Approaches to Validation of Methods for Regulatory Use

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Validation

* Establishing documented evidence that provides a high degree of assurance that a specific method and its ancillary instruments included in the method will consistently provide results that accurately reflect the quality characteristics of the product tested and demonstrate that the method is fit-for-purpose.

Clause 5.4.2 ISO/IEC 17025:2005

* “The laboratory shall use test and/or calibration methods, including methods of sampling, which meet the needs of the customer and which are appropriate for the tests and/or calibrations it undertakes..” and further.
* “When the customer does not specify the method to be used, the laboratory shall select appropriate methods……”

Single Laboratory Validation (SLV) or Multi-Laboratory Validation (MLV)

* Methods may be validated to meet the requirements for
  * Single Laboratory Validation (SLV) also referred to as in-house validation, or
  * Multi-Laboratory Validation (MLV) or collaborative (inter-laboratory) validation.

Method Parameters for Evaluation in a SLV

* Recovery
* Limit of Detection (LOD) and Limit of Quantification (LOQ)
* Bias (Accuracy) of the Measurement
* Precision of the Analytical Method
* Intermediate precision of the Analytical Method
* Measurement Uncertainty (MU)

Repeatability (Within Laboratory Study)

* Prepare and homogenize 3 unknown samples at different concentrations to represent the full, claimed range of the method.
* Analyze each unknown sample by the candidate method seven (multiple) times, beginning each analysis from weighing out the test portion through to final result with no additional replication (unless stated to do so in the method).
* All of the analyses for one unknown sample should be performed within as short a period of time as is allowed by the method.
* The second and third unknowns may be analyzed in another short time period.
* Repeat for each claimed matrix.

Repeatability (RSD_r) Relative Standard Deviations

* Repeatability Standard Deviation (RSD_r) This is the relative standard deviation calculated from within-laboratory data measurements

Table A2: Expected Precision as a Function of Concentration-Repeatability

Analyte (%)	Analyte Ratio	Unit (%, mg/kg)	%RSD	% Mean Recovery
100	1.0	100	1.3	98-102
10	10^{-1}	10	1.9	98-102
1	10^{-2}	1	2.7	97-103
0.01	10^{-3}	0.1	3.7	95-105
0.001	10^{-4}	100 mg/kg (ppm)	5.3	90-107
0.0001	10^{-5}	10 mg/kg (ppm)	7.3	80-110
0.00001	10^{-6}	1 mg/kg (ppm)	11	80-110
0.000001	10^{-7}	100 µg/kg (ppb)	15	80-110
0.0000001	10^{-8}	10 µg/kg (ppb)	21	60-115
0.00000001	10^{-9}	1 µg/kg (1ppb)	30	40-120

This is where most of us end up with our method development, method validation exercises and perhaps apply the method to pharmacokinetic analysis, depletion study, publish the method and/or implement it in a regulatory monitoring control program.

How do you get your Single Laboratory Validated method published with a Final Official Action Method Status as a Reference Method published in the AOAC International's Official Methods of Analysis (OMA)?

Standard Method Performance Requirements (SMPRs) The Pathway to Adopted Reference Methods - The Association of Official Analytical Communities (AOAC)

International Voluntary Consensus Standards

* SMPRs are a *consensus* standards developed by stakeholders in a very controlled process that ensures that users, research organizations, government departments, and consumers work together to create a standard that meets the demands of the analytical community and technology.

SMPRs

* SMPRs are a unique and novel concept for the analytical methods community.
* In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable.

SMPRs

* Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria.
* However, as the *Eurachem Guide* points out: " . . . the judgment of method suitability for its intended use is equally important . . ." (1) to the evaluation process.

Appendix F: AOAC Guidelines for SMPRs

* AOAC Guidelines for Standard Methods Performance Requirements (SMPR) was drafted over the 2009 – 2010 timeframe.
* Since 2010 AOAC has developed and adopted more than 60 SMPRs.
* The SMPR process has evolved since 2010.

Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis

Method Classification

Type I: Quantitative methods (generates a continuous number as a result)

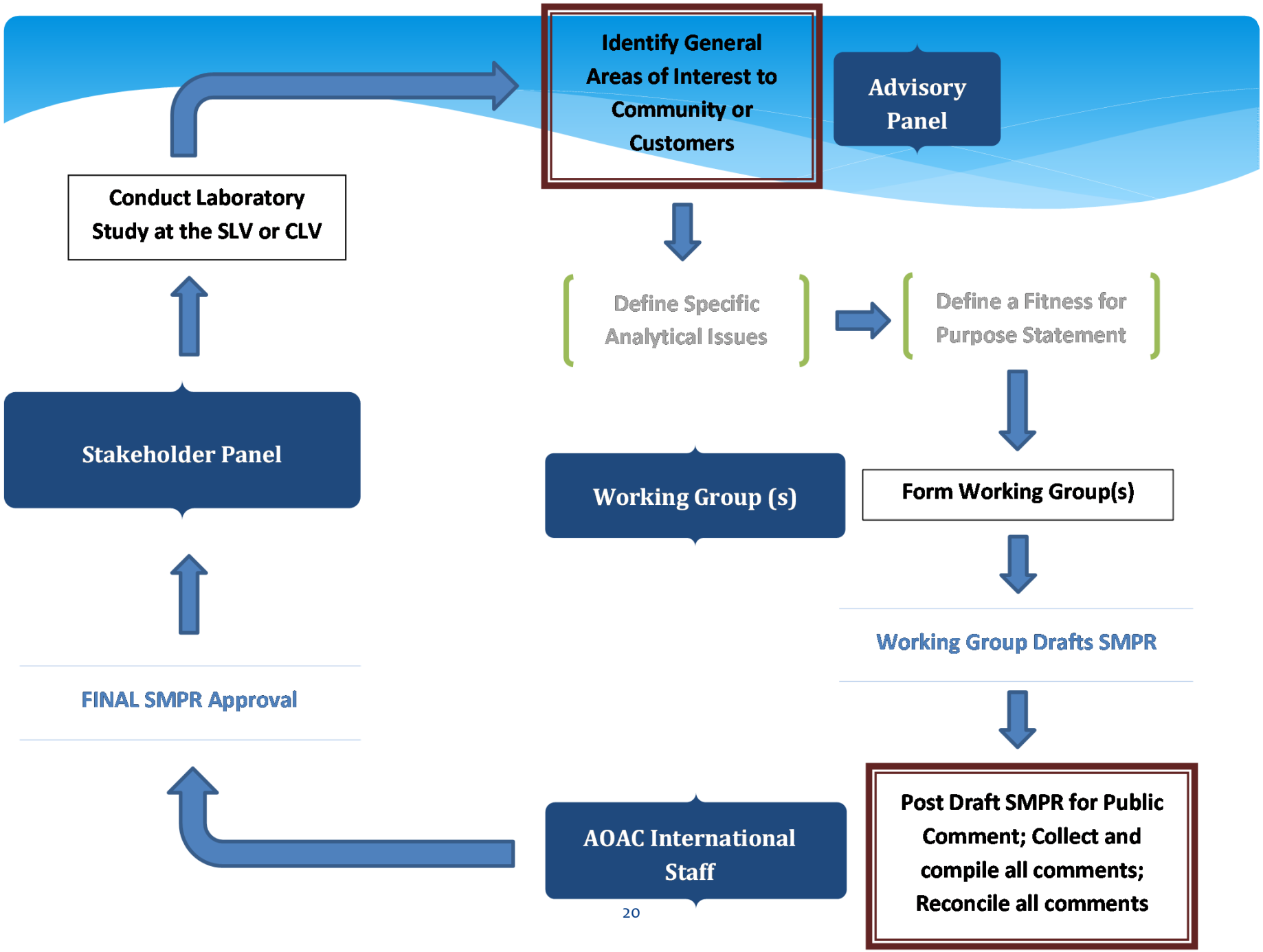
Type II: Methods that Report Ranges (generates a "range" indicator such as 0, low, moderate, and high)

Type III: Methods with Cut-Off Values (method may generate a continuous number as an interim result (such as a CT value for a PCR method), which is not reported but converted to a qualitative result (presence/ absence) with the use of a cut-off value.

Method Classification

* Type IV: Qualitative Methods (method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a specified test portion)
* Type V: Identification/Confirmatory Methods (method of analysis whose purpose is to determine the identity of an analyte).

