



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

John Austad
Sean Austin
Sneh Bhandari
Esther Campos-Giménez/Adrienne McMahon
Scott Christiansen
Hans Cruijssen/Wil van Loon
Jon DeVries
Sarwar Gilani
Brendon Gill
Don Gilliland/Karen Schimpf
Estela Kneeteman
Bill Mindak
Maria Ofitserova
Melissa Phillips/Kate Rimmer
Shay Phillips
Günther Raffler
Jinchuan Yang

Covance Labs (Chair)

Covance Labs
Nestlé Research Centre
Mérieux NutriSciences & OMB
Nestlé/Wyeth Nutrition
Perrigo Nutritionals
FrieslandCampina Domo
Independent Consultant
Consultant
Fonterra Cooperative
Abbott Nutrition
INTI
FDA CFSAN
Pickering Labs, Inc.
NIST
Mead Johnson Nutrition
Eurofins/CLF
Waters Corp.

Expert Review Panel Members (unable to attend):

Min Huang
Harvey Indyk

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

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	<i>Carn-06 - Carnitine Quantitated by liquid chromatography and isotope dilution and Choline quantitated by liquid chromatography and isotope dilution mass spectroscopy</i>	Sneh Bhandari John Austad	Method not adopted/ moved to First Action <i>Official Method</i> SM status	<ul style="list-style-type: none"> ▪ 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	<i>Amino-02 - HPLC Determination of Total Tryptophan in Infant Formula and Adult/Pediatric Nutritional Formula Following Enzymatic Hydrolysis</i>	Maria Ofitserova Shay Phillips	Method not adopted/ moved to First Action <i>Official Method</i> SM status	<ul style="list-style-type: none"> ▪ 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. <ul style="list-style-type: none"> ○ 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: <ul style="list-style-type: none"> ○ Analytical range ○ wide calibration range ○ system suitability
Carotenoids	<i>Carot-01 - Determination of Carotenoids in Infant Formula and Adult/Pediatric Nutritional Formula using High-Performance Liquid Chromatography with Photo Diode Array Detection.</i>	Jon DeVries Adrienne McMahon	Method not adopted/ moved to First Action <i>Official Method</i> SM status	<ul style="list-style-type: none"> ▪ 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 <ul style="list-style-type: none"> ○ Information provided on six (6) samples and one (1) lab ○ 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	<i>Chlor-03 - Single Laboratory Validation for Chloride Analysis in Infant Formula and Adult Nutritionals: AOAC SMPR 2014.015</i>	Bill Mindak Günther Raffler Karen Schimpf	Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ 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	<i>Fluor-02 - Single Laboratory Validation for Fluoride Analysis in Infant Formula and Adult Nutritionals: AOAC SMPR 2014.016</i>	John Austad Melissa Phillips Bill Mindak	Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ 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)	<i>Fos-04 - Determination of Fructans in Infant and Adult/Pediatric Nutritional Formulas as well as ingredient commodities</i>	Jon DeVries Sean Austin Hans Cruijssen	Jon DeVries (moved) Brendon Gill (second) Yes-4/ No-10/Abstain-1 <hr/> Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ Data will meet SMPR ▪ Spiked recovery <ul style="list-style-type: none"> ○ Part 1 - oligosaccharide (need to see data) ▪ The units used in the report are not clearly described
GOS	<i>Gos-02 - Determination of trans-Galacto-oligosaccharides (TGOS) in Infant Milk Formula (Ion-Exchange Chromatography)</i>	Sean Austin Maria Ofitserova Estela Kneeteman	Maria Ofitserova (moved) Estela Kneeteman (second) Yes-2/ No-13/Abstain-1 <hr/> Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ 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	<i>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)</i>	Bill Mindak Jinchuan Yang	Jinchuan Yang (moved) Scott Christiansen (second) Yes-6/ No-7/Abstain-1 <hr/> Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ Method has a simple microwave digestion ▪ No ionization buffer is required <ul style="list-style-type: none"> ○ should be required & stated ○ do background correction ▪ Specify the wavelength ▪ LOQ for three (3) elements <ul style="list-style-type: none"> ○ copper ○ iron ○ manganese ▪ No data on SPIFAN matrices
B Vitamins (1, 2, 3, 6)	<i>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</i>	Esther Campos-Gimenez Brendon Gill Scott Christiansen	Method not adopted/ moved to First Action <i>Official MethodSM</i> status	<ul style="list-style-type: none"> ▪ 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
	<i>BVit-02 - Simultaneous Determination of Total Vitamin B6, B2, B3 and B1 in Infant Formula Products by LC-MS/MS Using Enzymatic Digestion</i>	Esther Campos-Gimenez Scott Christiansen Estela Kneeteman	Scott Christiansen (moved) Sarwar Gilani (second) Yes-6/ No-9/Abstain-1 <hr/> Melissa Phillips (moved) Esther Campos-Gimenez (second) Yes-11/ No-2/Abstain-1 <hr/> Yes-12/ No-2/Abstain-1 **Method adopted/ moved to First Action <i>Official MethodSM</i> status for B₁, 2, 6 only	<ul style="list-style-type: none"> ▪ FAD/FAB <ul style="list-style-type: none"> ○ 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 <i>Official Method</i> SM status with a change in the applicability for infant formula	<ul style="list-style-type: none"> ▪ 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 <i>Official Method</i> SM status	<ul style="list-style-type: none"> ▪ Only one (1) sample was used for D₂ ▪ Exclude HPLC ▪ 8/16 had performance issues ▪ Next steps <ul style="list-style-type: none"> ○ Open call for methods ○ 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	<ul style="list-style-type: none"> ▪ 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	<ul style="list-style-type: none"> ▪ 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	<i>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)</i>	Melissa Phillips (Moved) Bill Mindak (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	<ul style="list-style-type: none"> ▪ Complete accuracy study in single lab
Minerals & Trace Elements	<i>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</i>	Shay Phillips (Moved) Hans Cruijisen (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	<ul style="list-style-type: none"> ▪ Complete MLT data on SPIFAN matrices
	<i>OMA# 2015.06 (MTE-02) - Determination of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo by ICP-MS</i>	Shay Phillips (Moved) Hans Cruijisen (Second)	~Motion: move to reproducibility testing Yes-13/ No-1/Abstain-1	
Vitamin K	<i>OMA# 2015.09 (VitK-02) - Determination of Trans Vitamin K1 by HPLC and Fluorescence Detection</i>	Brendon Gill (Moved) John Austad (Second)	~Motion: move to reproducibility testing Yes-14/ No-0/Abstain-1	<ul style="list-style-type: none"> ▪ 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.