. **Staff: Review and work on the ERP's request for clarification of the SMPR**

III. Kratom (*Mitragyna speciosa)*

Kratom ERP Members Present: Darryl Sullivan, Covance (Chair); Joseph Betz, NIH; Nour Eddine Es-Safi, Mohammed V University in Rabat; Charles Metcalfe, Custom Analytics; Tom Phillips, State of MD; John Spzylka, Mérieux Nutrisciences; Yan-Hong Wang, University of Mississippi

Kratom ERP Members Absent: Christine Casey, FDA; Catherine Rimmer, NIST

AOAC Method #	Manuscript Title	ERP Recommendations on Methods	Consensus	Decision Date
KRA-01	**Quantitative and Qualitative Analysis of Mitragynine in Kratom (*Mitragyna speciosa)* by GC-MS, LC-MS/MS and UPLC-PDA**	• Commercially available standards from Chromadex for 7 OH • Need to see recovery data • Include 7 OH data • Range of quantification must cover whole range from SMPR • Precision and accuracy data is needed across entire range of quantification • Interest from ERP is in the tandem MS method	**MOTION to accept the requested actions as written for KRA-01, KRA-02, KRA-03, KRA-04. Motion by Szpylka, second by Metcalfe. 7 in favor, 0 opposed, 0 abstain. MOTION PASSED.**	**December 9, 2015**
KRA-02	**Quantification of Mitragynine in Kratom Raw Materials and Finished Products by High-Performance Liquid Chromatography: Singly Laboratory Validation**	• LOD and LOQ information for 7-OH required. • Repeatability must be improved • More accuracy and recovery data • Peak purity PDA • Examine additional wavelength data		**December 9, 2015**
KRA-03	**Identification and Characterization of Indole and Oxindole Alkaloids from Leaves of *Mitragyna speciosa* Korth Using Liquid Chromatography-Accurate QToF Mass Spectrometry**	• Need precision and accuracy to demonstrate compliance with the SMPR.		**December 9, 2015**
KRA-04	**LC/MS Method for the Identification of Mitragyna speciose (Kratom) and**	• Feedback on recoveries outside the SMPR • Demonstration of precision data		**December 9, 2015**

	Quantitation of Mitragynine Using Linear Ion Trap Mass Spectrometer	• Additional details around sample preparation • Clarification of ability to measure 7-OH • Clarify the procedure used to gather accuracy data • Calibration curve range needs to be defined.		

ACTION ITEMS:

1. **Staff: Send notification to method authors respectively of the ERP decision and relevant ERP's recommendations for each Kratom method to be considered for First Action Official Methods status.**

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPSFAM HEAVY METAL METHODS

ERP Name	AOAC Expert Review Panel for SPSFAM Heavy Metal Methods			**Chair(s)**	Rick Reba (Nestle)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPSFAM.				
Roster	Rick Reba, Nestle (Chair) Sneh Bhandari, Merieux NutriSciences Michele Briscoe, Brooks Applied Labs Min Huang, Aegis Sciences Corporation Ferry Maniei, The Coca-Cola Company Bill Mindak, US FDA Cory Murphy, CFIA Jenny Nelson, Agilent Technologies Jenny Scifres, USDA Li Sheng, EPL Bioanalytical Christopher Smith, The Coca-Cola Company Darryl Sullivan, Covance				
Technical Documents created/used	1. SMPR for Total Heavy Metals 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2015.01 – Heavy Metals in Food				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Heavy Metals Expert Review Panel held on August 27, 2013.

Rick Reba, Expert Review Panel Chair

11/21/13
Date

Please sign and date this document and fax to La'Kia Phillips at 301-924-7089.

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed six (6) heavy metals methods. **Methods Reviewed:** Each heavy metal method collected by AOAC for consideration by this ERP under consideration is assigned a primary and secondary reviewer, the decisions of the ERP are shown below.

NO ACTION - FURTHER DOCUMENTATION REQUIRED

METHOD NO.	MANUSCRIPT TITLE	ERP DECISION(S) *(ERP Motions, Actions For Other & Additional Final Action Requirements)*	CONSENSUS	DECISION DATE
HVYM-01	DETERMINATION OF HEAVY METALS IN FOOD BY INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY *AUTHOR(S): MR. TAMAS UGRAI, DR. JOEL CRESWELL, AND MS. MICHELLE BRISCOE, BROOKS RAND LABS, 3958 6TH AVENUE NW, SEATTLE, WA 98107*	Motion: To advance the method to First Action Official Method status. *Amended: Method can be recommended to First Action Official Method status if the following requirements can be met:* 1. Revision of microwave digestion in section 5.5 to include method parameters. 2. Section 5.7: delete pressure control. 3. Clarification of dilution protocol. 4. Clarification of LOQ. 5. Explanation of positive bias for NIST SRM 1946. 6. Additional details for tables 12.3, 12.4, and 12.5. 7. Clarify the statements to include various IRT for arsenic. 8. Explanation of MSA Requirements for Final Action Official Method Status: 1. Provide additional data for spike recovery.	MOTION PASSED 9 APPROVED 0 OPPOSED 1 ABSTAIN (MB) *Bhandari/Huang*	August 23, 2013

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

NO ACTION - FURTHER DOCUMENTATION REQUIRED

METHOD NO.	MANUSCRIPT TITLE	ERP DECISION(S) *(ERP Motions, Actions For Other & Additional Final Action Requirements)*	CONSENSUS	DECISION DATE
HVYM-06	**METHOD VALIDATION FOR THE ANALYSIS OF ARSENIC, LEAD, AND CADMIUM IN JUICE CONCENTRATE** *AUTHOR(S): FARZANEH MANIEI, DR. JOE RAINEY, TIMOTHY BEASLEY, AND JAMES VAN SLATE , COCA COLA COMPANY*	Motion: Request to the Study Director to condense and resubmit the method and data as one document.	MOTION PASSED 9 APPROVED 0 OPPOSED 1 ABSTAIN (CS) *Sullivan/Bhandari*	August 23, 2013

NO ACTION

METHOD NO.	MANUSCRIPT TITLE	ERP DECISION(S) *(ERP Motions, Actions For Other & Additional Final Action Requirements)*	VOTE	DECISION DATE
HYVM-02	**DETERMINATION OF ARSENIC, CADMIUM, LEAD AND MERCURY IN RICE, CHOCOLATE, FRUIT JUICE AND INFANT FORMULA BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRIC METHOD** *AUTHOR(S): JIANHUI GAO, YABING XIAO, JUAN'E SONG, BAOKUN GE, TIANJIN EXIT-ENTRY INSPECTION AND QUARANTINE BUREAU THE P.R.C, AGILENT TECHNOLOGIES (CHINA) CO.,LTD*	Motion: Not to move forward to First Action Official Method status due to a lack of information provided.	MOTION PASSED 10 APPROVED 0 OPPOSED 0 ABSTAIN *Bhandari/Scifres*	August 23, 2013

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

NO ACTION

METHOD NO.	MANUSCRIPT TITLE	ERP DECISION(S) *(ERP Motions, Actions For Other & Additional Final Action Requirements)*	VOTE	DECISION DATE
HVYM-03	**IC-ICP-MS SPECIATION ANALYSIS OF AS IN APPLE JUICE USING THE THERMO SCIENTIFIC ICAP Q ICP-MS** *AUTHOR(S): DANIEL KUTSCHER, SHONA MCSHEEHY, JULIAN WILLS, DETLEF JENSEN, THERMO FISHER SCIENTIFIC, GERMANY AND THERMO FISHER SCIENTIFIC, SWITZERLAND*	Motion: Not to move forward to First Action Official Method status.	MOTION PASSED 10 APPROVED 0 OPPOSED 0 ABSTAIN *Reba/Bhandari*	August 23, 2013
HVYM-04	**IC-ICP-MS SPECIATION ANALYSIS OF AS IN ORGANIC BROWN RICE SYRUP (OBRS) USING THE THERMO SCIENTIFIC ICAP Q ICP-MS** *AUTHOR(S): DANIEL KUTSCHER, JULIAN WILLS AND LOTHAR ROTTMANN, AND DETLEF JENSEN, THERMO FISHER SCIENTIFIC, GERMANY AND THERMO FISHER SCIENTIFIC, SWITZERLAND*	Motion: Not to move forward to First Action Official Method status.	MOTION PASSED 10 APPROVED 0 OPPOSED 0 ABSTAIN *Mindak/Murphy*	August 23, 2013
HVYM-05	**MULTI-ELEMENT DETERMINATION IN FOOD SAMPLES USING THE THERMO SCIENTIFIC ICAP Q ICP-MS** *AUTHOR(S): SIMON LOFTHOUSE, THERMO FISHER SCIENTIFIC, UK*	Motion: Not to be further considered at this time.	MOTION PASSED 10 APPROVED 0 OPPOSED 0 ABSTAIN *Sullivan/Murphy*	August 23, 2013

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

EXPERT REVIEW PANEL MEMBERS

Name	*Company*
Rick Reba, Chair	**Nestle**
Sneh Bhandari	Silliker Laboratories
Michele Briscoe	Brooks Rand Laboratories
Min Huang	Aegis Sciences Corporation
Ferry Maniei	The Coca-Cola Company *(ALT)*
Bill Mindak	FDA-CFSAN
Cory Murphy	Canadian Food Inspection Agency
Jenny Scifres	USDA FSIS
Li Sheng	EPL Bioanalytical
Christopher Smith	The Coca-Cola Company
Darryl Sullivan	Covance Laboratories
Not Present	
Jenny Nelson	Agilent Technologies
Jameel Baig	International Islamic University

AOAC STAFF

Dawn Frazier
Deborah McKenzie
Tien Milor
La'Kia Phillips

OBSERVERS

Name	*Company*
Chris Blake	Nestle
David Boaz	Corbion Caravan Ingredients
France Cho	Maxxam
Scott Christiansen	PBM Nutritionals
Robert Clifford	Shimadzu
Xiaojun Deng	SHCIQ
David Ellington	Covance Laboratories
Greg Hostetler	Perrigo
Jung-Chen Johnson	FMC BioPolymer
Kristie Laurvick	US Pharmacopeia (USP)
Edwin Phifer	FDA
Eural Porter	FDA
Steve Royce	Agilent Technologies
Brian Schaneberg	Starbucks
Ru-Chia Shih	Taiwan FDA
Tiffany Stilwater	Brooks Rand Laboratories
Steve Wall	Agilent Technologies
Yang Zhou	Eurofins
Joyce Zhu	Jamieson Laboratories
Richard Zywicki	Covance Laboratories

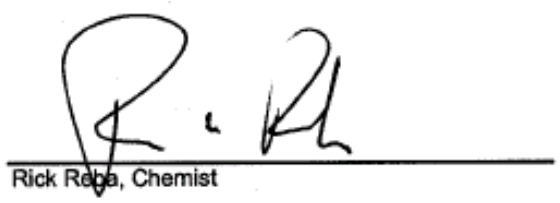
STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Heavy Metals Expert Review Panel held on February 11, 2015.



Rick Reba, Expert Review Panel Chair

STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS

EXPERT REVIEW PANEL ON HEAVY METALS

METHODS FOR CONSIDERATION:

Conclusion: The Expert Review Panel reviewed one (1) heavy metals method, which had been resubmitted as recommended at the December 2, 2014 Heavy Metals ERP Meeting.

Methods Reviewed: Each heavy metals method collected by AOAC for consideration by this ERP was assigned a primary and secondary reviewer. The decisions of the February 11, 2015 ERP are shown below.

Method No.	Manuscript Title	ERP Decisions (ERP Motions, Actions for Other & Additional Final Action Requirements)	Consensus	Decision Date
HVYM-001	Determination of Heavy Metals in Food by Inductively Coupled Plasma – Mass Spectrometry	Required prior to First Action vote as per December 2, 2014 ERP teleconference:: 1. Data for Infant Formula (See #6 in "Recommended Actions") 2. Internal standard clarification (Lu) 3. Verbiage around microwave digestion parameters 4. Ionization buffers carbon effect *February 11, 2015: All four requirements met as agreed by consensus.* Follow Up Actions: • AOAC staff to move method forward to publication. • Members of the ERP can independently volunteer to assist with the provision of data in support of a final action method.	ERP voted to accept HVYM-001 as a First Action Official Method of Analysis (9 in favor, 0 oppose, 1 abstain.)	02/11/2015

PROFILE OF AOAC EXPERT REVIEW PANEL FOR SPSFAM ST. JOHN'S WORT

ERP Name	AOAC Expert Review Panel for SPSFAM St. John's wort Methods			**Chair(s)**	Shauna Roman (RB)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from SMPRs developed by SPSFAM.				
Roster	Shauna Roman, Reckitt Benckiser (Chair) Paula Brown, British Columbia Institute of Technology Nour Eddine Es-Safi, Mohammed V-Agdal University Ikhlas Khan, University of Mississippi Elizabeth Mudge, British Columbia Institute of Technology Klaus Reif, PhytoLab GmbH & Co. KG Brian Schaneberg, Starbucks Maged Sharaf, US Pharmacopeia Darryl Sullivan, Covance Roy Upton, American Herbal Pharmacopeia				
Technical Documents created/used	1. SMPR for St. John's wort				
Methods Adopted *First Action and Final Action status*	AOAC 2013.15 – Hypericin and Pseudohypericin in St. John's wort				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

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STAKEHOLDER PANEL ON STRATEGIC FOOD ANALYTICAL METHODS
Final Report of the Expert Review Panel for St. John's wort

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT
ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on St. John's wort held on Wednesday, December 11, 2013 at AOAC INTERNATIONAL Headquarters located at 481 N. Frederick Avenue, Gaithersburg, Maryland 20877.

SHAUNA ROMAN
Expert Review Panel Chair

04-16-2014
Date

Please sign and date this document and fax to La'Kia Phillips at 301-924-7089.

Criteria for Vetting Methods to be Considered:
The Stakeholder Panel on Strategic Food Analytical Methods previously issued a Call for Methods for St. John's wort based upon the criteria set forth in the approved Standard Method Performance Requirements (SMPRs). Sixteen (16) methods were received via method authors and publication searches.

Criteria for Vetting Experts and Selection Process:
The Stakeholder Panel on Strategic Food Analytical Methods previously issued a Call for Experts for St. John's wort. The following names were submitted to the AOAC Official Methods Board to evaluate candidate methods for St. John's wort: Paula Brown, Nour Eddine Es-Safi, Ikhlas Khan, Elizabeth Mudge, Klaus Reif, Brian Schaneberg, Maged Sharaf, Darryl Sullivan, and Roy Upton. Shauna Roman was vetted as the St. John's wort Expert Review Panel Chair and the Official Methods Board liaison. The approved candidates have previously participated in AOAC activities, including but not limited to, the Methods Committee on Dietary Supplements, Dietary Supplements Community, Dietary Supplements Task Group, and the working group for St. John's wort.

ERP Orientation:
The AOAC Expert Review Panel Orientation Webinar was held on Friday, November 1, 2013.

Standard Method Performance Requirements (SMPRs):
The St. John's wort Working Group completed the draft Standard Method Performance Requirements (SMPRs) in August, 2012. The Stakeholder Panel endorsed the SMPR on March 14, 2013 (AOAC SMPR 2013.001). A copy of the SMPR is shown on the following page.

Conclusion:
The Expert Review Panel reviewed all St. John's wort methods. The following St. John's wort method was approved for First Action Official Method status for the analytes (hypericin and pseudohypericin) and matrices (plant material and powdered extracts) specified by the method and supported by the validation report:

- **SJW- 10: Determination of hypericin and pseudohypericin in St. John's wort by high-performance liquid chromatography.**

Subsequent ERP Activities:
ERP members will evaluate the method for 2 years. AOAC will build into the system a way of responding to method developers of submitted methods, to inform them if the ERP requires more data to make a decision. AOAC will establish a ranking system to provide feedback to method developers for submitted methods.

Action Items:
The following are action items for the specific methods as noted below as determined by the Expert Review Panel for St. John's wort (SJW).

1) **SJW-01**
   Robert Rathbone of AOAC will obtain the single laboratory validation data for this method.

2) **SJW-03**
   AOAC to seek and obtain the validation data for this method.

3) **SJW-07**
   Expert Review Panel member, Klaus Reif, will seek and obtain the validation data for this method.

4) **SJW-08**
   Expert Review Panel member, Brian Schaneberg and Robert Rathbone of AOAC will contact USP to obtain validation data for this method.

5) **SJW-09**
   Expert Review Panel member, Brian Schaneberg and Robert Rathbone of AOAC will contact USP to obtain validation data for this method.

6) **SJW-13**
   Robert Rathbone of AOAC will contact Mark Roman to obtain validation data for this method.

AOAC SMPR 2013.001

Standard Method Performance Requirements for Hypericins, Hyperforins, and Flavonoids in St. John's Wort (*Hypericum perforatum*) and Other *Hypericum* spp.

1 Applicability

Determination of hypericins, and/or hyperforins, and/or flavonoids [According to Herbal Drugs and Phytopharmaceuticals (3rd Ed.), the main flavonoids in St. John's wort are hyperoside, rutoside, and the biflavones I3, II8-biapigenin, and amentoflavone. Quercetin is also present. (http://www.ncbi.nlm.nih.gov/pubmed/11842341)] in St. John's wort (*Hypericum perforatum*) and other *Hypericum* spp. in powdered extracts, tablets, hard-shell capsules, and liquid alcohol extracts.

2 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable. It is acceptable to have a different analytical method for each class of analytes.

3 Definitions

Limit of quantitation (LOQ).—The minimum analyte concentration for 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.—The fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range, and a protocol to demonstrate suitability.

6 Reference Material(s)

Use an appropriate Certified Reference Material (CRM) where available.

7 Validation Guidance

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

8 Maximum Time-to-Result

Analysis time must be less than the established stability time of the analytes in solution.

Approved by the AOAC Stakeholder Panel on Strategic Foods Analytical Methods (SPSFAM). Final Version Date: March 13, 2013. Effective Date: March 14, 2013.

Table 1. Method performance requirements

Performance parameter		Target analyte: Hypercin	Hyperforin	Flavonoids
Analytical range[a], %		0.05–1	0.05–10	0.05–10
Limit of quantitation (LOQ)[a], %		≤0.02	≤0.02	≤0.02
Repeatability (RSD_r), %	0.05 to ≤1	≤5	≤5	≤5
	1 to ≤5	NA	≤3	≤3
	5 to ≤10	NA	≤3	≤3
Recovery[a], %	0.05 to ≤1	95–105	95–105	95–105
	1 to ≤5	NA	97–103	97–103
	5 to ≤10	NA	98–102	98–102
Reproducibility (RSD_R), %[b]	0.05 to ≤1	≤8	≤8	≤8
	1 to ≤5	NA	≤5	≤5
	5 to ≤10	NA	≤4	≤4

[a] % (w/w) for the starting material prior to sample preparation.

[b] RSD_R calculated as 1.2*$PRSD_R$ where $PRSD_R = 2C^{-0.15}$, where C is the mass fraction of the lower limit of each range, i.e., C = 0.0005 for the 0.05 to <1% range. $PRSD_R$ is the predicted relative standard deviation. Information on the $PRSD_R$ can be found in Annex D of Appendix F: Guidelines for Standard Method Performance Requirements in the *Official Methods of Analysis of AOAC INTERNATIONAL*, 19th Ed. (2012).

Methods Reviewed: Each St. John's wort method collected by AOAC for consideration by this ERP under consideration is assigned a primary and secondary reviewer as shown below on Table 1.

METHOD NO.	METHOD TITLE	PRIMARY REVIEWER	SECONDARY REVIEWER
SJW-01	Hypericumperforatum-Chemical Profling and Quantitative Results of St. John's wort Products by an Improved High-Performance Liquid Chromatography Method	Shauna Roman (Chair)	Darryl Sullivan*
SJW-02	Evaluation of major active components in St. John's Wort dietary supplements by high-performance liquid chromatography with photodiode array detection and electrospray mass spectrometric confirmation	Paula Brown	Brian Schaneberg
SJW-03	Determination of St. John's wort Components in Dietary Supplements and Functional Foods by Liquid Chromatography	Nour-EddineEs-Safi	Ikhlas Khan*
SJW-04	Simultaneous Determination of the Predominant Hyperforins and Hypericins in St. John's wort (Hypericum perforatum L.) by Liquid Chromatograph	Elizabeth Mudge	Klaus Reif
SJW-05	Simultaneous Determination of Protopseudohypericin, Pseudohypericin, Protohypericin, and Hypericin Without Light Exposure	Paula Brown	Darryl Sullivan*
SJW-06	St. John's wort - Hypericiherba	Ikhlas Khan*	Roy Upton (NP)
SJW-07	St. John's wort Dry Extract, Quantified Hypericiherbaeextractumsiccumquantificatum	Maged Sharaf	Roy Upton (NP)
SJW-08	Powdered St. John's wort Extract	Maged Sharaf	Roy Upton (NP)
SJW-09	St. John's wort	Maged Sharaf	Roy Upton (NP)
SJW-10	Determination of hypericin and pseudohypericin in St. John's wort by high-performance liquid chromatography.	Shauna Roman (Chair)	Klaus Reif
SJW-11	Determination of hyperforin in St. John's wort Extract and Plant Material by High-performance Liquid Chromatography (INA Method 112.001)	Shauna Roman (Chair)	Klaus Reif
SJW-12	Determination of naphthodiantrones in St. John's wort extracts and herbal drugs (SOP No. DC-185)	Nour-EddineEs-Safi	Brian Schaneberg
SJW-13	Determination of hyperforin in St. John's wort extracts and herbal drugs (SOP No. HPLC-352)	Ikhlas Khan*	Darryl Sullivan*
SJW-14	Chemical Composition of*Hypericumrumeliacum* BIOSS. Essential Oil. A new Chemotype of This pharmolgically valuable species?	Nour-EddineEs-Safi	Elizabeth Mudge
SJW-15	Simultaneous Determination of Total Hypericin and Hyperforin in St. John's wort Extracts by HPLC with Electrochemical Detection	Paula Brown	Ikhlas Khan*
SJW-16	St. John's wort	Elizabeth Mudge	Brian Schaneberg

**Expert Review Panel Members who were assigned methods and were not present during the session and/or did not submit their method review forms. These methods were reviewed by all present Expert Review Panel members.*

Primary and Secondary Evaluation of Method SJW-01

SJW-01: **Hypericumperforatum-Chemical Profling and Quantitative Results of St. John's wort Products by an Improved High-Performance Liquid Chromatography Method**

Author(s): *M. Ganzera, J. Zhao, I.A. Khan, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, Mississippi 38677, Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677*

SUMMARY OF METHOD:

GENERAL COMMENTS:

The method is a fairly typical HPLC method used to quantify nine (9) main Hypericumperforatum compounds in 35 minutes. The indicated compounds are hypericin, hyperforin, pseudohypericin, and six (6) flavonoids: rutin, hyperoside, isoquercitrin, quercitrin, quercetin and 3, II8-Biapigenin. The identity of each of the compounds was confirmed in an LC-MS experiment. The sample preparation for this method involves methanol extraction (4 times), centrifugation and filtration.

PROS/STRENGTHS:

The method is simple and straightforward HPLC method and sample preparation. It was noted that the manuscript has method validation data and the majority of well equipped analytical labs could run this assay. The laboratory used five (5) commercially available standards and isolated three (3). At least two of these standards, hypericin and hyperforin, are commercially available from Addipharma in Germany. The I3, II8-biapigenin was quantified utilizing a relative response factor (according to literature and relative to rutin).

CONS/WEAKNESSES:

The manuscript does not contain detail regarding the types of samples tested (i.e., matrices not well defined). The manuscript refers to commercial samples but does not give details of the type of samples.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION:Not to make a decision on the method at this time pending review of the SLV data.
Roman, Brown (Unanimous) – Motion Passed

Primary and Secondary Evaluation of Method SJW-02

SJW-02: **Evaluation of major active components in St. John's wort dietary supplements by high-performance liquid chromatography with photodiode array detection and electrospray mass spectrometric confirmation**

Author(s): *Frances F. Liu\ Catharina Y.W. Ang*, Thomas M. Heinze, Joshua D. Rankin2, Richard D. Beger, James P. Freeman, Jackson O. Lay Jr., US Food and Drug Administration, National Center for Toxicological Research, Division of Chemistry, 3900 NCTR Road, Jefferson, AR 72079, USA*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This method was published in 2000 and used RP-HPLC/DAD for the quantification of the flavonoids (rutin, hyperoside, isoquercitrin, quercitrin, and quercetin), phloroglucinol (hyperforin), and naphthodianthrones (hypericin and pseudohypericin) in five capsule products of St. John's wort. LC-ESI MS was used for confirmation of the detected compounds. Samples are extracted with an ethanol-acetone solution via shaking in a water bath at 55°C for 5.6 hours. Extract is filtered through filter paper into volumetric flask and diluted to 50.00 mL prior to analysis by reversed phase HPLC. Chromatographic separation is achieved on a 3 µm 250 x 4 mm C18 column at 25°C employing a gradient with 0.5%TFA/water and 0.5% TFA in methanol/acetonitrile (13:7). Analytes are detected by photodiode array at 284 and 590 nm and quantified using luteolin as an internal standard with peak confirmation by subsequent electrospray ionization mass spectrometry. The LC-MS method is 125 minutes long, employs a different chromatographic conditions, as opposed to detection in series, and as such offers no real advantage.

PROS/STRENGTHS:

This method used LC-ESI MS to confirm the identity of the peaks and the purity of the standards was confirmed by UV, MS and NMR. It detects and quantifies the analytes of interest and has a simple extraction procedure.

CONS/WEAKNESSES:

This method has a long extraction procedure and HPLC run time. Only capsules were used in this method and the composition of those capsules was not detailed. There were challenges regarding the precision and stability. The LOQ, RSD_r, RSD_R did not appear to meet the SMPR. There was no recovery or LOD data provided.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Brown, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-03

SJW-03: Determination of St. John's wort Components in Dietary Supplements and Functional Foods by Liquid Chromatography

Author(s): *Catharina Y.W. Ang, Yanyan Cui, Hebron C. Chang, WenhongLuo, Thomas M. Heinze, Lawrence J. Lin, and Antonia Mattia, U.S. Food and Drug Administration, National Center for Toxicological Research, Division of Chemistry, HFT-230, 3900, NCTR Rd, Jefferson, AR 72079,, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, Division of Petition Review, HFS-265, 5100 Paint Branch Pkwy, College Park, MD 20740*

SUMMARY OF METHOD:

GENERAL COMMENTS:

The method presents results dealing with the extraction and the determination of four (4) bioactive St. John's wort compounds (pseudohypericin (PHP), hypericin (HP), hyperforin (HF), and adhyperforin (AHF)) in SJW aerial parts (leaves and flowers), dietary supplements and functional foods such as SJW capsules, tea bag, snack bar, puff and drinks. The compounds were well separated through the developed HPLC method using an isocratic elution program. The starting material was sonicated in a methanolic solution and the obtained extract was analyzed through LC/UV at 2 wavelengths (290 & 590 nm) and LC/MS analysis. Two standard calibration curves with high and lowlevel ranges were constructed for the quantitative determination of target compounds using BKF as an internal standard.

PROS/STRENGTHS:

This method has good separation and detection techniques such as HPLC, UV, and MS allowing the separation and quantitative analysis of individual SJW bioactive compounds. This method could be applied to several matrixes.

CONS/WEAKNESSES:

No internal standard was used for hypericin, pseudohypericin, and some samples (Snack bar). Confirmation by LC/ESI/MS should be performed using the same chromatographic conditions. The retention time changes due to the column and room temperature variation and also to the used mobile phase pH variation. Recovery test should be performed on blank matrixes.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to make a decision on this method regarding First Action Official Method status pending review of results validation data for method suitability for hyperforin determination. ***(Brown, Sharaf – Unanimous)***

Amended: This method is not recommended for First Action official Method status based on the data ***presented. (Brown, Sharaf – Unanimous) Motion Passed***

Primary and Secondary Evaluation of Method SJW-04

SJW-04: Simultaneous Determination of the Predominant Hyperforins and Hypericins in St. John's wort (Hypericum perforatum L.) by Liquid Chromatograph

Author(s): *Dean E. Gray, George E. Rotiinghaus, H.E. Gene Garrett, and Stephen G. Pallardy, University of Missouri, Department of Forestry, 203 A-B Natural Resources Building, Columbia, MO 65211, Veterinary Medical Diagnostic Laboratory, PO Box 6023, Columbia, MO 65205, University of Missouri, Department of Forestry, 203 A-B Natural Resources Building, Columbia, MO 65211*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This method is used for the simultaneous determination of both hyperforins and hypericins in leaf/flower with isocratic HPLC and UV-FLD containing high, medium and low concentrations of the analytes. The method is not applicable to extracts and dietary supplements and flavonoids are not included into the method. St. John's wort aerials were extracted in methanol for two hours and diluted with acetonitrile prior to cleanup with mixed solid phase column. Most impurities which can be minimized by MSP elute much earlier than the analytes of interest. Hypericin is not shown in fluorescence detection nor at 590 nm. Quantitation was calculated using forced through origin external calibration curves. There chromatographic separation is short, although there is slight peak tailing.

PROS/STRENGTHS:

This method has fast chromatographic separation, good recovery for hyperforins, and short isocratic run (8 minutes). Hyperforins and hypericinsare both within one method and analyze two (2) classes of compounds as noted in the SMPR. Fluorescence sensitivity increased for hypericin and pseudohypericin compared with UV absorbanceIsocratic separation.

CONS/WEAKNESSES:

The use of in-house prepared mixed solid phase clean-up columns, could lead to variability between laboratories. Quantitation of pseudohypericin and adhyperforin calculated from hypericin and hyperforinusing forced through origin calibration curves can lead to errors in calculations due to poor fitting. Time consuming clean-up (2 h extraction time, MSP = Mixed Solid Phase), 2 detectors are necessary (UV and FLD), FLD though hypericin could be measured selectively at 588 nm. This method has very poor precision for hyperforins.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Mudge, Reif (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-05

SJW-05: Simultaneous Determination of Protopseudohypericin, Pseudohypericin, Protohypericin, and HypericinWithout Light Exposure

Author(s): *Steven F. Baugh, Industrial Laboratories, 4045 Youngfield St, Wheat Ridge, CO 80033*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This manuscript does not describe a method of analysis for determination of analytes in St. John's wort per se, but rather examines the feasibility of employing a mathematical conversion for accurate determination of the naphthodianthrones without light exposure by employing a simplified protocol in combination with using a conversion factor.

PROS/STRENGTHS:

This method could be a way to simplify sample preparation.

CONS/WEAKNESSES:

The paper does not describe a method of analysis. Data supporting use of a mathematical conversion with a simplified extraction protocol is presented. The focus is naphthodianthrones and does not apply to any other stated compounds of interest in St. John's wort.The method does not meet the requirements stated in the SMPR.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Brown, Reif(Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-06

SJW-06: St. John's wort - Hypericiherba

Author(s): *EUROPEAN PHARMACOPOEIA 6.0 (pp 2958-2959)*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This method is spectrophotometric quantitation of hypericins for regulatory compliance of St. John's wort raw material in the European Union. Quantitation of the sum of hypericin, pseudohypericin, protohypericin and protopseudohypericin calculated as total napthodianthrones expressed as "hypericin" for crude herbal St. John's wort material.

PROS/STRENGTHS:

Based on historical use of this method and its widespread utility by industry in the US and EU, this is a rapid and effective screening tool for basic quality assessment of St. John's wort raw material that meets the identification requirements of the European Pharmacopoeia. It has been officially accepted in formal compendium since at least 1991 and continues to be the officially accepted screening method for St. John's wort raw material. Once identification has been appropriately established, this method is a robust tool for the basic quality analysis of St. John's wort raw material.

CONS/WEAKNESSES:

For the analytical endpoint of quantifying total napthodianthrones inappropriately identified material this method is well suited with no deficiencies of which I am aware. Some may state that UV will not detect the presence of dyes that are viewed in the same wavelength and therefore cannot detect adulterants, but the stated purpose is for quantitation of material that has been appropriately identified. Does not quantify flavonoids and hyperforin as desired by AOAC.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Brown, Sharaf (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-07

SJW-07: **St. John's wort Dry Extract, Quantified Hyperici herbae extractumsiccum quantificatum**

Author(s): EUROPEAN PHARMACOPOEIA 7.6 (pp 4878-4880)

SUMMARY OF METHOD:

GENERAL COMMENTS:

Two methods are included in the monograph. The first is used for the quantification, after light conversion, of total hypericins (pseudohypericin and hypericin) expressed as hypericin. The second method is used for the quantification of hyperforin, using a provided correction factor, and flavonoids, all expressed as rutin.

PROS/STRENGTHS:

This method allows for the quantification of the various components of interest and marker components in St. John's wort. The methods are specific and stability-indicating compared to spectrometry methods.The methods provide a complementary tool for the article identification along with the TLC test included in the monograph. The methods include the use of quantitative and qualitative reference standards.The methods employ materials and chemicals that are readily available. The strength of the methods is that they are purported to run all analytes of interest to AOAC and that they represent the methods required for regulatory compliance in the EU. The LC method for hypericins appears sound and uses a St. John's wort dry extract as a reference standard, rather than the relatively unstable hypericin reference materials.

CONS/WEAKNESSES:

Users will need to do two set up and prepare materials for two methods.Information is not provided concerning the applicability of the methods to St. John's wort plant material; forms, preparations and products other than the extracts subject of the monograph; and to other Hypericum spp. The EP monograph for St. John's wort plant material includes a spectrometry method for the content of hypericins. Supporting data required to ensure method performance requirements are not provided.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to make a decision on this method at this time pending receipt and review of validation data.

Sharaf, Brown (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-08

SJW-08: Powdered St. John's wort Extract

Author(s): *USP 36 (pp 1582-1584)*

SUMMARY OF METHOD:

GENERAL COMMENTS:

One RP LC method is included in the monograph for the quantification of the content of hypericin and pseudohypericin and hyperforin. Protection from light is recommended. A surrogate reference standard is used for quantitative purposes and correction factors are provided. Another extract reference standard is used for qualitative purposes and to establish system suitability.

PROS/STRENGTHS:

The method makes it possible the quantification of some of the components of interest and marker components of in St. John's wort. The method is specific and stability-indicating compared to spectrometry methods.The method provide a complementary tool for the article identification along with the TLC test included in the monograph. The method includes the use of quantitative and qualitative reference standards.The method employs materials and chemicals that are readily available. The same method is used for the plant materials.

CONS/WEAKNESSES:

Information is not provided concerning the applicability of the method to St. John's wort products and other Hypericum spp. No information was provided concerning the applicability of the method for the quantification of the flavonoids content only hyperforin is quantified. Supporting data required to ensure method performance requirements are not provided.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to make a decision on this method at this time pending receipt and review of validation data.

Sharaf, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-09

SJW-09: **St. John's wort**

Author(s): *USP 36 (pp 1579-1581)*

SUMMARY OF METHOD:

GENERAL COMMENTS:

One RP LC method is included in the monograph for the quantification of the content of hypericin and pseudohypericin and hyperforin. Protection from light is recommended. A surrogate reference standard is used for quantitative purposes and correction factors are provided. Another extract reference standard is used for qualitative purposes and to establish system suitability.

PROS/STRENGTHS:

The method makes it possible the quantification of some of the components of interest and marker components of in St. John's wort. The method is specific and stability-indicating compared to spectrometry methods.The method provides a complementary tool for the article identification along with the TLC test included in themonograph. The method includes the use of quantitative and qualitative reference standards. The method employs materials and chemicals that are readily available. The same method is used for the plant materials.

CONS/WEAKNESSES:

Information is not provided concerning the applicability of the method to St. John's wort products and other Hypericum spp. No information was provided concerning the applicability of the method for the quantification of the flavonoids content. Only hyperforin is quantified. Supporting data required to ensure method performance requirements are not provided.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to make a decision on this method at this time pending receipt and review of validation data.

Sharaf, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-10

SJW-10: Determination of hypericin and pseudohypericin in St. John's wort by high-performance liquid chromatography

Author(s): *Institute for Nutraceutical Advancement Methods Validation Program*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This is a HPLC/UV method used to test the hypericin and pseudophypericin content in St. John's wort. The powdered extract sample preparation involves sonication, centrifugation, and a light exposure conversion step prior to analysis. The herb sample preparation is a reflux, rotary evaporator concentration, dilution step and a light exposure conversion step prior to analysis.

PROS/STRENGTHS:

Simple and straightforward HPLC method. Most analytical labs would have the analytical equipment needed to run this method.

