**CEN REPORT ON INFORMATION TO AND PROCEDURES FOR CEN TC 275 WORKING GROUPS TO CONSIDER WHEN SPECIFIC STANDARDS ARE BEING DEVELOPED AND ADOPTED BY THE TC AND ITS WORKING GROUPS**

***Informal Cover Information***

Sometime ago it was decided at CEN TC 275 that there were a number of issues which it would be useful to address on a horizontal basis within CEN TC 275 Working Groups.

The attached (very) draft Report was discussed briefly at the CEN TC 275 Plenary Session which was held 3rd/4th May 2012 in Berlin.

I gave a presentation on this draft – which I had prepared just before the meeting (sounds familiar?).

It was agreed that this paper, together with this covering note, would be circulated to all interested people the convenors/secretariat of the 13 (14) CEN TC 275 Working Groups. Some, of course, have been adjourned *sine die* in which case it may be assumed that the convenorship is held by DIN.

Carola (Seiler) circulated as requested and comments were received. They have not yet been incorporated into this draft to any significant extent but will be before the next CEN TC Plenary Session.

I have updated and where comment/information is needed, I have marked in red on the attached draft.

The document is intended to be “living”, i.e. to constantly revised in the light of experience and deficiencies.

I have been unable to progress significantly in the past few months, but in view of the 14th March Brussels meeting, the current draft is circulated.

Roger Wood

12th March, 2013

**CEN REPORT ON INFORMATION TO AND PROCEDURES FOR CEN TC 275 WORKING GROUPS TO CONSIDER WHEN SPECIFIC STANDARDS ARE BEING DEVELOPED AND ADOPTED BY THE TC AND ITS WORKING GROUPS**

**INTRODUCTION AND PURPOSE OF DOCUMENT**

The objective of CEN TC 275 is to prepare, approve and publish methods of analysis in the food sector. Such methods are “horizontal” in nature, i.e. not commodity specific. CEN has a number of TCs which are responsible for producing commodity specific methods of analysis, e.g. milk (CEN TC 302), fruit juice (CEN TC 174) etc.

CEN TC 275 aims to produce methods which have been “fully validated”, i.e. methods where the precision performance characteristics have been assessed through a collaborative trial which itself conforms to one of the International Protocols/Guidelines (normally ISO 5725: 19941 or the IUPAC Harmonised Protocol2. These are essentially the same except for the probabilities of the outlier tests. ISO 5725 is currently being revised.)

The driving force for the “quality” of CEN TC 275 methods of analysis is now the methods of analysis requirements given in the EU Official Feed and Food Control Diriective3. These are outlined in Appendix I. These are essentially the same as given in the Procedural Manual of the Codex Alimentarius Commission4. This means that CEN published methods will have a clear applicability outside of the EU.

However, during the development and publication of CEN TC 275 Standards a number of issues have arisen and this Report aims to comment on a number of them.

If CEN methods are to be used in the context of “official control” within the EU, then it must be appreciated that such users will have to undertake other considerations, e.g. use methods accredited to ISO 17025, know the performance characteristics of methods if applying the criteria approach, estimate and use the measurement uncertainty of the result etc.

With the introduction of the criteria approach it is important that CEN, when publishing its methods of analysis, gives all the information that will be of interest to the method user, particularly in the context of official control.

Question: should the document cover just chemical analysis or extend to microbiological procedures? How are qualitative methods of analysis to be treated?

Question: is the order of sections given in the document the most appropriate?

**1. COLLABORATIVE TRIALS**

Collaborative trials should be carried out according to one of the International Protocols (see above).

**1.1 Minimum requirement**

The minimum requirement for a collaborative trial is:

* 8 sets of valid data per test material, i.e. after removal of outliers and aberrant data. This means 10 non-aberrant data sets must be reported as the number of outliers should be no greater than 2/9 of the total data.
* 5 test materials if of significantly different concentrations (can be 3 if analyte concentration is restricted, e.g. tablet preparations).
* Test materials should be dispatched randomly coded and each participant should receive as either blind or split level samples. The use of known duplicates is to be discouraged. Test materials should ideally be disguised but this is often quite difficult to achieve in practice.
* Ideally test materials should be assessed for homogeneity before distribution but neither protocol specifies how this is to be carried out. Some collaborative trial coordinators use the procedures outlined in the International Harmonised Protocol of Proficiency Testing5 for this purpose.
* Participants should be competent but not necessarily the world-wide experts in the subject.

**1.2 Statistical Analysis of Data using Robust or Classical Statistics**

The ISO protocol permits the use of robust statistics for the analysis of data. The IUPAC protocol only describes the use of classical statistics. The application of robust and classical statistics when applied to the same set of data may result in different values of precision data being reported.

From the CEN view-point it is immaterial which type of statistics is used but it is important that the same one is used for all test materials in any one collaborative trial. It is not permitted to analyse some materials using classical statistics and others in the same trial using robust statistics because the resulting values are “better”.

**1.3 Use of Recovery Corrections when Calculating Method Performance Characteristics**

In many sectors of CEN TC 275 the results are to be used on a recovery corrected basis (see EU contaminants legislation as examples6, 7, 8). It should also be noted that some sectors, most notably the pesticide sector, does not require (expressly prohibits) correction.

