Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)
Meeting at The LaLiT Hotel
New Delhi, India

India Regional Meeting
DRAFT MEETING PROCEEDINGS
February 13 – 15, 2012

MEETING ATTENDEES:

Manjeet Agrawal, Shriram Institute for Industrial Research
Martin Alewijn, RIKILT
Nilesh S. Amritkar, Envirocare Labs Pvt. Ltd.
Priti Nilesh Amritkar, Envirocare Labs Pvt. Ltd.
Jayashree Arcot, University of New South Wales
Saurabh Arora, Arbro Pharmaceuticals Ltd.
Vishal Arora, AES Laboratories (P) Ltd.
R.K. Bajaj, Bureau of Indian Standards
Krishnamurthy Balasubramanian, Microchem Laboratory Private Ltd.
Sneh Bhandari, Silliker, Inc.
Esther Campos-Gimenez, Nestlé Research Center
Dinesh Goel, Delhi Test House
Lalitha Ramakrishna Gowda, CFTRI
Arti Gupta, Abbott Laboratories
Ramesh Jampala, Eurofins Analytical Services India Pvt. Ltd.
Amrit Kaur, Nestlé India Ltd. Moga
Imran Khan, Intertek
Erik Konings, Nestlé Research Center
Roopak Kumar, Arbro Pharmaceuticals Ltd.
Mihir Kumar Kundu, FSSAI
Adrienne McMahon, Pfizer Nutrition, Ireland
Anita R. Mishra, AOAC INTERNATIONAL
Deepali Mohindra, Dionex- Part of Thermo Fisher Scientific
Mardi Mountford, International Formula Council (IFC)
Deepthi Madura Sri Munasinghe, University of Peradeniya
Norriel Nipales, Pfizer Nutrition
Jitendra Parmar, Thermo Fisher Scientific
Rupali Pillai, Abbot Laboratories
Guenther Raffler, Central Laboratories Friedrichsdorf GmbH
Robert Rankin, International Formula Council
Vinod Singh Rawat, Shriram Institute for Industrial Research
Daniel Schmitz, Abbott Nutrition
Darryl Sullivan, Covance Laboratories
Joseph J. Thompson, Abbott Laboratories
Suneeti Toteja, Bureau of Indian Standards
David Woollard, New Zealand Laboratory Services Ltd.
Sudhakar Yadlapalli, First Source Laboratory Solutions Ltd.

DAY I: February 13, 2012

I. WELCOME AND INTRODUCTION:

Darryl Sullivan, chairman, called the meeting to order at 9:33 a.m. and welcomed the participants to stakeholder panel for infant formula and adult nutritionals (SPIFAN) India regional meeting.

All the participants introduce themselves beginning with voting panel. Deepa Bhajekar, announced the scheduled opening of AOAC section in India on April 5, 2012 and invited interested participants to attend the meeting. Sullivan provided background on how AOAC functions and its standards development process. He emphasized the different perspectives including India's perspective, the actions and proposed representations of the voting panel, the policies and procedures of AOAC including antitrust and conflict of interest policies and procedures.

Anita Mishra summarized minor revisions to the table of contents, but clarified that the agenda was correct as presented. Also, two presentations on India's Standards Development Process and ISO Standardization Update were not included in the meeting materials, but will be available on the SPIFAN website.

II. AOAC STANDARDS DEVELOPMENT PROCESS

Mishra presented an overview of the standards development process of AOAC. She provided a series of definitions used in the process including dominance, stakeholder panel, and key stakeholder. Mishra emphasized that a 2/3rd majority is required for approval of any motion presented before the panel. AOAC standards development process follows the following principles: transparency, balance of interests, and parliamentary procedure.

III. BACKGROUND ON THE SPIFAN PROJECT

Dan Schmitz provided a history of SPIFAN project. He shared that twenty (20) countries are involved and have participated since the project started in 2010. SPIFAN is an international community and believes in a global stakeholder process. He further explained that the purpose of the SPIFAN initiative is to establish reliable dispute resolution methods for infant formula and adult nutritionals. These methods would not necessarily supersede old methods, but will provide a definitive method. Currently, the focus is on nutritional analysis. For nutrient analysis, a set of unambiguous and approved validated reference methods are not available. He shared information about nutrients already in queue.

IV. INDIA'S STANDARDS DEVELOPMENT PROCESS

Dr. R.K. Bajaj presented the process of standard setting in India. He explained that the Bureau of Indian standards (BIS) came into being in 1986 with the objective of harmonization of standard development activities. He explained that Indian standards follow the consensus process with transparency. Indian standards are generally voluntary in nature except in legal situations or when there are contract stipulations. The standards development process follows a committee structure. Dr. Bajaj explained the principles of standardization and the standards development process via a flow chart.