CONS/WEAKNESSES:

The plant material (herb) sample preparation is time consuming (reflux, rotovap, light conversion).

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Adopt this method as First Action Official Method status for the analytes (hypericin and pseudohypericin) and matrices (plant material and powdered extracts) specified by the method and supported by the validation report.
Roman, Sharaf (Unanimous) Motion Passed

MOTION: Requirements for Final Action Official Method status:

1. **Optimize method with consideration given to sample preparation for plant material, additional matrices, and column technology.**
    - **Changes to the method may require additional performance data to ensure the method meets the AOAC SMPR 2013.001.**
2. **Statistically significant reproducibility data will be required to consider this method for final action.**
    - **Use of Certified Reference Material to acquire PT data, or**
    - **Full collaborative study (minimum of 6 blind duplicates, 8 laboratories)**

Schaneberg, Brown (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-11

SJW-11: Determination of hyperforin in St. John's wort Extract and Plant Material by High-performance Liquid Chromatography (INA Method 112.001)

Author(s): *Institute for Nutraceutical Advancement Methods Validation Program*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This is a HPLC/UV method used to test the hyperforin content in St. John's wort. The sample preparation involves sonication and centrifugation prior to analysis. The method is a fairly typical HPLC method; however, it does not include any validation data. The majority of well equipped analytical labs could run this assay.

PROS/STRENGTHS:

This is a simple and straightforward HPLC method that most analytical labs would have the analytical equipment needed to run this method.

CONS/WEAKNESSES:

The Hyperforin in the solution is not stable. This method does not include any validation data.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION:Not to consider this method for First Action Official Method status.
Reif, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-12

SJW-12: Determination of naphthodiantrones in St. John's wort extracts and herbal drugs (SOP No. DC-185)

Author(s): *Euromed USA*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This is a spectrophotometric method for the determination of hypericins in St. John's wort extracts and herbal plant preparations. The method concerns qualitative determination of hypericins through spectrometry analysis at 590 nm. Extraction method is straight forward and allows for exposure time under white light prior to analysis. White light is used to ensure conversion to protohypericin to hypericin. A blank is always run with each sample and samples are prepared in duplicate. Although the method is simple and straight forward, it only looks at the total hypericins without actual separation of the individual compounds. As this is a nonspecific method, it would not give good information on the individual compounds for use in a clinical research program. It would be beneficial possibly in a QC program internally of raw material verification.

PROS/STRENGTHS:

This method is simple and easy to follow and is good for internal quality controls.

CONS/WEAKNESSES:

The use of hypericin specific absorption which could vary according to the operating conditions.The presence of other compounds absorbing in the used 590 nm range could conduct to erroneous results. As a nonspecific method, it does not quantify for the individual hypericin compounds. As 590nm is based on color analysis, the method would unlikely detect adulteration.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Es-Safi, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-13

SJW-13: **Determination of hyperforin in St. John's wort extracts and herbal drugs (SOP No. HPLC-352)**

Author(s): *Euromed USA*

SUMMARY OF METHOD:

GENERAL COMMENTS:

Method review forms were not received for this method due to the absence of the reviewers. However, the Expert Review Panel discussed this method during the meeting and requested further information and validation data for this method.

PROS/STRENGTHS:

N/A

CONS/WEAKNESSES:

N/A

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to make a decision on this method at this time pending receipt and review of validation data.

Brown, Mudge (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-14

SJW-14: **Chemical Composition of*Hypericumrumeliacum* BIOSS. Essential Oil.A new Chemotype of This pharmologicallyvaluble species?**

Author(s): *Chemistry & Biodiversity – Vol. 9 (2012), Niko S. Radulovic´* and Polina D. Blagojevic*

SUMMARY OF METHOD:

GENERAL COMMENTS:

The method presented results dealing with the extraction of the essential oil of Hypericum rumeliacum aerial parts and analysis of its constituents through GC and GC/MS techniques. The results were used with MVA to determine if there are chemo type differences that exist within H. rumeliacum.

PROS/STRENGTHS:

This method used nitrogen to evaporate solvent which reduces loses of volatiles.

CONS/WEAKNESSES:

This method pertains to only plant tissues and not supplements. It's used for H. rumelaicum, not St. Johns Wort (H. perforatum). This is not a quantitative method and is not applicable to the SMPR.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Es-Safi, Mudge (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-15

SJW-15: **Simultaneous Determination of Total Hypericin and Hyperforin in St. John's wort Extracts by HPLC with Electrochemical Detection**

Author(s): *Phytochem. Anal. 18: 204–208 (2007), Ulla Rückert, Werner Likussar And Astrid Michelitsch**

SUMMARY OF METHOD:

GENERAL COMMENTS:

This method allows for simultaneous analysis of hypericin and hyperforin. The detection is accomplished with electrochemical detector and quantification performed using external standards of hypericin and hyperforin. St. John's wort extract and a finished product (90 mg) are extracted with 50 mL of ethanol and water solution (80:20 v/v) via sonication, filtered and frozen, all performed while protected from light. Samples are thawed, placed into amber vials and exposed to 380-700 nm light for 30 minutes. The resulting sample is diluted with methanol in a ratio of 1:10 (v/v) and analyzed by HPLC. The chromatographic separation is achieved isocratically on a RP-18 column (125 x 4 mm i.d.; 5um) with 10% ammonium acetate: acetic acid buffer (0.5 M: pH 3.7), methanol and acetonitrile (10:40:50 v/v/v). Flow rate of 0.8 mL/min. Run time is 15 min.

PROS/STRENGTHS:

This method allows for simultaneous analysis of hypericin and hyperforin with a short run time. The sample preparation is straight forward, although light-protection required. EC detector is relatively in expensive.

CONS/WEAKNESSES:

This method does not detect or quantify the flavonoids. Many SLV parameters were not evaluated.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Brown, Schaneberg (Unanimous) Motion Passed

Primary and Secondary Evaluation of Method SJW-16

SJW-16: **St. John's wort**

Author(s): *USP32–NF27 Page 1066*

SUMMARY OF METHOD:

GENERAL COMMENTS:

This is an HPLC method for total hypericins and hyperforin at 270nm using a response factor based on oxybenzone. This is a general method for identity and quality control, but lacks any detail around the actual validation data. This method uses an external calibrant without calibration curves or calibrant of the same structure as the analytes of interest. The mobile phase also uses a three phase mobile phase and long separation time, both of which are undesirable for routine laboratory analysis. The actual hypericin and hyperforin standards are not used; instead a response factor is used based on oxybenzone. The sample preparation states minimal light exposure and use of low-actinic glassware. This is the USP monograph for St. Johns Wort, including botanical identification, qualitative and quantitative tests. The content of hypericin, pseudohypericin and hyperforin are quantified by extracting 1 gram of dried aerials with 50 mL acetone: methanol (1:1 v/v) at 60C for 2 hours. The extract was separated with C18 column (250 x 4.6 mm) in 66 minutes. Quantitation is performed using oxybenzone and response factors.

PROS/STRENGTHS:

This method analyzes three (3) analytes of interest in the SMPR.

CONS/WEAKNESSES:

This method lacked detail, used one point calibration curve with response factors, and used three (3) solution mobile phases. The 66 minute separation is too long for routine analysis. Quantitation is not done with reference standards of the identical analytes. No chromatograms were provided. There is also no information on measuring at 588 nm in the experimental section.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to consider this method for First Action Official Method status.
Mudge, Schaneberg (Unanimous) Motion Passed

PROFILE OF AOAC EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUE METHODS

ERP Name	AOAC Expert Review Panel for Veterinary Drug Residue Methods			**Chair(s)**	Joe Boison (Canadian Food Inspection Agency)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	1 method
Scope:	Review and adopt methods resulting from SMPRs developed by SPMFF.				
Roster	1. Joe Boison, Canadian Food Inspection Agency (Chair) 2. Haejung An, U.S. FDA 3. Martin Danaher, TEAGASC 4. Doug Hite, State of Tennessee - Retired 5. Brian Kinsella, UCT, Inc. 6. Perry Martos, University of Guelph 7. Katerina Mastovska, Covance Laboratories 8. Cory Murphy, Canadian Food Inspection Agency 9. Sherry Turnipseed, U.S. FDA 10. Jon Reuther, Eurofins				
Technical Documents created/used	1. SMPR for Drug Residues in Fish and Seafood 2. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2012.25 – Residues of Three Triphenylmethane Dyes and Their Metabolites (Malachite Green, Leucomalachite Green, Crystal Violet, Leucocrystal Violet, and Brilliant Green) in Aquaculture Products				
Final Action Methods Recommended	Method listed above.				
Additional Input	Chemical Contaminants Community Veterinary Drug Residues Subgroup				
Awards/Recognitions	ERP of the Year in 2013				

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT
ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on Veterinary Drug Residues

Joe Boison

NOV 30, 2012

Date

Please sign and date this document and fax to La'Kia Phillips at 301-924-7089.

Report of the Expert Review Panel for Veterinary Drug Residues

Date: October 1, 2012

Location: Las Vegas, Nevada

ERP Members:

Name	*Affililation*
Joe Boison, ***Chair***	Canadian Food Inspection Agency
Haejung An	US FDA
Louis Bluhm	USDA FSIS
Martin Danaher	TEAGASC
Doug Hite	State of Tennessee, Department of Agriculture
John Kadavil	US FDA Center for Veterinary Medicine
Glenn Kennedy	AFBI Vet Sciences Division
Brian Kinsella	UCT, Inc.
Alex MacDonald	Pharma Sciences Consultant
Perry Martos	University of Guelph
Katerina Mastovska	Covance Laboratories
Cory Murphy	Canadian Food Inspection Agency
Guo Fang Pang	Chinese Academy of Inspection and Quarantine
John Reuther	Eurofins
Weilin Shelver	USDA ARS
Sherri Turnipseed	US FDA
Eric Verdon	French Agency for Food, Environmental and Occupational Health Safety (ANSES)
Victoria Siegel	Office of the Indiana State Chemist / Official Methods Board Representative

AOAC Staff

Jim Bradford
Dawn Frazier
Delia Boyd
Deborah McKenzie
Anita Mishra
Nora Marshall
La'Kia Phillips

Observing Attendees:

Lauren Bailey – AB SciEx
Ferenc Bencsath – US FDA
Sharon Brunelle – Contractor
Tom Burnett – Elanco Animal Health
Mark Coleman – Elanco Animal Health
Paul Connelly - Reztek
Jo Marie Cook – Florida Dept. of Agriculture
Phillipe Delahaut CER Groupe (Belgium)
Pat DeLicio – MPI Research
Rodrigo Granja – Microbioticos (Brazil)
Steve Lehotay - USDA ARS
Kim Lombardi – Elanco Animal Health
Mark Neely – MPI Research
Maria Nelson – Contractor
Tom Phillips – Maryland Dept. of Agriculture
Matt Rodewald – Covance Laboratories
Cheryl Stephenson - Eurofins
John Szpylka - Silliker
Michael Turburg – Elanco
Dennis Ulrey - Elanco
Mike Wallace – MPI Research
Jian Wang – Canadian Food Inspection Agency
Doug Winter – Covance Laboratories

SUMMARY METHODS FOR CONSIDERATION AND ERP ACTIONS

Method ID	Title of Method Under Review by the ERP	ERP Action
NIC-33:	Determination and Confirmation of Nicarbazin in Chicken Tissues by Liquid Chromatography with Tandem Mass Spectrometry.	Additional information needed – not approved
AOAC OMA 2011.23	Determination and Confirmation of Parent and Total Ractopamine in Bovine, Swine and Turkey Tissues by Liquid Chromatography with Tandem Mass Spectrometry: Multi-Laboratory Study.	Recommend to OMB for Final Action status
AOAC OMA 2011.24	Determination and Confirmation of Narasin and Monensin in Chicken, Swine and Bovine Tissues by LC-MS/MS: Multi-Laboratory Study.	Recommend to OMB for Final Action status
#22	Determination of 3 Triphenylmethane Dyes Residues and their metabolites in Aquaculture Products by LC MS MS.	Approve as First Action Official Methods of Analysis
AOAC OMA 2011.22	Determination of Ractopamine in Swine, Bovine and Turkey Tissues by HPLC with Fluorescence Detection.	Retain as First Action Official Methoc for another year and await any new information

EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUES

Evaluation of Method NIC-33

NIC-33: **Determination and Confirmation of Nicarbazin in Chicken Tissues by Liquid Chromatography with Tandem Mass Spectrometry**

Author(s): Mark R. Coleman, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
John M. Rodewald, Covance Laboratories, 671 S. Meridian Rd, Greenfield, IN 46140
Sharon Brunelle, Brunelle Biotech Consulting, 14104 194th Ave NE, Woodinville, WA 98077
Maria Nelson, Independent Consultant, Savage, MN
Thomas J. Burnett, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140

Reviewers: Alex MacDonald, Martin Danaher, Glenn Kennedy, Guo Fang Pang, John Reuther

ERP OVERALL COMMENTS

1. The method has proved to work in multiple laboratories. Eurofins, Covance and three analysts in China have successfully run the method.
2. Laboratories with data after having run the method should provide the additional data and information to AOAC so that ERP members can see it and consider any other changes prior to the next meeting.
3. Information on the following are needed:
    a. Use of an internal standard
        i. Include or Justify the non-inclusion of the internal standard
        ii. Not clear if it should be added at the start or end
    b. Please provide clarification on the recovery data for liver
    c. Investigate the bias graphs to see if there is detector saturation or some an intrinsic error being reflected in the negative bias data.
    d. Specify or clarify selectivity studies and clarify the standard and sample preparation procedures

METHOD AUTHOR(S) COMMENTS

1. Method authors received little guidance on which studies would be required.
2. Internal standard was not used due to sourcing issues; there is data with and without the standard and the data appears to have no significant differences.
3. Consideration of bias was included at the recommendation of an AOAC statistical advisor.

ERP CONSENSUS AND RECOMMENDATIONS

MOTION: Consider holding NIC-33 for further review following submission of additional data by the sponsor and subsequent review by the ERP. MacDonald moved and Danaher seconded. Vote: Passed

Action Items and Next Steps:

1. AOAC to collect any additional studies that were referred to from Eurofins, Covance and China and provide them to the ERP.
2. The ERP will review the information the additional submitted information to see if major issues are addressed;

namely, the lack of internal standard and the data supporting the recoveries for liver. The ERP will review the interpretation of the additional data recommend any action items.

3. AOAC will look into potential for an alternative meeting format to discuss this issue.

EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUES

Evaluation of AOAC OMA 2011.23

AOAC OMA 2011.23 **Determination and Confirmation of Parent and Total Ractopamine in Bovine, Swine and Turkey Tissues by Liquid Chromatography with Tandem Mass Spectrometry: Multi-Laboratory Study.**

Author(s): W. Dennis Ulrey, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Thomas J. Burnett, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Sharon Brunelle, Brunelle Biotech Consulting, 14104 194th Ave NE, Woodinville, WA 98077
Kimberly R. Lombardi, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Mark R. Coleman, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140

Reviewers: Weillin Shelver, Joe Boison, Guo Fang Pang

ERP OVERALL COMMENTS

1. The method has proved to work successfully by analysts in China.
2. Not much guidance provided by the earlier ERP in how to conduct the study.
3. Collaborators provided editorial comments with their responses that were included in a tracked changes version of the multi-laboratory manuscript.
4. The stakeholder and expert review panel acceptance criteria were met.
5. Acceptable method reproducibility was demonstrated.
6. Additional data can be submitted, but it will not change how well the method works.

METHOD AUTHOR(S) COMMENTS

ERP CONSENSUS AND RECOMMENDATIONS

MOTION: To recommend moving the method, *Determination and Confirmation of Parent and Total Ractopamine in Bovine, Swine and Turkey Tissues by Liquid Chromatography with Tandem Mass Spectrometry,* **AOAC OMA 2011.23** to Final Action to the Official Methods Board. Shelver moved and Pang seconded. Vote: Passed with two abstentions. Abstentions: were not due to scientific reasons related to the reviewed manuscript.

EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUES

Evaluation of AOAC OMA 2011.24

AOAC OMA 2011.24 **Determination and Confirmation of Narasin and Monensin in Chicken, Swine and Bovine Tissues by LC-MS/MS: Multi-Laboratory Study**

Author(s): Kimberly R. Lombardi, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Thomas J. Burnett, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Sharon Brunelle, Brunelle Biotech Consulting, 14104 194th Ave NE, Woodinville, WA 98077

W. Dennis Ulrey, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Mark R. Coleman, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140

Reviewers: Katerina Mastovska and Guo Fang Pang

ERP OVERALL COMMENTS

1. The method demonstrated good recovery and precision when run by analysts in Canada, China, and in the US.
2. Additionally, collaborator feedback included editorial modifications that are included in a tracked changes version of the study manuscript.
3. Data originally excluded because ion ratios in one laboratory differed from those of other collaborators is to be added back to the overall data set. Statistics to be reanalyzed.
4. No reference materials for narasin and monensin.
5. Stakeholder criteria were met.
6. Acceptable method reproducibility demonstrated.

METHOD AUTHOR(S) COMMENTS

ERP CONSENSUS AND RECOMMENDATIONS

MOTION: To recommend the method, *Determination and Confirmation of Narasin and Monensin in Chicken, Swine and Bovine Tissues by LC-MS/MS* for Final Action status to the Official Methods Board. Mastovska moved and Pang seconded. Vote: Passed.

EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUES

Evaluation of Method #22

Method #22 **Determination of 3 Triphenylmethane Dyes Residues and their metabolites in Aquaculture Products b MS MS**

Author(s): Dominique Hurtaud-Pessel, ANSES LERMVD 27-31 Avenue du General Leclerc 94701 Maisons-Alfort Fra
Pierrick Couëdor, ANSES LERMVD 27-31 Avenue du General Leclerc 94701 Maisons-Alfort France
Eric Verdon, ANSES LERMVD 27-31 Avenue du General Leclerc 94701 Maisons-Alfort France

Reviewers: Brian Kinsella, Perry Martos, Haejung An, Doug Hite

ERP OVERALL COMMENTS

1. The method is simple and quick and uses stable isotopes.
2. Meets EU and SMPR general criteria
3. Recovery and precision are good
4. Use of chlorinated solvents is an issues
5. Clarify or correct clerical errors or inconsistencies
6. Should consider the impact of sample volume and specify the required time. This can be done with a small study b spiking with the isotope and adding another 100 microliters of acetonitrile.
7. Use of twotransition ions meets the EU criteria and not US FDA criteria. US FDA criteria require a third ion is need for confirmation of unknown samples.
8. US FDA criteria requires $r^2 = 0.995$.
9. Specify limits on samples for the recommended injection sequences in the middle.
10. Various members of the ERP and observers have used the method.
    a. Method has been used with an expanded range, additional ion(s) and on catfish.
    b. Method has proficiency testing data.
11. ERP should be clear on what the applicable matrixes are for the method.

METHOD AUTHOR(S) COMMENTS

1. When we are controlling for these drugs, we are changing the method to address the new drugs. Within 2 yea new analytes will need to be added.
2. The method is used in proficiency testing for shrimp, trout, salmon, tilapia, and etc..., there is also validation d for some of these matrixes.
3. Good matrix effects using the deuterated /isotopic standards.

ERP CONSENSUS AND RECOMMENDATIONS

MOTION: To adopt this method as First Action Official Methods of Analysis – An moved and Hite seconded.

Action Items and Next Steps:

1. Review and revise manuscript for clerical edits.
2. Submit additional data to AOAC on additional matrixes.
3. Community subgroup to provide assistance on reproducibility data collection.

EXPERT REVIEW PANEL FOR VETERINARY DRUG RESIDUES

Evaluation of AOAC OMA 2011.22

AOAC OMA 2011.22 **Determination of Ractopamine in Swine, Bovine and Turkey Tissues by HPLC with Fluorescence Detection**

Author(s): Thomas J. Burnett, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
John M. Rodewald, Covance Laboratories, 671 S. Meridian Rd, Greenfield, IN 46140
John Moran, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Michael P. Turberg, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140
Sharon L. Brunelle, Brunelle Biotech Consulting, 14104 194th Ave NE, Woodinville, WA 98077
Mark R. Coleman, Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140

Reviewers: Weilin Shelver and Joe Boison

ERP OVERALL COMMENTS

No new data since it was adopted as First Action Official Methods of Analysis.

METHOD AUTHOR(S) COMMENTS

The method is a resource for under developed countries where MS MS is not an option.

ERP CONSENSUS AND RECOMMENDATIONS

ACTION ITEM: Retain AOAC OMA 2011.22 for another year and see if laboratories present new or additional information to AOAC.

Expert Review Panel
Veterinary Drug Residues - Dyes in Seafood
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned Chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the AOAC Expert Review Panel for Veterinary Drug Residues - Dyes in Seafood that was held via teleconference and Adobe web connect on Monday, December 7, 2015.

DR. JOE BOISON
Expert Review Panel Chair

_____

Date

AOAC RESEARCH INSTITUTE
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Members - Present
Joe Boison, Canadian Food Inspection Agency (Chair)
Haejung An, U.S. FDA
Brian Kinsella, UCT, Inc.
Perry Martos, University of Guelph
Katerina Mastovska, Covance Laboratories
Cory Murphy, Canadian Food Inspection Agency
Sherry Turnipseed, U.S. FDA

Expert Review Panel Members – Not Present
Jon Reuther, Eurofins
Martin Danaher, TEAGASC
Doug Hite, State of Tennessee - Retired

Method Authors
Eric Verdon, ANSES France
Wendy Andersen, U.S. FDA

AOAC Staff
Deborah McKenzie
La'Kia Phillips

Observers
N/A

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*℠ (OMA) Expert Review Panel (ERP) for Veterinary Drug Residues - Dyes in Seafood, that was held via teleconference and Adobe web connect on Monday, December 7, 2015 at 11:00am Eastern Standard Time (EST).

The purpose of the meeting was to review and evaluate AOAC 2012.25: Three Triphenylmethane Dyes Residues and Their Metabolites (Malachite Green, Leuco Malachite Green, Crystal Violet, Leuco Crystal Violet, and Brilliant Green) in Aquaculture Product, LC-MS/MS, First Action 2012, for recommendation of AOAC Final Action Official Methods status. Supplemental information was provided in an e-book[1] to the reviewers which included the following documents:

1) Collaborative Study Manuscript
2) AOAC Expert Review Panel Report (dated May 7, 2013)
3) AOAC 2012.25: Three Triphenylmethane Dyes Residues And Their Metabolites (Malachite Green, Leuco Malachite Green, Crystal Violet, Leuco Crystal Violet, Brilliant Green) In Aquaculture Product, LC-MS/MS, First Action 2012
4) an Article: The Monitoring of Triphenylmethane Dyes in Aquaculture Products Through the European Union Network of Official Control Laboratories
5) Method Feedback – Wendy Andersen, U.S. Food and Drug Administration,
6) Article: Expansion of the Scope Of AOAC First Action Method 2012.25—Single-Laboratory Validation of Triphenylmethane Dye and Leuco Metabolite Analysis in Shrimp, Tilapia, Catfish, and Salmon by LC-MS/MS
7) Article: Determination Of Triphenylmethane Dyes And Their Metabolites In Salmon, Catfish, and Shrimp by LC-MS/MS Using AOAC First Action Method 2012.25: Collaborative Study
8) Method Feedback – Robert Burger, U.S. Food And Drug Administration
9) Method Feedback – Yanxuan Cai, U.S. Food And Drug Administration
10) Method Feedback – Ross Potter, Canadian Food Inspection Agency
11) Method Feedback – Steven Lehotay, United States Department Of Agriculture

Criteria for Vetting Experts and Selection Process:
The current Expert Review Panel (ERP) consisting of ten (10) experts that were submitted for consideration by the Official Methods Board to evaluate candidate methods for Veterinary Drug Residues - Dyes in Seafood as per the Expert Review Panel (ERP) Policies and Procedures. The experts are Joe Boison (Chair), Haejung An, Martin Danaher, Doug Hite, Brian Kinsella, Perry Martos, Katerina Mastovska, Cory Murphy, Jon Reuther, and Sherry Turnipseed.

ERP Orientation:
All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar and/or have participated in previous AOAC Expert Review Panel meetings.

Expert Review Panel Meeting Quorum

[1] Expert Review Panel E-Book - http://griegler-aoac-org.cld.bz/AOAC-RI-ERP-Book-VDR

The meeting of the Expert Review Panel was held via teleconference and Adobe Web Connect. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Seven (7) members out of the ten (10) voting members were present and met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs):
The collaborative study was reviewed against the attached AOAC SMPR 2009.001: Standard Method Performance Requirements for Quantitative Methods for Drug Residues in Shrimp, Tilapia, Catfish, and Salmon that was approved by the AOAC Stakeholder Panel on Marine and Freshwater Foods on March 31, 2010.

Conclusion:
The ERP reviewed AOAC 2012.25: Three Triphenylmethane Dyes Residues And Their Metabolites (Malachite Green, Leuco Malachite Green, Crystal Violet, Leuco Crystal Violet, Brilliant Green) In Aquaculture Product, LC-MS/MS, First Action 2012 against the AOAC SMPR 2009.001:Standard Method Performance Requirements for Quantitative Methods for Drug Residues in Shrimp, Tilapia, Catfish, and Salmon. The ERP also reviewed the method against the optional and required items noted for consideration of AOAC Final Action status from the AOAC Expert Review Panel report of May 7, 2013.

1) Select a 3rd confirmation ion (optional).
2) Add a 5th non-zero calibration point (optional).
3) Clarify shelf life for the standard and provide data to support.
4) Edit typo in the title of the draft First Action paper.

The ERP concluded that AOAC 2012.25 should be recommended for AOAC Final Action Official Methods status by consensus as noted in the meeting minutes.

Subsequent ERP Activities:
The ERP will draft a recommendation report for AOAC 2012.25 and submit the completed report to the AOAC Official Methods Board for AOAC Final Action Official Methods status.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair, Dr. Joe Boison of the Canadian Food Inspection Agency (CFIA) members, and discussed the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Discuss Final Action Requirements for First Action Official Methods

All ERP members presented a review and discussed the information received from the method author for the AOAC First Action method AOAC 2012.25: Three Triphenylmethane Dyes Residues and Their Metabolites (Malachite Green, Leuco Malachite Green, Crystal Violet, Leuco Crystal Violet, Brilliant Green) In Aquaculture Product, LC-MS/MS, First Action 2012, which has approached the 2-year tracking period. The method authors, Wendy Andersen of United States Food and Drug Administration, and Eric Verdon of ANSES - French Agency for Food, Environmental and Occupational Health & Safety, were present and able to address the questions and concerns of the ERP. A summary of comments was provided to the ERP and the method authors.[2] The ERP is required to make a recommendation on AOAC Official First Action methods, 2 years after adoption, to the AOAC Official Methods Board regarding Final Action status. By consensus the ERP presented the following motions for AOAC 2012.25**:**

> **Motion by An; Second by Martos,** to recommend to the AOAC Official Methods Board, First Action Method AOAC 2012.25 for AOAC Final Action Official Methods status.
> **Consensus demonstrated by: 7 in favor, 0 opposed, and 0 abstentions. Unanimous, *Motion Passed.***

The ERP members noted additional discussion items regarding the details of the recommendation for AOAC Final Action Official Methods status that will assist in the AOAC Official Methods Board review.

Strengths, Weakness, and Community Needs

The method is easy to follow, speedy, flexible, has a broad scope, and can be evaluated amongst different matrices that will benefit the community that it serves. In addition, there are many publications available that help support the method. It was also noted that the method will be moving forward as an FDA Regulatory standard due to the data being easily reliable.

Reference Materials

The ERP noted that there are currently no reference materials available for these types of drugs and may need someone to generate reference materials in the future. The compounds are declared not acceptable for daily intake and cannot be used in fish production. The ERP should continue to monitor for future implementation in regulatory environments.

[2] Attachment 1: Summary of Expert Reviewer Comments for AOAC 2012.25

Comparison to SMPR

The ERP noted that the LOQ is above the minimum requirement as stated in SMPR 2009.001 and requested the method authors to redefine how the LOQ was determined. The ERP also requested that the method author should reference in the manuscript, the stability data information that is currently provided in the previous publications.

Recommended Editorial Changes

In the article published by J. AOAC Int., 96, 1152 (2013): First Action 2012.25, the following editorial changes are recommended:

1) Page 1153 under section C. Chemicals and Reagents, subsection(r), "250 L" should be changed to "250 mL".
2) Page 1155 under section E. Sample Preparation, subsection (i), "(1 g/L) and ascorbic acid" should be changed to "(1 g/L) ascorbic acid". This should also be changed in the published OMA method.

IV. Adjournment: Meeting concluded at 12:26pm EST.

PROFILE OF AOAC EXPERT REVIEW PANEL FOR MICROBIOLOGY METHODS FOR FOODS AND ENVIRONMENTAL SURFACES

ERP Name	AOAC Expert Review Panel for Microbiology Methods for Foods and Environmental Surfaces			**Chair(s)**	Michael Brodsky (Brodsky Consultants) and Wendy McMahon (Mérieux NutriSciences)
ERP Formed:	2012	**Number of Methods Adopted**	10 as First Action status	**Number of Methods Recommended**	7
Scope:	Review and adopt methods resulting from sole source submission of methods for pathogenic and nonpathogenic microbial detection or determination in foods and on environmental surfaces				
Roster	1. Michael Brodsky, Brodsky Consulting (Co-Chair) 2. Wendy McMahon, Silliker Laboratories (Co-Chair) 3. Maya Achen, Abbott Nutrition 4. Patrice Arbault, BioAdvantage 5. Mark Carter, MC2E 6. Yi Chen, U.S. Food & Drug Administration (FDA) 7. Peyman Fatemi, The Acheson Group LLC 8. Maria Christina Fernandez, University of Buenos Aires 9. Tom Hammack, U.S. Food & Drug Administration (FDA) 10. Tony Hitchins, U.S. Food & Drug Administration/CFSAN (Retired) 11. Yvonne Salfinger				
Technical Documents created/used	OMA Appendix J				
Methods Adopted *First Action and Final Action status*	AOAC 2012.02 - Gram-Positive Bacteria Identification AOAC 2013.01 – *Salmonella* in Variety of Foods AOAC 2013.02 – *Salmonella* Species in a Variety of Foods and Environmental Surfaces AOAC 2013.09 – *Salmonella* in Selected Foods AOAC 2013.10 – *Listeria* species in a Variety of Foods and Environmental Surfaces AOAC 2013.11 – *Listeria monocytogenes* in a Variety of Foods AOAC 2013.14 – Identification of *Salmonella* spp from Colony Picks *Red indicates Final Action OMA status*		AOAC 2014.01 – *Salmonella* in Selected Foods AOAC 2014.05 – Enumeration of Yeast of Mold in Food AOAC 2014.06 – *Listeria* species in Selected Foods and Environmental Surfaces AOAC 2014.07 – *Listeria monocytogenes* in Selected Foods and Environmental Surfaces AOAC 2015.13 – Enumeration of Aerobic Bacteria in Food		
Final Action Methods Recommended					
Additional Input	ISPAM				
Awards/Recognitions	Seven methods have Final Action status; ERP of the Year in 2014; AOAC 2013.10 /2013.11 -joint Multi-Laboratory of the Year in 2014; AOAC 2013.14 – Award in Technical and Scientific Excellence in 2014.				

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for OMAMAN-01 "Detection of *Salmonella* species in a Variety of Foods by the DuPont™ BAX® System Real-Time PCR Assay for *Salmonella*". **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Tuesday, March 12, 2013.

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-01	**DETECTION OF *SALMONELLA* SPECIES IN A VARIETY OF FOODS BY THE DUPONT™ BAX® SYSTEM REAL-TIME PCR ASSAY FOR *SALMONELLA*: COLLABORATIVE STUDY** *AUTHORS* *F. MORGAN WALLACE, BRIDGET ANDALORO, DAWN FALLON, NISHA CORRIGAN, STEPHEN VARKEY, DANIEL DEMARCO, ANDREW FARNUM, MONICA TADLER, STEVEN HOELZER, JULIE WELLER, EUGENE DAVIS, JEFFREY ROHRBECK AND GEORGE TICE, DUPONT NUTRITION & HEALTH, ESL BUILDING 400, ROUTE 141 & HENRY CLAY ROAD, WILMINGTON, DELAWARE 19880, PATRICK BIRD, ERIN CROWLEY, JONATHAN FLANNERY, KIEL FISHER, TRAVIS HUFFMAN, MEGAN BOYLE, M. JOSEPH BENZINGER, JR., PAIGE BEDINGHAUS, KATIE GOETZ, WILLIAM JUDD, JIM AGIN AND DAVID GOINS, Q LABORATORIES, INC., 1400 HARRISON AVENUE, CINCINNATI, OHIO 45214* *COLLABORATORS* *D. CLARK; B. DIECKELMAN; T. DONOHUE; H. ELGAALI; W. FEDIO; E. GALBRAITH; B. KUPSKI; K. MCCALLUM; G. MCWHORTER; J. MEYER; D. SWIFT; R. RADCLIFF; D. RODGERS; M. STEELE; L. THOMPSON*

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Method Status.	The following revisions are recommended for the Detection of *Salmonella* species in a Variety of Foods by the DuPont™ BAX® System Real-Time PCR Assay for *Salmonella*: Collaborative Study (revision 2-Redline) as submitted on March 11, 2013: *Please refer to next page.*	MOTION PASSED *UNANIMOUS* *Hitchins/Chen*	March 12, 2013
Motion to not require a statistics review of the Collaborative Study Protocol.	The ERP recommended that a statistics review maybe required only if there is a modification to an approved Collaborative Study Protocol.	MOTION PASSED *UNANIMOUS* *Hitchins/Chen*	March 12, 2013

ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS				
Page No.	**Line No.**	**Section**	**General Recommendation**	**Editorial Changes**
Page 2	Lines 23-28	Introduction Section	Move specifics from applicability sections to introduction section.	
Page 3	Lines 6-8	Study Design	Verify reference method for matrices, frankfurters and orange juice.	
Page 3	Lines 11-37	Test Sample Inoculation and Distribution	Add specifics to test sample matrices (i.e., 85% lean).	
Page 3	Lines 11-37	Test Sample Inoculation and Distribution	Add description on how it is done.	
Page 3	Lines 11-37	Test Sample Inoculation and Distribution	Add table showing stressed matrices.	
Page 3	Lines 11-37	Test Sample Inoculation and Distribution	Indicate stressed acidifying portion for orange juice.	
Page 3	Lines 11-37	Test Sample Inoculation and Distribution	Include data that shows heat stress (table stress % injury).	
Page 4	Line 7	AOAC Official Method Section		Add PTM Certification Number and Date
Page 4	Lines 10-15	Applicability Statement		Remove “85% lean”, “12% fat”, and “instant”.
Page 8	Line 7	Sample Enrichment/Cream Cheese (25g)		Change “22-26 hours” to “12-26 hours”
Page 13	Lines 25-26	Results and Discussion		Change “significant differences” to “no detectable variations detected”
Page 13	Lines 2-26	Results and Discussion	Add section discussing figures.	
Pages 15-24	-------	Tables 1 - 7	Transfer AOAC acronyms from Pre-collaborative Report to Collaborative Report; Tables 1-7 add acronyms so each table can stand on its own.	

ERP PROFILE SUMMARIES

EXPERT REVIEW PANEL MEMBERS

Name	*Company*
Michael Brodsky, Chair	**Brodsky Consultants**
Yi Chen	US FDA-CFSAN
Donna Douey	CFIA
Tony Hitchins	US FDA-CFSAN (retired)
Not Present	
Wendy McMahon	Silliker, Inc.
Maya Achen	Ohio Dept. of Agriculture
Maria C. Fernandez	ANMAT/Ministry of Health Univ. of Buenos Aires
Dermot Hayes	Chair, Committee on Safety
Robert LaBudde	Member, Committee on Statistics
Thomas Hammack	US FDA-CFSAN

AOAC STAFF

Jim Bradford, Executive Director
Deborah McKenzie
Tien Milor
La'Kia Phillips

OBSERVERS

Name	*Company*
Patrick Bird	Q Laboratories
Joe Boison	CFIA
Jim Harnly	USDA
Steven Hoelzer	DuPont
Ronald Johnson	bioMerieux, Inc.
John Szpylka	Silliker Inc.
Morgan Wallace	DuPont

Below are the recommendations from the voting members of the ERP based upon their submitted method review forms for the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*.

