AOAC INTERNATIONAL

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Stakeholder Panel on Infant Formula and Adult Nutritionals

SINGLE LABORATORY VALIDATION (SLV) INFORMATION



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SPIFAN II Single Laboratory Validation Study Outline

This SPIFAN Single Laboratory Validation (SLV) Study Outline is intended for all SPIFAN II SLV Study Directors and explains the process by which SPIFAN SLV Kits must be requested and processed.

- All requests for SPIFAN II SLV Kits must be directed via email to Donna Smith at the Infant Nutrition Council of America (dsmith@kellencompany.com).

- INCA will use its discretion in processing requests for SPIFAN II SLV Kits.

- Individuals may request one (1) SPIFAN II SLV Kit per method being studied.

- SPIFAN II SLV Kits will be shipped by Covance via Federal Express.

- Included with the kits will be a SPIFAN II SLV Kit Worksheet, which will outline the contents of the kit (i.e., amounts of fortified and non-fortified products) and instructions for storage and handling of the kits.

SPIFAN II Single Laboratory Validation Kit

I. Contents of Single Laboratory Validation (SLV) Kits

A. Placebo Products
1. Child Formula Powder: one (1) 365 g container
2. Infant Elemental Powder: one (1) 325 g container
3. Adult Nutritional RTF, High Protein: one (1) 250 g container
4. Adult Nutritional RTF, High Fat: one (1) - 250 g container
5. Infant Formula RTF, Milk Based: twelve (12) 60 g containers

Placebo products can be used to establish method specificity and to complete repeatability and recovery experiments at levels specified in SMPR. For most methods there is enough placebo product to complete precision (duplicate analyses on six days) and/or recovery (duplicate analyses on three days) experiments. There is enough placebo powder to make at least 9 reconstitutions by diluting 25 grams of powder to 225 grams with water and enough placebo liquid to prepare at least 8 samples if the sample size is 25 grams or less.

B. Fortified Products
1. SRM 1849a: six (6) 10 g pouches
2. Infant Formula Powder Partially Hydrolyzed Milk Based: two (2) 360 g containers
3. Infant Formula Powder Partially Hydrolyzed Soy Based: two (2) 360 g containers
4. Toddler Formula Powder Milk-Based: two (2) 400 g containers
5. Infant Formula Powder Milk-Based: two (2) 400 g containers
6. Adult Nutritional Powder Low Fat: two (2) 453 g containers
7. Child Formula Powder: two (2) 365 g containers
8. Infant Elemental Powder: two (2) 325 g containers
9. Infant Formula Powder FOS/GOS Based: two (2) 350 g containers
10. Infant Formula Powder Milk Based: two (2) 352 g containers
11. Infant Formula Powder Soy Based: two (2) 730 g containers
12. Infant Formula RTF Milk Based: twelve (12) 60 g containers
13. Adult Nutritional RTF High Protein: three (3) 250 g containers
14. Adult Nutritional RTF High Fat: three (3) 250 g containers

Fortified products will be used to complete repeatability and recovery experiments and may be used to establish method specificity. For all methods there should be enough fortified product to complete precision (duplicate analyses on six days) and/or recovery (duplicate analyses on three days) experiments. There is enough fortified powder to make at least 9 reconstitutions by diluting 25 grams of powder to 225 grams with water and enough placebo liquid to prepare at least 24 samples if the sample size is 25 grams or less.

II. Instructions for SLV Kits

A. Store products in a cool dry place, protected from light.
B. For all nutrient groups except vitamin C, samples can be taken from opened liquid product containers for up to 1 week after the containers are opened if the product is flushed with nitrogen and stored refrigerated in sealed containers. For vitamin C new containers of fortified liquid should be opened each day, but for placebo liquids, samples can be taken from opened liquid product containers for up to 1 week after the containers are opened if the product is flushed with nitrogen and stored refrigerated in sealed containers.
C. For all nutrient groups, samples can be taken from opened powder product containers for up to 2 weeks after the containers are opened if the product is flushed with nitrogen and stored frozen in sealed containers.
D. Per SPIFAN Standard Method Performance Requirements, all powder products except SRM 1849a should be reconstituted by dissolving 25 grams of powder in 200 grams of water. SRM 1849 can be reconstituted by dissolving 10 grams of powder in 90 grams of water or SRM 1849a powder can be weighed directly.
E. Do not throw away any unopened product containers. Store these in a cool dry place, protected from light.