There is the argument that if that is clearly defined then the calculations and information/decision on whether the precision parameters on methods should also be calculated and then expressed on a recovered or non-recovered basis.

Current work carried out in the area is inconclusive as to the better approach.

Question: how should CEN treat recovery when statistically analysing collaborative data if it is known that the methods will be used on a recovered basis?

**1.4** **Assessment of method performance statistics - use and definition of the HorRat Value**

It is recognised that the determination and reporting of HorRat Values within CEN/TC 275 standards is not applicable within all areas of CEN/TC 275 standardisation (e.g. GMOs and allergens). Nevertheless, it is recommended that HorRat Values should routinely be calculated, be reported in CEN/TC 275 standards and be ≤2. This notwithstanding this it is recognised that in some methods, where validation data indicates the method to be fit-for-purpose, calculated HorRat Values for one or more matrices collaboratively trialled may be >2 and in these instances the decision to accept the method for standardisation purposes should be made by the individual working groups on a case-by-case basis. If all HorRat Values are >2, for a conventional determination, then some consideration should be given as to whether the method is fit-for-purpose.

The EU and Codex criteria approach makes this an effective “quality standard” that such methods must meet if to be used in the context of official control.

**1.5 Full Collaborative Trial not Possible/or Practical/or too Expensive etc**

There are AOAC International discussions on the future direction that AOAC International should take in this area.

An explanatory paper is given in Appendix II but it is subject to constant review.

Critically, the system requires some element of inter-laboratory validation before designating a method “Final Action” on promotion from “First Action”.

This validation needed not be through a collaborative trial but may be obtained through the results of proficiency test schemes. AOAC are currently interested in this aspect as it is perceived as being a means of obviating the need for a full collaborative trial.

Note: the UK CCMAS paper showing the application of this approach to the Enumeration Of *Listeria Monocytogenes* In Meat And Meat Products has been forwarded to the AOAC for information.

Question: should CEN consider such an approach, or rely on the full collaborative trial approach? The latter is required by legislation!

**1.6 Modular Validation**

The use of modular validation is currently restricted to discreet areas of CEN/TC 275 standardisation (i.e. work undertaken within WG 10 and WG 11). Work has been undertaken to describe its applicability and this is given in Appendix III.

Note: considered important and needs expanding. To “how-to-do” information?

**2.0 METHOD SCOPES**

**2.1 Format**

This has been defined within CEN TC 275. The standard format is list the scope of the collaborative trial, e.g. in EN 15891:2010 dealing with the “Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and UV detection” it states:

This European Standard specifies a method for the determination of deoxynivalenol (DON) in cereals (grain and flour), cereal based foods and cereal based foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and UV detection. This method has been validated in three interlaboratory studies. The first study was for the analysis of samples of wheat, rice flour, oat flour, maize, polenta, and wheat based breakfast cereal ranging from 85,4 µg/kg to 1 768 µg/kg, the second study was for wheat and maize ranging from 165 µg/kg to 4 700 µg/kg and the third study was for cereal based foods for infants and young children ranging from 58 µg/kg to 452 µg/kg.

Thus the user is made very aware of the validation that has been carried out.

**2.2 Extension of Method Scopes**

Practical guidance on how the scope of CEN/TC 275 standards can be extended to cover new topic areas (change of matrix and concentration from that used in original collaborative trial) is required.

Comment: practical guidance needs to be provided on how this is to be achieved within a single laboratory. This needs to link nwth Section 2.1 above.

3. **ADDITIONAL PERFORMANCE PARAMETERS**

The additional method performance parameters (e.g. sensitivity, limits, recovery etc) obtained during method development and validation need to be readily available to the method purchaser. This then leads to the marrying of performance parameters in methods to the needs from the adoption of the criteria approach by many Regulatory Agencies.

Ideally the various parameters (excluding measurement uncertainty) detailed in Annex 3 of Regulation 882/2004 should be addressed within CEN/TC 275 standards. However, it is recognised that this will not be possible (or appropriate) in all instances.

**4. MEASUREMENT UNCERTAINTY WITHIN CEN/TC 275 STANDARDS**

It is recommended that measurement uncertainty should not be included within CEN/TC 275 standards and that, as an initial approach, collaborative trials results may be used to estimate measurement uncertainty.

Measurement uncertainty is not only a function of the method but much more significantly how it (the method) is used by individual analysts.

**5. DEFINITIONS**

These have now been adopted by the Codex Alimentarius Commission and are reproduced as Appendix IV to this Report. It is recommended that they are used by all the Working Groups of CEN TC 275.

Note: need some discussion about a new initiative to help analysts estimate these provisions in a short-snappy half-page description for each. And there have been some comment about the definitions given – this is an internationally agreed document in an area where there are many different opinions!

**6. PROPRIETARY METHODS OF ANALYSIS**

There has been extensive international discussion on the use of propriety methods of analysis in International Standards and in legislation.