Reviewer	Recommendation
Michael Brodsky, Chair	First Action
Yi Chen	First Action
Donna Douey	First Action w/revisions
Tony Hitchins	First Action
Wendy McMahon	First Action
Maya Achen	First Action
Maria C. Fernandez	First Action

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for OMAMAN-02 **"**Detection of Listeria monocytogenes in a Variety of Foods by the **VIDAS®** L. monocytogenes Xpress (LMX) Method: Collaborative Study" and OMAMAN-03 "**D**etection of *Listeria* species in a Variety of Foods by the **VIDAS®** UP *Listeria* (LPT) Method: Collaborative Study". **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Monday, June 24, 2013.

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-02	**EVALUATION OF VIDAS® *LISTERIA MONOCYTOGENES* XPRESS (LMX) FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* IN A VARIETY OF FOODS: COLLABORATIVE STUDY** *AUTHORS* Erin Crowley, Patrick Bird, Jonathan Flannery, M. Joseph Benzinger, Jr., Kiel Fisher, Megan Boyle, Travis Huffman, Ben Bastin, Paige Bedinghaus, Will Judd, Thao Hoang, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214, Ronald L. Johnson, bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042 *COLLABORATORS* J. Mills, P. Rule, B. Howard, N. Rogman, J. Cannon , B. Paul, M. Sala-Rhatigan, S. Josephs, N. Palen, A. Stegmann, B. Perry, R. Hiles, T. Stubblefield, N. Nagassar, Sylvanus Owusu, J. Zimmerman, B. Brahmanda, H. Elgaali, A. Capps, G. Rosario, D. Davis, L. Parker, C. Said, J. Li, K. Klemms, B. May, B. Hand, R. Burkhart, J. Pickett, J. Adams, A. Bollenbacher, K. Wiggins, L. Cesanas, J. Jolly, S. Moore, D. Ebbing, M. Michels, A. Kehres, J. Hirsch

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Method Status.		MOTION PASSED ***UNANIMOUS*** *Brodsky/Hitchins*	June 24, 2013

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-03	**EVALUATION OF VIDAS® UP *LISTERIA* ASSAY (LPT) FOR THE DETECTION OF *LISTERIA* IN A VARIETY OF FOODS AND ENVIRONMENTAL SURFACES: COLLABORATIVE STUDY** *AUTHORS* Erin Crowley, Patrick Bird, Jonathan Flannery, M. Joseph Benzinger, Jr., Kiel Fisher, Megan Boyle, Travis Huffman, Ben Bastin, Paige Bedinghaus, William Judd, Thao Hoang, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214, Ronald L. Johnson, bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042 *COLLABORATORS* J. Mills, P. Rule, B. Howard, N. Rogman, J. Cannon, B. Paul, M. Sala-Rhatigan, S. Josephs, N. Palen, A. Stegmann, B. Perry, R. Hiles, T. Stubblefield, N. Nagassar, Sylvanus Owusu , J. Zimmerman, B. Brahmanda, H. Elgaali, A. Capps, G. Rosario, D. Davis, L. Parker, C. Said, J. Li, K. Klemms, B. May, B. Hand, R. Burkhart, J. Pickett, , A. Bollenbacher, K. Wiggins, L. Cesanas, J. Jolly, S. Moore, D. Ebbing, M. Michels, A. Kehres, J. Hirsch

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Method Status.		MOTION PASSED ***UNANIMOUS*** *Brodsky/Hitchins*	June 24, 2013

ERP PROFILE SUMMARIES

EXPERT REVIEW PANEL MEMBERS

Name	***Organization***
Michael Brodsky, Chair	**Brodsky Consultants**
Yi Chen	US FDA-CFSAN
Maria C. Fernandez	ANMAT/Ministry of Health Univ. of Buenos Aires
Tony Hitchins	US FDA-CFSAN (retired)
Yvonne Salfinger	

Not Present

Wendy McMahon	Silliker, Inc.
Maya Achen	Ohio Dept. of Agriculture
Donna Douey	CFIA
Dermot Hayes	Chair, Committee on Safety
Robert LaBudde	Member, Committee on Statistics
Thomas Hammack	US FDA-CFSAN

AOAC STAFF

Jim Bradford, Executive Director

Deborah McKenzie
Tien Milor
La'Kia Phillips

OBSERVERS

Name	***Organization***
Patrick Bird	Q Laboratories
Ronald Johnson	bioMérieux, Inc.
John Mills	bioMérieux, Inc.
Ray Turnley	bioMérieux, Inc.

Below are the recommendations from the voting members of the ERP based upon their submitted method review forms for the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*.

	Recommendation	
Reviewer	**OMAMAN-02**	**OMAMAN-03**
Michael Brodsky, Chair	First Action	First Action w/editorial changes
Yi Chen	First Action	First Action w/data
Donna Douey	First Action, if concerns are addressed by ERP	First Action, if concerns are addressed by ERP
Tony Hitchins	First Action	First Action
Maya Achen	First Action	First Action
Maria C. Fernandez	First Action	First Action
Yvonne Salfinger	First Action w/minor changes	First Action w/changes

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on Microbiology for Food and Environmental Surfaces (*Salmonella* spp.) held on December 12, 2013.

M.H. Brodsky

_____

Michael Brodsky, Expert Review Panel Chair
Wendy McMahon, Expert Review Panel Chair

December 18, 2013

_____

Date

Please sign, date and fax this document to La'Kia Phillips at 301-924-7089.

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for **OMAMAN-07:** Evaluation of the ANSR® for Salmonella Assay for Identification of *Salmonella* spp. from Colony Picks from Selective/Differential Agar Media. **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Thursday, December 12, 2013.

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-07	**EVALUATION OF THE ANSR® FOR SALMONELLA ASSAY FOR IDENTIFICATION OF SALMONELLA SPP. FROM COLONY PICKS FROM SELECTIVE/DIFFERENTIAL AGAR MEDIA** *AUTHORS* Mark Mozola[1], Oscar Caballero, Nicole Enslin, Preetha Biswas, and Jennifer Rice, Neogen Corp., 620 Lesher Place, Lansing, MI 48912, [1] Corresponding author's email: mmozola@neogen.com *COLLABORATORS* E.S. Adams, H. Alnughaymishi, J.B. Barrett, J. Benzinger, M.E. Berrang, M. Boyle, J. Cannon, D. Clark, A. Copeland, M. Corebello, D.E. Cosby, N.A. Cox, N. Cuthbert, H. Dammann, J. Dyszel, E. Feldpausch, J. Flannery, C. Flores, J.G. Frye, R. Fuller, V. Gill, L.M. Hiott, B. Howard, M. Hudgens, C.R. Jackson, W. Jones, S.W. Knapp, L. Kuepfer, P. Kulkarni, B. Kupski, C. Pidgeon, A. Quenneville, L.L. Rigsby, N. Rogman, E. Sai, A. Scollon, M. Sisemore, J. Stepnitz, J. Van Brunt, M. Vross, D. Waltman, H. Wang, G. Whitbeck, S. York, L. Zhang

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion that this method be adopted for First Action Official Method status.	*Requirements for Final Action:* 1. *Actively solicit feedback and submit to the Expert Review Panel.*	MOTION PASSED ***UNANIMOUS*** *Brodsky, Salfinger*	December 12, 2013

ERP PROFILE SUMMARIES

EXPERT REVIEW PANEL MEMBERS

Name	***Organization***
Michael Brodsky, Chair	**Brodsky Consultants**
Wendy McMahon, Chair	**Silliker, Inc.**
Maria C. Fernandez	ANMAT/ Univ. of Buenos Aires
Tony Hitchins	US FDA-CFSAN (retired)
Yvonne Salfinger	
Donna Douey	CFIA
Thomas Hammack	US FDA-CFSAN

Not Present

Maya Achen	Ohio Dept. of Agriculture
Yi Chen	US FDA-CFSAN
Robert LaBudde	Member, Committee on Statistics

AOAC STAFF

Jim Bradford, Executive Director
Deborah McKenzie
Tien Milor
La'Kia Phillips

OBSERVERS

Name	***Organization***
Mark Mozola	Neogen Corp.
Erin Crowley (OMB Rep.)	Q Laboratories

Below are the recommendations from the voting members of the ERP based upon their submitted method review forms for the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*.

Reviewer	**OMAMAN-07**
Maya Achen	First Action
Michael Brodsky, Chair	First Action, if concerns are addressed by ERP
Donna Douey	First Action, if concerns are addressed by ERP
Maria C. Fernandez	First Action
Tom Hammack	First Action
Tony Hitchins	First Action
Wendy McMahon	First Action with Revisions
Yvonne Salfinger	First Action, if concerns are addressed by ERP.

AOAC RESEARCH INSTITUTE
Official Methods of Analysis℠ (OMA)
Expert Review Panel on Microbiology for Food and Environmental Surfaces

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on Microbiology for Food and Environmental Surfaces held on March 20, 2014.

Wendy McMahon

Wendy McMahon, Expert Review Panel Chair

April 10, 2014

Date

Please sign, date and fax this document to La'Kia Phillips at 301-924-7089.

AOAC RESEARCH INSTITUTE

Official Methods of Analysis[SM] (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for OMAMAN-08: Evaluation of the 3M™ Petrifilm™ Salmonella Express System for the Detection of Salmonella in Selected Foods: Collaborative Study and Review the OMA Modification for 2013.09: 3M™ Molecular Detection Assay (MDA) Salmonella. **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Thursday, March 20, 2014.

METHOD NO.	MANUSCRIPT TITLE
OMA Modification for 2013.09	**EVALUATION OF MODIFICATION OF THE 3M™ MOLECULAR DETECTION ASSAY (MDA) SALMONELLA FOR THE DETECTION OF SALMONELLA IN SELECTED FOODS: COLLABORATIVE STUDY** *AUTHORS* Patrick Bird, Kiel Fisher, Megan Boyle, Travis Huffman, M. Joseph Benzinger, Jr., Paige Bedinghaus, Jonathon Flannery, Erin Crowley, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214, DeAnn Benesh, John David, 3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144 *COLLABORATORS* K. Newman, V. Gill, I. Mello, M. Ontiberos, C. Gwinn, S. Moosekian, J. Marchent, J. Dyszel, M. Vross, K. Blanchard, D. Lewis, M. Horan, B. Stawick, J. Ruebl, K. Rajkowski, A. Morey, S. Montez, J. Jurgens, L. Thompson, M. Bandu, M. Oltman, D. Bosco, R. Brooks, S. Luce, H. Dammann, D. Clark Jr., W. McMahon, D. Awad, M. Kelly, M. Greenwell

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Methods status.	*N/A*	MOTION PASSED ***UNANIMOUS*** *Salfinger, Hammack*	March 20, 2014

AOAC RESEARCH INSTITUTE

Official Methods of Analysis℠ (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-08	**EVALUATION OF THE 3M™PETRIFILM™SALMONELLA EXPRESS SYSTEM FOR THE DETECTION OF SALMONELLA SPECIES IN SELECTED FOODS: COLLABORATIVE STUDY** *AUTHORS* Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH45214, Robert Jechorek, 3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144 *COLLABORATORS* K. Newman, J. Pickett, J. Adams, A. Martin, J. Meyer, H. Elgaali, J. Marchant-Tambone, K. Blanchard, D. Lewis, R. Colvin, B. Stawick, K. Rajkowski, D. Rodgers, K. Beers, A. Morris, K. McCallum, A. Morey, W. Fedio, R. Brooks, M. Boyle

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Methods status.	*N/A*	MOTION PASSED ***UNANIMOUS*** *Brodsky, Douey*	March 20, 2014

AOAC RESEARCH INSTITUTE

Official Methods of Analysis[SM] (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

EXPERT REVIEW PANEL MEMBERS

Wendy McMahon, Silliker, Inc. (CHAIR)
Michael Brodsky, Brodsky Consultants
Maya Achen, Ohio Dept. of Agriculture
Donna Douey, CFIA
Maria C. Fernandez, ANMAT/Ministry of Health Univ. of Buenos Aires
Thomas Hammack, US FDA-CFSAN
Tony Hitchins, US FDA-CFSAN (retired)
Yvonne Salfinger

Not Present

Yi Chen, US FDA-CFSAN (Alternate)

AOAC STAFF

Jim Bradford, Executive Director
Delia Boyd
Deborah McKenzie
La'Kia Phillips

OBSERVERS

DeAnn Benesh, 3M
Patrick Bird, Q Laboratories
Farpan Bower, Sample 6
Erin Crowley, Q Laboratories
Steven Hoelzer, DuPont Nutrition & Health
Robert Jechorek, 3M
Mike Koeris, Sample 6
Todd Marrow, University of Guelph
Sam Mohajer, CFIA
Karen Silbernagel, AOAC Technical Consultant

OMA Modification of 2013.09 Reviewers

Michael Brodsky	Primary Reviewer
Yvonne Salfinger	Secondary Reviewer
Yvonne Salfinger	Safety Reviewer
Hilde Skaar-Norli	Statistical Reviewer

OMAMAN-08 Reviewers

Maria Christina Fernandez	Primary Reviewer
Maya Achen	Secondary Reviewer
Yvonne Salfinger	Safety Reviewer
Caryn Thompson	Statistical Reviewer

Expert Review Panel
Microbiology for Food and Environmental Surfaces
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Microbiology for Food and Environmental Surfaces was held on Wednesday, December 10, 2014 at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

WENDY MCMAHON, SILLIKER LABORATORIES
Expert Review Panel Co-Chair

MICHAEL BRODSKY, BRODSKY CONSULTING
Expert Review Panel Co-Chair

Wendy McMahon
Date Jan 23, 2015

AOAC RESEARCH INSTITUTE
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair (s)
Michael Brodsky, Brodsky Consulting
Wendy McMahon, Silliker Laboratories

Expert Review Panel Members
Patrice Arbault, BioAdvantage
Yi Chen, FDA/ Tom Hammack, FDA
Maria Christina Fernandez, University of Buenos Aires
Tony Hitchins, FDA/CFSAN (Retired)
Sam Mohajer, Canadian Food Inspection Agency
Paul Wehling, General Mills

Method Authors
DeAnn Benesh, 3M Food Safety
Robert Jechorek, 3M Food Safety
Lisa Montereso, 3M Food Safety
Erin Crowley, Q Labs
Patrick Bird, Q Labs

AOAC Staff
Jim Bradford, Executive Director
Deborah McKenzie
Tien Milor
La'Kia Phillips

Observers
N/A

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*℠ (OMA) Expert Review Panel for Microbiology for Foods and Environmental Surfaces on Wednesday, December 10, 2014 from 8:00am to 12:00pm at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

The purpose of the meeting was to review and evaluate OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast and Mold Collaborative Study submitted by Bob Jechorek of 3M Food Safety located at 3M Center, Building 260-06-B-01, St. Paul, MN 55144 and Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, *Performance Tested Methods*℠ protocol and package insert.

OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria for the Detection of Listeria Species submitted by Lisa Monteroso of 3M Food Safety Department located at 3M Center, Building 0260-06-B-01, Saint Paul, Minnesota 55144-1000 U.S.A. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, two (2) *Performance Tested Methods*℠ modification reports and package insert.

OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes for the Detection of Listeria Monocytogenes submitted by Lisa Monteroso of 3M Food Safety Department located at 3M Center, Building 0260-06-B-01, Saint Paul, Minnesota 55144-1000 U.S.A. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, and package insert.

Criteria for Vetting Experts and Selection Process:
The following seven (7) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for Pesticide Residues methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Chemical Contaminants and Residues in Foods Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Amy Brown, Jo Marie Cook (Alternate), Julie Kowalski, John Reuther, Marina Torres, Jian Wang, and Xiaoyan Wang.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, November 5, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the eleven (11) voting members were present and therefore met a quorum to conduct the meeting. It was also noted that Jim Agin who was not present, will not participate on this Expert Review Panel.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast and Mold, OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria For The Detection Of Listeria Species, and OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes For The Detection Of Listeria Monocytogenes and have adopted these methods for AOAC First Action Official Method status by unanimous decision as noted in the meeting minutes.

Subsequent ERP Activities:
ERP members will continue to evaluate the method for 2 years.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-chairs, Michael Brodsky and Wendy McMahon welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

A brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a quick overview of the Expert Review panel process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

IV. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast And Mold Collaborative Study. The method authors Robert Jechorek of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[1]

> **MOTION:**
> **Motion by Brodsky; Second by McMahon, for the method to move forward for First Action Official Method Status.**
> **Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous).** ***Motion Passed.***

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria For The Detection Of Listeria Species. The method authors Lisa Montereso of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[2]

> **MOTION:**
> **Motion by Brodsky; Second by Arbault, for the method to move forward for First Action Official Method Status.**
> **Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous).** ***Motion Passed.***

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes for The Detection Of Listeria Monocytogenes. The method authors Lisa Montereso of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-16
[2] Attachment 2: Summary of Expert Reviewer Comments for OMAMAN-17

address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[3]

MOTION:
Motion by Hitchins, Second by Mohajer, for the method to move forward for First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). ***Motion Passed.***

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)
No further action was discussed at this time.

VI. Adjournment

[3] Attachment 3: Summary of Expert Reviewer Comments for OMAMAN-18

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
ER 7	yes
ER 8	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	No

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
General Comments about the Method Scope/Applicability:	
ER 1	The method scope and applicability are clearly described, and the Petrifilm protocols have been clearly presented. Both incubation temperature options were also clearly stipulated. Description of the look like colonies were sufficiently explained.
ER 2	A statement about growth curve for the yeast selected was made on page 3, row 14-15. This culture incubated for 48h while there are strains requiring 5-7 days incubation. The method includes up to 60 h incubation. Reviewer recommends applicability statement to this point. The background section could include this point and then the discussion could address it.
ER 3	Almonds and Ground Beef have been used for the collaborative study which are both appropriate matrices and were used in the pre-collaborative study.
ER 4	No additional comments
ER 5	The method is useful for rapid quantification of spoilage microorganisms
ER 6	Accurately reflects breadth and depth of study
ER 7	None
ER 8	1. Page 5, lines 27-29, Sterile Diluent. The method lists several diluents that can be used with this method, including 0.1% peptone water. All of the diluents, other than 0.1% peptone water, should be removed from the method, since the method was validated with 0.1 peptone water and no other diluent. The implied conclusion is that the method, as given by the draft official method, is that it can be run with any diluent even though it was only validated with 0.1 % peptone water. 2. Page 5, lines, 7 - 9, applicability (here and elsewhere in the report). The method refers to yogurt, ready-made-pie, frozen ground beef patties, sandwiches, and dehydrated soup. These are not specific enough. Yogurt and beef should have fat percentages. The types of pie, sandwiches and dehydrated soup should be specified. For example, apple pie, chicken soup, tuna sandwich... The applicability statement is too broad as written.

General comments about the method:	
ER 1	The method is very well described in the various documents.
ER 2	NA
ER 3	The method is much simpler compared to traditional method and the space savings in the use of Petrifilm are significant as compared to traditional agar plates.
ER 4	No additional comments
ER 5	The method is really useful due to it significantly reduces test times
ER 6	Well written and easy to follow
ER 7	none
ER 8	1. Page 7, line 17. There should be references directing the analyst to methods for the further identification of yeast/fungal isolates. They should be ISO, BAM, MLG and others.
Pros/Strengths of the Manuscript:	
ER 1	Good description of the sample preparation protocols. Efficient description of the collab study workflow and organization. Tables are very useful for summarizing the results.
ER 2	Very well written.
ER 3	The Manuscript is well written and the information flow is in an understandable order.
ER 4	Generally well written.
ER 5	It is a simple method for working
ER 6	Very thoroughly written and detailed
ER 7	Well written in general.
ER 8	It is well written.
Cons/Weaknesses of the Manuscript:	
ER 1	Very minor edits: page 7, line 25, reports lab 5 as one of the 4 labs with deviations, but in table 1 page 13, lab 6 is marked as the lab showing deviations for ground beef??? Reading through the report and the pack insert, it remains unclear if the minimum incubation is 48 hours or 46 hours since it is stipulated that incubation shall be 48+/-2 hours but reading is required at 48 hours: is minimal time of 48 hours of incubation is required?
ER 2	NA
ER 3	Table 1 describes which data sets were not used in the statistical analysis; however, there are not indications as such in tables 2 – 9 where raw data is presented. It may help to identify the labs who's data sets were excluded in each table using a superscript letter.
ER 4	Need to elaborate on the issues and possible causes of those issues of laboratories whose data were not used in the study.
ER 5	no
ER 6	None
ER 7	Page 1, line 24 states "unpaired study design" but page 4 line, line 22 states "paired study design". Please clarify in the manuscript. Page 3, line 29: Clarify that after lyophilization dilutions were done with sterile NFDM powder or reconstituted NFDM. Page 4, line 38: Increase font size. Page 4, line47: Justify or omit reverse transformed mean difference here and in Tables 2014.1 and 2014.2.[Continued] Page 7, line 24: Insert "valid" before both "data" words. Page 7, line 33: Omit Figs 1-4 which are somewhat redundant. Add statement about acceptability of Youden plots. Page 7, line 37: Remove "reverse transformed difference" here and in Tables 2014.1 &.2. Page 8, lines 31-33: State the repeatability SD values supporting this assertion. Page 8, lines 44-46: State the repeatability SD values supporting this assertion.

	Page 9, lines 8-10: State the repeatability SD values supporting this assertion. Page 9, lines 30-32: State the repeatability SD values supporting this assertion. Page 9, lines 42-44: State the repeatability SD values supporting this assertion. Page 10, lines 7-9: State the repeatability SD values supporting this assertion. Page 10, lines 21-23: State the repeatability SD values supporting this assertion. Page 10 lines 42-44: State the repeatability SD values supporting this assertion. Add the requested repeatability SD values as footnotes to relevant Tables.
ER 8	Applicability statement is too general. Tables are not properly footnoted.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	yes
ER 6	Yes
ER 7	Yes, in general.
ER 8	Yes.
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	The data supports the criteria.
ER 2	Yes
ER 3	No
ER 4	yes
ER 5	yes
ER 6	Yes
ER 7	Yes.
ER 8	Yes.
Are there any concerns regarding the safety of the method?	
ER 1	To be covered once Safety Advisor review is presented.
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	No
ER 7	No.
ER 8	No.
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No concerns.
ER 2	No
ER 3	The manuscript would be much stronger if a more detail rational is given as to why some of the collaborative labs were excluded. It is understandable to have such occurrences in a large collaborative study.
ER 4	No
ER 5	No
ER 6	No

ER 7	In Table 2014.1 for the RYM method it is not clear what data from Tables 2, 3, 4 & 5 is used to get the SD values for repeatability and reproducibility. In Table 2014.2 for the RYM method it is not clear what data from Tables 6 7, 8 & 9 is used to get the SD values presented for repeatability and reproducibility. Typically the SD for reproducibility is noticeably larger than that for repeatability. This is true for the 2014.2 (almond) results by the RYM and the FDA/ISO methods. However, it is not so for the 2014.1 (beef) results by the RYM or reference method. So perhaps the calculations and data inputs should be checked. [Continued] In many cases, especially in Table 2014.1 the mean RYM and reference methods' mean counts are not significantly different and yet the differences, although not very different, are often significantly different. In Table 2014.1 perhaps this is related to the fact that only 11 collaborators provided valid results.
ER 8	None.
General Comments (2)	
ER 1	
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	
ER 7	None
ER 8	Tables 2014.1 & 2014.2. It should be footnoted that the BAM and ISO methods are identical when using 0.1 % peptone water. as was done in this collaborative study.
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes

ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	No
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	Yes
ER 7	Yes
ER 8	No
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes

ER 8	Yes
RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*?**	
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	yes
ER 6	Yes
ER 7	This method is potentially recommendable for adoption after manuscript clarifications are made.
ER 8	Yes, if my above comments regarding applicability and diluent are addressed. The package insert also needs to be revised in light of these comments. If multiple diluents are recommended, even though only 1 was validated, then I would recommend against giving the method First Action Status.
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	No.
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	No
ER 6	User comments
ER 7	No
ER 8	Two years worth of use in the field, without substantial problems, should be the criteria for making this a final action method.
General Comments (3)	
ER 1	
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	
ER 7	None
ER 8	1. Package insert, page 4 of 8, Sterile Diluent. The method lists several diluents that can be used with this method, including 0.1% peptone water. All of the diluents, other than 0.1% peptone water, should be removed from the method, since the method was validated with 0.1 peptone water and no other diluent. 2. Package insert, page 6 of 8, applicability. The method refers to yogurt, ready-made-pie, frozen ground beef patties, sandwiches, and dehydrated soup. These are not specific enough. Yogurt and beef should have fat percentages. The types of pie, sandwiches and dehydrated soup should be specified. For example, apple pie, chicken soup, tuna sandwich... The applicability is too broad as written.

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
ER 7	yes
ER 8	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	No
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	Yes
ER 7	No
ER 8	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	Yes
ER 7	No
ER 8	
General Comments about the Method Scope/Applicability:	
ER 1	Method is applicable to a wide variety of foods judging from the PTM study.
ER 2	NA
ER 3	No additional comments
ER 4	Cottage cheese has been used for this collaborative study which is an appropriate matrix since it was also used in the pre-collaborative study.
ER 5	The method scope and applicability are clearly described, and the various enrichment protocols have been extensively presented and summarized in tables. The possibility to use different enrichment media, with one or two enrichment steps according to the matrix, makes the overall analysis more complex, but all information can be found in the document. Nonetheless, no indication has been provided regarding the choice of mLRB versus Demi-Fraser for the user: does it matter?
ER 6	Listeria is a bacterium frequently isolated from different types of food and surfaces. This method allows to detect Listeria in an easy and rapid way with a high sensitivity and specificity.
ER 7	Overall, the method looks good. My main question concerns the application of the guidelines (Appendix J, section 4.3.4, "Matrix Selection"), which affects applicability. Only Demi-Fraser broth was used in the collaborative study, but the official method calls for both Demi-Fraser and mLRB. Section 4.3.4 states "For methods with more than one sample preparation/enrichment, one matrix per procedure may be required in the collaborative study. The determination if the procedures differ significantly to warrant expanding the collaborative study is made by the appropriate method volunteer(s) in consultation with the Study Director." Was a decision reached between the study director and volunteers? If not, then it seems to me that the method may only be validated for use with Demi-Fraser broth.
ER 8	Appropriate

General comments about the method:	
ER 1	Clearly the isothermal feature of the method is very convenient. It would be nice if a few sentences were added outlining the biochemical/molecular basis of the method in general terms.
ER 2	Page 3, row 21-22, Remove shipped to collaborators as it is described at row 35 along with randomizing and blind coding.
ER 3	No additional comments
ER 4	The method appears to be a very rapid method with a simple work flow that allows for the rapid detection of Listeria species.
ER 5	The method is very well described in the various documents , and the various steps of the sample preparation and MDA assay are exhaustively presented, including the ciritical steps. The method can be applied to a large variety of matrices covering different food categories and environmental surfaces. Enrichment media and procedures may vary according to the matrix, and therefore, users shall be clearly informed about the enrichment conditions for given matrices. Enrichment times usually mention a upper limit of incubation: what does happen if the user exceeds this upper value?
ER 6	This is a method very useful for the industry and it allows more short times for detection of Listeria than with culture methods . So, this is very useful for facilitate regional trade.
ER 7	None.
ER 8	Direct and easy to follow
Pros/Strengths of the Manuscript:	
ER 1	Generally a very clearly written manuscript.
ER 2	Very well written.
ER 3	Generally well written.
ER 4	Manuscript is well written and the information flow is in an understandable order.
ER 5	Exhaustive presentation of the various AOAC-PTM validations/extensions. Complete description of the sample preparation protocols. Efficient description of the collab study workflow and organization. Tables are very useful for summarizing the various enrichment protocols of the method, for presenting the results.
ER 6	The method has a high sensitivity and specificity.
ER 7	Well written.
ER 8	Well written with full details

Cons/Weaknesses of the Manuscript:	
ER 1	Some editorial suggestions are made below.
ER 2	NA
ER 3	Need to elaborate on the issues and possible causes of those issues of laboratories whose data were not used in the study.
ER 4	• Correction on table 2 page 17, AOAC OMA 993.12, lab 6 is excluded in the analysis and should have a superscript b. • It is unclear as to why the reference method chosen is not the BAM reference method which would be in this reviewer's opinion a better and more comprehensive method to use. While the reference method used is an OMA method, the use of the BAM method would have given more confidence to regulatory bodies reviewing this method. • The uses of positive and negative controls are not well defined. • There may be some value in stating the ISO status of the testing labs to add more confidence on the results. • The reasons for the exclusion of labs 6 and 13 may need more detail. In both cases the data set suggests that these labs were simply excluded since they detected false negative results which I am sure is not the case; however, no detail scientific explanation is provided. • While many of the acronyms used in the data and statistical tables are well known and are described in the Appendix J of the AOAC method validation guidelines, it may be beneficial to include a section on the explanation of these terms. Since this is my first time reviewing such a manuscript, I am not sure if these were included in the past but they may help some understand the tables.
ER 5	The manuscript does not discuss the reasons behind the selection of DF broth enrichment protocol for the collaborative study: mLRB vs DF? 1 step enrichment vs 2 step enrichment? There are very few mistakes in the report among which one is more relevant: table 2 presenting individual collaborator results seem wrong for the low level test portion results as the total from the table give 67 positive when the report mentions 73. Comparing data with table 2014.2A (p21), it seems that numbers for labs 2 and 8 are wrong in table 2: is that right? Please advise.
ER 6	NO
ER 7	None observed.
ER 8	None
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	Since the cottage cheese is a posturized product, a heat stress inoculum would have been more appropriate; however, this was also not done during the pre-collaborative study.
ER 5	Yes & No. Yes when it relates to the DF-1 step enrichment protocol. No because mLRB and DF-2 step enrichment protocols have not been evaluated during this collaborative study. That is my understanding that each of the different enrichment protocols must be evaluated during a collaborative study to be submitted to OMA first action: am I mistaken? Some of the claimed matrices require a 2-step enrichment protocol (bagged raw spinach and whole cantaloupe)
ER 6	yes
ER 7	I marked "no" for questions 3 - 6 above, because Tables A and B make "claims" that are not supported by the PTM or collaborative studies. Both tables have a section for "other matrices" that include dairy, vegetables, meat, poultry, seafood, and fruit, but none of these categories have been fully validated (only 1 or 2 matrices per category; traditionally, you need at least 3 matrices to claim a category). Please remove the "For Other Matrices" sections from both Tables A and B, so that the Tables will be aligned

	with the applicability statement.
ER 8	Yes
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes.
ER 2	Yes
ER 3	yes
ER 4	YES, the data has been generated in accordance to the collaborative study protocol.
ER 5	The data supports the criteria regarding the use of DF broth and one step enrichment protocol.
ER 6	yes
ER 7	Please remove the "For Other Matrices" sections from both Tables A and B, so that the Tables will be aligned with the applicability statement (see above).
ER 8	Yes
Are there any concerns regarding the safety of the method?	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	To be covered once Safety Advisor review is presented.
ER 6	No
ER 7	No.
ER 8	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No
ER 2	No
ER 3	No
ER 4	I would like to bring the following points for discussion by the group: • While the AOAC guidelines under Appendix J, Annex F prescribe the analysis of the data set as a whole for the POD analysis, is there a need to also consider the equivalency of the methods in each individual lab. This may be outside of the realm of the guidelines, but I believe that this type of evaluation could be useful. For example in this method, overall the combined data set produced 66 and 64 positive results for the presumptive and confirmed candidate results respectively, while a total of 73 positives were detected using the reference method. As a whole in this case the LCL and the UCL of the dPOD encompasses the 0 value showing no statistical differences. This is however not true for two of the participating laboratories,laboratory 7 and 9 both produced differences between the candidate and the reference method which are statistically significant. In both cases the candidate method produced results that were much lower than the reference method. Does this indicate that there was a particular problem with these sample sets, the method or the analysis? The table below shows an example of the results in lab 7 where the candidate method detected 4 confirmed positives whereas the reference method detected 10. • It is interesting to see that sample 9 in the low sample set of the candidate method for labs 7-15 all produced negative results and overall only one lab that in the included sample set produced a positive sample. To illustrate this point, 1 out of 11 labs had a positive sample giving a POD of 9%. This is much lower than the overall POD of about 50% for the sample set and falls outside of the statistical (normal) distribution expected with a 50 % fractional positive result. All other sample sets exhibit a more normal distribution of fractional positive results as would be expected. The same pattern does not repeat anywhere in the reference

	method data set. • The APC counts for the sample sets seem to have a broad range of values where the range covers 3 logs of counts. I was not expecting such a difference given the method that is used to enumerate these results is a well validated and used quantitative method.
ER 5	No concerns.
ER 6	No
ER 7	No.
ER 8	No
General Comments (2)	
ER 1	None.
ER 2	NA
ER 3	No additional comments
ER 4	NA
ER 5	
ER 6	
ER 7	None.
ER 8	
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	No
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are all the figures and tables pertinent?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Could some be omitted and covered by a simple statement?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	
ER 5	No
ER 6	No
ER 7	No
ER 8	Yes
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
General Comments (3)	
ER 1	1.Page 19 is titled Appendix. What is the intent here? 1. An appendix with data? 2. Appended data is not to be published? 2. Figures: omit as they are repetitious. 3. Page 1, line 44: Replace "climates" with "conditions". 4. Page 1, Line 44: Define "high pH" and provide reference. [CONTINUED] Is this an allusion to resistance to alkaline sanitizers or to the high end of the pH 4.3-9.4 growth range of listeria? Page 1, lines 40 & 42: Change "Listeriosis" to "listeriosis". Page 2, line 33: Insert "the reproducibility among different laboratories of" between "compare" and "the". Page3, lines 24-26: Sentence is awkward - restructure. Page 4, lines 3 and 4:Change "(12 high, 12 low and 12 controls for each method)" to "(12 high inoculum, 12 low inoculum and 12 uninoculated controls for each method)". Page 5, line 12: "Appendix"? See comment above. Page 14, lines 42-43: Provide website address. Page 18, Table 2014.1A: Readability of the table could be enhanced by delineating the 4 subsections. For example, one could add blank lines between the Candidate Presumptive, Candidate Confirmed, Positive Reference and dLPOD subsections. Pages 21-22, Table 2014.2A: Suggest making this into 3 tables.
ER 2	NA
ER 3	No additional comments
ER 4	
ER 5	Few edits to be covered. Will be discussed during the meeting.
ER 6	On Page 12 line 30 “to the” is mentioned twice
ER 7	None.
ER 8	

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*?**	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	YES, while some issues may require discussion, the method has proven its performance.
ER 5	Yes but limited to DF and the one step enrichment protocol. Therefore, matrix claim shall be narrowed. To be discussed during the ERP meeting.
ER 6	Yes
ER 7	Yes, as long as Tables A & B are corrected as specified above.
ER 8	Yes
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	Why was only one food collaboratively studied?
ER 2	NA
ER 3	No additional comments
ER 4	NA
ER 5	Recommendation to monitor the false positive rates generated by the method.
ER 6	No
ER 7	None, other than 2 years of field use.
ER 8	User feedback

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
ER 7	yes
ER 8	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	No
ER 7	Yes
ER 8	Yes

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	Yes
ER 8	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	Yes
ER 8	Yes
General Comments about the Method Scope/Applicability:	
ER 1	Table A, pp 8 & 9 of the report, should not have an entry entitled "For Other Matrices", since "other matrices" were not validated in either the pre-collaborative (PTM) or collaborative studies. It should only include validated matrices. Table A, in its current form, implies that the applicability is broader than is substantiated by the validation study.
ER 2	This isothermal nucleic acid amplification method detects L. monocytogenes in a variety of foods and on a variety of environmental surfaces found in food processing plants.
ER 3	NA
ER 4	No additional comments
ER 5	Cottage cheese and deli turkey have been used for the collaborative study which are appropriate matrices and were both used in the pre-collaborative study.
ER 6	The method scope and applicability are clearly described, and the enrichment protocols have been properly presented and summarized in a table. The possibility to use one or two enrichment steps according to the matrix, makes the overall analysis more complex, but all information can be found in the document.
ER 7	Listeria is a bacterium frequently isolated from different types of food and surfaces. This method allows to detect Listeria in an easy and rapid way with a high sensitivity and specificity.
ER 8	Accurately reflects breadth and depth of PTM and collaborative studies