Stakeholders discussed Indian infant formula products consisting of buffalo milk and that SPIFAN needs to consider this as one of the matrices.

V. TECHNICAL PERSPECTIVE: GENERAL OVERVIEW

Stakeholders discussed the technical perspective and challenges regarding analytical methods for infant formula and adult nutritionals in India. Stakeholders commented that validation work needs to be completed on new methods because current methods are lengthy and tedious. In some cases newer ICP methods are needed. New modern methods are required to replace old traditional methods. Methods are available, but should be prescribed and made mandatory. Methods exist but do not get applied and hence are not functional. It is important to know how to extract the vitamin for maximum benefits of recovery and to choose between levels which are nutritionally significant.

Priority nutrients discussed include: amino acids, carotenes, non-protein nitrogen, lacto albumin, glucosamine, and other various nutrients that are present in mother's milk but absent in formulated products. These priority nutrients are similar to those listed as priority on BIS's list.

Stakeholders commented that methods should be harmonized with ISO and BIS. Stakeholders also commented that harmonization is needed for energy content between regulatory agencies.

Stakeholders commented that in India, infant formula can be cow or buffalo milk as defined by BIS. The major difference between buffalo and cow milk is fat content. Methods should determine milk fat, vegetable fat and animal fat.

VI. STANDARD METHOD PERFORMANCE REQUIREMENT (SMPR) DEVELOPMENT

Sullivan provided an overview and guidance on developing standard method performance requirements (SMPRs). His presentation included an explanation of the purpose/objectives of SMPR, the process to develop SMPRs, and the alternative path to official first action status.

VII. DISCUSS TECHNICAL AND REGULATORY BACKGROUND:

The first working group presentation was given by co-chairs: Drs. Jayashree Arcot and Lalitha Gowda on Vitamin C:

Background information on the history of vitamin C and ascorbic acid were presented. They shared the molecular structure, biochemistry, metabolism, physiological functions, recommended dietary allowance & intake, sources, and stability information. Information on currently available analytical methods was presented. Methods presented included:

1. 2,6-dichloroindophenol titrimetry method (AOAC Method 967.21) where L-dehydroascorbic acid can be determined by first converting it to L-ascorbic acid
2. 2,4-dinitrophenylhydrazine method (ascorbic acid, dehydro form+di keto gluconic acid)

3. Microfluorometric method (AOAC method 967.22) which measures both ascorbic acid and dehydroascorbic acid (DHAA)
4. HPLC where ascorbic acid and dehydroascorbic acid are separated from the rest of the components of the sample

They concluded by presenting a fitness-for-purpose statement for Vitamin C.

The second working group presentation was given by co-chairs: Drs. Sneh Bhandari & Rajesh Girdhar on choline:

Background information on the history of choline was presented. They shared the chemical structure, metabolism, biological function, recommended daily amounts.
Information on the currently available analytical methods was presented. Methods presented included:

1. AOAC 999.14-CODEX TYPE 2 method for infant formula and milk.
2. AACC METHOD 86-45.01 is not validated for infant formula.
3. China NFSS-GB5413.20.2010 is an enzymatic colorimetric method and is very similar to AOAC 994.14.
4. China-NFSS-GB5413.20.2010 is Reinecke's salt spectrophotometric method, not collaboratively studied for infant formula.
5. China-NFSS-GB 5413.20.2010, is an iron chromatography method, but has not been validated or collaboratively studied over the range of milk based infant formulas.
6. Dionex method is similar to the GB method, but it has not been validated or collaboratively studied over the range of milk based infant formulas.
7. HPLC method
8. HPLC-MS method
9. Liquid chromatography-ESI-IDMS Method, not validated for milk based infant formula
10. LC –MS method
11. Biosensor method

VIII. REGULATORY AND INTERNATIONAL TRADE OF INFANT FORMULA AND ADULT NUTRITIONALS

Sullivan led a discussion with stakeholders on the definition of infant formula and adult nutritionals in India. Discussions included mechanisms in which to have the SPIFAN methods and standards facilitated through the BIS process.

Stakeholders discussed the possibility of investigating having SPIFAN methods being adopted by ISO and then adopted by BIS, because BIS would adopt ISO methods without question.

AOAC requested BIS to put SPIFAN methods as an option. It was suggested that if BIS does not have a standard with a method then a SPIFAN method can fill the void.

Bhajekar of Microchem volunteered to organize with the BIS laboratories recommendations for inclusion of AOAC methods into BIS.

IX. WRAP UP

The first day session wrapped up summarizing the excellent presentations and information that were delivered by all the speakers. In addition, stakeholders were reminded that during the second day, the working group co-chairs would begin developing SMPRs for vitamin C and choline.