Appendix L: AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation

1 General

(**a**) All methods for a given nutrient or nutrient group will be subjected to a common single-laboratory validation (SLV) protocol utilizing the available SPIFAN matrices.

(**b**) SLV protocols may vary somewhat *between* nutrients, depending on the specific demands associated with each.

(**c**) Study directors (SDs) for each nutrient or nutrient group will agree on final details of the required SLV protocol.

(**d**) Suitability criteria indicating method/system performance is acceptable will be generated during SLV.

2 Linearity/Calibration Fit

(**a**) Minimum of six levels (levels to be agreed upon by SDs) that span the desired working range.

(**b**) Relative error of back-calculated concentrations determined within the desired working range. (No specific criterion in standard method performance requirement. Recommend calibration errors to be <5%.)

(**c**) Minimum of three independent experiments. (Independently prepared standards, if feasible.)

3 LOD/LOQ

Ten independent analyses of blank or blank spiked at low level (to be agreed upon by SDs) (if there is no detectable blank signal):

LOD = blank mean + 3 standard deviations

LOQ = blank mean + 10 standard deviations
(concentration of blank to be <10% of the estimated LOQ)

4 Specificity

(**a**) No explicit proposals for evaluating specificity have been suggested.

(**b**) Because useful strategies for doing this vary from analyte to analyte, SDs for each nutrient will agree on acceptable practice.

(**c**) An adequate evaluation of specificity may have already been done for some methods, in which case it would not have to be repeated.

5 Precision

(**a**) All samples selected for precision studies will be analyzed in duplicate on each of 6 days using multiple analysts and instruments as practical for the different days. Fresh reagents and working standards will be used each day. Reports will include information of number of analysts, instruments, etc.

(**b**) Precision data using SRM 1849a should be included for *all* methods. For each nutrient or nutrient group, precision data shall be collected using an appropriate variety of SPIFAN matrices that contain the nutrient or nutrient group (as agreed upon by the SDs). The number of matrices may vary between nutrients.

(**c**) Estimate within-day (repeatability), day-to-day, and overall (intermediate precision) for each sample type. Estimates pooled across sample types may also be useful.

6 Accuracy (Trueness)

(**a**) *Analysis of SRM 1849a*.—Comparison to SRM values may not always be applicable because nutrient definitions are not aligned. SDs will agree on whether this should be part of the accuracy assessment.

(**b**) *Spike recovery.*—(*1*) Recovery will be determined from an appropriate sampling of SPIFAN matrices. Either unfortified (preferably) and/or fully fortified products may be used.

(*2*) Each selected matrix will be spiked at two levels. Recommended spike levels are 50 and 150% of typical target; or 50 and 100% overspikes. SDs will agree on levels used.

(*3*) Spiked and unspiked samples will be analyzed in duplicate on each of 3 days.

(*4*) The overall mean of unspiked samples will be used for computing recoveries.

(*5*) Matrices used for estimating recoveries may or may not coincide with one or more of those selected for precision studies. If there is overlap, then a single 2 × 6 replication of the unspiked matrix covers both requirements for that sample type.

(**c**) *Comparison to reference methods*.—(*1*) This is not required as matter of routine, because the additional effort and lack of appropriate reference methods.

(*2*) SDs may choose to collect reference method comparison data.

The SPIFAN SLV guidelines were approved by the AOAC Expert Review Panel on Infant Formula and Adult Nutritionals in September 2011.