General comments about the method:	
ER 1	None.
ER 2	The inter-laboratory reproducibility of the method is evaluated in two foods, cottage cheese and deli turkey. Heat stressed cells of L. monocytogenes were used for the latter matrix.
ER 3	Page 3, row 27-28, Remove shipped to collaborators as it is described at page 4 row 1 along with randomizing and blind coding.
ER 4	No additional comments
ER 5	The method appears to be a very rapid method with a simple work flow that allows for the rapid detection of Listeria monocytogenes.
ER 6	The method is very well described in the various documents , and the various steps of the sample preparation and MDA assay are exhaustively presented, including the ciritical steps. The method can be applied to a large variety of matrices covering different food categories and environmental surfaces. Enrichment protocol may vary according to the matrix, and therefore, users shall be clearly informed about the enrichment conditions for given matrices. Enrichment times usually mention a upper limit of incubation: what does happen if the user exceeds this upper value?
ER 7	This is a method very useful for the industry and it allows more short times for detection of Listeria than with culture methods . So, this is very useful for facilitate regional trade.
ER 8	Well conceived and scientifically sound
Pros/Strengths of the Manuscript:	
ER 1	Well written.
ER 2	The validation is generally clearly described.
ER 3	Very well written.
ER 4	Generally well written.
ER 5	Manuscript is well written and the information flow is in an understandable order.
ER 6	Exhaustive presentation of the various AOAC-PTM validations/extensions. Complete description of the sample preparation protocols. Efficient description of the collab study workflow and organization. Tables are very useful for summarizing the various enrichment protocols of the method, for presenting the results.
ER 7	The method has a high sensitivity and specificity.
ER 8	Clearly written and explained

Cons/Weaknesses of the Manuscript:	
ER 1	None.
ER 2	The manuscript is lengthy.
ER 3	NA
ER 4	Need to elaborate on the issues and possible causes of those issues of laboratories whose data were not used in the study.
ER 5	• Correction on table 2 page 19 for cottage cheese matrix, AOAC OMA 993.12, lab 6 is excluded in the analysis and should have a superscript b. • It is unclear as to why the reference method chosen is not the BAM reference method which would be in this reviewer's opinion a better and more comprehensive method to use. While the reference method used is an OMA method, the use of the BAM method would have given more confidence to regulatory bodies reviewing this method. • The uses of positive and negative controls are not well defined. • There may be some value in stating the ISO status of the testing labs to add more confidence on the results. • The reasons for the exclusion of labs 6 and 13 for the cottage cheese sample set and lab 8 and 10 for the deli turkey sample set may need more detail. In all cases the data set suggests that these labs were simply excluded since they detected false negative results which I am sure is not the case; however, no detail scientific explanation is provided. • While many of the acronyms used in the data and statistical tables are well known and are described in the Appendix J of the AOAC method validation guidelines, it may be beneficial to include a section on the explanation of these terms. Since this is my first time reviewing such a manuscript, I am not sure if these were included in the past but they may help some understand the tables.
ER 6	There are some mistakes in the report especially regarding the total number of positive samples for the 2 methods and that creates some controversy between text and results. For cottage cheese, page 13, from line 27, candidate method is claimed at 65/132 presumptive positive for low level when 66 shall be accounted (64 confirmed & 2 false positive, according to table 3). The report claimed 63 were confirmed when 64 were confirmed from the presumptive positive (from table 3) + 1 false negative (lab 8).The same applies to the reference method for which it's reported 73/132 positive for low level, but table 3 gave only 67 (the same number as for the MDA Listeria spp study which was using the same samples and therefore the same reference method and so results). These numbers will modify all the calculations reported in table 2014.1A. For deli turkey, page 14, from line 16, the false positive and false negative results obtained at low level for the candidate method shall be clearly disclosed: 66 presumptive positive results of which 64 were confirmed and 2 were not confirmed and so were false positive results. Additionally, 3 other samples were found negative by MDA Listeria monocytogenes but confirmed positive (3 false negative: one from lab 12 and 2 from lab 15). Did lab 12 participate in the ring trial (it has a note "b" in table 4 stipulating that results were not used ... If so the text shall be corrected accordingly; page 20 from line 8). Table 2014.2A, page 26, for lab 1, X = 5 for CP column: from table 3 page 19, I conclude that X = 6 as table 3 reports 5 MDA confirmed positive and 1 false positive (noted "c" & footnote claims false positive results). Same remark applies to lab 3 for which X = 7 when it shall be 8.
ER 7	NO

ER 8	None
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes, except that Table A should be revised as described above.
ER 2	Yes.
ER 3	Yes
ER 4	Yes
ER 5	Since the cottage cheese is a posturized product, a heat stress inoculum would have been more appropriate; however, this was also not done during the pre-collaborative study. The deli turkey appears to have been stressed to an acceptable level.
ER 6	Yes & No. Yes when it relates to the DF-1 step enrichment protocol. No because DF-2 step enrichment protocols have not been evaluated during this collaborative study. That is my understanding that each of the different enrichment protocols must be evaluated during a collaborative study to be submitted to OMA first action: am I mistaken? Some of the claimed matrices require a 2-step enrichment protocol (bagged raw spinach and whole cantaloupe)
ER 7	yes
ER 8	Yes
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes
ER 2	Yes.
ER 3	Yes
ER 4	yes
ER 5	YES, the data has been generated in accordance to the collaborative study protocol.
ER 6	The data supports the criteria regarding the use of DF broth and one step enrichment protocol.
ER 7	yes
ER 8	Yes
Are there any concerns regarding the safety of the method?	
ER 1	No.
ER 2	No.
ER 3	No
ER 4	No
ER 5	No
ER 6	To be covered once Safety Advisor review is presented.
ER 7	No
ER 8	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	N/A.
ER 2	No.
ER 3	No
ER 4	No
ER 5	I would like to bring the following points for discussion by the group: • While the AOAC

	guidelines under Appendix J, Annex F prescribe the analysis of the data set as a whole for the POD analysis, is there a need to also consider the equivalency of the methods in each individual lab. This may be outside of the realm of the guidelines, but I believe that this type of evaluation could be useful. For example in this method for the cottage chese, overall the combined data set produced 66 and 64 positive results for the presumptive and confirmed candidate results respectively, while a total of 73 positives were detected using the reference method. As a whole in this case the LCL and the UCL of the dPOD encompasses the 0 value showing no statistical differences. This is however not true for two of the participating laboratories, laboratory 7 and 9 both produced differences between the candidate and the reference method which are statistically significant. In both cases the candidate method produced results that were much lower than the reference method. Does this indicate that there was a particular problem with these sample sets, the method or the analysis? The table below shows an example of the results in lab 7 where the candidate method detected 4 confirmed positives whereas the reference method detected 10.The deli turkey sample set does not appear to have the same issue above. • It is interesting to see that sample 9 in the low sample set for cottage cheese of the candidate method for labs 7-15 all produced negative results and overall only one lab that in the included sample set produced a positive sample. To illustrate this point, 1 out of 11 labs had a positive sample giving a POD of 9%. This is much lower than the overall POD of about 50% for the sample set and falls outside of the statistical (normal) distribution expected with a 50 % fractional positive result. All other sample sets exhibit a more normal distribution of fractional positive results as would be expected. The same pattern does not repeat anywhere in the reference method data set. • The APC counts for the sample sets seem to have a broad range of values where the range covers 3 logs for cottage cheese and 4 logs for the deli turkey of counts. I was not expecting such a difference given the method that is used to enumerate these results is a well validated and used quantitative method.
ER 6	Some of the numbers need to be checked and edited accordingly.
ER 7	No
ER 8	No
General Comments (2)	
ER 1	None.
ER 2	Suggestions are given below for shortening the manuscript
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	
ER 7	
ER 8	
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes

ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	Yes
ER 3	No
ER 4	No
ER 5	
ER 6	No
ER 7	No
ER 8	Yes
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Does the method contain adequate safety precaution reference and/or statements?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
General Comments (3)	
ER 1	There should be a reminder in the cautionary statement that this test may generate high levels of L. monocytogenes, which can particularly dangerous to the immunocompromised and pregnant women.
ER 2	Page 1, lines 27-28: add "using heat stressed cells" to this sentence. Page 1, lines 28-29: add "using non-stressed cells" to this sentence. Page 2, line 24: change "compare the" to " compare the reproducibility of the". Page 2, line 25: change "to the" to "to the reproducibilities of the methods of the". [CONTINUED] Page 2, line 38: change "with Listeria" to "with heat stressed Listeria". Page 4, line 16: change "(12 high, 12 low, and 12 controls for each method)" to "(12 high inoculum, 12 low inoculum, and 12 uninoculated controls for each method)". Page 7, line 29 to page 12 line 32: Omit this material. Refer to the 3M MDA Listeria spp. manuscript for the relevant material taking care to emphasize the use of the L. monocytogenes reagent already listed in section B(b). Page 13, lines 12 and 14: Omit reference to Appendix. Page 16, references 5, 6, 8: These will not necessarily be accessible to the manuscript readers even if the website were properly provided. The corresponding J. AOAC Int. manuscripts might be more readily available. Page 18, Table 2: Add a footnote detailing the stress temperature and time. Page 21: Omit this blank page. Pages 22 and 23, Tables 2014.1A and .1B: These would be clearer if blank lines separated the five subsections. Also, in both Tables the Candidate Confirmed Positive (CC) sections could well be omitted and the results described in the narrative. It is somewhat confusing that they are not already mentioned in the narrative as are CP and C section results. Page 24, Appendix: Omit this page. Pages 32-37, Fig. titles and Figs: Omit figures
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	Few more edits to be covered. Will be discussed during the meeting.
ER 7	
ER 8	

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*?**	
ER 1	Yes.
ER 2	Yes.
ER 3	Yes
ER 4	Yes
ER 5	YES, while some issues may require discussion, the method has proven its performance.
ER 6	Yes but limited to DF and the one step enrichment protocol. Therefore, matrix claim shall be narrowed. To be discussed during the ERP meeting.
ER 7	Yes
ER 8	Yes
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	Two years of use in the greater food micro community.
ER 2	No.
ER 3	NA
ER 4	No additional comments
ER 5	NA
ER 6	Recommendation to monitor the false positive rates generated by the method.
ER 7	No
ER 8	User comments/concerns

Expert Review Panel
Microbiology for Food and Environmental Surfaces
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned co-chair(s) hereby confirm that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Microbiology for Food and Environmental Surfaces that was held on Wednesday, March 18, 2015 during the AOAC Mid-Year Meeting located at the Hilton Washington DC North, 620 Perry Parkway, Gaithersburg, Maryland 20877.

Wendy McMahon

WENDY MCMAHON, SILLIKER LABORATORIES
Expert Review Panel Co-Chair

MICHAEL BRODSKY

MICHAEL BRODSKY, BRODSKY CONSULTING
Expert Review Panel Co-Chair

April 3, 2015
Date

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Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Members
Michael Brodsky, Brodsky Consulting (Co-Chair)
Wendy McMahon, Silliker Laboratories (Co-Chair)
Maya Achen, Abbott Nutrition
Patrice Arbault, BioAdvantage
Mark Carter, MC2E, Inc.
Yi Chen, U.S. Food & Drug Administration (FDA)
Peyman Fatemi, The Acheson Group LLC
Maria Christina Fernandez, University of Buenos Aires
Tom Hammack, U.S. Food & Drug Administration (FDA)
Tony Hitchins, U.S. Food & Drug Administration/CFSAN (Retired)
Yvonne Salfinger
Sam Mohajer, Canadian Food Inspection Agency[1]

AOAC Official Methods Board Liaisons
Erin Crowley, Q Laboratories
Brad Stawick, Microbac *(Not present)*

Method Authors
Phil Feldsine, BioControl

AOAC Staff
Jim Bradford, Executive Director
Scott Coates
Chris Dent
Kyrstyna McIver
Deborah McKenzie
Nora Marshall
La'Kia Phillips
Karen Silbernagel, AOAC Technical Consultant

Observers
Joe Boison, Canadian Food Inspection Agency
Mike Clark, BioRad
Imola Ferro, MicroVal
Tony Lupo, Neogen Corporation
Kyle Rhoden, DuPont Nutrition
Brooke Schwartz, Brooke Schwartz Consulting
Morgan Wallace, DuPont Nutrition
Paul Wehling, General Mills

[1] Sam Mohajer of the Canadian Food Inspection Agency has changed positions and is no longer serving on the Expert Review Panel.

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Microbiology for Foods and Environmental Surfaces on Wednesday, March 18, 2015 from 8:30 a.m. to 5:00 p.m. during the AOAC Mid-Year Meeting located at Hilton Washington DC North, 620 Perry Parkway, Gaithersburg, Maryland 20877.

The purpose of the meeting was to review and evaluate an OMA modification of AOAC Official Method 2009.03 *Salmonella* in Foods and Environmental Surfaces, Assurance GDS for *Salmonella* submitted by Andrew Lienau of BioControl Systems, Inc., located at 12822 SE 32nd Street, Bellevue, Washington 98005. Supplemental information was also provided to the reviewers which included the single laboratory validation report for the matrix extension of 2009.03 to validate the application of the method for selected spices, specifically curry powder, cumin powder, and chili powder for the detection of *Salmonella*, the OMA Method 2009.03 modification from 2012, the AOAC 2009.03 method, and the method safety checklist.

Criteria for Vetting Experts and Selection Process:
The current Expert Review Panel (ERP) consisting of ten (10) experts and one (1) alternate were submitted for consideration by the Official Methods Board to evaluate candidate methods for microbiology methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Microbiology Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Wendy McMahon (Co-Chair), Michael Brodsky (Co-Chair), Maya Achen, Patrice Arbault, Mark Carter, Peyman Fatimi, Maria Christina Fernandez, Tom Hammack/Yi Chen (alternate), Tony Hitchins, and Yvonne Salfinger.

ERP Orientation:
All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Ten (10) members and one (1) alternate, out of the ten (10) voting members, were present and therefore met a quorum to conduct the meeting. It was also noted that Sam Mohajer of the Canadian Food Inspection Agency has changed positions within his organization and is no longer able to serve on the Expert Review Panel or participate in any AOAC activities.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The ERP reviewed the OMA modification of AOAC Official Method 2009.03, *Salmonella* in Foods and Environmental Surfaces and has approved the matrix extension to include selected spices, curry powder, cumin powder, and chili powder and have re-adopted this method for AOAC First Action Official Method status by consensus as noted in the meeting minutes.

Subsequent ERP Activities:
ERP members are required to track the performance of the recently approved First Action method for a 2 year period effective as of March 18, 2015.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-chairs, Michael Brodsky and Wendy McMahon, welcomed Expert Review Panel (ERP) members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Review of Methods

All ERP members presented a review and discussed the proposed OMA modification matrix extension *of* AOAC Official Method 2009.03, *Salmonella* in Foods and Environmental Surfaces, Assurance GDS for *Salmonella.* On behalf of the method developer, Phil Feldsine was present and able to address the questions and concerns of the ERP members. A summary of comments was provided to the ERP members and the method author.[2] By consensus the ERP presented the following motions for OMAMOD-03 **(AOAC 2009.03).**

MOTION:
Motion by Hitchins; Second by Salfinger, to move method OMAMOD-03 (AOAC 2009.03) to First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 2 opposed, and 1 abstention. *Motion Did Not Pass.**

**Method must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons. Negative vote(s) can be overridden by 2/3 of voting ERP members after due consideration.*

Delineation of Negative Votes/ Discussion
The negative votes were regarding concerns of whether the method was identified as a modification or a matrix extension. There were concerns regarding the TSB, the performance of the PCR, the BHI subculture being used before the TSB, and false negative results. It was noted that there were enough samples and data to run the method with data presented to draw conclusion of the method performance and that there is currently no literature for PCR in spices.

MOTION:
Motion by Hitchins; Second by Salfinger, to move method OMAMOD-03 (AOAC 2009.03) to First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 2 opposed, and 1 abstention. *Motion Passed.*
Negative votes were overridden by 2/3 of voting ERP members.

[2] Attachment 1: Summary of Expert Reviewer Comments for OMAMOD-03 (AOAC 2009.03)

MOTION:
Motion by Hitchins; Second by Salfinger, to have the method developer provide single laboratory validation for at least 1 of the 3 spices with the false negative result.
Consensus demonstrated by: 8 in favor, 1 opposed, and 1 abstention. *Motion Passed.*
Negative votes were overridden by 2/3 of voting ERP members.

IV. Discuss Final Action Requirements for First Action Official Methods (if applicable)
The ERP will discuss, review any additional information (i.e., additional collaborative study data, proficiency testing, and other feedback), track AOAC Official First Action methods for 2 years after adoption, and make recommendations to the Official Methods Board regarding Final Action status.

AOAC staff presented the AOAC First Action Official Method to Final Action Feedback Mechanism for Method Use and Performance Evaluation fo**r** proprietary/sole source AOAC *Official Method*^{SM} (OMA).[3] In accordance to Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis the following criteria must be submitted for AOAC Final Action Official Method consideration regarding the following aspects of the method: method applicability, safety concerns, reference materials, single-laboratory validation, reproducibility/uncertainty and probability of detection, additional feedback from users of method, and ERP additional required information (if applicable). AOAC is continuously seeking performance feedback from the method authors, method developers, and method end users. AOAC staff will revise the feedback form to reflect yes/no questions with options to explain if the answer was marked "no".

V. Discussion on ERP Review of Methods for OMA and PTM Programs
ERP members discussed their roles as ERP members and as Expert Reviewers for the PTM program and how to become more actively involved in the review processes earlier. The ERP members agreed that everyone will be copied on PTM/OMA harmonized collaborative study protocol reviews, in addition to the assigned (1) AOAC Expert, and (2) ERP members.

VI. Harmonization and Implementation
Deborah McKenzie presented the current harmonization activities of the AOAC Research Institute and the AOAC International Stakeholder Panel for Alternative Methods (ISPAM). It was also noted that there will be a symposium at the AOAC Annual Meeting & Exposition in Los Angeles, California on the harmonization programs and how they can work together; this includes MicroVal, Afnor, and NMKL. The ERP was tasked to consider scenarios and a path forward for harmonization, implementation of the use of Broad Range of Foods vs. Variety of Foods claims, and to consider the acceptance criteria for validation studies for ISO 16140.

VII. Action Items
1) Due to the discrepancies of whether or not additional data is required, the ERP will revisit the following AOAC First Action Official Methods:
    a. AOAC Official Method 2013.02: Salmonella Species in a Variety of Foods and Environmental Surfaces (BAX® System Real-Time PCR Assay for Salmonella)
    b. AOAC Official Method 2014.06: Listeria species in Selected Foods and Environmental Surfaces (3M™ Molecular Detection Assay (MDA) Listeria Method)

[3] Attachment 2: First to Final Action Feedback Instructions

   c. AOAC Official Method 2014.07: Listeria monocytogenes in Selected Foods and Environmental Surfaces (3M™ Molecular Detection Assay (MDA) Listeria monocytogenes Method)
2) AOAC Staff will revise the AOAC First Action Official Method to Final Action Feedback Mechanism for Method Use and Performance Evaluation for proprietary/sole source AOAC *Official Method*^{SM} (OMA).
3) AOAC Staff will circulate AOAC Technical Questions to the ERP members for feedback.[4]
4) ERP members to review ISO 16140 along with AOAC Organizational Affiliates.

VIII. Adjournment

MOTION by Hammack, Second by Arbault to adjourn the meeting at 1:22 p.m.
Consensus demonstrated by: 10 in favor, 0 opposed, and 0 abstentions. Unanimous, *Motion Passed.*

[4] Attachment 3: AOAC Technical Questions

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	No
ER 2	NA
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	NA
ER 6	No

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	NA
ER 6	Yes
General Comments about the Method Scope/Applicability:	
ER 1	The method is marketed for the detection of hydrolyzed gluten. Standards for the use of the term gluten-free are focused on 20 ppm. As such, the method's repeated use of different units (prolamin) confuse. Further, the product is marketed for the quantitative detection of hydrolyzed gluten yet the number of gluten concentrations examined do not properly bracket & include 20 ppm. The number of food matrices are insufficient and none of the products had gluten incurred prior to processing at defined levels. The 'incurred' food samples were commercially acquired and the true gluten content was unknown necessitating using the assay to determine content, circular logic more appropriate for a proficiency test and not validation.
ER 2	Can the applicability or scope be moved out of the "Principle" section higher up under the title?
ER 3	A significantly improved write-up for this collaborative study
ER 4	The R5 competitive ELISA is the only method so far that can detect and quantify partially hydrolyzed gluten. It is clear from literature that R5 sandwich ELISA will not work properly. Some criticism may be given on the calibrator used and the calibration using method software (cubic spline) and fixed cut-off. Accuracy may be questioned, but considering we are dealing with an ELISA method dealing with a complex analyte, and looking at previously published results with ELISA methods, the "inaccuracy" is within the scope of other results reported. Still the method shows an LOD that is well-below action level, for most allergen ELISA the emphasis is on LOD. For example Codex 118-1979 (rev 2008) states an LOD of 10 mg/kg, there is no mentioning of an LOQ requirement in Codex. Technicality: Immuno-stimulatory may be right, but immuno-stimulatory does not mean it will cause an adverse reaction, from QQPFP we know it always will cause an adverse reaction (hence called "toxic").

ER 5	RIDASCREEN Gliadin competitive assay can be used for the analysis in fermented and hydrolyzed foods (e.g. beer, starch syrup, malt extract, sourdough, soy sauce etc. The method is based on enzyme immunoassay format using a monoclonal antibody that can react gliadin derived from wheat and related prolamins derived from rye and barley. The antibody binds to the potentially immunostimulatory amino acid sequence QQWPFP which exists as motifs on all the prolamin subunits. The antibody detects intact and partially hydrolyzed prolamins. No cross reactivity has been observed with non-gluten cereals and millets. The calibration curve covers gliadin concentration in a sample of 10 to 270 mg/kg.
ER 6	As mentioned previously, the applicability is limited to the enzyme used for hydrolysis since other hydrolysates are not used in the method validation study.
General comments about the method:	
ER 1	The method has the potential to be of considerable use to the scientific community. However, as multi-laboratory examined, it is at best misleading and unacceptable. There is also a serious problem associated with the lack of a proper standard material and the approach taken of mixing three different grain digests. This counter-productive to meaningful future interpretations.
ER 2	I think it is ok. Fundamentally I'm ok with it, just a few nagging issues with the manuscript and the statistics tables.
ER 3	The authors have addressed the difficulties encountered in reading the original study report in this revision.
ER 4	Looking at the SLV or in-house study of the method, the R5 competitive comes out “pretty good". Just based on such an SLV one could already strongly consider the potential of the method for a 1st action. Next to the in-house validation there is data of the AACC collaborative study. This data is good to have, but it did reveal some of the weaknesses when the method is strongly challenged with difficult matrices. We see more variation and higher LOD, these are all logical outcomes and collaborative studies are needed for a "full acceptance" of a new method in most organizations (AOAC, AACC, CEN). Another point that can be considered is that the AACC results were obtained in 2011. When after a 1st action the final action takes place 2 yrs further the AACC collab document is from > 6 years ago.
ER 5	The method is useful in detecting partially hydrolyzed gluten in foods. The other available test kits for gluten don't have the ability of this analysis. The validation of the method could not establish its accuracy in the lack of availability of a certified reference material. The possibility do exist that the assay could be biased in the lack of proof of its accuracy. The accuracy of the method can be reduced by potential specific enzymes (i.e., proline specific endpetidases) which may be present in fermented and hydrolyzed foods samples. There is a possibility that activities of these types of enzymes may cause false negative. The manuscript states that 90% of the secalins in rye sour dough was not detectable by the assay after fermentation. The lack of an alternate method to estimate secalins in the fermented rye doesn’t allow establishing a true level of secalins in this sample. The secalins were spiked in gluten free quinoa sourdough by fortifying this sample with the fermented sour dough at the levels so that secalin

	concentration in spiked sample is calculated to be 35 and 75 mg/kg. In the absence of true value of secalins in the fermented sourdough the spike values as well as the spike recoveries calculated in these spike materials remain questionable.
ER 6	The method is a good initiative towards hydrolysed gluten detection in foods and uses well characterized R5 antibody in the competitive ELISA.
Pros/Strengths of the Manuscript:	
ER 1	Pro: uses the R5 monoclonal antibody. R-biopharm is a respectable company.
ER 2	ok
ER 3	While the authors clarify that the accuracy of the test cannot be determined, they indicate however, that the method is precise and is therefore suitable and fit-for-purpose.
ER 4	The strength of the manuscript is that it shows that the R5 competitive ELISA is suited for partially hydrolyzed gluten. The reported AACC collab study published in CFW also shows that the method sufficiently met guidelines on recovery and LOD when challenging matrices were used (incurred vs spiked). The manuscript is very technical, but correct. The manuscript describes the current state-of-the-art in detecting hydrolyzed gluten. On the other hand the collab challenge also revealed a weakness - the AACC collab demonstrated high RSDs.
ER 5	The method is useful in detecting partially hydrolyzed gluten in foods. The other available test kits for gluten don't have the ability of this analysis.
ER 6	The method shows good precision. Method will be helpful in hydrolyzed gluten (pepsin-trypsin digested) detection since there is no currently validated method available.
Cons/Weaknesses of the Manuscript:	
ER 1	Cons: work as presented is very misleading. Gluten has a specific target level making the design of quantitative analytical methods straightforward. The validation should have focused on 20 ppm and bracketed this concentration. The use of a mixture of wheat, barley, and rye hydrolysate as a standard makes meaningful /accurate quantification impossible. The food samples should have been made with incurred gluten and subjected to processing/hydrolysis versus spiking with arbitrarily pre-hydrolyzed gluten. Discussion of these limitations might help, but none presented.
ER 2	Data tables still need to be in units of mg/kg gluten, not prolamins.
ER 3	None that I can point out in the revised manuscript.
ER 4	The very technical style does make the manuscript not easy to read. Accuracy and/or high RSD may be identified as weakness - a high lab to lab variation is there. There are however no strict criteria for the allowed RSDs or Horrats of ELISA methods, by definition - e.g. due to the complexity of the analyte (no single molecule) it will fall typically in a Type I. The AOAC guidelines & best practices focus on LOD and recovery of allergen ELISAs. Concerns about high RSD could be valid, but allowing a gluten ELISA with relatively high RSD in AOAC methods is not unprecedented: AOAC 991.19 (2001) for intact gluten in foods.
ER 5	The validation of the method could not establish its accuracy in the lack of availability of a certified reference material. The possibility do exist that the assay could be biased in the lack

	of proof of its accuracy. The accuracy of the method can be reduced by potential specific enzymes (i. e., proline specific endpetidases) which may be present in fermented and hydrolyzed foods samples. There is a possibility that activities of these types of enzymes may cause false negative. The manuscript states that 90% of the secalins in rye sour dough was not detectable by the assay after fermentation. The lack of an alternate method to estimate secalins in the fermented rye doesn't allow establishing its true level in the sample. The secalins were spiked in gluten free quinoa sourdough by fortifying the sample with the fermented sour dough at the levels so that secalin concentration in spiked samples is calculated to be 35 and 75 mg/kg. In the absence of true value of secalins in the fermented sourdough the spike values as well as the spike recoveries calculated in these spike materials may remain questionable.
ER 6	The accuracy of the method may be affected as the standard uses pepsin-trypsin digested prolamins, which may be different than the hydrolyzed gluten in foods. The method may overestimate intact gluten and may not accurately measure gluten in foods containing mixture of intact and hydrolyzed gluten.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	no
ER 2	No collaborative study protocol was given to the ERP
ER 3	Yes
ER 4	This could be better, but is sufficient
ER 5	The ERP was not consulted in creating protocols for the study. I am not finding those ready accessible.
ER 6	Yes
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	not as presented. Makes the claim can detect reliably down to the LOQ, but insufficient data to establish such.
ER 2	yes
ER 3	Yes
ER 4	The SLV/in house study shows the potential strength of the method The AACC collab supports the method, but also revealed weaknesses / points of attention.
ER 5	Yes. The protocols used in the study to hydrolyze the prolamins may not reflect the process which take place in the fermented and hydrolyzes samples with respect to the prolamins. But in the lack of availability of a method or estimation of gluten in fermented and hydrolyzed the method under review may be valuable.
ER 6	Yes

Are there any concerns regarding the safety of the method?	
ER 1	none
ER 2	none
ER 3	No
ER 4	No concerns all is well described
ER 5	No.
ER 6	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	With a %CV of 10%, I would expect only two significant figures. Also, why were only duplicate analyses performed? We routinely use triplicates and report a mean. Here, both individual values are given resulting in tables giving the impression of massive amounts of data, but such is actually not the case.
ER 2	units need changing
ER 3	No
ER 4	No concerns, perhaps that the authors can look at AOAC guidelines only - it is "nice to know" that you come to a lower LOD in the SLV/in house study with IUPAC guidelines; but would you have reported this when the LOD's with an alternative calculation to AOAC's came out with 30-40% higher LOD's?
ER 5	Yes. The manipulations of data to calculate spike recovery may have some questions. The estimations of the true value of the secalins in the fermented sourdough were not confirmed by an alternate established method in this as well as the spiked samples. This may result in question to the values used for calculation of spike recovery. The manuscript throughout except few places in text provides result as mg prolamin/kg. The title of the method states partially hydrolyzed (analysis) in fermented cereal- based products. The kit insert declares it as gliadin analysis. It will be useful to provide results to fit to the main objective (analysis of gluten/gliadin) of the method. The LOD of the method requires clarification mss states 5 mg/kg as LOD determined by manufacturer and they also provide alternate calculation of the LOD = 6.5 mg/Kg. Which value is the represents the method's LOD?
ER 6	No
General Comments (2)	
ER 1	The manuscript presented does not demonstrate the method as valid-for purpose.
ER 2	N/A
ER 3	N/A
ER 4	N/A
ER 5	The manuscript throughout except few places in text provides result as mg prolamin/kg. The title of the method states partially hydrolyzed (analysis) in fermented cereal- based products. The kit insert declares it as gliadin analysis. It will be useful to provide results to fit to the main objective (analysis of gluten/gliadin) of the method.
ER 6	N/A

EDITORIAL EVALUATION CRITERIA	
Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	No
ER 5	Yes
ER 6	Yes
Could some be omitted and covered by a simple statement?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	Yes
ER 5	No
ER 6	No

Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	NA
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
General Comments (3) Editorial	
ER 1	see above
ER 2	Can I get a clean copy of the manuscript? I can't read this thing.
ER 3	There are a couple of reference citations that are still incomplete. There are some with periods in the wrong places. There is one citation with et al. not acceptable in this Journal.
ER 4	NA
ER 5	The method is provided is in good format with enough details.
ER 6	N/A

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*?**	
ER 1	no
ER 2	no - manuscript still has too many deficiencies
ER 3	Yes
ER 4	Yes, but under the condition that some things are done /can be done by the method developer between the stage of 1st Action to Final Action
ER 5	Yes. After the gliadin (Gluten) correction is made throughout mss. The LOD of the method requires clarification. Manuscript states 5 mg/kg as LOD determined by manufacturer and they also provide alternate calculation of the LOD = 6.5 mg/Kg. Which value is the represents the method's LOD? This needs to be clarified before method can be recommended to the first action.
ER 6	No
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	n/a
ER 2	yes. Need to collect more data at other levels (20 mg/kg gluten) before final action.
ER 3	Feedback from users of the method
ER 4	My concern is that by the time to move to final action / accept as final action (2 yrs after 2015) some of the important interlab data presented from AACC will have some years on it since 2011. It may not be a strict rule in AOAC acceptance guidelines to have fresher results, but personally I would like to see some updated results for difficult matrices in multiple labs before we take the final action decision.
ER 5	The estimations of the true value of the secalins in the fermented sourdough were not confirmed by an alternate established method in this as well as the spiked samples. This may result in question to the values used for calculation of spike recovery. This can be further clarified. The authors may provide additional data in different cereal based fermented foods to rule out the possibility of under estimation and false negative in samples which may have endopetidases active towards prolamins.
ER 6	N/A

REVIEWERS

ER 1	Eric A.E. Garber, Ph.D.
ER 2	Paul Wehling
ER 3	Dr. Joe Boison
ER 4	Clyde Don
ER 5	Sneh D. Bhandari
ER 6	Girdhari Sharma

AOAC First Action Official Method to Final Action Feedback Mechanism
Method Use and Performance Evaluation

AOAC INTERNATIONAL and the AOAC Research Institute invite's your feedback regarding the method use and performance of the proprietary/sole source AOAC *Official Method*^{SM} (OMA). In accordance to Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis the following criteria must be submitted for AOAC Final Action Official Method consideration. We are seeking feedback from the method authors, method developers, and method end users regarding the following aspects of the method:

- Method Applicability
- Safety Concerns
- Reference Materials
- Single-Laboratory Validation
- Reproducibility/Uncertainty and Probability of Detection
- Additional Feedback from Users of Method
- Expert Review Panel Additional Required Information (if applicable)

Please read the instructions thoroughly. Review and verify that your information is complete, accurate and inclusive of all required documentation (i.e., supporting data, etc.) to support your feedback.

All OMA methods are accessible in e-OMA available at the AOAC website (www.aoac.org). If you should have any issues with completing this form, please contact La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org.

STEP 1: COMPLETE THE ONLINE FEEDBACK FORM
To Submit Method Feedback: Go to https://adobeformscentral.com/?f=GxxnupYNi3OUPcrTXXDHFw
Please note that you may submit feedback for up to three (3) methods.

STEP 2: INFORMATION TO INCLUDE IN FEEDBACK FORM

- **AOAC Official Methods Number (i.e. 2012.01)**
- **Method Name or Manuscript Title**
  - Manuscript publication reference, if available.
  - OMA methods have manuscripts unless otherwise noted in the OMA. Author(s) name(s), Journal Name, Volume number, Issue number (if applicable), Page numbers (if applicable).
- **Method Applicability**
  - In your experience using the method, does the method perform according to the method's applicability as written?
  - Does the applicability of the method need to be improved, such as potential method scope expansions or are there potential points of concern?
- **Safety Concerns**
  - In your experience with the method, are there any safety concerns that were identified while using or regarding use of the method?
  - All safety concerns identified during the 2-year evaluation period must be addressed.
  - Guidance and support can be obtained from the AOAC Safety Committee.

- **Reference Materials**
  - Document efforts undertaken to locate reference materials. Methods may still progress to Final Action even if reference materials are not available.
  - Guidance and support can be obtained from the AOAC Technical Division on Reference Materials.
- **Single-Laboratory Validation**
  - Data demonstrating response linearity, accuracy, repeatability, LOD/LOQ, and matrix scope must be present. Experimental designs to collect this data may vary with the method protocol and the intended use of the method.
  - Resources can be identified by the AOAC Statistics Committee.
- **Reproducibility/Uncertainty and Probability of Detection**
  - Do you have any information that supports regarding the reproducibility of this method as written? If so, please specify and submit information.
  - For quantitative methods, data demonstrating reproducibility and uncertainty must be present. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method (i.e., collaborative studies, proficiency testing, etc.).
  - For qualitative methods, data must be present demonstrating the probability of detection at specified concentration levels as defined by the SMPR. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method.
  - Guidance and support can be obtained from the AOAC Statistics Committee.
- **Additional Feedback from Users of Method**
  - Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?
  - Document positive and negative feedback from users of the method during the trial period regarding the apparatus and reagents, general instructions, enrichment, results and interpretation, confirmation, etc.
  - Feedback from users demonstrating method ruggedness should be documented.
  - Access to the future availability of vital equipment, reference materials, and supplies.

STEP 4: SUBMIT YOUR FEEDBACK

After you submit your feedback via our online form, you will receive a confirmation email of your completed from that was received by the AOAC Research Institute. We thank you for your feedback.

IMPORTANT THINGS TO REMEMBER

- All documents are required to be submitted electronically through the online feedback form. Scanned copies must be clearly legible.
- This form allows feedback for three (3) separate methods.
- If you have supporting data, other supporting documentation or formal recommendation(s) regarding the method, please attach essential documents.
- Attachment file sizes must be less than 20MB each. Multiple files can be uploaded.

TECHNICAL QUESTIONS AND CONCERNS
EXPERT REVIEW PANEL FOR MICROBIOLOGY FOR FOODS AND ENVIRONMENTAL SURFACES

1. If a company wants to claim multiple test portion sizes of more than one matrix, what are the requirements for PTM AND OMA testing?

   Example: The method developer wants to claim 375g, 325g and 25g ground beef, 375g and 25g spinach - what are the requirements for the collaborative study to get all of these test portion sizes claimed in the OMA? Test 375g ground beef or spinach, test 325g ground beef, AND 25g ground beef or spinach? Is there every an understanding that if you test the largest test portion size that the others are covered?