DAY 2: February 14, 2012

X. DISCUSS TECHNICAL STANDARD METHOD PERFORMANCE REQUIREMENTS (SMPRs)

Vitamin C

Arcot and Gowda, working group co-chairs for vitamin C led the discussion on establishing the SMPR. The group began by discussing the applicability for the methods. Ascorbyl palmate was considered by the working group. The group agreed that it will not be included in defining vitamin C because it is not used as a nutrient but as an antioxidant. The group was reminded that dehydroascorbic acid may or may not have the same biological activity as ascorbic acid. The group reiterated the need to consider buffalo milk, goat milk and sheep milk as future matrices.

The working group reached general agreement on the following applicability statement: Determination of vitamin C in all forms of infant, adult or Pediatric formula (powders, ready to feed liquids, and liquid concentrates). For the purpose of this SMPR the Vitamin C is defined as the sum of L-ascorbic acid or its salts and dehydroascorbic acid.

The working group reached general agreement on the method performance requirements:

Analytical range	1.0-250 mg/100g	
Limit of detection (LOD)	0.3 mg	
Limit of quantitation (LOQ)	1.0 mg	
Repeatability (RSD)	1.0 mg	≤ 10%
	10 mg	≤5%
	75 mg	
	200 mg	
Recovery	90-110%of mean spiked recovery over the range of the assay	
Reproducibility (RSD)	1.0 mg	≤15%
	10 mg	≤10%
	75 mg	
	200 mg	
Concentrations apply to (1) 'ready-to feed' liquids "as is", (2) reconstituted powders (25 g into 200 g water, and (3) liquid concentrates diluted 1:1 by weight		

The working group reached general consensus on the SMPR for Vitamin C. See Attachment 1. The working group co-chairs reviewed several methods submitted to the working group. The working group endorsed methods 2, 3, 4 to go forward. Below are the comments on each method. AOAC will extend the call for methods for a couple more weeks to allow other stakeholders to submit their methods for consideration.

Method	Title	Author	Comments
VitC-01	Determination of Total Vitamin C in Fruit Juices and Related Products by Liquid Chromatography: Interlaboratory Study	Brause/Woollard/Indyk	Method is outdated, method will advance with a modified method.
VitC-02	Determination of Vitamin C by HPLC with UV Detection	Submitted by: Abbott Nutrition	May not include de hydro and they may submit an updated method, has good in house data. **(Method will advance)**

VitC-03	HPLC-UV determination of total vitamin C in a wide range of fortified food products	Fontannaz/Kilinc/Heudi	Has good in house validation data. **(Method will advance)**
VitC-04	Determination of ascorbic acid in different food and pharma applications - validation of analytical method	K. Schäfer/Kessler	No infant formula data. Has good in house data. **(Method will advance)**
VitC-05	Determination of Vitamin C Content in Food Sample by HPLC	Submitted by: M. Mariappan	Not enough information
Vit-06	Withdrawn		
VitC-07	Analysis of ascorbic acid, citric acid and benzoic acid in orange juice	S. Lateef	In house data but on fruit juice and doesn't measure de hydro

Choline

Bhandari and Girdhar, working group co-chairs for choline led the discussion on establishing the SMPR. Bhandari presented the fitness for purpose statement for choline before the table was opened for discussion on choline.

The working group reached general agreement on the applicability statement for choline: Determination of total choline in all forms of infant/adult/pediatric formula (powders, ready to feed liquids and liquid concentrates). For the purpose of this SMPR total choline is defined as free and bound forms. The bound forms include choline esters, phosphocholine, sphingomyelin, phosphatidylcholine, glycerophosphocholine. Choline will be reported as choline hydroxide.

There was one objection by Raffler who would prefer forms to be specified.

The working group reached general agreement on the method performance requirements for choline:

Analytical range	2-250 mg/100g	
Limit of detection (LOD)	0.7	
Limit of quantitation (LOQ)	2.0	
Repeatability (RSD)	2.0	≤10%
	20	≤5%
	100	
	200	
Recovery	90-110%	
Reproducibility (RSD)	2	≤15%
	20	≤10%
	100	
	200	
Concentrations apply to (1) 'ready-to feed' liquids "as is", (2) reconstituted powders (25 g into 200 g water, and (3) liquid concentrates diluted 1:1 by weight		

For all concentrations, choline will be expressed as mg/100g of reconstituted liquids. Choline will be reported as choline hydroxide.

The working group reached general consensus on the SMPR for choline ***(see Attachment 2)***. The working group co-chairs reviewed several methods submitted to the working group. Below are the comments on each method. The working group suggested that when the call for methods is re-issued, that it should include carnitine. Methods that can measure both carnitine and choline will be given higher priority. The working group discussed that they have not been able to determine the efficiency of extraction of sphingomyelin. AOAC will extend the call for methods for a couple of weeks to allow other stakeholders to submit their methods for consideration. The working group agreed to advance methods 1, 3, 6 to move forward.

