Introduction

This protocol describes the requirements for setting up and conducting Multi-Laboratory Precision Testing for the purpose of validating an Official AOAC First Action Method for its further designation as an AOAC Final Action Method.

The approved SPIFAN - SMPR for the parameter(s) of interest defines:

- applicability statement
- parameters to be tested
- acceptability criteria

Note: This protocol is proposed as a basis for Codex, ISO, IDF acceptance [1,2,3,6,7] of the final action method.

Abbreviations

SPIFAN = Stakeholder Panel on Infant Formula and Adult Nutritionals
MLT = Multi Lab Test
ERP = expert review panel
OMB = official methods board
PL = participating laboratory
SD = study director
SLV = single laboratory validation
SMPR = standard method performance requirements

Principles:

- An Official AOAC First Action method must be available and published.
- Before MLT is undertaken, the respective ERP should have approved the results of a SLV study using the SPIFAN SLV kit.
- If Multiple methods for the same analyte will proceed for MLT testing, SD's should compare the results of the SPIFAN kit samples among methods, and demonstrate no statistical difference.
- SLV covers: precision (*recovery*, *repeatability, intermediate reproducibility*) and accuracy for as many matrices as possible. The MLT then focuses on collecting *reproducibility* data. *Within-laboratory repeatability* will be based on blind duplicates, while *accuracy* is evaluated by analyzing a NIST SRM. MLT provides a more robust and practical estimate of repeatability because it yields an estimate that is pooled across multiple laboratories. Accuracy (Trueness) is not part of the MLT, and is not considered in the AOAC statistical analysis of MLT data.
- The SD is responsible to organize MLT.
- A check sample, e.g NIST SRM, must be tested by each PL in duplicate to demonstrate its ability to perform the method and obtain true results, before starting the main MLT. The SLV LOQ should also be demonstrated.
- As recovery data (by spiking blank matrices) will be collected during SLV, and absence of the analytes is not relevant in the case of nutrient analysis, blank matrices will not be included in the set of MLT materials.

Design of Multi-Laboratory Testing Process. [1,2,3,6,7]

1. Number of laboratories and samples

- It is recommended to enrol 10 PL'. These laboratories should be instructed that analyses must be performed simultaneously or in rapid succession by the same analyst using the same materials / solutions / apparatus. At least 8 PL's must submit valid data.

- NOTE: The participating laboratories should not consist exclusively of those that have gained special experience during the process of standardizing the method. Multiple organizations should take part in a MLT. However, the use of multiple labs at different locations in the world from one organization is allowed. It is recommended the laboratories be chosen in different regions or countries to cover various climate conditions.

- It is strongly recommended to include at least 6 sample matrices, representing different levels of the test property. It is preferred to select three levels (low, medium, high), with at least two sample materials, only slightly different in composition, so that involuntary censoring of the results by the analyst (matching of the blind duplicate laboratory samples, by comparing the results obtained) is avoided.

- From each sample material blind duplicate laboratory samples should be provided. All samples should be coded randomly.

2. Sample labeling

- It has been agreed that the SPIFAN materials can be included as such in the MLT. However, efforts should be undertaken to take off current labels, and/or repack samples for MLT study to be able to include blind duplicates.
- In some cases, there are not enough materials containing the nutrient of interest. In this case, the respective SD is responsible to complete the set of study materials from other sources.

3. SPIFAN matrices for MLT

- A suite of SPIFAN sample matrices has been developed and stored at Covance Laboratories. The homogeneity of theses matrices will be verified. The following principles must be applied:

    - Heterogeneity between test samples from a single test material must be negligible compared to the analytical variability.
    - To ensure homogeneity, Abbott Nutrition will analyze some typical analytes.
    - In principle, after homogenization a randomly-selected set of 10 samples is analyzed in duplicate (as two separate test portions), for some of the analytes mentioned above.
    - Within-sample (analytical) standard deviation, and between-sample standard deviation are calculated and compared according to ISO 13528:2005 annex B [4] and the IUPAC Harmonized Protocol [5].
- The SRM 1849a (infant formula) is included as one of the 6 in the suite of MLT materials.

4. Practice sample

   One month before the start of the MLT, SD's must send one or more practice samples to PL's. It is recommended to use the SRM 1849a material, unless there is specific justification for an alternate matrix as a better measure of method execution.

5. Calibration standard

   The MLT protocol should specify the source, and if applicable, purity and concentration check of calibration standard to be used.
   Also, sources for reagents, solvents, supplies, etc., that are considered by SD to be critical for optimal performance of the method should be specified.

6. Protocol/timelines

- The Official AOAC First Action method must be followed exactly as described. Note that there might be a difference between the AOAC first action method as published and the actual method description for MLT (e.g. sample preparation as agreed 25 g into 200 g water). The method to be used should be provided by the SD.
- No modifications must be made to the method and a PL must contact the SD in case of any problems and to receive assistance.
- All PL's must report their results within two months from receipt of the suite of test samples.

7. Statistical evaluation of data:

- Data should be statistically evaluated according to AOAC guidelines for collaborative study procedures [(3)].

8. Reporting MLT

- Final MLT manuscript must be submitted to AOAC and reviewed and approved by ERP to ensure that it meets the SPIFAN-SMPR acceptability requirements.
- The approved MLT must be published in J.AOAC International.

9. Final Action Status.

- ERP recommends method for Official Final Action status to OMB
- OMB grants Final Action status

10. ISO/IDF acceptance.

- The protocol will allow publication of the method as an ISO/IDF Standard.

References:

1. ISO 5725-1; Accuracy (trueness and precision) of measurement methods and results- Part 1: General principles and definitions.
2. ISO 5725-2, Accuracy (trueness and precision) of measurement methods and results- Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.
3. AOAC Official Methods of Analysis 2005, Appendix D: Guidelines for collaborative Study Procedures to validate characteristics of a method of analysis.
4. ISO 13528:2005 annex B; Statistical methods for use in proficiency testing by inter-laboratory comparisons.
5. IUPAC harmonized protocol for the Proficiency testing of Analytical Chemistry Laboratories: Thompson et al. Pure Applied Chemistry 78:145-196, 2006.
6. Codex Alimentarius Commission. Procedural Manual, nineteenth edition. Rome, 2010.
7. IDF bulletin 453/2012. Guidance for the evaluation of precision characteristics of physicochemical quantitative analytical methods for milk and milk products.