2. Are unique test portions covered under the OMA if tested in the PTM - carcass rinse, carcass sponge/swab, environmental surface testing? Is there an official response to this?

3. I want to confirm that ANY alternate enrichments tested in the PTM would be required in the collaborative study. If there is a transfer step prior to testing of the alternative method, that would also be considered an alternate enrichment.

4. A recent paper has described 5 new Listeria species, and shown how these species may be associated or not to the existing top 6 Listeria species we usually refer too in our inclusivity studies (L. monocytogenes, L. innocua, L. welshimeri, L. grayi, L. ivanovii and Listeria seeligeri), plus the some species which have been described for more recent years, L. marthi, L. fleischmannii, L. weihenstephanensis and L. rocourtiae.

   Listeria floridensis sp. nov., Listeria aquatica sp. nov., Listeria cornellensis sp. nov., Listeria riparia sp. nov. and Listeria grandensis sp. nov., from agricultural and natural environments
   Reference: International Journal of Systematic and Evolutionary Microbiology (2014), 64, 1882–1889 DOI 10.1099/ijs.0.052720-0

PTM/OMA Review and Protocol development

Expert Review Panel
Microbiology for Food and Environmental Surfaces
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned co-chair(s) hereby confirm that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Microbiology for Food and Environmental Surfaces that was held on Sunday, September 27, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071 USA.

WENDY MCMAHON, SILLIKER LABORATORIES
Expert Review Panel Co-Chair

M. H. Brodsky

MICHAEL BRODSKY, BRODSKY CONSULTING
Expert Review Panel Co-Chair

February 8, 2016

Date

AOAC RESEARCH INSTITUTE
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UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Members - Present
Michael Brodsky, Brodsky Consulting (Co-Chair)
Wendy McMahon, Silliker Laboratories (Co-Chair)
Maya Achen, Abbott Nutrition
Patrice Arbault, BioAdvantage
Peyman Fatemi, The Acheson Group LLC
Maria Christina Fernandez, University of Buenos Aires
Tom Hammack, U.S. Food & Drug Administration (FDA)
Tony Hitchins, U.S. Food & Drug Administration/CFSAN (Retired)

Expert Review Panel Members – Not Present
Mark Carter, MC2E, Inc.
Yi Chen, U.S. Food & Drug Administration (FDA)
Yvonne Salfinger

AOAC Official Methods Board Liaisons
Erin Crowley, Q Laboratories
Brad Stawick, Microbac *(Not present)*

Method Authors
Robert Jechorek, 3M Food Safety
De Ann Benesh, 3M Food Safety
Ron Johnson, BioMerieux
Mark Mozola, Neogen Corporation
Morgan Wallace, DuPont Health and Nutrition

AOAC Staff
Deborah McKenzie
La'Kia Phillips

Observers
Sharon Brunelle, AOAC Technical Consultant
Zerlinde Johnson, AOAC Technical Consultant
Karen Silbernagel, AOAC Technical Consultant

Marcia Armstrong, Qiagen
Christopher Bahrdt, Eurofins GeneScan
Brian Beck, Microbiologics
Patrick Bird, Q Laboratories
Francis Bourdichan, Danone
Brianna Buschbach, Cascade Analytical, Inc.
Catharine Carlin, Merieux Nutrisciences
Jonathan Cloke, Thermo Fisher Scientific
Virendra Gohil, Maxxam
Sunee Himsthongkham, FDA
Hussein Hussein, Health Canada
Irene Iugovaz, Health Canada
Hilde Skar Norli, NMKL
Paul Wehling, Medallion Labs
Guodong Zhang, FDA/CFSAN

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Microbiology for Foods and Environmental Surfaces on Sunday, September 27, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071.

The purpose of the meeting was to review and evaluate OMAMAN-25: Evaluation of the 3M™Petrifilm™Rapid Aerobic Count Plate for the Enumeration of Aerobic Bacteria: Collaborative Study submitted by Bob Jechorek of 3M Food Safety, located at 3M Center, Building 260-06-B-01, St. Paul, MN 55144 and Erin Crowley of Q Laboratories, located at 1400 Harrison Avenue, Cincinnati, OH 45214.

Supplemental information was also provided to the reviewers which included the collaborative study manuscript, collaborative study protocol, method safety checklist, method user guide, and the AOAC *Performance Tested Methods*^{SM} validation report for #121403.

Criteria for Vetting Experts and Selection Process:
The current Expert Review Panel (ERP) consisting of ten (10) experts and one (1) alternate were submitted for consideration by the Official Methods Board to evaluate candidate methods for microbiology methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Microbiology Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Wendy McMahon (Co-Chair), Michael Brodsky (Co-Chair), Maya Achen, Patrice Arbault, Mark Carter, Peyman Fatemi, Maria Christina Fernandez, Tom Hammack/Yi Chen (alternate), Tony Hitchins, and Yvonne Salfinger.

ERP Orientation:
All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) members, out of the ten (10) voting members and one (1) alternate, were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The ERP reviewed OMAMAN-25: Evaluation of the 3M™Petrifilm™Rapid Aerobic Count Plate for the Enumeration of Aerobic Bacteria: Collaborative Study and adopted the method for AOAC First Action Official Method status. In addition, seven (7) AOAC First Action Official Methods were recommended for AOAC Final Action Official Methods status. Those methods are AOAC 2012.02, AOAC 2013.01, AOAC 2013.02, AOAC 2013.09, AOAC 2013.10, AOAC 2013.011, and AOAC 2013.14. These methods were reviewed by consensus as noted in the meeting minutes.

Subsequent ERP Activities:

ERP members are required to track the performance of the recently approved First Action method for a 2 year period effective as of September 27, 2015. ERP to draft recommendation reports to the AOAC Official Methods Board for the methods recommended for AOAC Final Action Official Methods status.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-chairs, Michael Brodsky and Wendy McMahon, welcomed Expert Review Panel (ERP) members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Review of Methods

All ERP members presented a review and discussed OMAMAN-25: Evaluation of the 3M™Petrifilm™Rapid Aerobic Count Plate for the Enumeration of Aerobic Bacteria: Collaborative Study. The method author, Robert Jechorek of 3M Food Safety, was present and able to address the questions and concerns of the ERP members. A summary of comments was provided to the ERP and the method author.[1] By consensus the ERP presented the following motions for OMAMAN-23**.**

Motion by Brodsky; Second by Arbault, to move OMAMAN-25 to AOAC First Action Official Methods status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

Motion by Brodsky; Second by Arbault, to request statistical advisors to come to an agreement on how quantitative microbiological methods are reviewed and to amend the workbooks accordingly**.**
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

Motion by McMahon; Second by Brodsky, method feedback must be submitted during the 2-year tracking period.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

IV. REVIEW OF AOAC RESEARCH INSTITUTE TECHNICAL RECOMMENDATIONS

The ERP reviewed and discussed the responses to the technical consultant questions to assist the AOAC Research Institute Technical Consultants in the development of protocols and studies for independent laboratory testing for the AOAC *Performance Tested Methods*^{SM} (PTM) program and Consulting Services. The Expert Review Panel members previously submitted their feedback regarding specific questions as provided by the AOAC Technical Consultant. The Expert Review Panel discussed the following areas of interest regarding unique test portion sizes, use of expensive equipment for alternative collaborative study design and the use of the new *Listeria* species in the inclusivity studies.

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMOD-03 (AOAC 2009.03)

In regards to multiple and unique test portion sizes, and alternate enrichments, the ERP discussed that the information be considered on a case by case basis. However, it was noted that this information should be captured for future use and the tracking of exceptions regarding sample sizes, etc, should be noted.

The ERP discussed that the use of the 5 new *Listeria* species in the inclusivity studies, the ERP suggested that it should be included in the study, wherever available.

The ERP discussed available information regarding the use of the alternative collaborative study design for expensive equipment. It was noted that ISO 16140 and AOAC Appendix I: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures, both allow for up to three collaborators in the same laboratory, as long as it is independent with separate sample sets. However, AOAC Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis allows for up to five collaborators.

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)

The ERP discussed and reviewed the information received from the method author for the AOAC First Action methods that are or have approached their 2-year tracking period. The method author's were present to answer questions and comments from the ERP. The ERP is required to make a recommendation on AOAC Official First Action methods, 2 years after adoption, to the AOAC Official Methods Board regarding Final Action status. By consensus of the ERP the following motions were noted.

1) **AOAC Official Method 2012.02: Gram-Positive Bacteria Identification**
   Kit Name: VITEK® 2 Gram Positive (GP) Biochemical Identification Method
   Company: bioMérieux
   Method Status: First Action 2012

   Motion by McMahon; Second by Fernandez, to recommend 2012.02 for AOAC Final Action status.
   Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

2) **AOAC Official Method 2013.01: *Salmonella* in a Variety of Foods**
   Kit Name: VIDAS® UP *Salmonella* (SPT) Method
   Company: bioMérieux
   Method Status: First Action 2013

   Motion by McMahon; Second by Fatemi, to recommend 2013.01 for AOAC Final Action status.
   Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

3) **AOAC Official Method 2013.02**: ***Salmonella* Species in a Variety of Foods and Environmental Surfaces**
   Kit Name: BAX® System Real-Time PCR Assay for *Salmonella*
   Company: DuPont Nutrition & Health
   Method Status: AOAC First Action 2013

   Motion by Brodsky; Second by Arbault, to recommend 2013.02 for AOAC Final Action status.
   Consensus demonstrated by: 7 in favor, 0 opposed, and 1 abstention. *Motion Passed.*

4) **AOAC Official Method 2013.09: *Salmonella* in Selected Foods**
Kit Name: 3M™ Molecular Detection Assay (MDA) *Salmonella* Method
Company: 3M Food Safety
Method Status: AOAC First Action 2013, Revised First Action 2014

Motion by McMahon; Second by Hitchins, to recommend 2013.09 for AOAC Final Action status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

5) **AOAC Official Method 2013.10: *Listeria* species in a Variety of Foods and Environmental Surfaces**
Kit Name: VIDAS® UP *Listeria* (LPT) Method
Company: bioMérieux
Method Status: AOAC First Action 2013

Motion by McMahon; Second by Hammack, to recommend 2013.10 for AOAC Final Action status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

6) **AOAC Official Method 2013.11: *Listeria monocytogenes* in a Variety of Foods**
Kit Name: VIDAS® *Listeria monocytogenes* Xpress (LMX) Method
Company: bioMérieux
Method Status: AOAC First Action 2013

Motion by McMahon; Second by Fernandez, to recommend 2013.11 for AOAC Final Action status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

7) **AOAC Official Method 2013.14: Identification of *Salmonella* spp. from Colony Picks**
Kit Name: ANSR® *Salmonella* Confirmation Test
Company: Neogen Corp.
Method Status: AOAC First Action 2013

Motion by McMahon; Second by Hammack, to recommend 2013.14 for AOAC Final Action status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

VI. Discuss Implementation of the AOAC INTERNATIONAL Stakeholder Panel for Alternative Methods (ISPAM) Guidance for Food Categories
ERP will discuss, review and decide on an implementation date for the use of the ISPAM approved guidance documentation.

Motion by McMahon; Second by Arbault, to recommend to the AOAC Official Methods Board (OMB) to adopt Annex A of ISO 16140.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

Motion by McMahon; Second by Achen, to recommend to the AOAC Official Methods Board (OMB) to add definitions of all the AOAC claims for PTM and OMA to Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

IV. Action Items

1) AOAC Staff will update the First to Final Action mechanism and information to include additional validations as part of additional feedback requirements.

V. Adjournment: Meeting concluded at 6:00pm.

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PROPRIETARY VITAMIN METHODS

ERP Name	AOAC Expert Review Panel for Proprietary Vitamin Methods			**Chair(s)**	Shang-Jing (Jean) Pan (Abbott Nutrition)
ERP Formed:	2013	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	1 method
Scope:	Review and adopt methods resulting from sole source method developer				
Roster	1. Shang-Jing (Jean) Pan, Abbott Nutrition (Chair) 2. John Austad, Covance 3. Sneh Bhandari, Merieux NutriSciences 4. Johanna Camera, NIST 5. Sarwar Gilani, Health Canada (retired) 6. Erik Konings, Nestle 7. John Szpylka, Merieux NutriSciences 8. Dave Woollard, Eurofins				
Technical Documents created/used	1. OMA Appendix D				
Methods Adopted *First Action and Final Action status*	*Method not published as ERP requested revisions made by method developer prior to publication and revisions were not completed. No OMA number assigned.*				
Final Action Methods Recommended	Method listed above.				
Additional Input					
Awards/Recognitions					

PROPRIETARY VITAMIN METHODS FOR CONSIDERATION

Meeting minutes:
The Expert Review Panel (ERP) for Proprietary Vitamin Methods convened on Monday, August 26, 2013 during the AOAC Annual Meeting and Exposition at the Palmer House Hilton in Chicago, IL. The ultimate goal of this ERP was to review candidate methods for adoption as AOAC First Action *Official Methods*. ERP members are requested to review methods and to discuss their reviews with the other ERP members during the meeting. Two (2) ERP members, a primary and secondary reviewer, are assigned to review each method. The methods under consideration are folic acid, biotin and pantothenic acid.

Criteria for Vetting Methods to be Considered:
The Proprietary Vitamin methods were reviewed against the approved combined protocol for folic acid, biotin and pantothenic acid and Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis.

Other considerations:
The method for folic acid previously had an initial review and revisions were made to their submitted manuscript. A copy of the tracked changes version of the folic acid method was provided to the ERP.

Conclusion:
The Expert Review Panel reviewed the methods for Folic Acid, Biotin and Pantothenic Acid. One (1) method was approved for First Action Official Method status (OMAMAN-05 Biotin).

Methods Reviewed: Each proprietary vitamin method collected by AOAC for consideration by this ERP is assigned a primary and secondary reviewer as shown below on Table 2.

Table 2

Method No.	Manuscript Title	Primary Reviewer	Secondary Reviewer
OMAMAN-04	Determination of Folic Acid in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study	John Szpylka	Erik Konings
	Method Author(s): Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast		
OMAMAN-05	Determination of Biotin in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study	Johanna Camara	Sawar Gilani
	Method Author(s): Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast		
OMAMAN-06	Determination of Pantothenic Acid in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study	Shang-Jing (Jean) Pan	John Austad
	Method Author(s): Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast		

Safety Reviews of all three methods was completed by Dr. Sneh Bhandari.

Primary and Secondary Evaluation of Method OMAMAN-04

OMAMAN-04: **DETERMINATION OF FOLIC ACID IN FORTIFIED BOVINE MILK-BASED INFANT FORMULA POWDER, FORTIFIED SOYA-BASED INFANT FORMULA POWDER, FORTIFIED CEREALS, UNFORTIFIED CEREALS, VITAMIN TABLETS AND DIETARY SUPPLEMENTS BY SURFACE PLASMON RESONANCE: COLLABORATIVE STUDY**

Author(s): *Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast*
Primary Reviewer: John Szpylka
Secondary Reviewer: Erik Konings

SUMMARY OF METHOD:

A collaborative study was conducted on an inhibition-based protein binding assay using the Biacore Q biosensor instrument and the Biacore Qflex kit folic acid. The samples included infant formula, cereals, premix, vitamin tablet, dietary supplement and baby food. Folic acid from samples within the matrix scope is extracted into water utilizing sonication. Amylase and/or autoclaving are addition extraction steps depending on the matrix. The remaining steps are not listed in the manuscript, but the introduction section summarizes the measurement involving the a mixture of the extraction solution and a competition solution being flowed across an HBS-EP folic acid sensor chip and the reduction in signal measured by surface Plasmon resonance. The level of folic acid in the extraction solution is calculated using external standardization.

GENERAL COMMENTS:

No mention of the method's ability to detect or be influenced by the presence of naturally-occurring folates. The study contained many errors demonstrated by the removal of 36 out of 198 data points (18%). Omitted data should be listed with accompanying reasons. The removal of one of the duplicates reported by the lab impacts determining the method's repeatability for the matrix being studied. Trace folic acid levels were measured in the "blank" matrix by nine of the eleven labs (the other two were "NA" for both replicates). Since the blank material was not tested for folic by an alternate method, the source of the trace positives is unknown. Results from NIST 1849, Soy IF, and NIST 3244 are acceptable in accuracy and precision (assuming the Soy if reference value is accurate – source not reported). Results from the unfortified cereal appear accurate assuming the expected range is accurate – source is not reported. The vitamin premix sample did not show acceptable accuracy or precision (assuming accuracy based on independent measurement).

PROS/STRENGTHS:

The assay employs simple extractions and rapid analyses.

CONS/WEAKNESSES:

The study experienced a number of issues that prohibit interpretation of results. Mold contamination of NIST 2383 compromised the samples resulting in an average measurement ~1/2 of the certified value. Also, only four of the eleven labs have replicate data, therefore repeatability cannot be reliably calculated. For future consideration, the study needs to be repeated. A discussion about the forms analyzed is missing in the manuscript. Intermediate Folic acid solution: gram is probably not the correct unit. (2.5 g/100 ml). Also concentration is expressed in micro liter/mil. The report states that on request of the General Referee a blank sample was included. However, AOAC's Collaborative Study protocol describes the need of a blank sample. This should be corrected. The bias between the certification value of IRMM CRM BCR 421 and the Biacore value needs to be explained.
This might be related to the fact that the certified value is based on total folate determined by microbiological assay. However, when mentioned this should be properly described and discussed.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to move forward to First Action Official Method status.

Entered By:	Szpylka			
Second By:	Konings			
Vote:	7 Approved	**0** Opposed	**0** Abstain	***MOTION PASSED***

Primary and Secondary Evaluation of Method OMAMAN-05

OMAMAN-05: **DETERMINATION OF BIOTIN IN FORTIFIED BOVINE MILK-BASED INFANT FORMULA POWDER, FORTIFIED SOYA-BASED INFANT FORMULA POWDER, FORTIFIED CEREALS, UNFORTIFIED CEREALS, VITAMIN TABLETS AND DIETARY SUPPLEMENTS BY SURFACE PLASMON RESONANCE: COLLABORATIVE STUDY**

Author(s): *Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast*
Primary Reviewer: Johanna Camara
Secondary Reviewer: Sawar Gilani

SUMMARY OF METHOD:

This method uses the Biacore Q instrument. The Biacore Q utilizes bio-molecular interaction with anti-biotin monoclonal antibody and detection by SPR. The Biacore Q uses a fluidics cartridge which consists of a sensor chip coated with gold, which is turn coated with a dextran hydrogel. When the sample is introduced into fluidics cartridge, it allows the analyte to pass over the sensor chip. As an inhibition assay, the excess antibody binds to the biotin-immobilized sensor chip generating the SPR responses, which is inversely related to the biotin content. The method is an enzymatic and/or autoclave extraction of biotin from food and vitamin materials followed by dilution and analysis on the Biacore QTM biosensor instrument (label-free protein binding based assay for surface plasmon resonance). Quantitative determination of biotin due to SPR response that is proportional to remaining free binding protein as mixture of biotin extract and excess binding protein binds to immobilized surface of SPR chip.

GENERAL COMMENTS:

The sensitivity of the SPR assay technology coupled with the specificity of the ligand-binding protein interaction should provide reliable results. However, there is some possibility of non-specific binding which may result in inaccurate results. The samples should have enough dilution to avoid this possibility. Therefore, this method may not be robust enough like other established methods and one can get unpredictable results once a while. When reviewing this method against the specifications found in Appendix D, it appears that the study contains the appropriate number of labs (11) and studies an appropriate number of samples (9 blind duplicates) and that the samples represent a variety of types (commercial products, reference materials, and blanks). The study also included a variety of international laboratories (service, government, corporate, etc.)

PROS/STRENGTHS:

This method is rapid (11 hours for up to 40 samples) compared to traditional microbiological methods, which can take 2-3 days. It is a cost-efficient assay in laboratories in developed countries and correlates well with the established microbiological assay (R2 = 0.9805).

CONS/WEAKNESSES:
There are low recoveries (72-76%) for milk powder, milk-based infant formula and soy-based infant formula. Further work may be required to improve low recoveries such as sample preparation by sonication versus autoclaving. Potential negative cost impact on laboratories in developing countries is not known. The blank material used in this study was manufactured from assumed biotin-free components, but the blank was not verified as "biotin-free" by a secondary method. Method was not able to be applied to baby food due to spoilage of study samples prior to analysis.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: To move forward to First Action Official Method status.
Amendment: To define the scope to be fortified milk based infant formula powder, fortified soy based infant formula powder, and vitamin tablets.

Entered By:	Camara
Second By:	Gilani
Vote:	7 Approved **0** Opposed **0** Abstain ***MOTION PASSED***

RECOMMENDATION FOR OMAMAN-05:
A precautionary note regarding the corrosiveness and safe handling of concentrated HCl and sodium hydroxide may be added to the method.

Primary and Secondary Evaluation of Method OMAMAN-06

OMAMAN-06: DETERMINATION OF PANTOTHENIC ACID IN FORTIFIED BOVINE MILK-BASED INFANT FORMULA POWDER, FORTIFIED SOYA-BASED INFANT FORMULA POWDER, FORTIFIED CEREALS, UNFORTIFIED CEREALS, VITAMIN TABLETS AND DIETARY SUPPLEMENTS BY SURFACE PLASMON RESONANCE: COLLABORATIVE STUDY

Author(s): *Dr. Anthony O'Kane, MSB, Institute for Global Food Security (IGFS), Queens University Belfast*

Primary Reviewer: Shang-Jing (Jean) Pan

Secondary Reviewer: John Austad

SUMMARY OF METHOD:

This is an inhibition assay, pantothenic acid binds to poly (A)-binding protein (PABP) and inhibits the interaction between PABP and immobilized PA on sensor chip. PABP binds to PA on sensor chip and generates surface plasmon resonance (SPR) response which is inversely related to PA level in sample. Using a simple sample preparation, Vitamin B5 (Pantothenic Acid) can be determined using SPR that uses the specificity of the ligand binding protein interaction to determine the concentration Pantothenic Acid.

GENERAL COMMENTS:

This method is a quick assay, more time-efficient than micro-assay and chromatography based methods. Due to the use of proprietary instrumentation and kits, it limits the number of vendors a laboratory could use to purchase. The manuscript indicates the costs are less than HPLC and MS instrumentation, but no actual costs are used. There is limited applicability of the instrument.

PROS/STRENGTHS:

This method is very fast and there is a quick determination of B5 in specified matrices versus legacy microbiological methods.

CONS/WEAKNESSES:

It is a proprietary assay and it has a limited scope. The method only uses specific instrumentation and kits. It was also noted that there are high RSDs in some of the matrices.

EXPERT REVIEW PANEL VOTE AND RECOMMENDATION

MOTION: Not to move forward for First Action Official Methods status.

Entered By: Austad
Second By: Pan
Vote: **7** Approved **0** Opposed **0** Abstain ***MOTION PASSED***

ATTENDEE LIST

EXPERT REVIEW PANEL MEMBERS

Name	*Company*
Shang-Jing (Jean) Pan, Chair	**Abbott Laboratories**
John Austad	Covance Laboratories
Sneh Bhandari	Silliker Laboratories
Johanna Camara	NIST
Sawar Gilani	(Retired)
Erik Konings	Nestle
John Szpylka	Silliker Laboratories
Not Present	
David Woollard	Eurofins NZ

AOAC STAFF

Deborah McKenzie
Tien Milor
La'Kia Phillips

OBSERVERS

Name	*Company*
Simon Bevis	R-Biopharm
Chris Blake	Nestle
Robert Clifford	Shimadzu
Jon Devries	Medallion Labs/Gen Mills
Harvey Indyk	Fonterra
Greg Jaudzems	Nestle
Elaine Marley	R-Biopharm
Edwin Phifer	FDA/SRL
Eural Porter	FDA/SRL
Aniko Solyom	GAAS Analytical
Nancy Thiex	AOAC Consultant
Xun Yan	Amway

PROFILE OF AOAC EXPERT REVIEW PANEL FOR FOOD ALLERGEN METHODS - GLUTEN

ERP Name	AOAC Expert Review Panel for Food Allergens - Gluten Methods			**Chair(s)**	Terry Koerner (Health Canada) and Shang-Jing (Jean) Pan (Abbott Nutrition)
ERP Formed:	2014	**Number of Methods Adopted**	3 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the detection or determination of food allergen compounds in food products				
Roster	1. Terry Koerner, Health Canada 2. Shang-Jing Pan, Abbott Nutrition 3. Joe Boison, Canadian Food Inspection Agency 4. Clyde Don, Foodphysica 5. Bert Popping, Mérieux NutriSciences 6. Girdhari Sharma, US FDA 7. Paul Wehling, Medallion Labs / General Mills 8. Jupiter Yeung, Nestle Nutrition 9. Sneh Bhandari, Silliker 10. Eric Garber, US FDA				
Technical Documents created/used	OMA Appendix L OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.03 - Gluten in Rice Flour and Rice-Based Food Products AOAC 2015.05 – Partially Hydrolyzed Gluten in Fermented Cereal-Based Products AOAC 2015.16 – Gluten in Processed and Nonprocessed Corn Products				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions					

AOAC RESEARCH INSTITUTE
Official Methods of Analysis℠ (OMA)
Expert Review Panel on Food Allergens - Gluten

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on Food Allergens - Gluten held on March 20, 2014.

Shang-Jing (Jean) Pan, Expert Review Panel Chair

4/7/2014
Date

Please sign, date and fax this document to La'Kia Phillips at 301-924-7089.

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for **OMAMAN-09: Detection Of Gluten In Food By Enzyme Immunoassay Method Based On A Specific Monoclonal G12 Antibody To The Celiac Toxic Amino Acid Prolamin Sequences**. **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Thursday, March 20, 2014.

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-09	**DETECTION OF GLUTEN IN FOOD BY ENZYME IMMUNOASSAY METHOD BASED ON A SPECIFIC MONOCLONAL G12 ANTIBODY TO THE CELIAC TOXIC AMINO ACID PROLAMIN SEQUENCES: COLLABORATIVE STUDY** *AUTHORS* Elisabeth Halbmayr-Jech, Adrian Rogers, Clyde Don, Michael Prinster, Romer Labs Division Holding GmbH, Technopark 1, 3430 Tulln, Austria, Romer Labs UK Ltd, Block 5, The Heath Technical & Business Park, Runcorn, Cheshire WA7 4QX, United Kingdom, Foodphysica, Vogelwikke 12, 6665 HP Driel, The Netherlands, Romer Labs Inc, 1301 Stylemaster Drive, Union, MO 63084-1156, USA *COLLABORATORS* G. Augustin, C. Brewe, Z. Bugyi, S. Tomoszi, D. Clarke, P. Cressey, A. Firzinger, J. Gelroth, M. Hemingway, R. Hochegger, J. Jolly, P. Kasturi, P. Koehler, C. Poirier, T. Koerner, A. Rogers, G. Sharma, R. Sherlock, C. Sousa, S. Taylor, J. Topping, P. Wehling, M. Marquard

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Methods status based upon the revisions to the manuscript and the supplemental information.	*N/A*	MOTION PASSED ***UNANIMOUS*** *Wheling, Garber*	March 20, 2014

EXPERT REVIEW PANEL MEMBERS

Shang-Jing Pan, Abbott Nutrition
Sneh Bhandari, Silliker, Inc.
Joe Boison, Canadian Food Inspection Agency
Eric Garber, US FDA
Todd Marrow, University of Guelph
Girdhari Sharma, FDA
Paul Wehling, General Mills, Inc.

Not Present

Bert Popping, Eurofins Scientific, Inc.
Terry Koerner, Health Canada

AOAC STAFF

Jim Bradford, Executive Director
Delia Boyd
Deborah McKenzie
La'Kia Phillips

OBSERVERS

Michael Prinster, Romer Labs

Below are the noted reviewers for OMAMAN-09. Expert Review Panel members are required to review the method for discussion.

Reviewer	
Joe Boison	Primary Reviewer
Todd Marrow	Secondary Reviewer
Julie Drotz	Safety Reviewer
Sidney Sudberg*	Statistical Reviewer

**Statistical Review was not completed as assigned. Paul Wehling completed the review on behalf of Sidney Sudberg.*

Expert Review Panel
Microbiology for Food Allergens - Gluten
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Food Allergens – Gluten was held on Wednesday, December 10, 2014 at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

SHANG JING (JEAN) PAN, ABBOTT NUTRITION
Expert Review Panel Chair

_____

Date

AOAC RESEARCH INSTITUTE
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair (s)
Shang-Jing Pan, Abbott Nutrition

Expert Review Panel Members
Joe Boison, Canadian Food Inspection Agency
Girdhari Sharma, US FDA
Paul Wehling, General Mills, Inc.
Terry Koerner, Health Canada

Sneh Bhandari, Silliker, Inc. *(Not Present)*
Eric Garber, US FDA *(Not Present)*
Todd Marrow, University of Guelph *(Not Present)*
Bert Popping, Eurofins Scientific, Inc. *(Not Present)*

Method Authors
Dr. Markus Lacorn, R-Biopharm
Patricia Meinhardt, R-Biopharm
Sean Tinkey, Executive Vice President, R-Biopharm

AOAC Staff
Jim Bradford, Executive Director
Scott Coates
Deborah McKenzie
Tien Milor
La'Kia Phillips

Observers
N/A

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Microbiology for Foods and Environmental Surfaces on Wednesday, December 10, 2014 from 1:00pm to 5:00pm at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

The purpose of the meeting was to review and evaluate OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based on the R5 Monoclonal Antibody to Determine Partially Hydrolysed Gluten in Foods Containing Wheat, Rye, and Barley. The co-study directors were Dr. Markus Lacorn from R-Biopharm AG, located at An der neuen Bergstraße 17, 64297 Darmstadt, Germany and Patricia Meinhardt of R-Biopharm Inc. located at 870 Vossbrink Drive, Washington, MO 63090.

The candidate method was reviewed and supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, MSDS Conjugate, MSDS Sample Dilute, MSDS Standard, AACC Published Article (includes protocol), and In House Validation report, and the package insert.

Criteria for Vetting Experts and Selection Process:
The following eight (8) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for food allergens (gluten) as per the Expert Review Panel (ERP) Policies and Procedures. The following candidates are highly recommended by the Food Allergens Community and other Food Allergen experts. Many of the following candidates have participated in various AOAC activities, including but limited to, members of Committee H, and expert reviewers for the AOAC Research Institute's PTM Program. The members are Shang Jing Pan (Chair), Sneh Bhandari, Joe Boison, Eric Garber/ Girdhari Sharma (Alternate), Terrence Koerner, Todd Marrow, Bert Popping, and Paul Wehling.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, November 5, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Five (5) out of the eight (8) voting members were present and therefore did not met a quorum to conduct the meeting and present a vote. It was also noted that Bert Popping, who was not present, will not participate on this Expert Review Panel until after March 2015.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based On The R5 Monoclonal Antibody To Determine Partially Hydrolysed Gluten In Foods Containing Wheat, Rye, And Barley and request recommended changes, clarifications and/or revisions to the manuscript during the meeting. This information is noted in the meeting minutes.

Subsequent ERP Activities:
A follow-up teleconference is scheduled for Tuesday, December 16, 2014 at 11:00am EST. The purpose will be to review the revised edits and present a vote of the method as long as a quorum is present.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair Shang-Jing (Jean) Pan welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

A brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement, and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a quick overview of the Expert Review panel process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

IV. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based On The R5 Monoclonal Antibody To Determine Partially Hydrolysed Gluten In Foods Containing Wheat, Rye, and Barley. The method author Dr. Markus Lacorn of R-Biopharm was present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[1] In addition, Sean Tinkey, the Executive Director President of R-Biopharm was also present. The following comments noted below are the recommendations of the Expert Review Panel members who were present.

1) This sentence: "RIDASCREEN® Gliadin competitive is used for the analysis of fermented and hydrolyzed food (e.g. beer, starch syrup, starch, malt extract, sourdough, soy sauce) which are declared as "gluten-free"." Must be moved above the Principle section of the OMA.
2) Change "celiac toxic sequence QQPFP" to "immuno-stimulatory" and provide additional clarification.
3) Bracket all mentions of "prolamins" with gluten measurement.
    a. Add additional statement to tables in reference to both prolamins and gluten.
4) Accuracy and recovery statistics should have a paragraph explaining the nature of the sour dough samples specifically and also generally the difficulty estimating trueness in general.
    a. Provide paragraph regarding the hordein digest. How much of the extraction is the hordein? Indicate % hordein
5) Insist level at 20 for gluten collaborative study.
6) Concentration range? Bracket level at or below 20. – Additional work?
7) Remove: "The celiac activity of oats is still under discussion, and the Codex standard notes that the allowable level for oats in foods not contaminated with wheat, rye or barley may be determined at the national level(1)."
8) Remove: "besides intact prolamins,"

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-19

9) We may suggest additional data which may change the definition of the LOD. (6.5-75 LOD):
    a. Discussion Section: "The competitive ELISA is able to determine partially hydrolyzed fragments in samples with concentrations as low as 5 mg gliadin/kg (10 mg gluten/kg)."
    b. Conclusion Section: "The competitive ELISA is able to determine partially hydrolyzed fragments in samples with concentrations as low as 5 mg gliadin/kg (10 mg gluten/kg)."
10) Remove from Table 3 (last sentence)
    a. HORRAT values

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)

No further action was discussed at this time.

VI. Adjournment

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	No
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	No
ER 7	
ER 8	
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	No
ER 7	
ER 8	
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	No
ER 2	Yes
ER 3	No
ER 4	Yes
ER 5	No
ER 6	Yes
ER 7	
ER 8	
Are the conclusions statements valid based upon data presented?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	
ER 8	

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	
ER 8	
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	No
ER 6	Yes
ER 7	
ER 8	
General Comments about the Method Scope/Applicability:	
ER 1	There is a fundamental problem with the language used to describe the method. Though nothing is technically wrong, the description has led to end-users concluding a suitability that at times is neither valid or proper. Correction of the language to properly delineate the method's utility and accuracy should solve these problems. However, as it currently stands, this method has resulted unfortunately with some end-users making incorrect conclusions and causing unnecessary problems in dealings with other organization
ER 2	LOQ of 5 ppm is suitable for testing foods containing partially hydrolyzed gluten.
ER 3	This method is for determination of gluten in fermented and hydrolyzed "gluten free" foods.
ER 4	The method has been validated for testing fermented foods and beverages to determine whether they comply with the Codex threshold of 20 mg of gluten per kg in total for products for which a gluten-free label is claimed. It is applicable to beer, starch syrup, starch, malt extract, sourdough and soy sauce all of which are declared as gluten-free.
ER 5	The scope of the method under investigation is appropriate for the intended use. The matrices selected in this investigation are some of the matrices that will be of interest and applicable to laboratories using this method.
ER 6	The method may be suitable for hydrolyzed gluten produced by pepsin trypsin (PT) digestion, but does not provide enough evidence for broader applicability to other forms of gluten in hydrolyzed or fermented form.
ER 7	
ER 8	

General comments about the method:	
ER 1	The method onto itself performs as would be expected for a Competitive ELISA. This means, that the precision of the results and sensitivity are acceptable . However, in the documentation language is at times used that with a few minor tweaks may correct any misleading aspects (e.g., the sensitivity of the Competitive ELISA is not better than that of the Sandwich. Instead, the Sandwich ELISA is inappropriate for the detect not hydrolyzed gluten making it unreliable due to variability in the number of antigenic epitopes associated with proteolysis.
ER 2	Good that all labs that participated in the study were familiar with ELISA tests, but probably would be valuable to have experience with competitive ELISA tests. Also, the RIDASOFT cubic spline function calculation is much different than the typical gliadin/gluten tests and requires a higher understanding of what is going into this calculation.
ER 3	data indicates this is a fit-for-purpose method.
ER 4	Samples are extracted by a simple sample preparation and analyzed within 40 minutes.
ER 5	This configuration of the R5 ELISA format has a definite advantage over the current sandwich format and will be a valuable tool for measuring gluten in certain matrices. This method should provide a more accurate assessment of the gluten in products where gluten is randomly hydrolyzed.
ER 6	The competitive ELISA based on R5 antibody may be better than the sandwich format for hydrolyzed gluten as it requires one epitope for effective binding. The accuracy of the method needs to be evaluated towards not only the PT digest, but other hydrolyzed form with known gluten concentration as well.
ER 7	
ER 8	
Pros/Strengths of the Manuscript:	
ER 1	The existence of competitive ELISAs is a strength and needed format for gluten methodology.
ER 2	Naturally gluten-contaminated starch syrup was included as one of the samples.
ER 3	all required sections are included
ER 4	The R5 ELISA is commercially available. The Competitive ELISA which was selected for the determination of partially hydrolysed gluten is more sensitive than the R5 Sandwich ELISA. The Competitive Assay quantitates beside intact prolamins, peptide fragments of prolamins from wheat (gladins), rye (secalin), and barley (hordein).
ER 5	I found the manuscript concise and ordered appropriately.
ER 6	Good approach towards detection of hydrolyzed gluten as currently this is a big challenge. Uses R5 antibody which is well characterized. The in-house validation is extensive and provides useful information on the method. The method shows good precision.
ER 7	
ER 8	

Cons/Weaknesses of the Manuscript:	
ER 1	The field is a sensitive one. Meaning, anything with the potential to mislead can cause more problems than it would solve. Unfortunately, the manuscript needs serious tweaking to its language.
ER 2	Missing the results of labs E, F, and K that have been listed in the AACCI validation report. I have some concerns that 3 out of 16 labs were not technically competent to perform this method. I can't tell if the average calculation of the 5.3 ppm gliadin in the naturally gluten-contaminated starch syrup (Sample 5) includes results that are all from the cubic spline calculation or a combination of cubic spline and second-order polynomial curve as some of the lab results are lower than 5 ppm. I'm assuming that even the results below 5 ppm were calculated by cubic spline.
ER 3	data is not complete
ER 4	None that could be identified even though I wondered if the method will always have to be operated in a separate test room under normal circumstances. For the Collaborative study, participants were advised to carry out the test in a separate room due to the low detection limit and the possibility of contamination.
ER 5	There are some typos and broken links to references.
ER 6	The hydrolyzed standards may not detect intact gluten accurately. The accuracy of the method towards hydrolyzed gluten can not be ascertained from recoveries since the gluten concentration used for spiking was also determined by this method for sourdough and the gluten in starch syrup was unknown. Also it is not clear if the gluten spiked in beer was PT-hordein or mixture of PT-digests from wheat, rye and barley. The LOD and LOQ is not calculated from this study, though discussed in the in-house validation and the AACCI paper.
ER 7	
ER 8	
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	technically yes, however the need to drop 3 labs from the collaborative study and upon reviewing the data the observation that a significant variance still occurred with a few labs, makes the issue of method precision real (though not unexpected considering such is common with competitive ELISAs)
ER 2	Yes
ER 3	yes
ER 4	Yes.
ER 5	I believe that he data collected supports the AACCI guidelines as written.
ER 6	The method is unclear as the PT-hordein is discussed as used in beer spiking, while the section F of the method mentions the spiking material was identical to standard solution (PT fragments from wheat, rye, barley). Also the actual gluten in rye sourdough cannot be determined by the method used for validation. What was the gluten concentration based on the amount in rye used for sourdough preparation.
ER 7	
ER 8	
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes
ER 2	Yes
ER 3	yes
ER 4	Yes.

