# 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

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**Expert Review Panel Members** (in attendance):

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| ***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):

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|---|---|
| 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)<br><br>*Method retains First Action *Official Method*$^{SM}$ status | ▪ Method meets SMPR<br>▪ Detects the poly glutamate<br>▪ Process is long<br>▪ SLV data is available<br>▪ Folate reported as total folate<br>▪ Needs % of folate<br>▪ Standard corrective for water; 8%<br>▪ Accuracy that doesn't have methyl; non folic<br>▪ 5 methyl being different |
| **Carnitine** | *Carn-07 - Analysis of Free and Total Carnitine and Choline in Infant Formula and Adult Nutritionals* | Günther Raffler<br>Sneh Bhandari (by evaluation form) | Günther Raffler moved & Melissa Phillips second<br><br>*Motion: recommended method to First Action *Official Method*$^{SM}$ status & proceed in AOAC SPIFAN process (DRM)<br>**Yes- 11/ No-0 /Abstain-1** | ▪ Recovery rates are good<br>▪ SLV report complete<br>▪ 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<br>Shay Phillips | Method not recommended for First Action at this time | ▪ Method is well written; sound methodology<br>▪ Study author should use all AOAC SPIFAN samples and provided the information<br>▪ Analytical range; LOQ<br>▪ Lysteine has limited range<br>▪ Title change – total amino acid<br>▪ Does not capture tryptophan<br>▪ Proprietary technique<br>▪ Recovery over 110%<br>▪ 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<br>Scott Christiansen | Method not recommended for First Action at this time | ▪ Five (5) different samples used<br>○ Infant milk powder<br>▪ Simple method<br>▪ No LOQ data or recovery<br>▪ 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<br>Karen Schimpf | Method not recommended for First Action at this time | ▪ Semi proprietary method<br>▪ No chromatography<br>▪ Two (2) samples used<br>▪ Free or total?<br>▪ Use NIST 1849a as reference<br>▪ Not enough SLV data<br>▪ Columns from two (2) different manufacturers |
| | *Boi-03 - Simultaneous Determination of Seven Water Soluble Vitamins in Products by LC-MS/MS* | Adrienne McMahon<br>Estela Kneeteman | Method not recommended for First Action at this time | ▪ Lower LOQ<br>▪ Needs full recovery<br>▪ Needs more AOAC SPIFAN samples<br>▪ 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<br>Karen Schimpf<br>Bill Mindak | Method not recommended for First Action at this time | ▪ Applicable to milk based infant formula<br>▪ May help to have figures; can't distinguish<br>▪ No AOAC SPIFAN matrices<br>▪ For medical nutritionals please provide information |
| | *#Chlor-02 - Infant Formula and Adult Nutritionals Chloride by Potentiometry* | Günther Raffler<br>Shay Phillips<br>Bill Mindak | Scott Christiansen moved<br>Jon DeVries second<br><br>*Motion: move to First Action<br>Yes- 10/ No-2 /Abstain-1<br>———————<br>Second vote<br>Yes- 10/ No-2 /Abstain-1 | ▪ Hydrolyzed method<br>▪ No SLV data included<br>▪ Simple method<br>▪ Address issue with high protein & fat in adult nutritionals<br>▪ Recovery/repeatability meets the SMPR |
| | *Chlor-03 - Determination of Chloride in Infant Formula* | Shay Phillips<br>Günther Raffler<br>Bill Mindak | Method not recommended for First Action at this time | ▪ Different approach<br>▪ Needs AOAC SPIFAN SLV<br>▪ Newer technology<br>▪ Needs reagents<br>▪ Performance based method is needed not equipment |