Method	Title	Author	Comments
Chol-01	Determination of Choline in Milk and Infant Formulas by Enzymatic Analysis: Collaborative Study	D. Woollard/H. Indyk	Good method but concerned about vitamin C interference, and underestimates choline from phosphatidylcholine **(Method will advance)**
Chol-02	The Routine, Enzymatic Estimation of Total Choline in Milk and Infant Formulas	D. Woollard/H. Indyk	Method removed since it is outdated.
Chol-03	Simultaneous Determination of Free Carnitine and Total Choline by Liquid Chromatography/Mass Spectrometry in Infant Formula and Health-Care Products: SLV	Submitted by: Nestlé	The method is considered good. **(Method will advance)**
Chol-04	Enzymatic determination of the total choline content in foodstuffs	Submitted by: Danone	Method was removed since there is nothing new or done before.
Chol-05	Determination of Choline in Dry Milk and Infant Formula	Submitted by: ThermoScientific	Wait to see what the GB method looks like, method is not going forward
Chol-06	Method development for determination of total and free choline in nutritional products by LC-MS/MS	Submitted by: Abbott Nutrition	Method is good. It measures free and total, uses UPLC MSMS, there is some validation data. **(Method will advance)**

XI. WRAP UP

Sullivan summarized activities of the day and explained to the working group what would take place the next day.

DAY 3: February 15, 2012

XII. STAKEHOLDER PANEL

Sullivan opened up the stakeholder panel meeting. He informed the group that they will be reviewing the SMPRs developed by the working groups and making a recommendation to the broader stakeholder panel that will be meeting in September at the AOAC Annual Meeting.

Vitamin C:

Working group co-chairs Gowda and Arcot shared the summary of the SMPR discussed the previous day. One stakeholder suggested adding hydrolyzed lactose to the definition of pediatric formula.

Schmitz moved/Konings seconded the motion to approve the SMPR for Vitamin C. The motion passed unanimously.

The stakeholders unanimously agreed to advance VITC-01 revised method.

In summary, stakeholders unanimously agreed that revised method 1, 2, 3, 4 will advance and that there is a proposal to combine one or more of these methods together.

Choline:

Working group co-chair Bhandari shared the summary of SMPR discussed the previous day.

Campos-Gimenez motion/Adrienne McMahon seconded the motion for approval of SMPRs. The motion was unanimously approved in the house

The stakeholders recommended that all methods should demonstrate the recovery of phosphatidylcholine. It was noted that all the methods lack information in the context of recovery of phosphatidylcholine so to advance any method this fact has to be understood.

In summary, stakeholders unanimously agreed that methods 1, 3, 6 will advance.

XIII. PRESENTATION ON SPIFAN MATRICES

Schmitz provided information on SPIFAN based materials for validation studies. He shared that the manufacturers provided product matrices, representative products, key representative macro nutrient formula and product matrix components for the SLV studies and the reproducibility studies. He provided additional information on the product classification and its use.

XIV. TECHNICAL PRESENTATION ON SLV REQUIREMENTS

Sullivan presented the technical aspects of single laboratory validation approved guidelines. He reviewed the requirements for validation, including precision, accuracy, specificity, LOD, LOQ, linearity, and acceptability criteria. He also described the next steps for study authors. Methods that are selected based on the SLV as “dispute resolution” will continue to multi-lab studies.

XV. ISO STANDARDIZATION UPDATE

Konings provided an update on ISO standardization and global acceptance of analytical standards through SPIFAN. He provided a description of ISO, what it is, and the eligibility criteria to be member. Konings discussed the reasons for ISO and Codex Alimentarius standards. He also mentioned that there is discussion between AOAC and ISO on a proposal.

XVI. ENGAGEMENT IN OVERALL SPIFAN PROJECT

Mishra informed all stakeholders that regular up dated information on the SPIFAN project can be found on its website. The next working group meetings will be held in Gaithersburg, Maryland, USA in June 2012 for carnitine, iodine, and Pantothenic acid. There will be a stakeholder meeting in Las Vegas, Nevada USA in September 2012. There will also be an ERP meeting at the September meeting to consider methods for Official Methods, First Actions status. AOAC will extend the call for methods for choline and vitamin C for a couple of weeks. Working group co-chairs will review the new method submissions. Study authors can begin collecting SLV data.

XVII. NEXT STEPS/ADJOURN

See above, Item XVI.

Attachments:

Attachment 1: Draft SMPR for Vitamin C
Attachment 2: Draft SMPR for Choline