ER 5	I believe that the data collected in the collaborative study does not meet the AOAC guidelines or the guidance developed from the AOAC food allergen community for gluten analysis. The data collected for the beer matrix is not appropriate for Appendix D is the strictest sense. I believe the method should be tested at the CODEX recommended level for gluten free foods (20mg/kg of gluten or 10mg/kg prolamin) and the sample concentration should backed this concentration. The AOAC guidance on gluten ELISA methods suggest even more concentration levels should be studied. The same criteria should be used for the other two matrices tested.
ER 6	Yes, the data collected from various laboratories are in accordance with the study protocol.
ER 7	
ER 8	
Are there any concerns regarding the safety of the method?	
ER 1	No
ER 2	No
ER 3	no
ER 4	No.
ER 5	No
ER 6	No
ER 7	
ER 8	
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	yes, see item above regarding the dropping of three labs and the remaining variance in the data.
ER 2	As previously mentioned, how was the 5.3 ppm result for the naturally contaminated sample derived. I'm assuming all results were calculated with the RIDASOFT software.
ER 3	yes. the original data table (including lab E, F, K, table 1 in AACCI publication) should also be included in the manuscript
ER 4	The statistical advisor's review was not available at the time of this review.
ER 5	No
ER 6	No
ER 7	
ER 8	
General Comments (2)	
ER 1	see above
ER 2	
ER 3	
ER 4	
ER 5	NA
ER 6	
ER 7	
ER 8	
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	

ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	
ER 8	
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	
ER 8	

Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	No
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	
ER 8	
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	
ER 8	
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	
ER 7	
ER 8	
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	Yes
ER 3	No
ER 4	No
ER 5	No
ER 6	No
ER 7	
ER 8	

Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	
ER 8	
General Comments (3)	
ER 1	NA
ER 2	More Tables are included in the AACCI validation report.
ER 3	p.15, FDA regulation reference is missing
ER 4	The references need to be revised to align with AOAC journal reference citation.
ER 5	The references need to be reformatted to the AOAC requirements.
ER 6	Yes
ER 7	
ER 8	

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*?**	
ER 1	NOT WITHOUT CORRECTIONS TO LANGUAGE!
ER 2	Yes
ER 3	yes
ER 4	Recommend adoption as First Action Method Status
ER 5	No, I believe more work needs to be done to assess this method around the Codex Gluten level in all of the selected matrices.
ER 6	No
ER 7	
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	NA
ER 2	There could be end-user issues with this kit. Unfortunately, with 3 out of 16 labs not able to complete the testing appropriately, there would appear to be a higher level of competence required to use this test kit.
ER 3	user feedback
ER 4	The Collaborative study has been completed and so it remains for AOAC to monitor and obtain feedback from method users for the next 2-year grace period.
ER 5	This method needs to be tested with proline specific protease because the target epitope for this monoclonal has two proline residues.
ER 6	NA
ER 7	

Expert Review Panel for Food Allergens - Gluten

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned co-chair(s) hereby confirm that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Food Allergens – Gluten that was held on Thursday, March 19, 2015 during the AOAC Mid-Year Meeting located at the Hilton Washington DC North, 620 Perry Parkway, Gaithersburg, Maryland 20877.

Terry Koerner

TERRY KOERNER, HEALTH CANADA
Expert Review Panel Co-Chair

(Not Present)

SHANG JING (JEAN) PAN, ABBOTT NUTRITION
Expert Review Panel Co-Chair

April 13, 2015

Date

AOAC RESEARCH INSTITUTE
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UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Co-Chairs
Terry Koerner, Health Canada
Shang-Jing Pan, Abbott Nutrition *(Not Present)*

Expert Review Panel Members
Sneh Bhandari, Silliker, Inc. *(Not Present)*
Joe Boison, Canadian Food Inspection Agency
Clyde Don, Foodphysica
Eric Garber, US FDA
Todd Marrow, University of Guelph
Bert Popping, Mérieux NutriSciences
Girdhari Sharma, US FDA
Paul Wehling, Medallion Labs / General Mills
Jupiter Yeung, Nestle Nutrition

Method Authors
Dr. Markus Lacorn, R-Biopharm
Patricia Meinhardt, R-Biopharm

AOAC Staff
Jim Bradford, Executive Director
Delia Boyd
Deborah McKenzie
La'Kia Phillips

Observers
Carmen Diaz
Mary Trucksess, US FDA (Retired)
Tony Lupo, Neogen Corporation

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:

AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Food Allergens – Gluten on Thursday, March 19, 2015 during the AOAC Mid-Year Meeting located at the Hilton Washington DC North, 620 Perry Parkway, Gaithersburg, Maryland 20877.

The purpose of the meeting was to review and evaluate OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based on the R5 Monoclonal Antibody to Determine Partially Hydrolysed Gluten in Foods Containing Wheat, Rye, and Barley and OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick.

The co-study directors for OMAMAN-19 were Dr. Markus Lacorn from R-Biopharm AG, located at An der neuen Bergstraße 17, 64297 Darmstadt, Germany and Patricia Meinhardt of R-Biopharm Inc. located at 870 Vossbrink Drive, Washington, MO 63090. The candidate method (OMAMAN-19) was reviewed and supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, MSDS for Conjugate, MSDS for Sample Dilute, MSDS for Standard, AACC Published Article (includes protocol), In-house validation report, the package insert, Expert Review Panel Chair Report, AOAC CSO Memorandum, second set of reviewer comments and a presentation of next steps for OMAMAN-19.

The co-study directors for OMAMAN-20 were Dr. Markus Lacorn and Thomas Weiss from R-Biopharm AG, located at An der neuen Bergstraße 17, 64297 Darmstadt, Germany. The candidate method (OMAMAN-20) was reviewed and supplemental information was also provided to the reviewers which included the collaborative study manuscript, AACC Technical Committee report, method safety checklist, method user guide, MSDS for the cocktail solution, MSDS for sample dilute, MSDS for Ethanol, Preamble of Corporate Design, and the In-house validation report.

Criteria for Vetting Experts and Selection Process:

The following ten (10) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for food allergens (gluten) as per the Expert Review Panel (ERP) Policies and Procedures. The following candidates are highly recommended by the Food Allergens Community and other Food Allergen experts. Many of the following candidates have participated in various AOAC activities, including but limited to, members of Committee H, and expert reviewers for the AOAC Research Institute's PTM Program. The members are Shang Jing Pan (Co-Chair), Terrence Koerner (Co-Chair), Sneh Bhandari, Joe Boison, Clyde Don, Eric Garber/ Girdhari Sharma (Alternate), Todd Marrow, Bert Popping, Paul Wehling, and Jupiter Yeung.

ERP Orientation:

All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar.

Expert Review Panel Meeting Quorum

The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the ten (10) members and one (1) alternate, were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The ERP reviewed OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based On The R5 Monoclonal Antibody to Determine Partially Hydrolysed Gluten in Foods Containing Wheat, Rye, And Barley and have adopted this method for AOAC First Action Official Method status by consensus.

In addition, the ERP reviewed OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick and have tabled this method for a decision at a future date as reflected in the meeting minutes.

The decisions above have been captured and reflected in the meeting minutes.

Subsequent ERP Activities:
ERP members are required to track the performance of the recently approved First Action method for a 2 year period effective as of March 19, 2015.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-Chair Terry Koerner welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-19: R-Biopharm, Competitive Enzyme Immunoassay Based on the R5 Monoclonal Antibody to Determine Partially Hydrolysed Gluten in Foods Containing Wheat, Rye, and Barley. The method author Dr. Markus Lacorn and Patricia Meinhardt of R-Biopharm were present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[1] By consensus, the ERP presented the following motions for OMAMAN-19.

MOTION:
Motion by Wehling; Second by Boison, to move OMAMAN-19 to AOAC First Action Official Methods status.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

MOTION:
Motion by Garber; Second by Wehling, First to Final action requirements for OMAMAN-19 require single laboratory validation data showing at and below 20 mg/kg.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick. The method author Dr. Markus Lacorn of R-Biopharm was present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[2] By consensus, the ERP presented the following motions for OMAMAN-20.

MOTION:
Motion by Don; Second by Yeung, to table the discussion and decision on OMAMAN-20 pending R-Biopharm's response to the ERP comments.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

IV. Discuss Final Action Requirements for First Action Official Methods (if applicable)

No further action was discussed at this time. The First to Final Action requirements set for OMAMAN-19 were noted in the meeting minutes above.

V. Adjournment: Meeting adjourned at 5:22pm

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-19

[2] Attachment 2: Summary of Expert Reviewer Comments for OMAMAN-20

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	No
ER 2	NA
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	NA
ER 6	No

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	No
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	No
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	NA
ER 6	Yes
General Comments about the Method Scope/Applicability:	
ER 1	The method is marketed for the detection of hydrolyzed gluten. Standards for the use of the term gluten-free are focused on 20 ppm. As such, the method's repeated use of different units (prolamin) confuse. Further, the product is marketed for the quantitative detection of hydrolyzed gluten yet the number of gluten concentrations examined do not properly bracket & include 20 ppm. The number of food matrices are insufficient and none of the products had gluten incurred prior to processing at defined levels. The 'incurred' food samples were commercially acquired and the true gluten content was unknown necessitating using the assay to determine content, circular logic more appropriate for a proficiency test and not validation.
ER 2	Can the applicability or scope be moved out of the "Principle" section higher up under the title?
ER 3	A significantly improved write-up for this collaborative study
ER 4	The R5 competitive ELISA is the only method so far that can detect and quantify partially hydrolyzed gluten. It is clear from literature that R5 sandwich ELISA will not work properly. Some criticism may be given on the calibrator used and the calibration using method software (cubic spline) and fixed cut-off. Accuracy may be questioned, but considering we are dealing with an ELISA method dealing with a complex analyte, and looking at previously published results with ELISA methods, the "inaccuracy" is within the scope of other results reported. Still the method shows an LOD that is well-below action level, for most allergen ELISA the emphasis is on LOD. For example Codex 118-1979 (rev 2008) states an LOD of 10 mg/kg, there is no mentioning of an LOQ requirement in Codex. Technicality: Immuno-stimulatory may be right, but immuno-stimulatory does not mean it will cause an adverse reaction, from QQPFP we know it always will cause an adverse reaction (hence called "toxic").

ER 5	RIDASCREEN Gliadin competitive assay can be used for the analysis in fermented and hydrolyzed foods (e.g. beer, starch syrup, malt extract, sourdough, soy sauce etc. The method is based on enzyme immunoassay format using a monoclonal antibody that can react gliadin derived from wheat and related prolamins derived from rye and barley. The antibody binds to the potentially immunostimulatory amino acid sequence QQWPFP which exists as motifs on all the prolamin subunits. The antibody detects intact and partially hydrolyzed prolamins. No cross reactivity has been observed with non-gluten cereals and millets. The calibration curve covers gliadin concentration in a sample of 10 to 270 mg/kg.
ER 6	As mentioned previously, the applicability is limited to the enzyme used for hydrolysis since other hydrolysates are not used in the method validation study.
General comments about the method:	
ER 1	The method has the potential to be of considerable use to the scientific community. However, as multi-laboratory examined, it is at best misleading and unacceptable. There is also a serious problem associated with the lack of a proper standard material and the approach taken of mixing three different grain digests. This counter-productive to meaningful future interpretations.
ER 2	I think it is ok. Fundamentally I'm ok with it, just a few nagging issues with the manuscript and the statistics tables.
ER 3	The authors have addressed the difficulties encountered in reading the original study report in this revision.
ER 4	Looking at the SLV or in-house study of the method, the R5 competitive comes out “pretty good". Just based on such an SLV one could already strongly consider the potential of the method for a 1st action. Next to the in-house validation there is data of the AACC collaborative study. This data is good to have, but it did reveal some of the weaknesses when the method is strongly challenged with difficult matrices. We see more variation and higher LOD, these are all logical outcomes and collaborative studies are needed for a "full acceptance" of a new method in most organizations (AOAC, AACC, CEN). Another point that can be considered is that the AACC results were obtained in 2011. When after a 1st action the final action takes place 2 yrs further the AACC collab document is from > 6 years ago.
ER 5	The method is useful in detecting partially hydrolyzed gluten in foods. The other available test kits for gluten don't have the ability of this analysis. The validation of the method could not establish its accuracy in the lack of availability of a certified reference material. The possibility do exist that the assay could be biased in the lack of proof of its accuracy. The accuracy of the method can be reduced by potential specific enzymes (i.e., proline specific endpetidases) which may be present in fermented and hydrolyzed foods samples. There is a possibility that activities of these types of enzymes may cause false negative. The manuscript states that 90% of the secalins in rye sour dough was not detectable by the assay after fermentation. The lack of an alternate method to estimate secalins in the fermented rye doesn’t allow establishing a true level of secalins in this sample. The secalins were spiked in gluten free quinoa sourdough by fortifying this sample with the fermented sour dough at the levels so that secalin

	concentration in spiked sample is calculated to be 35 and 75 mg/kg. In the absence of true value of secalins in the fermented sourdough the spike values as well as the spike recoveries calculated in these spike materials remain questionable.
ER 6	The method is a good initiative towards hydrolysed gluten detection in foods and uses well characterized R5 antibody in the competitive ELISA.
Pros/Strengths of the Manuscript:	
ER 1	Pro: uses the R5 monoclonal antibody. R-biopharm is a respectable company.
ER 2	ok
ER 3	While the authors clarify that the accuracy of the test cannot be determined, they indicate however, that the method is precise and is therefore suitable and fit-for-purpose.
ER 4	The strength of the manuscript is that it shows that the R5 competitive ELISA is suited for partially hydrolyzed gluten. The reported AACC collab study published in CFW also shows that the method sufficiently met guidelines on recovery and LOD when challenging matrices were used (incurred vs spiked). The manuscript is very technical, but correct. The manuscript describes the current state-of-the-art in detecting hydrolyzed gluten. On the other hand the collab challenge also revealed a weakness - the AACC collab demonstrated high RSDs.
ER 5	The method is useful in detecting partially hydrolyzed gluten in foods. The other available test kits for gluten don't have the ability of this analysis.
ER 6	The method shows good precision. Method will be helpful in hydrolyzed gluten (pepsin-trypsin digested) detection since there is no currently validated method available.
Cons/Weaknesses of the Manuscript:	
ER 1	Cons: work as presented is very misleading. Gluten has a specific target level making the design of quantitative analytical methods straightforward. The validation should have focused on 20 ppm and bracketed this concentration. The use of a mixture of wheat, barley, and rye hydrolysate as a standard makes meaningful /accurate quantification impossible. The food samples should have been made with incurred gluten and subjected to processing/hydrolysis versus spiking with arbitrarily pre-hydrolyzed gluten. Discussion of these limitations might help, but none presented.
ER 2	Data tables still need to be in units of mg/kg gluten, not prolamins.
ER 3	None that I can point out in the revised manuscript.
ER 4	The very technical style does make the manuscript not easy to read. Accuracy and/or high RSD may be identified as weakness - a high lab to lab variation is there. There are however no strict criteria for the allowed RSDs or Horrats of ELISA methods, by definition - e.g. due to the complexity of the analyte (no single molecule) it will fall typically in a Type I. The AOAC guidelines & best practices focus on LOD and recovery of allergen ELISAs. Concerns about high RSD could be valid, but allowing a gluten ELISA with relatively high RSD in AOAC methods is not unprecedented: AOAC 991.19 (2001) for intact gluten in foods.
ER 5	The validation of the method could not establish its accuracy in the lack of availability of a certified reference material. The possibility do exist that the assay could be biased in the lack

	of proof of its accuracy. The accuracy of the method can be reduced by potential specific enzymes (i. e., proline specific endpetidases) which may be present in fermented and hydrolyzed foods samples. There is a possibility that activities of these types of enzymes may cause false negative. The manuscript states that 90% of the secalins in rye sour dough was not detectable by the assay after fermentation. The lack of an alternate method to estimate secalins in the fermented rye doesn't allow establishing its true level in the sample. The secalins were spiked in gluten free quinoa sourdough by fortifying the sample with the fermented sour dough at the levels so that secalin concentration in spiked samples is calculated to be 35 and 75 mg/kg. In the absence of true value of secalins in the fermented sourdough the spike values as well as the spike recoveries calculated in these spike materials may remain questionable.
ER 6	The accuracy of the method may be affected as the standard uses pepsin-trypsin digested prolamins, which may be different than the hydrolyzed gluten in foods. The method may overestimate intact gluten and may not accurately measure gluten in foods containing mixture of intact and hydrolyzed gluten.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	no
ER 2	No collaborative study protocol was given to the ERP
ER 3	Yes
ER 4	This could be better, but is sufficient
ER 5	The ERP was not consulted in creating protocols for the study. I am not finding those ready accessible.
ER 6	Yes
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	not as presented. Makes the claim can detect reliably down to the LOQ, but insufficient data to establish such.
ER 2	yes
ER 3	Yes
ER 4	The SLV/in house study shows the potential strength of the method The AACC collab supports the method, but also revealed weaknesses / points of attention.
ER 5	Yes. The protocols used in the study to hydrolyze the prolamins may not reflect the process which take place in the fermented and hydrolyzes samples with respect to the prolamins. But in the lack of availability of a method or estimation of gluten in fermented and hydrolyzed the method under review may be valuable.
ER 6	Yes

Are there any concerns regarding the safety of the method?	
ER 1	none
ER 2	none
ER 3	No
ER 4	No concerns all is well described
ER 5	No.
ER 6	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	With a %CV of 10%, I would expect only two significant figures. Also, why were only duplicate analyses performed? We routinely use triplicates and report a mean. Here, both individual values are given resulting in tables giving the impression of massive amounts of data, but such is actually not the case.
ER 2	units need changing
ER 3	No
ER 4	No concerns, perhaps that the authors can look at AOAC guidelines only - it is "nice to know" that you come to a lower LOD in the SLV/in house study with IUPAC guidelines; but would you have reported this when the LOD's with an alternative calculation to AOAC's came out with 30-40% higher LOD's?
ER 5	Yes. The manipulations of data to calculate spike recovery may have some questions. The estimations of the true value of the secalins in the fermented sourdough were not confirmed by an alternate established method in this as well as the spiked samples. This may result in question to the values used for calculation of spike recovery. The manuscript throughout except few places in text provides result as mg prolamin/kg. The title of the method states partially hydrolyzed (analysis) in fermented cereal- based products. The kit insert declares it as gliadin analysis. It will be useful to provide results to fit to the main objective (analysis of gluten/gliadin) of the method. The LOD of the method requires clarification mss states 5 mg/kg as LOD determined by manufacturer and they also provide alternate calculation of the LOD = 6.5 mg/Kg. Which value is the represents the method's LOD?
ER 6	No
General Comments (2)	
ER 1	The manuscript presented does not demonstrate the method as valid-for purpose.
ER 2	N/A
ER 3	N/A
ER 4	N/A
ER 5	The manuscript throughout except few places in text provides result as mg prolamin/kg. The title of the method states partially hydrolyzed (analysis) in fermented cereal- based products. The kit insert declares it as gliadin analysis. It will be useful to provide results to fit to the main objective (analysis of gluten/gliadin) of the method.
ER 6	N/A

EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	No
ER 5	Yes
ER 6	Yes
Could some be omitted and covered by a simple statement?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	Yes
ER 5	No
ER 6	No

Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	NA
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
General Comments (3) Editorial	
ER 1	see above
ER 2	Can I get a clean copy of the manuscript? I can't read this thing.
ER 3	There are a couple of reference citations that are still incomplete. There are some with periods in the wrong places. There is one citation with et al. not acceptable in this Journal.
ER 4	NA
ER 5	The method is provided is in good format with enough details.
ER 6	N/A

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*?**	
ER 1	no
ER 2	no - manuscript still has too many deficiencies
ER 3	Yes
ER 4	Yes, but under the condition that some things are done /can be done by the method developer between the stage of 1st Action to Final Action
ER 5	Yes. After the gliadin (Gluten) correction is made throughout mss. The LOD of the method requires clarification. Manuscript states 5 mg/kg as LOD determined by manufacturer and they also provide alternate calculation of the LOD = 6.5 mg/Kg. Which value is the represents the method's LOD? This needs to be clarified before method can be recommended to the first action.
ER 6	No
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	n/a
ER 2	yes. Need to collect more data at other levels (20 mg/kg gluten) before final action.
ER 3	Feedback from users of the method
ER 4	My concern is that by the time to move to final action / accept as final action (2 yrs after 2015) some of the important interlab data presented from AACC will have some years on it since 2011. It may not be a strict rule in AOAC acceptance guidelines to have fresher results, but personally I would like to see some updated results for difficult matrices in multiple labs before we take the final action decision.
ER 5	The estimations of the true value of the secalins in the fermented sourdough were not confirmed by an alternate established method in this as well as the spiked samples. This may result in question to the values used for calculation of spike recovery. This can be further clarified. The authors may provide additional data in different cereal based fermented foods to rule out the possibility of under estimation and false negative in samples which may have endopetidases active towards prolamins.
ER 6	N/A

REVIEWERS

ER 1	Eric A.E. Garber, Ph.D.
ER 2	Paul Wehling
ER 3	Dr. Joe Boison
ER 4	Clyde Don
ER 5	Sneh D. Bhandari
ER 6	Girdhari Sharma

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
ER 7	yes
ER 8	No
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	No
ER 3	NA
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	No
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	No

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	No
ER 8	No
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
General Comments about the Method Scope/Applicability:	
ER 1	In-house validation report was not available to review the method.
ER 2	The method is well established and is known to generate false positives with soy products (e.g., soy milk) that the use of milk powder does not eliminate. This is never mentioned but instead the use of milk powder is presented as a solution~ .The authors should re-write the documents with a more open / critical perspective. -There is also a serious question of why they did not use corn flour that was totally free of gluten and prepare cookies with known levels of 0, etc etc.
ER 3	none.
ER 4	Method is well written and the experimental details and instructions provided to participants were very clearly laid out. The pre-collaborative tests were organized properly and sufficient time was given to participants to demonstrate proficiency prior to undertaking to analyze the collaborative method samples.
ER 5	The method is very sensitive, I wonder that maybe -looking at the action level of 20 mg/kg- the LFD method could be too sensitive? Another point as with the R5 sandwich, the gluten content = 2 x gliadin. Unifying results to gluten content will be helpful instead of using mg/kg gliadin
ER 6	The dip stick assay based on R5 antibody is meant for a rapid qualitative visual detection of gluten in processed and non-processed samples. The scope of the method to detect intact gluten (prolamins) includes matrices like corn, wheat, rye and barley. The non-processed samples are extracted in 60% ethanol and processed samples are extracted in a proprietary cocktail provided in the kit. The assay has been designed to detect gluten well below the

	threshold level of 20 mg/Kg. The reproducibility of the assay has been evaluated in corn sample and processed samples like cookies and corn snacks. The assay provides positive results above 2.5 mg/kg in non-processed foods and above 4 mg/kg in processed foods. The positive results are produced if a surface contains more than 1to 2 mcg gliadin/100 cm2. .
ER 7	NA
ER 8	The R5 dipstick has been in the market for a long time. It's applicability to food matrices are recognized. However, food surfaces application appears to be an afterthought. No validation data were presented or reported elsewhere. As mentioned in the manuscript, a positive band can have non-uniform color intensity due to in-homogenous distribution of gluten on the dipstick sample pad. This may be a sampling protocol flaw and may give an inconsistent result.

General comments about the method:	
ER 1	In-house validation report was not available to review the method.
ER 2	The method is well established and is known to generate false positives with soy products (e.g., soy milk) that the use of milk powder does not eliminate. This is never mentioned but instead the use of milk powder is presented as a solution~ .The authors should re-write the documents with a more open / critical perspective. -There is also a serious question of why they did not use corn flour that was totally free of gluten and prepare cookies with known levels of 0, etc etc.
ER 3	none.
ER 4	Method is well written and the experimental details and instructions provided to participants were very clearly laid out. The pre-collaborative tests were organized properly and sufficient time was given to participants to demonstrate proficiency prior to undertaking to analyze the collaborative method samples.
ER 5	The method is very sensitive, I wonder that maybe -looking at the action level of 20 mg/kg- the LFD method could be too sensitive? Another point as with the R5 sandwich, the gluten content = 2 x gliadin. Unifying results to gluten content will be helpful instead of using mg/kg gliadin
ER 6	The dip stick assay based on R5 antibody is meant for a rapid qualitative visual detection of gluten in processed and non-processed samples. The scope of the method to detect intact gluten (prolamins) includes matrices like corn, wheat, rye and barley. The non-processed samples are extracted in 60% ethanol and processed samples are extracted in a proprietary cocktail provided in the kit. The assay has been designed to detect gluten well below the threshold level of 20 mg/Kg. The reproducibility of the assay has been evaluated in corn sample and processed samples like cookies and corn snacks. The assay provides positive results above 2.5 mg/kg in non-processed foods and above 4 mg/kg in processed foods. The positive results are produced if a surface contains more than 1to 2 mcg gliadin/100 cm2. .
ER 7	NA
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Pros/Strengths of the Manuscript:	
ER 1	Well designed study with appropriate controls. study includes validation of methods for both processed and non-processed foods
ER 2	none that was obvious. Other dipsticks have been more rigorously validated with inclusion of cross-reactivity (inclusivity / exclusivity agents). and use of surface testing.
ER 3	I think the method looks pretty good. Some issues with the manuscript
ER 4	The authors clearly identify gliadin as what is being measured by the test kit and this is made

	clear throughout the paper
ER 5	The manuscript is technically and scientifically sound
ER 6	The method is useful in qualitative detecting of gluten in non-processed and processed foods relatively rapidly. It's utility is also in rapid detection of surface contamination by gluten. Data presentation and the manuscript are easy to understand.
ER 7	NA
ER 8	The strength of the manuscript is also a weakness of the validation study. The authors offered an alternative statistic approach to define the minimum level in a positive result and a maximum level in a negative observation. This is important for the end users or manufacturers to make an informed risk based management decision, and this information is lacking in Appendix N.
Cons/Weaknesses of the Manuscript:	
ER 1	Study lacks validation of method for surfaces
ER 2	Considering the manufacturer's reputation and the quality of the product, a more serious submission was expected.
ER 3	Lack of clarity around use of gliadin and gluten - it seems the terms are used interchangeably. I feel the authors need to go one way or the other. Personally, I prefer gluten as a basis, since gluten is in the title of the method.
ER 4	My only concern is that since this is a test kit to detect gluten as the title says why is it that the authors are not reporting the detected as gluten when it is well known that there is always a factor of 2 to be applied to the gliadin concentration. Why do they have to leave it to the end user to do that simple arithmetic in order to define whether the test result is compliant with international (Codex) guidelines or not.
ER 5	Perhaps a very technical write-up which could be improved on readability.
ER 6	The reproducibility of the assay has been evaluated in corn sample and processed samples like cookies and corn snacks. The reproducibility of the assay performance was not done for non-processed samples like wheat, rye and barley which are indicated in the scope of the kit. The kit is suggested to have great utility in rapid detection of surface contamination by gluten. But the reproducibility of this type analysis was not undertaken in the collaborative study.
ER 7	NA
ER 8	The study design deviates from the Appendix N. There is no defined LOD. The study should also include product consistency, shelf-life or stability of the kit, Lot-to-lot consistency, and consistency within same lot studies.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes
ER 2	no
ER 3	yes
ER 4	Yes
ER 5	The collaborative study supports the potential of this method as shown in the SLV/in house study. Although corn is the most used base in the collab.

ER 6	No, The described scope and applicability of the kit is not supported for all the mentioned matrices or big categories.
ER 7	Yes
ER 8	No, no surface swab data. No data or reference cited to claim no high-dose hook effect in page 3, line 82.
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes
ER 2	no
ER 3	No protocol was provided to the ERP
ER 4	Yes
ER 5	Yes
ER 6	The ERP was not consulted in creating protocols for the study. I am not finding those ready accessible.
ER 7	Yes
ER 8	No, no surface swab data. No data or reference cited to claim no high-dose hook effect in page 3, line 82.
Are there any concerns regarding the safety of the method?	
ER 1	No
ER 2	none
ER 3	no
ER 4	No
ER 5	So far no comments, all necessary precautions are well described
ER 6	Yes, the use of ethanol and the cocktail may have some safety issues but those have been addressed by the authors in the manuscript.
ER 7	NA
ER 8	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No
ER 2	Yes, the authors undermine the statistical evaluation when an excuse is given that two samples were 'apparently' swapped by a lab instead of accepting the results and performing the statistical analysis.
ER 3	Yes - need to consult Stats Committee for further discussion of the procedures given here.
ER 4	No
ER 5	Some of the tables need editing (titles look blurred sometimes)
ER 6	The data presented in Table 2 suggest that for the sample 5 three of the labs. Could not detect it positive in most of their tested 10 replicates (0-1 out of 10) whereas the remaining labs did report 7-10 positives out of tested 10 replicates of the sample. The sample contains 3.2 mg gliadin/kg. The Result Reporting section of the manuscript states that a negative result is

	expected if the sample contains less than 4 mg gliadin/kg. 15 of the labs out of 18 reported positive result for this sample and 3 negative. Thus 83% of the participating lab found the sample positive and about 17% negative. Does this mean that Result reporting key presented in the manuscript need a revision or a review? Three labs which found sample negative reported 9-10 tested replicates of the samples negative and the labs which found positive most of the tested 10 replicates positive. This can lead to an issue when the assay is put in operation and the same sample if tested by different labs, 17% labs can come up with negative results while the remaining labs will call it a positive with respect to gluten.
ER 7	NA
ER 8	OMA Statistic Advisor should review the proposed statistical approach and justifications.
General Comments (2)	
ER 1	NA
ER 2	If there was a serious problem with the data analysis using by-eye examination, why not use a dip-stick reader?
ER 3	NA
ER 4	NA
ER 5	NA
ER 6	The manuscript throughout except few places in text provides result as mg gliadin/kg. Gliadin in parenthesis may be added in title of the manuscript after mention of Gluten.
ER 7	NA
ER 8	NA
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	No
ER 2	Yes
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	No
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	No
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	Yes
ER 5	Yes
ER 6	No
ER 7	No
ER 8	No
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	No
ER 3	NA
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	No
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes

General Comments (3) Editorial	
ER 1	NA
ER 2	NA
ER 3	I am concerned that this method, having come from AACC International has not been approved yet by the technical committees of AACCI. This ERP should wait until after AACC has vetted the data and report before considering first action on this method.
ER 4	
ER 5	Some editing needed in tables and use of gluten / gliadin (gluten = 2 x gliadin)
ER 6	The method is provided is in a good format with enough details.
ER 7	NA
ER 8	NA

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*?**	
ER 1	Yes
ER 2	no
ER 3	not at this time
ER 4	Yes
ER 5	Yes, but first some revisions (gluten / gliadin) need to be done in the manuscripts and check whether the collab provided from AACC is 'free' to use in publications when moving to final action
ER 6	The additional information is required from the authors before the manuscript can be recommended for First action. The information pertinent to why only corn samples selected and not other cereals, and no surface sample was part of the collaborative study as all these are in the scope and applicability of the method. Also authors comments about 83% labs found sample positive at around 3 mg/kg of gliadin. The gliadin content of less than 4 mg/kg in the processed samples are expected to be negative per results reporting section of the manuscript.
ER 7	Yes, I would recommend adoption as First Action Status.
ER 8	No, not at current state. However, if the authors consider the recommendations and revise the manuscript First Action status can be considered.
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	...
ER 2	Omission of known cross-reactvities and other limitations, along with a misleading title. are unacceptable.
ER 3	no
ER 4	Feedback from end users regarding how the method works in their hands in their respective laboratories
ER 5	The provided collab data from AACC has strong focus on corn flour / corn based matrices (snack). This may limit the scope of the method when moved to final action. If possible, additional matrices to broaden the (matrix) scope when moving towards final action.
ER 6	This will depend on authors' response to comments regarding getting the method to the First Action status.
ER 7	I believe the method should be tested on different surfaces and in a wider variety of matrices.
ER 8	No comment at this time.

REVIEWERS

ER 1	Girdhari Sharma
ER 2	Eric Garber
ER 3	Paul Wehling
ER 4	Dr. Joe BOISON
ER 5	Clyde Don
ER 6	Sneh D. Bhandari
ER 7	Terry Koerner
ER 8	Jupiter Yeung

Expert Review Panel for Food Allergens - Gluten

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned co-chair(s) hereby confirm that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Food Allergens – Gluten that was held on Wednesday, September 30, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071 USA.

TERRY KOERNER, HEALTH CANADA
Expert Review Panel Co-Chair

SHANG JING (JEAN) PAN, ABBOTT NUTRITION
Expert Review Panel Co-Chair

_____

Date

AOAC RESEARCH INSTITUTE
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Co-Chairs
Terry Koerner, Health Canada
Shang-Jing Pan, Abbott Nutrition

Expert Review Panel Members
Joe Boison, Canadian Food Inspection Agency
Clyde Don, Foodphysica
Bert Popping, Mérieux NutriSciences
Girdhari Sharma, US FDA
Paul Wehling, Medallion Labs / General Mills
Jupiter Yeung, Nestle Nutrition

Sneh Bhandari, Silliker, Inc. *(Not Present)*
Eric Garber, US FDA *(Not Present)*

Method Authors
Dr. Markus Lacorn, R-Biopharm
Patricia Meinhardt, R-Biopharm

AOAC Staff
Jim Bradford, Executive Director
Scott Coates
Deborah McKenzie
La'Kia Phillips

Observers
Zerlinde Johnson, AOAC Technical Consultant
Maria Nelson, AOAC Technical Consultant

Laura Allred, GFCO
Adam Bouchard, Elution Technologies
Michelle Colgrave, CSIRO
Melandie Dawns, FARRP-UNL
Mark Garkbuff, Sigma Aldrich
Thomas Grace, BiaDiagnostics/ET
Sigrid Haas-L, R-Biopharm
Tiffany Highben, The J.M. Smucker Co.
Phil Johnson, FARRP-UNL
Debra Lambchecht, FARRP-UNL
Eric Marceau, CFIA
Ivo Meier-Wiedenbach, Biotecon
Armen Mirzoian, TTB
Lynn Neimann, FARRP-UNL
Lars Reimann, Eurofins
Scott Radcliffe, Romer Labs
Eric Reyes, Sigma Aldrich
Andre Schruben, Sciex
Darsa Siantar, TTB
Ryan Viator, Neogen
Jennifer Sealey Voyksher, ImmunogenX
Jerry Zweigenbaum, Agilent

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:

AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Food Allergens – Gluten on Wednesday, September 30, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071.