- The Guidance may be used to determine which performance parameters in Table A1 apply to different classifications of methods.
- AOAC INTERNATIONAL does not recognize "*semi-quantitative*" as a method classification.
- Methods can be only qualitative or quantitative


Identify General Areas of Interest to Community or Customers
Advisory Panel
Conduct Laboratory Study at the SLV or CLV
Define Specific Analytical Issues
Define a Fitness for Purpose Statement
Stakeholder Panel
Working Group (s)
Form Working Group(s)
Working Group Drafts SMPR
FINAL SMPR Approval
AOAC International Staff
Post Draft SMPR for Public Comment; Collect and compile all comments; Reconcile all comments

Conducting a Multi-Laboratory Validation (MLV) Study

Table A1: Performance Requirements for SMPR Development

Performance parameters to be included into an SMPR based on five method classifications:

(1) main component quantitative methods

(2) trace or contaminant quantitative methods

(3) main component qualitative methods

(4) trace or contaminant qualitative methods and

(5) identification/confirmatory methods

Reproducibility (Collaborative or Inter-laboratory study)

* Quantitative methods: Recruit 10 –12 labs;
  * Must have at least 8 valid data sets; two blind duplicate replicates at five concentrations for each analyte/matrix combination to each collaborator.
* Qualitative methods: Recruit 12 –15 labs;
  * Must have at least 10 valid data sets; six replicates at five concentrations for each analyte/matrix combination to each collaborator.

SMPR Format

Information about method requirements is itemized into nine categories:

(1) intended use;
(2) applicability;
(3) analytical technique;
(4) definitions;
(5) method performance requirements;
(6) system suitability;
(7) reference materials;
(8) validation guidance; and
(9) maximum time-to-determination.

Table A1: Performance Requirements for MLV (Reproducibility)

Quantitative Methods		Qualitative Methods		Confirmatory/ Identification Methods
Main Component	**Trace or Contaminant**	**Main Component**	**Trace or Contaminant**	
Applicable Range	Applicable Range	Inclusivity/ Selectivity	Inclusivity/ Selectivity	Inclusivity/ Selectivity
Bias	Bias	Exclusivity/ cross –reactivity	Exclusivity/ cross –reactivity	Exclusivity/ cross –reactivity
Precision	Precision	Environmental interference	Environmental interference	Environmental interference
Recovery	Recovery	Laboratory Variance	Laboratory Variance	
LOQ	LOQ			
		PQD	POD @AMDL	POI

Table A1: Performance Requirements for MLV (Reproducibility)

Quantitative Methods		Qualitative Methods		Confirmatory Methods
Main Component	**Trace or Contaminant**	**Main Component**	**Trace or Contaminant**	
RSD_R or Target Measurement Uncertainty	RSD_R or Target Measurement Uncertainty	POD (o) POD (c)	POD (o) POD (c)	POI (c)
		Laboratory POD	Laboratory POD	Laboratory POI
POD = Probability of Detection; POI = Probability of identification; AMDL = Acceptable Minimum Detection Level				

Reproducibility (RSD_R) Relative Standard Deviations

* Reproducibility Relative Standard Deviation (RSD_R) This is the relative standard deviation calculated from among-laboratory data measurements

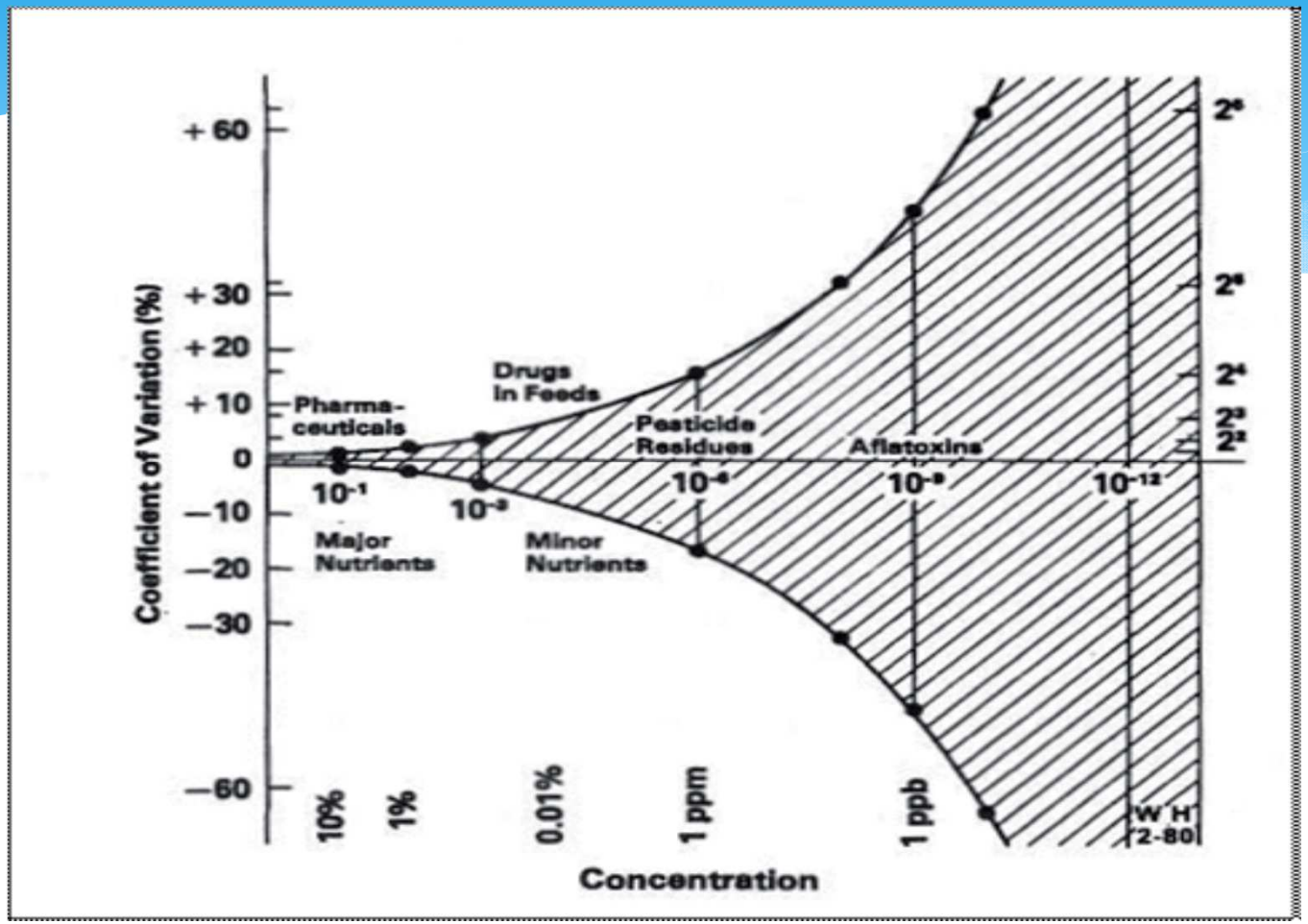
Predicted Relative Standard Deviation of Reproducibility ($PRSD_R$)