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

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



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

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|---|---|---|---|---|
| **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<br>Jinchuan Yang | *Method retains First Action *Official Method*$^{SM}$ status | ▪ Good method; great update<br>▪ SLV used pertinent samples<br>▪ Method meets most of the SMPR<br>▪ LOQ meets SMPR<br>▪ Spiked recovery meeting SMPR (90-110%)<br>▪ Analytical range recovered higher<br>▪ 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<br>Jinchuan Yang | Moved<br>second<br><br>*Motion: move to First Action<br>Yes- 9/ No-2 /Abstain-1<br>_____<br>Second vote<br>Yes- 10/ No-2 /Abstain-2 | ▪ Too much instrument details<br>▪ Internal standard added before<br>▪ Meets most of the SMPR<br>▪ Carbon buffer<br>▪ Used AOAC SPIFAN samples<br>▪ No scope applicability<br>▪ SRM too high<br>▪ Written for only one model<br>▪ Potassium has high RSD<br>▪ 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<br>Jinchuan Yang | Bill Mindak moved<br>Jinchuan Yang second<br><br>Method withdrawn/tabled until data can be reviewed by ERP | ▪ No internal standard<br>▪ Copper did not meet the low range<br>▪ Remove the "dry ash" from the method<br>▪ 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<br>Scott Christiansen | Method not recommended for First Action at this time | ▪ Peak in the chromatograms are not labeled correctly<br>▪ Extraction procedure; separation of the cis/trans could be compromised<br>▪ Method seems promising; has potential<br>▪ LOQ/accuracy meets the SMPR<br>▪ No primary stock standard used<br>▪ Variability & precision is a concern<br>▪ No purity check on the standard |
| | *VitK-02 - Determination of Trans Vitamin K1 by HPLC and Fluorescence Detection* | Scott Christiansen<br>Esther Campos-Gimenez | Scott Christiansen moved<br>Esther Campos-Gimenez second<br><br>*Motion: move to First Action<br>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<br>Jon DeVries second<br>*Motion: move to Final Action<br>Yes-11/ No-0 /Abstain-1 | ▪ Fourteen (14) labs participated; only thirteen (13) submitted data<br>▪ Used red dye #3 (strawberry drink mix)<br>▪ Six (6) matrices/one (1) SRM<br>▪ Used three (3) different suppliers<br>▪ Three (3) labs used microwave digestion<br>▪ Complete method<br>▪ May need maintenance<br>▪ Add precautions<br>○ Lens stack<br>⮝ 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<br>Don Gilliland/ Karen Schimpf | John Austad moved<br>Karen Schimpf second<br>*Motion: move to Final Action<br>Yes- 12/ No-0 /Abstain-1 | ▪ Sixteen (16) labs participated<br>○ One (1) lab dropped out<br>○ One (1) lab did not qualify<br>○ Fourteen (14) labs provided data<br>⮝ Two (2) labs provided data as one (1)<br>▪ Consider a way to dry<br>○ 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<br>Brendon Gill/ Harvey Indyk | Jon DeVries moved<br>Scott Christiansen second<br>Motion: move to Final Action<br>Yes- 4/ No-4 /Abstain-4<br>*Method retains First Action *Official Method*$^{SM}$ status | ▪ Twenty-six (26) labs originally participated<br>○ Two (2) labs failed<br>○ Twenty- four (24) labs provided data<br>⮝ Fourteen (14) labs provided data using UPLC<br>⮝ Nine (9) labs provided data using HPLC<br>⮝ Due to customs restrictions, one (1) lab used known samples<br>○ Two (2) labs slightly passed<br>▪ Method needs more guidance<br>○ Stability<br>○ Timing<br>▪ May be oxidized<br>▪ Samples should be in sealed containers with the same lot number<br>▪ System suitability included<br>▪ Precision data from the two (2) sample types<br>▪ Add a control |



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

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| ***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)<br><br>Brendon Gill/ Harvey Indyk | Method not recommended for Final Action<br><br>*Method retains First Action *Official Method*$^{SM}$ status | ▪ Method author needs to publish two (2) sets of data<br>○ Second set of data should stand alone<br>▪ Clearly identify which data was previously used and which in new<br>⅄ Do not exclude data<br>⅄ Need outliers in the report<br>▪ Clarify labs that participated in the study Remediation<br>○ Clean up data<br>⅄ UPLC<br>⅄ 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)**