The purpose of the meeting was to review and evaluate OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick that was tabled during the Expert Review Panel meeting held on March 19, 2015 during the AOAC Mid-Year Meeting.

The co-study directors for OMAMAN-20 were Dr. Markus Lacorn and Thomas Weiss from R-Biopharm AG, located at An der neuen Bergstraße 17, 64297 Darmstadt, Germany. Dr. Markus Lacorn and Patricia Meindhardt were present during the Expert Review Panel session. The candidate method (OMAMAN-20) was reviewed and supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, method user guide, material safety data sheet for cocktail solution, material safety data sheet for sample diluent, in-house validation – revised, method evaluation card, responses to Expert Review Panel comments, and responses to statistical review comments.

Criteria for Vetting Experts and Selection Process:

The following nine (9) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for food allergens (gluten) as per the Expert Review Panel (ERP) Policies and Procedures. The following candidates are highly recommended by the Food Allergens Community and other Food Allergen experts. Many of the following candidates have participated in various AOAC activities, including but limited to, members of Committee H, and expert reviewers for the AOAC Research Institute's PTM Program. The members are Shang Jing Pan (Co-Chair), Terrence Koerner (Co-Chair), Sneh Bhandari, Joe Boison, Clyde Don, Eric Garber/ Girdhari Sharma (Alternate), Bert Popping, Paul Wehling, and Jupiter Yeung. Please note that Todd Marrow has resigned from this Expert Review Panel.

ERP Orientation:

All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar.

Expert Review Panel Meeting Quorum

The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the nine (9) members/one (1) alternate, were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:

The ERP reviewed OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick and have adopted this method for AOAC First Action Official Method status by consensus. The decisions have been captured and reflected in the meeting minutes.

Subsequent ERP Activities:

ERP members are required to track the performance of the recently approved First Action method for a 2 year period effective as of September 30, 2015.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-Chair Terry Koerner welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-20: R-Biopharm, Qualitative Detection of Gluten on Surfaces and In Processed and Non-Processed Products by R5 Immuno-Chromatographic Dip Stick. The method author Dr. Markus Lacorn of R-Biopharm was present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[1] By consensus, the ERP presented the following motions for OMAMAN-20.

MOTION:
Motion by Wehling; Second by Koerner, to move OMAMAN-20 to AOAC First Action Official Methods status. Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

MOTION:
Motion by Boison; Second by Popping, For OMAMAN-20 to move from First Action to Final Action; Requirements are, 1) Feedback from Users, 2) To provide evidence of the application of the alternative approach. Recommended that the method author, 1) Add additional matrices in the collaborative. Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

IV. Discuss Final Action Requirements for First Action Official Methods (if applicable)

The Expert Review Panel will convene via teleconference to discuss the First Action to Final Action Official methods status for OMA 2012.01: Gliadin as a Measure of Gluten in Foods Containing Wheat, Rye, and Barley **(**RIDASCREEN® Gliadin**)**. The First to Final Action requirements set for OMAMAN-20 were noted in the meeting minutes above.

V. Adjournment: Meeting adjourned at 10:00am.

[1] Attachment 2: Summary of Expert Reviewer Comments for OMAMAN-20

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PAH METHODS

ERP Name	AOAC Expert Review Panel for PAH Methods			**Chair(s)**	Tom Phillips (Maryland Department of Agriculture)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of PAHs in seafood				
Roster	1. Tom Phillips, Maryland Department of Agriculture (Chair) 2. Mark Crosswhite, Florida Department of Agriculture 3. Julie Kowalski, RESTEK 4. Cheryl Lassitter, DOC, NOAA, NMFS, NSIL 5. Kai Liu, Eurofins 6. Jian Wang, Canadian Food Inspection Agency (CFIA) 7. Xiaoyan Wang, United Chemical Technologies, Inc. (UCT) 8. Stephen A. Wise, National Institute of Standards and Technology (NIST) 9. Tracy Collier - (Alternate), NOAA (retired) 10. Lowri de Jager, U.S. Food and Drug Administration				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.08 - Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions	Method of the Year in 2014				

Expert Review Panel on Polycyclic Aromatic Hydrocarbons (PAHs)

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel on Polycyclic Aromatic Hydrocarbons (PAHs) held on Monday, September 8, 2014 during the AOAC Annual Meeting and Exposition at the Boca Raton Resort and Club, 501 East Camino Reel, Boca Raton, Florida 33432.

TOM PHILLIPS, MD DEPARTMENT OF AGRICULTURE

Expert Review Panel Chair

30 October 2014

Date

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2275 Research Blvd, Suite 300
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UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair(s)
Tom Phillips, MD Department of Agriculture

Expert Review Panel Members
Mark Crosswhite, Florida Department of Agriculture
Julie Kowalski, RESTEK
Cheryl Lassitter, DOC, NOAA, NMFS, NSIL
Kai Liu, Eurofins
Jian Wang, Canadian Food Inspection Agency (CFIA)
Xiaoyan Wang, United Chemical Technologies, Inc. (UCT)
Stephen A. Wise, National Institute of Standards and Technology (NIST)

Not Present: Tracy Collier - (Alternate), NOAA (retired)
Not Present: Lowri de Jager, U.S. Food and Drug Administration

Method Authors
Kate Mastovska, Covance Laboratories
Jana Hajslova, Institute of Chemical Technology

AOAC Staff
Scott Coates
Deborah McKenzie
Tien Milor
La'Kia Phillips

Observers
Jana Huckrabolt, Institute of Chemical Technology
Nick Kosa, Bioo Scientific
Laszlo Torma,
Suramya Waidyanatha, NPT/NIEHS

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Polycyclic Aromatic Hydrocarbons (PAHs) on Monday, September 8, 2014 from 1:30pm to 4:00pm during the AOAC Annual Meeting and Exposition in Boca Raton, Florida. The purpose of the meeting was to 1) Review the Collaborative Study Manuscript/ OMAMAN-15: Determination Of Polycyclic Aromatic Hydrocarbons (PAHs) In Seafood Using Gas Chromatography-Mass Spectrometry (Study Director: Katerina Mastovska, Covance Laboratories Inc., Nutritional Chemistry and Food safety, 3301 Kinsman Boulevard, Madison, WI 53704, USA) and to 2) discuss First to Final Action requirements and feedback mechanisms. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, response to AOAC statistical review, summary shrimp corrected, DBAHA shrimp mid s9&s14 corrected, ICDP oyster mid o1&o7 corrected, and additional experiments with shrimp matrix.

Criteria for Vetting Experts and Selection Process:
The following nine (9) candidates and one (1) alternate were submitted for consideration by the Official Methods Board to evaluate candidate methods for Polycyclic Aromatic Hydrocarbons (PAHs) methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Chemical Contaminants and Residues in Foods Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Tom Phillips, Cheryl Lassitter, Tracy Collier (Alternate Member), Kai Liu, Mark Crosswhite, Jian Wang, Lowri de Jager, Xiaoyan Wang, Julie Kowalski, and Stephen Wise. Tom Philips was vetted as the Expert Review Panel Chair.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, July 16, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the nine (9) voting members were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-15: Determination of Polycyclic Aromatic Hydrocarbons (PAHs) In Seafood Using Gas Chromatography-Mass Spectrometry and adopted this method for First Action Official Method status by a unanimous decision.

Subsequent ERP Activities:
ERP members will continue to evaluate the method for 2 years.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair, Tom Phillips welcomed Expert Review Panel members and initiated introductions. The Chair discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

Deborah McKenzie presented an overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented an overview of the Expert Review panel process. The presentation included information regarding method submission, recruitment of ERP members, composition and vetting expertise, method assignments, meeting logistics, consensus, First Action to Final Action requirements, method modifications, publications, and documentation.

IV. Review of Methods

All members of the ERP presented a review and discussed the proposed collaborative study manuscript for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) In Seafood Using Gas Chromatography-Mass Spectrometry. The method authors are Katerina Mastovska of Covance Laboratories Inc., Nutritional Chemistry and Food safety, 3301 Kinsman Boulevard, Madison, WI 53704, USA. A summary of comments was provided to the ERP members.[1]

MOTION:
Motion by Kowalski; Second by Crosswhite to adopt this method as a First Action Official Method.
Consensus demonstrated by: 7 in favor, 1 opposed, and 0 abstentions.
Motion failed.[2]

Negative Vote Discussion: One member of the expert review panel voted against the motion. Due to the reviewer's comments, he inquired about the method not using a certified reference material for PAHs in seafood. Standard Reference Material (SRM) 1974b Mussel Tissue is mentioned as part of the qualification of the labs as a practice sample, but no data was reported using SRM 1974b for validation of the proposed method. The availability of SRM 1974c (which has replaced SRM 1974b) provided an excellent opportunity to use a CRM to validate an AOAC method. The discussion of the Expert Review Panel concluded that the use of a certified reference material was not required and did not delineate scientific reasoning to not move the method forward. This method was created in an effort to address an emergency response to the gulf oil spill. The information provided in reference to the selection of the 19 target PAH compounds and the matrices selected were noted in the Fitness for Purpose statement established by the Stakeholder Panel on Petroleum Contaminants in Seafood in 2010. The ERP captured a revote.

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-15

[2] *Method must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons. Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.*

MOTION:
Motion by Kowalski; Second by Crosswhite to adopt this method as a First Action Official Method.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous).
Motion Passed.

V. Discuss Final Action Requirements for First Action Official Methods *(if applicable)*
No further action was discussed at this time.

VI. Adjournment

	Summary of Method
ER 1	PAHs in homogenized seafood's are extracted with EtOAc:water. Extracts are cleaned with SPE technique before GC-MS analysis.
ER 2	This method utilizes solvent extraction of a homogenized sample followed by a silica-SPE procedure. The eluant is introduced into a GC-MS or GC-MS/MS in either SIM or MRM modes. Issues encountered by participating laboratories are typical of PAH analyses including loss of more volatile PAHs during evaporation and background PAH contamination. This method achieved good sensitivity, accuracy and precision and has Limits of detection/quantification that are lower than the levels of concern in seafood samples set by regulatory agencies.
ER 3	The method describes analysis of 19 PAHs and alkylated PAHs via GC-MS. Sample preparation involves solvent extraction followed by salting out partitioning with silica SPE cleanup. Method performance criteria are set instead of prescribing specific products/instruments needed to successfully complete analysis. Criteria address analytes recovery, matrix cleanup, calibration quality, chromatographic separation and detector sensitivity.
ER 4	This method presented a procedure to determine 19 selected polycyclic aromatic hydrocarbons (PAHs) and their relevant alkyl homologous in seafood using fast sample preparation followed by GC-MS analysis. The sample preparation included two steps: (1) extraction using water-ethyl acetate and salt-out by anhydrous magnesium sulfate and sodium chloride; (2) clean-up by silica gel SPE. GC-MS was very common and practical instrumentation for PAHs analysis. The method is simple, fast, accurate, robust and is easy to follow.
ER 5	Collaborative study conducted to determine selected PAHs and relevant alkyl homologues in seafood matrices using a fast sample preparation method followed by analysis with gas chromatography-mass spectrometry (GC-MS).
ER 6	This is a GC-MS method with C13 labeled internal standards; sample preparation is a QuEChERS approach followed by SPE cleanup. The method was designed around performance-based criteria regarding the GC separation, SPE, and evaporation steps.
ER 7	PAHs in seafood samples (10 g, hydrated with 5 or 10 mL water) are extracted into 10 mL ethyl acetate with the aid of partitioning salts (4 g magnesium sulfate and 2 g sodium chloride). 5 mL of the ethyl acetate extract is concentrated down and cleaned with 1 g silica gel SPE cartridge, the eluate is concentrated and solvent exchanged to 0.5 mL isooctane, and analyzed by GC/MS.
ER 8	good

	Method Scope/Applicability
ER 1	Applicable to seafood's such as mussel, oyster, and shrimp.
ER 2	This method provides determination of PAH and PAH analogues in shrimp, mussels and finfish which are representative of this class of compounds. The method achieves limits of detection well below the regulatory levels of concern.
ER 3	Scope of the method includes 19 specific PAHs in seafood. The matrices tested, shrimp, oyster and mussel, are typically 5% lipid content and below (USDA Nutrient database). Lipid content of commodities amendable for this method is an important consideration and should be addressed in the text of the method. Higher fat samples are addressed briefly in the method, indicating that a reduction of volume of extract should be applied to the silica SPE cartridge. This is a reasonable modification to the method but has implications for overall detectability, especially for BaP. It is possible that to meet fat removal criteria, modifications for calibration curves and/or sample preparation will need to be made. Modifications may be significant and therefore some comment on an upper limit of the lipid content applicable for the method as written would be useful.
ER 4	The method covers 19 selected PAHs in shrimp, mussel, and oyster with analytical ranges of fit for purpose. The scope should be easily expanded to include more analytes and applied to a verity of seafood or processed ones.
ER 5	All Federal, State and Commercial laboratories analyzing PAHs in seafood.
ER 6	The methods is intended for seafood that would accumulate PAHs and has been tested using mussel, oyster, and shrimp matrices.
ER 7	The method is capable to detect 19 selected PAHs quantitatively in shrimp, oyster and mussel samples.
ER 8	good

	General Comments
ER 1	This study successfully addressed the urgent need for a reliable approach to analyzing various PAHs in potentially contaminated seafood's. Method procedures are easy to follow and not time-consuming.
ER 2	The validation procedure outlined in this document was comprehensive and the results demonstrated that the method was accurate and rugged. This method is a significant improvement over current regulatory methods. It is significantly faster, requires less organic solvent and produces less waste, less labor intensive and is easier to perform that the current method, while fulfilling required method performance benchmarks.
ER 3	none
ER 4	The collaborative study report was a very clear presentation. The experiment or study was thorough and well designed. Performance-based criteria led to a robust method for analysis of PAHs in seafood. The method was practical and fit for purpose. Results from collaborative study supported the method performance and demonstrated that the method was fit-for-purpose to determine PAHs and their alkyl homologues in seafood.
ER 5	Method is well-written but needs more specificity in select sections. For example, on page 6, under (5), it is stated all analytes of reagent blanks must be below the concentrations in the lowest calibration standard. Needs more clarification....how far below? Also, since stability of some PAHs was questionable, a Stability Study needs to be carried out with PAH standards stored at varying temperatures and times. The Safety Section must be in the front of the method since safety is more important than any other part of the protocol.
ER 6	None
ER 7	The method is quick, easy to use, and allows flexibility in method development. It demonstrated good GC separations of isomer pairs, excellent recoveries and reproducibility were achieved except for 2 compounds in oyster which might degrade at -20 C, no degradation was observed when oyster was stored at -70 C, however it's not very practical for many labs to maintain such low storing temperature.
ER 8	Very good method

	Method Clarity
ER 1	Good clarity throughout the manuscript.
ER 2	The method is well written and easily understood. The instructions are clear and I found no ambiguities.
ER 3	The method as written is clean with only a few instances for improvement. Performance criteria and how to evaluate the criteria are nicely described.
ER 4	The method procedure is well described and steps are easy to follow.
ER 5	Method is generally clear but needs more specificity in select areas.
ER 6	The method is clear and all the necessary information provided to reproduce and use the method. The authors should use correct nomenclature for the PAHs, i.e., Benzo[ghi]perylene not benzo[g,h,i]perylene
ER 7	The method is clearly described in the manuscript.
ER 8	good

	Pros/Strengths
ER 1	The organizer took great effort setting up the procedures to allow for individual lab's choice of various instruments, columns, SPE vendors, and evaporation techniques, as long as the lab passed the performance requirements. The procedures are straight forward and not hard to follow.
ER 2	Fairly easy method that requires significantly less sample preparation compared to other methods. Utilizes equipment and instrumentation that is widely available. Uses less flammable and toxic solvents than other methods. Sample handling is minimized which decreases the probability of environmental contamination.
ER 3	Strengths include: 1. easy to follow criteria for sample preparation evaluation 2. easy to follow instructions for solution and calibration solution preparation 3. some allowance for environmental background of the naphthalene 4. importance of monitoring blank is clearly stated
ER 4	The method is simple, fast, accurate, robust and is easy to follow.
ER 5	Well written, encompasses analyses of PAH compounds deleterious to humans at low levels, the calculations outlines on pages 14-15 are well written.
ER 6	A major strength is that the method is an isotope dilution (ID) GC-MS methods using C13 labeled internal standards for 13 of the 19 target PAHs. The sample preparation appears to be simplified compared to normal solvent extraction methods (Soxhlet, ACE, MAE). Another strength is the performance criteria required for the choice of GC column and the requirements to separate critical PAH isomers such as the benzofluoranthenes.
ER 7	The method is simple, fast, and easy to use. High sample throughput with little lab ware needed. Applicable to a variety of seafood matrices. Overall method performance are acceptable.
ER 8	no comment

	Cons/Weaknesses
ER 1	Isotope-Labeled mixed standards may be expensive or could be unavailable occasionally. Precision of results (all three levels) may have some room for improvement.
ER 2	Still requires some sample clean up, including a dry down step which if performed incorrectly could cause artificially low calculated concentrations for low molecular weight PAHs. Does not incorporate many alkyl homolog PAH compounds. These are often present at higher concentration in oil contamination and have similar toxicity to the PAHs. Addition of these compounds to the GC-MS method would increase the applicability and impact of the method. This could perhaps be done in the future
ER 3	Weaknesses include: 1. Method scope of 1 ug/kg LOQ of BaP was not tested as a fortification level. As I read the method, the lowest fortification level for BaP was 2 ug/kg. 2. Polypropylene tubes used for extraction will likely cause users of the method issues with PAH contamination. Discussion of alternatives would be helpful. 3. PAH GC-MS analysis has significant differences than typical analysis of most other types of compounds. Guidance for GC-MS parameters would likely be helpful for users of the method. These include parameters like inlet temperature, transfer line temperature, ion source temperature, column loadability and efficient flow conditions. 4. There is no recommendation on how to report data on chrysene and triphenylene if the recommended, but not required, 50% valley separation is not met. Can chrysene and triphenylene be reported together? 5. Ion ratios are mentioned as a requirement for identification but there is no indication as to the RSD value that is acceptable or some other qualification. 6. When a linear calibration curve is not possible, allowance for a "well-characterized" quadratic formula is made but with no discussion of what "well-characterized" means. Some guidance would be useful because some user will not be accustomed using quadratic calibration curves.
ER 4	A commercially available mix of standards suitable for the method is beneficial.
ER 5	The Safety Section must be in the front of the method since safety is more important than any other part of the protocol. Method must be more specific. Under Degradation Issues on page 18, the discussion emphasizes the need for a Stability Study. 18.2 megaohm water should be used for any GC/MS method (page 9, Section C). Need a statement that documented calibrations/reference checks were performed on all analytical equipment and instrumentation used in the collaborative study.
ER 6	A major weakness is that there is no validation using a certified reference materials for PAHs in seafood. Standard Reference Material (SRM) 1974b Mussel Tissue is mentioned as part of the qualification of the labs (p. 6) as a practice sample, but no data are reported using SRM 1974b for validation of the proposed method. The availability of SRM 1974c (which has replaced SRM 1974b) provided an excellent opportunity to use a CRM to validate an AOAC method. The use of only fortified/spiked samples for the method validation is a weakness. Spiked samples are sometimes the only option but in this

	case with SRM 1974 available, it could have been handled differently. The selection of the 19 target PAHs to determine could be questioned. The authors comment on criteria for separation of chrysene and triphenylene and also BaP and BeP, which is good, but perhaps triphenylene and BeP should have been included. There were no performance criteria for the separation of the alkyl-PAHs from potential isomers, e.g., with 3-MeChr targeted, how do you know if you are separating it from other methylchrysene or methyl-BaA isomers? The inclusion of several alkyl-PAHs is good, but will this really provide a method to look at the alkyl-PAHs, which in petroleum contaminated samples may be more abundant than the parent PAHs. Should there be a provision for looking at the alkyl-PAHs as a group by MS? Perhaps this is beyond the intended scope of this method.
ER 7	Fish, one of the most common seafood, was not covered in this method. Oyster needs to be stored at -70 C, which is not very easy for many labs to maintain.
ER 8	no comment

	Supporting Data Comments
ER 1	Great summary of results and provided complete statistical information. Would like to see more on linearity results.
ER 2	The supporting data indicates that the method is rugged and accurate.
ER 3	Nicely organized.
ER 4	na
ER 5	More specificity needed in quantitative parameters.
ER 6	In general, this is a good method, but needs further validation.
ER 7	Tested real samples. Additional experiments with shrimp matrix performed.
ER 8	good

	Method Optimization
ER 1	N/A
ER 2	Was well described and performed appropriately.
ER 3	Good.
ER 4	na
ER 5	The Safety Section must be in the front of the method since safety is more important than any other part of the protocol. Method must be more specific when outlining quantitative parameters. Under Degradation Issues on page 18, the discussion emphasizes the need for a Stability Study. 18.2 megaohm water should be used for any GC/MS method (page 9, Section C). Need a statement that documented calibrations/reference checks were performed on all analytical equipment and instrumentation used in the collaborative study.
ER 6	The method optimization is well done in particular the SPE cleanup. Could the use of an aminopropyl SPE be less sensitive than silica regarding deactivation by moisture content?
ER 7	Elution solvent volume for silica gel SPE cleanup is optimized. Increased water amount (10 mL) is used in shrimp samples to help shake and extract PAHs from more viscous shrimp samples.
ER 8	good

	Analytical Range
ER 1	Varies depending on the analyte. Generally, low is from 2 ppb to 25 ppb. High is 20 ppb to 250 ppb.
ER 2	Range varies and is dependent on the specific analyte. This is appropriate as lower molecular weight PAHs are generally found in higher concentrations than higher molecular weight PAHs. It is acceptable for this application as levels of concern for low molecular weight PAHs are significantly higher.
ER 3	Sufficient.
ER 4	BaP: 0.5 - 100 µg/kg (0.5, 1, 2, 5, 10, 20, 50, 100) Other PAHs: 1.25 - 250 µg/kg (1.25, 2.5, 5, 12.5, 25, 50, 125, 250) naphthalene: 2.5 - 500 µg/kg (2.5, 5, 10, 25, 50, 100, 250, 500)
ER 5	Good
ER 6	Suitable
ER 7	0.5 to 100 ug/kg for BaP and other lower-level PAHs; 1.25 to 250 ug/kg for higher-level PAHs; and 2.5 to 500 ug/g for naphthalene.
ER 8	good

	LOQ
ER 1	Not discussed in the method. Likely in the low ppbs.
ER 2	LOQ varies and is dependent on the specific analyte. This is appropriate as lower molecular weight PAHs are generally found in higher concentrations than higher molecular weight PAHs. It is acceptable for this application as levels of concern for low molecular weight PAHs are significantly higher.
ER 3	Please see comment above about spike levels and LOQ of BaP.
ER 4	Bap: 0.5 µg/kg Other PAHs: 1.25 µg/kg naphthalene: 2.5 µg/kg
ER 5	Good
ER 6	Suitable
ER 7	1 ug/kg for BaP and other lower-level PAHs; 2.5 ug/g for higher-level PAHs; and 5 ug/g for naphtalene.
ER 8	good

	Accuracy/Recovery
ER 1	Varies depending on the analyte. 70-120% mostly.
ER 2	Accuracy/Recovery is high and meets validation criteria.
ER 3	Good
ER 4	In shrimp: 83.8-115% In mussel: 77.3-107% In oyster: 71.6-94.6%, except for a lower mean recovery of 68.6% for benzo[α]anthracene (BaA) in oyster, and 50.3-56.5% and 48.2-49.7% for anthracene and beno[α]pyrene, respectively.
ER 5	Good
ER 6	As noted by the authors, the recoveries for the oyster tissue are low and probably inadequate.
ER 7	After excluding the outliers, the mean recoveries (8-10 labs) are in the range of 70-120% with a few exceptions which may due to the compound degradation in oyster samples stored at -20 C.
ER 8	good

	Precision
ER 1	Varies. Mostly around or below 10%, with one exception of 27% for low level 1-MN.
ER 2	Precision and reproducibility varies and is dependent on the specific analyte. The reported values meet the validation criteria
ER 3	Good
ER 4	In Shrimp: 1.40-26.9% In mussel: 2.52-17.1% In oyster: 3.12-22.7%
ER 5	Good
ER 6	I am not familiar with the expectations for precision for an AOAC method; however, the precision here appears to be adequate.
ER 7	Precision was excellent.
ER 8	good

	Reproducibility
ER 1	Varies. Mostly between 10%-20%.
ER 2	Precision and reproducibility varies and is dependent on the specific analyte. The reported values meet the validation criteria
ER 3	Good
ER 4	In Shrimp: 5.41-29.4% In mussel: 4.19-32.5% In oyster: 8.41-31.8%
ER 5	Good
ER 6	I am not familiar with the expectations for reproducibility for AOAC method; however, the reproducibility for this study appears to be inadequate for many of the more volativle PAHs (e.g., naphthalene) and particularly in the oyster tissue.
ER 7	Reproducibility was good except for a few compounds in oyster stored at -20 C.
ER 8	good

	System Suitability
ER 1	Not discussed.
ER 2	System is suitable
ER 3	Good
ER 4	na
ER 5	Were IDLs, MDLs and PQLs carried out on all instrumentation used in the Collaborative Study? If not, this should be performed and documented in the Method. Are there records of Intra-day, Inter-day variability? Are there records of Analyst variability? If so, the Method should state.
ER 6	No comments
ER 7	System check samples were analyzed.
ER 8	very good

	First Action Recommendation
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	I recommend that the method, which has been gone through AOAC collaborative study successfully, for determination of PAHs in seafood using GC-MS be adopted Official Fist Action
ER 5	No
ER 6	Not yet....I think it needs validation with a natural matrix CRM such as SRM 1974c.
ER 7	Yes, with minor modifications (please see After First Action Recommendation)
ER 8	yes

	After First Action Recommendation
ER 1	Explore for ways to improve inter-lab precision RSD(R)%
ER 2	NO
ER 3	See comments above.
ER 4	na
ER 5	It may be helpful to refer to the FDA's LIB # 4475 to get a better feel for how the Method should be formatted and important quantitative data to include. The only exception here is the Safety Section is not in the front of this FDA LIB.
ER 6	As mentioned above, information on the method performance using SRM 1974c
ER 7	Fish samples should be analyzed in the future to see if this method is applicable to fish as well, especially those with high fat content. Was matrix effect significant? or the internal standards (13C PAHs) added to samples before extraction corrected the matrix effect of their corresponding PAHs? How about the alkyl PAHs that did not include their isotope labeled standards in this study? Should matrix matched calibration be more appropriate? I would suggest the study group to compare the PAH recoveries using this method and one of the other currently accepted methods to test an oyster reference material stored at - 20 C to show if the degradation of Ant and BaP is method dependent.
ER 8	no

PROFILE OF AOAC EXPERT REVIEW PANEL FOR PESTICIDE RESIDUE METHODS

ERP Name	AOAC Expert Review Panel for Pesticide Residue Methods			**Chair(s)**	Joe Boison (Canadian Food Inspection Agency)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of pesticide residues in teas				
Roster	1. Joe Boison, Canadian Food Inspection Agency (CFIA) 2. Amy Brown, Florida Department of Agriculture 3. Jo Marie Cook (Alternate), Florida Department of Agriculture 4. Julie Kowalski, Restek Corporation 5. John Reuther, Eurofins 6. Marina Torres, LATU 7. Jian Wang, Canadian Food Inspection Agency (CFIA) 8. Xiaoyan Wang, United Chemical Technologies, Inc. (UCT)				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.09 - Residues of 653 Multiclass Pesticides and Chemical Pollutants in Tea				
Final Action Methods Recommended					
Additional Input					
Awards/Recognitions	ERP of the Year in 2015; Method of the Year in 2014				

AOAC RESEARCH INSTITUTE

Expert Review Panel on Pesticide Residues
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel on Pesticide Residues held on Monday, September 8, 2014 during the AOAC Annual Meeting and Exposition at the Boca Raton Resort and Club, 501 East Camino Reel, Boca Raton, Florida 33432.

DR. JOE BOISON, CANADIAN FOOD INSPECTION AGENCY (CFIA)

Expert Review Panel Chair

October 14, 2014

Date

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UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair (s)
Joe Boison, Canadian Food Inspection Agency (CFIA)

Expert Review Panel Members
Amy Brown, Florida Department of Agriculture
Jo Marie Cook (Alternate), Florida Department of Agriculture
Julie Kowalski, Restek Corporation
John Reuther, Eurofins
Marina Torres, LATU
Jian Wang, Canadian Food Inspection Agency (CFIA)
Xiaoyan Wang, United Chemical Technologies, Inc. (UCT)

Method Authors
Dr. Guo-Fang Pang, Chinese Academy of Inspection and Quarantine

AOAC Staff
Deborah McKenzie
Tien Milor
La'Kia Phillips

Observers
Muna Aljabir, Central Food Lab
Tyson Friday, Frontier Coop
Jana Huckrabolt, Institute of Chemical Technology
Tom Phillips, MD Department of Agriculture
Jana Pugerhbovn, Institute of Chemical Technology
Nancy Thiex, AOAC RI Consultant
David Whitman, 3M Food Safety
David Woollard, Hill Laboratories (NZ)

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Pesticide Residues on Monday, September 8, 2014 from 4:30pm to 7:30pm during the AOAC Annual Meeting and Exposition in Boca Raton, Florida. The purpose of the meeting was to 1) Review the Collaborative Study Manuscript/ OMAMAN-14: High-Throughput Analytical Techniques For The Determination And Confirmation Of Residues Of 653 Multi-Class Pesticides And Chemical Pollutants In Tea By GC-MS, GC-MS/MS And LC-MS/MS (Study Director: Guo-Fang Pang, Chun-Lin Fan,Yan-Zhong Cao, Fang Yang, Yan Li, Jian Kang, Hui Chen, Qiao-Ying Chang, Chinese Academy of Inspection and Quarantine, No. 3 Gaobeidian North Rd 100123, Chaoyang District, Beijing, People's Republic of China) and to 2) discuss First to Final Action requirements and feedback mechanisms. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, Appendix 1.1-1.5 Analytical Instrumentation used in the Collaborative Study, Appendix 2 Determination Results of Collaborative Study, Appendix 3: Results of Practice Samples, Appendix 4: The Statistical Results, and an Journal Article: High-Throughput GC-MS and HPLC-MS/MS Techniques for the Multiclass, multiresidue Determination of 653 Pesticides and Chemical Pollutants in Tea.

Criteria for Vetting Experts and Selection Process:
The following seven (7) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for Pesticide Residues methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Chemical Contaminants and Residues in Foods Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Amy Brown, Jo Marie Cook (Alternate), Julie Kowalski, John Reuther, Marina Torres, Jian Wang, and Xiaoyan Wang.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, July 16, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Seven (7) out of the seven (7) voting members were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-14: High-Throughput Analytical Techniques For The Determination And Confirmation Of Residues Of 653 Multi-Class Pesticides And Chemical Pollutants In Tea By GC-MS, GC-MS/MS And LC-MS/MS and adopted this method for First Action Official Method status by a unanimous decision with additional revisions as noted in the meeting minutes.

Subsequent ERP Activities:
ERP members will continue to evaluate the method for 2 years.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair, Dr. Joe Boison welcomed Expert Review Panel members and initiated introductions. The Chair discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

Deborah McKenzie presented an overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented an overview of the Expert Review panel process. The presentation included information regarding method submission, recruitment of ERP members, composition and vetting expertise, method assignments, meeting logistics, consensus, First Action to Final Action requirements, method modifications, publications, and documentation.

IV. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for High-Throughput Analytical Techniques for the Determination and Confirmation of Residues of 653 Multi-Class Pesticides and Chemical Pollutants in Tea by GC-MS, GC-MS/MS and LC-MS/MS. The method author, Dr. Guo-Fang Pang of the Chinese Academy of Inspection and Quarantine, was present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[1]

MOTION:
Motion by Wang, J.; Second by Reuther that this method be recommended for First Action Official Method Status.
Consensus demonstrated by: 7 in favor, 0 opposed, and 0 abstentions (Unanimous). ***Motion Passed.***

MOTION:
Motion by Wang, J.; Second by Brown that the revisions requested by the ERP be provided for review.

- **Provide data on the parameters of the method for all of the 653 Analytes**
- **Provide clarity to the text**
- **Include the data on hydration**

Consensus demonstrated by: 7 in favor, 0 opposed, and 0 abstentions (Unanimous). ***Motion Passed.***

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)

No further action was discussed at this time.

VI. Adjournment

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-14

PROFILE OF AOAC EXPERT REVIEW PANEL FOR DIETARY STARCH METHODS

ERP Name	AOAC Expert Review Panel for Dietary Starch Methods			**Chair(s)**	Lars Reimann (Eurofins)
ERP Formed:	2014	**Number of Methods Adopted**	1 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for dietary starch determination in animal feed and pet food.				
Roster	1. Lars Reimann, Eurofins 2. Sean Austin, Nestle Research Centre 3. Sneh Bhandari, Silliker, Inc. 4. Kommer Brunt, Rotating Disc BV 5. Jon DeVries, Medallion Laboratories 6. Kai Liu, Eurofins 7. Barry McCleary, Megazyme International Ireland 8. Tom Phillips, MD Department of Agriculture 9. John Szpylka, Silliker, Inc. (Alternate)				
Technical Documents created/used	OMA Appendix D				
Methods Adopted *First Action and Final Action status*	AOAC 2014.10 - Dietary Starch in Animal Feed and Pet Food				
Final Action Methods Recommended					
Additional Input	AOAC Community on Agricultural Materials				
Awards/Recognitions					

AOAC RESEARCH INSTITUTE

Expert Review Panel on Dietary Starches
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel on Dietary Starches held on Wednesday, September 10, 2014 during the AOAC Annual Meeting and Exposition at the Boca Raton Resort and Club, 501 East Camino Reel, Boca Raton, Florida 33432.

Lars Reimann

LARS REIMANN, EUROFINS

Expert Review Panel Chair

Oct 02, 2014

Date

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Contact:
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Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair (s)
Lars Reimann, Eurofins

Expert Review Panel Members
Sean Austin, Nestle Research Centre
Sneh Bhandari, Silliker, Inc.
Kommer Brunt, Rotating Disc BV
Jon DeVries, Medallion Laboratories
Kai Liu, Eurofins
Barry McCleary, Megazyme International Ireland
Tom Phillips, MD Department of Agriculture
John Szpylka, Silliker, Inc. (Alternate)

Method Authors
Mary Beth Hall, U. S. Department of Agriculture

AOAC Staff
Deborah McKenzie
La'Kia Phillips

Observers
Jason Kong, Ohio Department of Agriculture
Maria Nelson, AOAC Technical Consultant
Ioannis Vrasidas, Eurofins Analytico

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the Official Methods of Analysis℠ (OMA) Expert Review Panel for Dietary Starches on Wednesday, September 10, 2014 from 8:00am to 10:00am during the AOAC Annual Meeting and Exposition in Boca Raton, Florida from September 7-10, 2014. The purpose of the meeting will be to 1) Review the Collaborative Study Manuscript/ OMAMAN-13: Determination of Dietary Starch in Animal Feeds and Pet Food by an Enzymatic-Colorimetric Method Collaborative Study (Study Director: Mary Beth Hall, U. S. Department of Agriculture – Agricultural Research Service, U.S. Dairy Forage Research Center, 1925 Linden Drive, Madison, WI 53706, USA) and to 2) discuss First to Final Action requirements and Feedback mechanisms. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, Method Safety Checklist, Collaborative Study Tables, Collaborative Study Figures and Captions, and the Collaborative Study Protocol.