The Predicted ($PRSD_R$) of REPRODUCIBILITY is calculated from the Horwitz equation

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction

Horwitz Curve



Horwitz Curve, illustrating the exponential increase in the coefficient of variation (% CV) as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095 (2006)].

The HorRat (R or r) Value

The predicted PRSD of reproducibility or repeatability are calculated from the Horwitz equation as follows:

$$\text{HorRat (repeatability, r)} = RSD_r/PRSD_R$$

$$\text{HorRat (Reproducibility, R)} = RSD_R/PRSD_R$$

For Inter-laboratory studies: acceptable HorRat (R) of 1 with limits of acceptability of 0.5 to 2

For Within-Laboratory studies: acceptable HorRat (r) of 0.3 – 1.3

Table A2: Expected Precision as a Function of Concentration-Reproducibility

Analyte (%)	Analyte Ratio	Unit (%, mg/kg)	%RSD	% Mean Recovery	% $PRSD_R$
100	1.0	100	1.3	98-102	2
10	10^{-1}	10	1.9	98-102	
1	10^{-2}	1	2.7	97-103	4
0.01	10^{-3}	0.1	3.7	95-105	8
0.001	10^{-4}	100 mg/kg	5.3	90-107	
0.0001	10^{-5}	10 mg/kg	7.3	80-110	
0.00001	10^{-6}	1 mg/kg	11	80-110	16
0.000001	10^{-7}	100 µg/kg	15	80-110	
0.0000001	10^{-8}	10 µg/kg	21	60-115	32
0.00000001	10^{-9}	1 µg/kg (1ppb)	30	40-120	45

$PRSD_R = 2C^{-0.15}$ where C is expressed as a Mass fraction

Limitations of the Use of the HorRat Values

* The HorRat value is a very rough but useful summary of the precision in analytical chemistry
* It overestimates the precision at the extremes:
  * At concentrations > 0.1 i.e., 10% and,
  * At concentrations < 10^{-8} i.e., 10 ng/g or 10 ppb

- Once the MLV Study is completed and the results of the study are shown to meet the reproducibility requirement following ERP approval and Stakeholder acceptance, the method is given **AOAC First Action Method Status** and subjected to further method trials in the hands of end users.

- The method authors are encouraged to obtain feedback from method users for a period of 2 years.
- If the feedback from users over that period are satisfactory, the method is adopted **AOAC Final Action Method Status** and published in the Official Methods of Analysis (OMA) as a Reference method.

Submit your Properly Validated MLV Method to the AOAC

THANK YOU
AOAC IS
WAITING FOR YOUR
METHOD