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.