Criteria for Vetting Experts and Selection Process:
The following eight (8) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for Dietary Starches methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Agricultural Materials Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Sean Austin, Sneh Bhandari, Kommer Brunt, Jon DeVries, Kai Liu, Barry McCleary, Tom Phillips, John Szpylka, and the Chair, Lars Reimann.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, July 16, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the eight (8) voting members were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-13: Determination of Dietary Starch in Animal Feeds and Pet Food by an Enzymatic-Colorimetric Method and adopted this method for First Action Official Method status by a unanimous decision with additional revisions as noted in the meeting minutes.

Subsequent ERP Activities:
ERP members have stated that no additional data is requested to move from First to Final Action. User Feedback and supporting documentation in support of the need for quadratic standard curve is expected for this method to move forward to Final Action Official Method status. ERP members will continue to evaluate the method for 2 years.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair, Lars Reimann welcomed Expert Review Panel members and initiated introductions. The Chair discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

Deborah McKenzie presented an overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. The Expert Review panel openly discussed any potential conflicts of interest. The group approved all of the members after disclaimers were noted.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented an overview of the Expert Review panel process. The presentation included information regarding method submission, recruitment of ERP members, composition and vetting expertise, method assignments, meeting logistics, consensus, First Action to Final Action requirements, method modifications, publications, and documentation.

IV. Review of Methods

All members of the ERP presented a review and discussed the proposed collaborative study manuscript for Determination of Dietary Starch in Animal Feeds and Pet Food by an Enzymatic-Colorimetric Method. The method author, Mary Beth Hall of the U. S. Department of Agriculture, was not present to address the concerns of the ERP members. A summary of comments was provided to the ERP members.[1]

MOTION:

Motion by DeVries; Second by Szpylka to adopt this method for First Action Official Methods Status with the requested revisions.

OMA METHOD: Line 376: Include “free from catalase activity”.

EDITORIAL: Line 351: Include “the enzymes should be of a purity meeting the specifications listed in OMA methods 985.29 and 991.43
Line 366: The “amylase” should be listed as “amyloglucosidase”
Line 344: Include activity definitions and assay procedures.
Line 118-122: Please clarify section.

The Expert Review Panel would like to know if GOPOD blank is used as instrument blank will the intercept disappear and negate the need for a quadratic standard curve?

Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). *Motion Passed.*

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-13

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)

MOTION:
Motion by DeVries, Second by Liu that no additional data is requested to move from First to Final Action. User Feedback and supporting documentation in support of the need for quadratic standard curve is expected.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). ***Motion Passed.***

VI. Adjournment

ERP PROFILE SUMMARIES

	Summary of Method
ER 1	Acceptable
ER 2	It consists of incubation of an aliquot of the sample with thermostable alpha-amylase in pH 5.0 acetate buffer for 1 hr at 100°C with periodic mixing to gelatinize and partially hydrolyze alpha-glucan. Amyloglucosidase is added and mixture is incubated at 50°C for 2 h and mixed. After subsequent addition of water, mixing, clarification, and dilution as needed, free + ezymetically released glucose are measured using a colorimetric glucose oxidase-peroxidase method. Values from a separate determination of free glucose are subtracted to give values of enzymatically-released glucose. Dietary starch = Enzymatically- released glucose multiplied by (162/180) or 0.9 and divided by the as received sample weight (g) used in the assay.
ER 3	Dietary starch is digested to glucose and the increase in glucose level is used to calculate %dietary starch. Potential interferences are either accounted for (inherent glucose) or excluded (deter inherent sucrose digestion and deter maltulose formation).
ER 4	Starch is digested by traditional amylase/amyloglucosidase using gelatinization conditions. Glucose released is measured colorimetrically with adjustment for free glucose in the sample.
ER 5	Ground or homogenized samples are digested with α-amylase and amyloglucosidate in acetate buffer to release glucose from dietary starch. The digestate, after optional dilution, is analyzed for its glucose content. A second sample portion is also assessed for free glucose by treatment with all reagents but the enzymes. The difference of the two glucose result is used to calculate dietary starch content in the sample.
ER 6	Sample (containing up to 100mg of starch) is weighed in duplicate. sodium acetate buffer (pH 5.0) is added to both tubes. Then to one tube alpha-amylase and amylglucosidase are added to hydrolyse the starch. To the other tube no enzymes are added. Samples are then clarified (centrifugation or filtration) and diluted. Aliquots from each tube are then taken for analysis of glucose using the glucose oxidase peroxidase (GOPOD) method, or other suitable validated method for glucose determination. Glucose determined in the untreated sample is subtracted from the glucose determined in the enzyme-treated sample. The result is then multiplied by 0.9 to correct for water uptake during hydrolysis to calculate starch content.
ER 7	good
ER 8	A well performed study. However, the advantages over AOAC Method 996.11 need to be more clearly identified. A significant contribution is the application to samples more relevant to the particular study, but some of the stated general advantages are not substantiated.

	Method Scope/Applicability
ER 1	Animal Feeds and pet foods. 1%-70% starch
ER 2	Animal feedstuffs and pet foods. Limitation in application: The method underestimates dietary starch in feeds and foods whose antioxidant content is known to exceed 10-20 micromol of hydrophilic antioxidant (as ascorbic acid) per 0.1 g of test dry matter. The method in the current format may not be easily applicable to foods/feeds high in phenolic compounds (e.g. beets, red sorghum grain).
ER 3	A wide range of animal and pet feeds were covered in the study. Dry and wet products were included along with a variety of grains as the base material.

ER 4	See method scope and applicability statement.
ER 5	Applicable to pet foods (wet and dry), animal feed, forage, as well as grains.
ER 6	method has been applied to a range of different animal feeds; canned dog food, low starch horse feed, ground corn, complete dairy feed, soybean meal, distillers grains, poultry feed, corn silage, dry dog kibble, alfaalfa pellets. It is applicable for the analysis of "dietary starch" as defined in the introduction of the paper.
ER 7	good
ER 8	Non-resistant starch in animal feeds

	General Comments
ER 1	Positive feedback from collaborators
ER 2	The manuscript describes a method and SLV and its performance in multilaboratory study for dietary Starch (glycogen, maltooligosaccharides, and other alpha-1,6-linked glucose carbohydrates, exclusive of resistant starch). This method is replacement of invalidated AOAC 920.14 due to unavailability of one of the enzyme required in the assay. The described method is more efficient than other methods considered.
ER 3	Measurement of carbohydrates by enzyme-digestion and analysis of the liberated mono-saccharides is an established approach which has worked well for a range of carbohydrates. The collaborative data from this study demonstrates this approach works well for dietary starches due to properly accounting for sucrose & inherent glucose interferences, and in deterring formation of maltulose.
ER 4	Excellent approach
ER 5	This method is similar to older, but now obsolete methods in principal, with better description in choice of enzymes and analysis approach of the glucose contents. This method also simplifies experimental procedures by adding reagents into the same tube until the final dilution step.
ER 6	The principles of the method are good. Enzymes are used to specifically hydrolyse the relevant alpha-glucans in feeds (i.e. starch, maltooligosaccharides, etc) composed of alpha-1,4 and alpha-1,6 linked glucose. Other poly- or oligosaccharides should not be hydrolysed. Resulting glucose is determined using a well established procedure (GOPOD) and free glucose which would interfere is accounted for by running a sample without enzymatic hydrolysis. I don't know if the concept of resistant starch is used in the animal feed world. If yes, it would be good to clarify if the methodology is expected to account for all the starch or only the available starch.
ER 7	none
ER 8	Page 2, line 25. In reference to AOAC Method 996.11, the author refers to the method being “quasi-empirical” and justifies this by stating that “glucose is the analyte detected, but its release is determined by run conditions and specification of enzymes.” The term “quasi-empirical” is unacceptable. This method was run through a full AOAC International interlaboratory evaluation involving 31 laboratories and over this number, the RSDr and RSDR values were similar to those reported in this paper. In reference to the comments about the run conditions and specification of enzymes, of course the method was defined. This is a requirement of any method. It is especially important to specify details of enzymes and particularly purity. This is the reason why so many enzyme based methods have failed in the past. It is dangerous to recommend industrial, or in fact any, enzymes that have not been analysed for activity and purity (contamination with other

	interfering enzymes or sugars etc). AOAC Method 996.11 has also been adapted to run at pH 5. This was evaluated after I had discussions with Mary Beth Hall in 2007 (or 2008). The method works fine at pH 5 and both enzymes are active and stable at this pH. The change to do both incubations at pH 5 is convenient. However, in our hands, the same analytical values were obtained for a number of starch containing samples when sam both incubations were run at pH 5 as compared to running the alpha-amylase incubation at pH 7 (as per 996.11). It is known that a small amount of maltulose can be formed on hydrolysis of starch by alpha-amylase at pH 7 or above. However, this occurs in the industrial hydrolysis of starch which is performed at a starch concentration of approx. 30% w/v. Starch analyses are performed at a starch concentration of just 0.03% (1,000-fold lower concentration). Page 4, line 79. "the use of mildly…excludes the use of alkali or DMSO and thus excludes resistant starch from inclusion in the dietary starch fraction". This is exactly what is measured in AOAC Method 996.11, unless there is a requirement to also measure RS. So where is the difference? Also, how can the author be sure that RS is not hydrolysed in the gut of horses or chickens (pigs will be much the same as humans). Page 4, line72. Dietary starch is defined and includes glycogen. Of course these methods also measure glycogen and maltodextrins, but glycogen is unlikely to be in an animal ration, and maltodextrins would be rare (perhaps some in distillers grains). Page 6, lines 119-121. Pure corn starch gave a recovery of 99.3%, but in the interlab results, this averaged at just 89,4%. Why? Page 7, point (6). In our laboratory, we have not experienced non-linear color formation with GOPOD reagent over t he range 0 – 1.2 absorbance units. Is this a problem with enzyme purity? Page 8, point (8). Ease of use/efficiency. The advantages claimed are exactly the same advantages as described in AOAC Method 996.11. Where is the difference? Page 9, lines 182-184. Method uses the same temperature for AMFG and glucose analysis. This is already done in 996.11. Page 9. Lines 195-197. Enzyme purity. Enzymes must be free of glucose, but it is essential that they are also free of other enzymes active on other glucose containing polymers e.g. beta-glucan. Industrial AMG preparations are highly contaminated with beta-glucanase and to a lesser extent beta-glucosidase. This requirement should be highlighted. Page 10, lines 214-215. For the participants in the interlab, did you state purity requirements for glucose oxidase and peroxidase. It is essential that high purity enzymes are used. Glucose oxidase is commonly contaminated with catalase and this results in instability and fading of the color formed in this reaction.

	Page 16. Point (b) is missing. Page 16 – Purity and source of alpha-amylase and AMG. Detailed specifications on the source of alpha-amylase are given. However, it must be remembered that these enzymes are made for industrial use. There may be variation from batch to batch in contaminants important in an analytical procedure but of no consequence in the intended industrial application. Industrial AMG cannot be used in analytical procedures because it contains glucose, but more importantly, because they contain contaminating activities that interfere with starch determination in plant samples. As far as I am aware, the Megazyme purified AMG (E-AMGDF) is the only AMG pure enough to use in such assays other than pure AMG, which is too costly to use in such assays. Page 17, line 336. Change “amylase” to “amyloglucosidase” Page 17 (e). A statement should be made about the required purity of glucose oxidase and peroxidase. Page 17, line 378. Phenol is generally not used in glucose determination reagents because it is carcinogenic and also is not very stable. The chemical most commonly used in its place is *p*-hydroxybenzoic acid.
ER 9	For me the term "dietary starch" is new, especailly in connection with animal feed and pet food. Fromenergetic viewpoint, I can agree to include maltodextrins, glycogen fromanimal and microbial origin in the new term dietary starch. However I have problems what to do with the4 different types of resistant starhes, the RS1, RS2, RS3 and RS4. Starch incubation with alpha-amylase at 100 C will hydrolyse the RS1, RS2 and RS4 resistant starch but certainly not the RS3 resistant starch, the so-called retrograded starch. Different animals have different intestinal tracks, for example, pets, pigs, cows some can digest resistant starches, others not. So the content of dietary starch in a feed sample depends also on which kind of animal consumes the feed.The for digestion available "dietary starch" in one sample containing resistant starch categories RS1+RS2+RS3+RS4 is most likely different for pets (originally carnivores and less capable to digest native starches), pigs, cows.

	Method Clarity
ER 1	Positive feedback from collaborators
ER 2	Good with the exception how the limitation of the method in application to matrices containing hydrophilic antioxidant contents/activity exceeding 10-20 micromol as ascorbic acid) per 0.1 g of test dry matter.
ER 3	Easy to read. No issues.
ER 4	Well written and understandable

ER 5	Satisfactory
ER 6	Method is clearly written I didn't have problems following it, with the exception of the units used for the enzyme activities. It would be preferable for the authors to define the units of activity for each enzyme since definitions vary from manufacturer to manufacturer. This will be fundamental if the enzymes used need to be replaced with others.
ER 7	good
ER 8	Well thought through study and well written

	Pros/Strengths
ER 1	Single vessel
ER 2	Relatively more efficient method. Very well studied and validated in SLV. 15 labs. collaboratively studied the method and analyzed 10 homogenous test materials (animal feeds and pet foods) using the described method for dietary starch (ranging starch contents of 1-70%). The average within lab. Repeatability as sr for % Dietary starch was 0.49 with a range of 0.03 to 1.56, and among –laboratory repeatability of standard deviation sR averaged 0.96 with a range of 0.09 to 2.69. HORRAT averaged 2.0 for all test samples and 1.9 for samples containing dietary starch more than 2%.
ER 3	Measurement of carbohydrates by enzyme-digestion and analysis of the liberated mono-saccharides is an established approach which has worked well for a range of carbohydrates. The collaborative data from this study demonstrates this approach works well for dietary starches due to properly accounting for sucrose & inherent glucose interferences, and in deterring formation of maltulose. Dietary starch is digested to glucose and the increase in glucose level is used to calculate %dietary starch. Potential interferences are either accounted for (inherent glucose) or excluded (deter inherent sucrose digestion and deter maltulose formation).
ER 4	Traditional chemistry that has been well studied. Can be carried out in modestly equipped laboratories by technical personnel with modest training.
ER 5	Relatively straightforward procedures Satisfactory recovery on glucose and corn starch. Low interference from sucrose , β-glucan and cellulose. Good repeatability and reproducibility.
ER 6	- A simple method that does not need specialized equipment. - option to use alternative methods for glucose analysis is mentioned if a lab does not wish to use the GOPOD assay
ER 7	no comment
ER 8	The specific advantages of this method over AOAC Method 996.11 are not clear. With both methods, good reproducibility and recovery of starch was obtained over a wide range of samples. This method is no easier to perform than 996.11.

	Cons/Weaknesses
ER 1	None
ER 2	The method underestimates dietary starch in feeds and foods whose antioxidant content is known to exceed 10-20 micromol of hydrophilic antioxidant (as ascorbic acid) per 0.1 g of test dry matter. The method in the current format may not be easily applicable to foods/feeds high in phenolic compounds (e.g. beets, red sorghum grain).

ER 3	Spectrophotometric measurement does work well and is easy, quick, and reliable. Quantitative of sugars by HPLC is also simple (a bit more expensive though) but will allow tracking of sucrose to assure its digestion did not occur. The method's steps do prevent sucrose digestion by relying on high-purity enzymes. Since these enzymes are more expensive, laboratories using lower cost enzymes would be at risk of reporting less accurate, higher values.
ER 4	Lack of sophisticated instrumentation will be unappealing to those inclined to high level tech methods.
ER 5	Quadratic fit may be difficult for some users to use, and automation of the whole quantitation process is somewhat difficult to achieve. Scaling up is somewhat limited because of the need to measure absorbency within 30 min of GOPOD reaction. High content of anti-oxidant will prevent accurate determination of glucose, forcing other glucose detection methods into consideration.
ER 6	- potential interference of substances with anti-oxidant activity (if this is unknown it needs to be assessed somehow, or an alternative glucose assay should be used) - although it is mentioned that glucose assays other than GOPOD can be used, it does not appear to have been tested or validated. - it is mentioned that leaving the sample (taking a break) after dilution of fully digested samples has an impact on recovery - but why should that be the case?
ER 7	none
ER 8	This is a good method, but would appear not to be an improvement over AOAC

	Supporting Data Comments
ER 1	Impressive data package
ER 2	15 labs. collaboratively studied the method and analyzed 10 homogenous test materials (animal feeds and pet foods) using the described method for dietary starch (ranging starch contents of 1-70%). The average within lab. Repeatability as sr for % Dietary starch was 0.49 with a range of 0.03 to 1.56, and among –laboratory repeatability of standard deviation sR averaged 0.96 with a range of 0.09 to 2.69. HORRAT averaged 2.0 for all test samples and 1.9 for samples containing dietary starch more than 2%.
ER 3	Excellent study
ER 4	Excellent data package. Well done study.
ER 5	Well-organized summary tables about statistics of all matrix results Good study on the glucose standard responses across different batches
ER 6	This looks to be a straight forward assay which did not appear to be problematic for most of the labs involved in the MLT. The authors have mentioned that alternative assays for glucose could be used instead of GOPOD (and may be essential for samples with high anti-oxidant contents). It would be interesting to know if this has been tested in any of the labs because although it is mentioned it does not appear to have been verified.
ER 7	good
ER 8	Method should be accepted with some changes to text

	Method Optimization
ER 1	Done
ER 2	The method has been optimized for its efficiency and better recovery of starch.
ER 3	Keep as written (see comment in Cons/Weaknesses for optional digestion)
ER 4	No further work needed.

ER 5	Same temperature for both enzymatic procedures, allowing better efficiency. Changed to quadratic curve due to the slight non-linearity of the standards.
ER 6	It would be interesting to understand why leaving the sample (taking a break) after dilution of fully digested samples has an impact on recovery (line 699, p31)
ER 7	good
ER 8	n/a

	Analytical Range
ER 1	1-100%
ER 2	0-100 mg starch in the assay
ER 3	Range studied was 1.00% - 69.6%. Corn starch was used as a spiking agent which suggests this material can be tested directly on this material (89% dietary starch) as long as enzymes are keep in sufficient excess/
ER 4	See method collaborative study report.
ER 5	~1% to 100%
ER 6	about 1 (lowest amount in samples tested in MLT) - 100% starch (considering corn starch used as control)
ER 7	good
ER 8	Acceptable

	LOQ
ER 1	Approx. 0.3% (probably a little larger)- definitely less than 1%
ER 2	0.9% of starch sample weight basis
ER 3	0.3%. Acceptable limit.
ER 4	See method collaborative study report.
ER 5	0.3%
ER 6	This has been estimated as 0.2% dietary starch by using reagent blanks. The approach seems reasonable, although one may expect the practical LoQ to be higher when applied to samples (and is probably not independent of the free glucose content of a sample)
ER 7	good
ER 8	Acceptable

	Accuracy/Recovery
ER 1	99.3 pure corn starch, 90@ control corn starch.
ER 2	89.9% +/- 3.7%
ER 3	993.8% wi+/- 0.8% is excellent
ER 4	See method collaborative study report.
ER 5	Pure corn starch: 99.3% ± 0.8% (Theoretical = 100%) Corn Starch: 89.9% ± 3.7% (Estimated = 89.4)
ER 6	This does not appear to have been extensively tested. Pure starch products have been assayed and the recoveries are greater than 95%, Dextrins appear to be more problematic, but this does not seem to have been discussed.
ER 7	good

ER 8	Good

	Precision
ER 1	Average RSDr 3.5%
ER 2	RSDr % = 1.23 - 8.61%
ER 3	Acceptable. Soybean meal was the highest but this was likely due to a possible lower degree of sample homogeneity.
ER 4	See method collaborative study report.
ER 5	2-3% most samples; >8% Alfalfa pellets (low level @ ~1%); ~6% Dry Dog Kibble; 5% Soybean Meal (low level @~1%)
ER 6	RSD(r) varies from 1.2 - 8.6 %, and is generally below 5% which I would generally regard as acceptable.
ER 7	good
ER 8	Good

	Reproducibility
ER 1	Average RSDR 6.1%
ER 2	RSDR% = 3.87 - 11.16%
ER 3	Acceptable. Soybean meal was the highest but this was likely due to a possible lower degree of sample homogeneity.
ER 4	See method collaborative study report.
ER 5	4-6% most samples; ~10% for Alfalfa pellets and Soybean Meal
ER 6	RSD(R) varies from 3.9- 11.2 %, and is generally below 6% which I would also consider acceptable.
ER 7	good
ER 8	Good

	System Suitability
ER 1	Good systems suggested (Starch, sucrose, glucose)
ER 2	The use of corn starch as control sample to evaluative quantitative recovery in the assay.
ER 3	see above
ER 4	Definitely suitable for purpose
ER 5	N/A
ER 6	The use of enzymatic hydrolysis to convert starch to glucose, and the GOPOD assay to specifically assay the starch means the method is very selective. Potential interferences have been identified and suitable controls are mentioned.
ER 7	good
ER 8	Acceptable

	First Action Recommendation
ER 1	Yes
ER 2	Yes, I do recommend the method to be adopted as First Action Method by ERP after authors have explained the following two limitation of the method in application. 1. The method in the current format is not be easily applicable to foods/feeds high in phenolic compounds (e.g. beets, red sorghum grain). 2. Oats beta-glucan interfere in the assay and provide values above LOD = 0.31 +/- 0.09%.
ER 3	Yes. Recommend consideration of allowing HPLC as an option to measure liberated glucose to calculate %dietary starch. This approach would also measure free, inherent glucose and track if sucrose-digestion has occurred.
ER 4	Yes
ER 5	Yes.
ER 6	Yes
ER 7	yes
ER 8	Yes

	After First Action Recommendation
ER 1	Use feedback
ER 2	NA
ER 3	Recommend consideration of allowing HPLC as an option to measure liberated glucose to calculate %dietary starch. This approach would also measure free, inherent glucose and track if sucrose-digestion has occurred. Decision needed if single or multiple lab work is needed to verify.
ER 4	Just the normal 2 year feedback period. Collaborative is completed and complete.
ER 5	N/A
ER 6	It would be good to test the performance of the method when an alternative glucose assay is used. Clarify the reason why dextrin recovery is low.
ER 7	no
ER 8	That included in the text above. (Please clarify)

PROFILE OF AOAC EXPERT REVIEW PANEL FOR FERTILIZER METHODS

ERP Name	AOAC Expert Review Panel for Fertilizer Methods			**Chair(s)**	Bill Hall (Mosaic)
ERP Formed:	2014	**Number of Methods Adopted**	2 as First Action status	**Number of Methods Recommended**	None Yet
Scope:	Review and adopt methods resulting from sole source submission of methods for the analysis of fertilizers				

Roster	*Slow and Controlled Release Fertilizers*	*P and K Inorganic Fertilizers*	*Total Sulfur in Fertilizers*	*Ar, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se, Zn in Fertilizers*
	1. Bartos, James 2. Hall, William 3. Hartshorn, Jon 4. Hojjatie, Michael 5. Nacharaju, Krishnamurthy (Murthy) 6. Nagarajan, Rajamani *7. Parisi, Salvatore* *8. Provance-Bowley, Mary*	1. Bartos, James 2. Hall, William 3. James, Barbara 4. Kariuki, Solomon 5. Parisi, Salvatore 6. Phillips, Heidi 7. Shelite, Kristopher 8. Tan, Rechel 9. Tsourides, Dion	1. Bartos, James 2. Hall, William 3. Kariuki, Solomon 4. Parisi, Salvatore 5. Phillips, Heidi *6. Provance-Bowley, Mary* 7. Wegner, Keith	1. Bartos, James 2. Hall, William 3. Kariuki, Solomon 4. Parisi, Salvatore 5. Phillips, Heidi 6. Provance-Bowley, Mary 7. Reba, Rick 8. Shelite, Kristopher 9. Shoemaker, Dirk D 10. Tan, Rechel 11. Tsourides, Dion 12. Wegner, Keith

Technical Documents created/used	Fertilizer Forum Documents OMA Appendix D
Methods Adopted *First Action and Final Action status*	AOAC 2015.15 - Nitrogen, Phosphorus, and Potassium Release of Slow- and Controlled Release Fertilizers AOAC 2015.18 - Phosphorus and Potassium in Commercial Inorganic Fertilizers
Final Action Methods Recommended	
Additional Input	AOAC Community on Agricultural Materials
Awards/Recognitions	

Expert Review Panel for Fertilizers

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned co-chair(s) hereby confirm that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Fertilizers that was held on Monday, September 28, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071 USA.

WILLIAM HALL, MOSIAC
Expert Review Panel Chair

_____

Date

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UNITED STATES

Contact:
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Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair
William Hall, Mosaic

Expert Review Panel Members - Present
James Bartos, Office of Indiana State Chemist
William Hall, Mosaic
Jon Hartshorn, Morral Companies, LLC
Michael Hojjatie, Tessenderlo Kerely, Inc.
Barbara James, PotashCorp
Solomon Kariuki, University of Kentucky Division of
Krishnamurthy (Murthy), ICL-SF
Rajamani Nagarajan, Hexion Inc.
Salvatore Parisi, Industrial Consultant
Heidi Phillips, Consultant
Mary C. Provance-Bowley, Harsco Metals & Minerals
Rick Reba, Nestle - USA
Kristopher Shelite, Compass Minerals Inc.
Dirk D. Shoemaker, Nebraska Dept. of Agriculture Labs
Rechel Tan, Abu Dhabi Fertilizer Ind. Co. W.L.L.
Dion Tsourides, Spectro A.I. Inc.
Keith Wegner, Colorado Department of Agriculture

Expert Review Panel Members – Not Present
Robert Clifford, Shimadzu
Anil Gopala, PerkinElmer
Elizabeth Guertal, Auburn University
Ana Rita Nogueira, Embrapa
Uwe Oppermann, Shimadzu
Medet Zor, Istanbul Food Control Laboratory

Expert Review Panel Members – Rescinded
Kai Liu, Eurofins Scientific

Method Authors
James Bartos, Office of Indiana State Chemist, Purdue
William Hall, Mosaic
Calum McCusker, Elementar Americas

AOAC Staff
Deborah McKenzie
La'Kia Phillips

Observers
Shauna Roman, OMB Liaison, Chair OMB

Jason S. Kong, Ohio Dept. of Agriculture
Hilde Skar-Norli, NMKL
Shah Zaman, New Mexico Dept. of Agriculture

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:

AOAC convened the *Official Methods of Analysis*^{SM} (OMA) Expert Review Panel for Fertilizers on Monday, September 28, 2015 during the AOAC Annual Meeting and Exposition held at the Westin Bonaventure Hotel, 404 South Figueroa Street, Los Angeles, California 90071.

The purpose of the meeting was to review and evaluate OMAMAN-22: Determination of Nitrogen, Phosphorus, Potassium and other nutrients release in Slow- and Controlled-Release Fertilizers, OMAMAN-23: Single Laboratory Validation of a Method for the Determination of Phosphorus and Potassium in Commercial Inorganic Fertilizers by ICP-OES, OMAMAN-24: Determination of Total Sulfur in Fertilizers by High Temperature Combustion, and OMAMAN-28: Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganeses, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single Laboratory Validation.

The study director for OMAMAN-22 was William Hall for Mosaic, located at 13830 Circa Crossing Drive, Lithia, Florida 33547. The candidate method (OMAMAN-22) was reviewed and supplemental information was also provided to the reviewers which included the Evaluation Of A Soil Incubation Method To Characterize Nitrogen Release Patterns of Slow- And Controlled-Release Fertilizers, Optimization And Validation Of An Accelerated Laboratory Extraction Method To Estimate Nitrogen Release Patterns Of Slow- And Controlled-Release Fertilizers, Statistical Correlation Of The Soil Incubation And The Accelerated Laboratory Extraction Methods To Estimate Nitrogen Release Rates Of Slow- And Controlled-Release Fertilizers, and the Method Safety Checklist. This method was reviewed as a single laboratory validation.

The study director for OMAMAN-23 was James Bartos of the Office of Indiana State Chemist, Purdue University, located at 175 South University Street, West Lafayette, Indiana 47907. The candidate method (OMAMAN-23) was reviewed and supplemental information was also provided to the reviewers which included the Determination of Phosphorus and Potassium in Commercial Inorganic Fertilizers by Inductively Coupled Plasma-Optical Emission Spectrometry: Single-Laboratory Validation. This method was reviewed as a single laboratory validation.

The study director for OMAMAN-24 was Calum McCusker of Elementar Americas, located at 520 Fellowship Road, Suite D-408, Mt. Laurel, New Jersey 08054. The candidate method (OMAMAN-24) was reviewed and supplemental information was also provided to the reviewers which included the Determination of Total Sulfur in Fertilizers by High Temperature Combustion: Single-Laboratory Validation, Material Safety Data Sheet for SICAPENT with Indicator, Method Safety Checklist, and the AOAC Official Method 980.02 Sulfur in Fertilizers- Final Action 1985. This method was reviewed as a single laboratory validation.

The study directors for OMAMAN-28 was Sharon Webb of the University of Kentucky, Division of Regulatory Services, located at 103 Regulatory Services Bldg, Lexington , Kentucky 40546-0275. The candidate method (OMAMAN-28) was reviewed and supplemental information was provided to the reviewers which included the Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganeses, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single Laboratory Validation modification and extension of AOAC 2006.03, Single Laboratory Information, Method Safety Checklist, and AOAC OMA 2006.03. This method was reviewed as a new method.

William Hall, Calum McCusker, and James Bartos were present during the Expert Review Panel session.

Criteria for Vetting Experts and Selection Process:
The original twenty-five (25) candidates are being submitted for consideration by the Official Methods Board to evaluate candidate methods for Fertilizers which include the subject matter areas of Metals, Nitrogen (slow release), Phosphorus & Potassium, Total Sulfur, and Urea as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Agricultural Materials Community, the Association of American Feed and Control Officials (AAFCO), the Association of Fertilizer and Phosphate Chemists (AFPC) and other subject matter experts. Many of the following candidates have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway. The following members were present to review methods OMAMAN-22, OMAMAN-23, OMAMAN-24, and OMAMAN-28.

OMAMAN-28
1. Bartos, James
2. Hall, William
3. Kariuki, Solomon
4. Parisi, Salvatore
5. Phillips, Heidi
6. Provance-Bowley, Mary
7. Reba, Rick
8. Shelite, Kristopher
9. Shoemaker, Dirk D
10. Tan, Rechel
11. Tsourides, Dion
12. Wegner, Keith

OMAMAN-22
1. Bartos, James
2. Hall, William
3. Hartshorn, Jon
4. Hojjatie, Michael
5. Nacharaju, Krishnamurthy (Murthy)
6. Nagarajan, Rajamani
7. *Parisi, Salvatore*
8. *Provance-Bowley, Mary*

OMAMAN-23
1. Bartos, James
2. Hall, William
3. James, Barbara
4. Kariuki, Solomon
5. Parisi, Salvatore
6. Phillips, Heidi
7. Shelite, Kristopher
8. Tan, Rechel
9. Tsourides, Dion

OMAMAN-24
1. Bartos, James
2. Hall, William
3. Kariuki, Solomon
4. Parisi, Salvatore
5. Phillips, Heidi
6. *Provance-Bowley, Mary*
7. Wegner, Keith

ERP Orientation:
All ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. OMAMAN-22: Eight (8) out of the nine (9) members were present and therefore met a quorum to conduct the meeting. OMAMAN-23: Nine (9) out of the fourteen (14) members were present and therefore met a quorum to conduct the meeting. OMAMAN-24: Seven (7) out of the eight (8) members were present and therefore met a quorum to conduct the meeting. OMAMAN-28: Twelve (12) out of the fifteen (15) members were present and therefore met a quorum to conduct the meeting.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The ERP adopted OMAMAN-22: Determination of Nitrogen, Phosphorus, Potassium and other nutrients release in Slow- and Controlled-Release Fertilizers, OMAMAN-23: Single Laboratory Validation of a Method for the Determination of Phosphorus and Potassium in Commercial Inorganic Fertilizers by ICP-OES, and OMAMAN-24: Determination of Total Sulfur in Fertilizers by High Temperature Combustion as AOAC First Action Official Methods by consensus. The decisions have been captured and reflected in the meeting minutes.

Subsequent ERP Activities:
ERP members are required to track the performance of the recently approved First Action method for a 2 year period effective as of September 28, 2015.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Chair, William Hall, welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form. In addition, she also presented an overview of the ERP process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

III. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-28: Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganeses, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single Laboratory Validation. The method author Sharon Webb of the University of Kentucky, Division of Regulatory Services was not present to address the questions and comments of the ERP members. A summary of comments were provided to the ERP members and the method author.[1] By consensus, the ERP presented the following motions for OMAMAN-28.

MOTION:
Motion by Phillips; Second by Provance-Bowley, to move to table to discussion for OMAMAN-28 until the method author responds to the ERP comments.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-24: Determination of Total Sulfur in Fertilizers by High Temperature Combustion. The method author Calum McCusker of Elementar Americas was present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[2] By consensus, the ERP presented the following motions for OMAMAN-24.

MOTION:
Motion by Phillips; Second by Wegner, to move OMAMAN-24 to AOAC First Action Official Methods status.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

MOTION:
Motion by Phillips; Second by Nagarajan, to review method to accommodate sample preparation, instrument conditions, safety, and robustness, and to allow for other instrument manufacturers.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

Motion by Phillips; Second by Wegner, to withdraw previous motion.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Withdrawn.*

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-28
[2] Attachment 2: Summary of Expert Reviewer Comments for OMAMAN-24

MOTION:
Motion by Phillips; Second by Wegner, to edit the method prior to First Action publication to accommodate sample preparation, instrument conditions, safety, and to allow for other instrument manufacturers.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-23: Single Laboratory Validation of a Method for the Determination of Phosphorus and Potassium in Commercial Inorganic Fertilizers by ICP-OES. The method author, James Bartos of the Office of Indiana State Chemist, Purdue University was present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[3] By consensus, the ERP presented the following motions for OMAMAN-23.

MOTION:
Motion by James; Second by Tsourides**,** to move OMAMAN-23 to First Action Official Methods status with editorial changes.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

MOTION:
Motion by Tsourides; Second by Shelite, to move OMAMAN-23 to First Action Official Methods status with editorial changes as follows prior to first action publication: 1) To consider empirical calibration and that the system optimization be based the instrument manufacturer's recommendation for an organic matrix (carbon).
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

MOTION:
Motion by Shelite; Second by James, for OMAMAN-23 to be considered for AOAC Final Action, the following is recommended: 1) for the method to be collaboratively studied, and 2) based on collaborative results, the ERP will determine whether the method is for screening or confirmatory.
Consensus demonstrated by: (Unanimous) in favor, 0 opposed, and 0 abstentions. *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for OMAMAN-22: Determination of Nitrogen, Phosphorus, Potassium and other nutrients release in Slow- and Controlled-Release Fertilizers. The method author, William Hall of Mosaic, was present and able to address questions and concerns of the ERP members. A summary of comments were provided to the ERP members and the method author.[4] By consensus, the ERP presented the following motions for OMAMAN-22.

MOTION:
Motion by Bartos; Second by Hartshorn, to move OMAMAN-22 to First Action Official Methods status.
Consensus demonstrated by: 5 in favor, 1 opposed, and 1 abstention. *Motion Did Not Pass.**
**Method must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons. Negative vote(s) can be overridden by 2/3 of voting ERP members after due consideration.*

MOTION:
Motion by Bartos; Second by Hartshorn, to move OMAMAN-22 to First Action Official Methods status.
Consensus demonstrated by: 5 in favor, 1 opposed, and 1 abstention. *Motion Passed.*
Negative votes were overridden by 2/3 of voting ERP members.

[3] Attachment 3: Summary of Expert Reviewer Comments for OMAMAN-23
[4] Attachment 4: Summary of Expert Reviewer Comments for OMAMAN-22

MOTION:
Motion by Murthy; Second by Nagarajan, for OMAMAN-22 to be considered for AOAC Final Action, the following is recommended: 1) to clarify the need for the ambient soil method for coated fertilizers. (Not including sulfur coated), and 2) to include liquid matrices.
Consensus demonstrated by: 5 in favor, 0 opposed, and 2 abstentions. *Motion Passed.*

IV. Discuss Final Action Requirements for First Action Official Methods (if applicable)
No further action was discussed at this time. The First to Final Action requirements set for OMAMAN-22 and OMAMAN-23 were noted in the meeting minutes above.

V. Adjournment:
Meeting adjourned at 7:31pm.