The most comprehensive discussion is that undertaken by the Codex Committee on Methods of Analysis and Sampling as a result of an initiative within the Inter-Agency Meeting, of which CEN is a member.

This is reproduced as Appendix V.

However, it is important for CEN TC 275 to evaluate the need in standardising proprietary methods on a case-by-case basis.

In particular there should be discussions as to whether standardising a particular proprietary method gives a commercial advantage and so effectively “kills” methods which are not so standardised.

**7 EXPRESSION OF ANALYTICAL RESULTS AND ROUNDING RULES**

It is considered necessary for information to be given on data rounding rules. Recommendations have been prepared and these are given in Appendix VI.

Note: these need to be further discussed.

**8. VALIDATION OF QUALITATIVE METHODS**

There are international discussions on the validation of qualitative methods of analysis.

There appears to be two approaches, from AOAC and IUPAC, but they are coming together. Based on a “Probability of Detection” approach.

A number of recent papers have been published, e.g.:

Characterising the performance of qualitative analytical methods: Statistics and terminology by S.L.R. Ellison, T. Fearn

Trends in Analytical Chemistry, Vol. 24, No. 6, 2005

A protocol for the validation of qualitative methods of detection by Roy Macarthur and Christoph von Holst

Anal. Methods, 2012, 4, 2744

Comment: need to decide how the consideration of such methods should be included in this document.

**9. FORMAT OF METHODS WITHIN CEN**

Note: already agreed in principle, but needs to be transferred to this document. Different parts of CEN will have different approaches – do they need to be brought together?

**10. METHOD VERIFICATION PROCEDURES**

It is considered important that the users of CEN TC 275 methods of analysis are also fully aware that they are being used correctly. Information on this aspect is given in Appendix VI and is taken from the approach being recommended on method verification within the European CRL/NRL network.

**11. RECOMMENTATIONS AND CONCLUSIONS**

To be completed

**12. REFERENCES**

To be completed.

**APPENDIX I: EXTRACT FROM THE METHODS OF ANALYSIS REQUIREMENTS GIVEN IN THE EU OFFICIAL FEED AND FOOD CONTROL DIRIECTIVE**

Article 11

Methods of sampling and analysis

1. Sampling and analysis methods used in the context of official controls shall comply with relevant Community rules or,

(a) if no such rules exist, with internationally recognised rules or protocols, for example those that the European Committee for standardisation (CEN) has accepted or those agreed in national legislation; or,

(b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

1. Where paragraph 1 does not apply, validation of methods of analysis may take place within a single laboratory according to an internationally accepted protocol.
2. Wherever possible, methods of analysis shall be characterised by the appropriate criteria set out in Annex III.
3. The following implementing measures may be taken in accordance with the procedure referred to in Article 62(3):
4. methods of sampling and analysis, including the confirmatory or reference methods to be used in the event of a dispute;
5. performance criteria, analysis parameters, measurement uncertainty and procedures for the validation of the methods referred to in (a); and
6. rules on the interpretation of results.

**CHARACTERISATION OF METHODS OF ANALYSIS**

1. Methods of analysis should be characterised by the following criteria:

1. accuracy;
2. applicability (matrix and concentration range);
3. limit of detection;
4. limit of determination;
5. precision;
6. repeatability;
7. reproducibility;
8. recovery;
9. selectivity;
10. sensitivity;
11. linearity;
12. measurement uncertainty;
13. other criteria that may be selected as required.
14. The precision values referred to in 1(e) shall either be obtained from a collaborative trial which has been conducted in accordance with an internationally recognised protocol on collaborative trials (e.g. ISO 5725:1994 or the IUPAC International Harmonised Protocol) or, where performance criteria for analytical methods have been established, be based on criteria compliance tests. The repeatability and reproducibility values shall be expressed in an internationally recognised form (e.g. the 95% confidence intervals as defined by ISO 5725:1994 or IUPAC). The results from the collaborative trial shall be published or freely available.
15. Methods of analysis which are applicable uniformly to various groups of commodities should be given preference over methods which apply only to individual commodities.
16. In situations where methods of analysis can only be validated within a single laboratory then they should be validated in accordance with e.g. IUPAC Harmonised Guidelines, or where performance criteria for analytical methods have been established, be based on criteria compliance tests.
17. Methods of analysis adopted under this Regulation should be edited in the standard layout for methods of analysis recommended by the ISO.

Note: some sectors in the food sector, e.g. pesticides, may have other requirements, but these are the “over-arching” requirements.

**APPENDIX II: AOAC INTERNATIONAL Alternative Pathway to First Action Status**

The Presidential Task Force on Increasing Method Output developed guidance documents, which include a process flowchart and process guidelines, for the alternative pathway to achieving Official First Action Method status. Approved by the AOAC Board of Directors on May 25, 2011, the guidance documents outline requirements for ERPs (role, composition), decision to First Action status, and transition from First to Final Action status.

Under the alternative pathway to achieving Official First Action status, ERPs have the authority—for the first time—to approve methods deemed satisfactory as AOAC Official Methods of AnalysisSM based on evaluation of the validation data available.

As outlined in the guidance documents, ERPs, which are managed by AOAC Headquarters and whose members are properly vetted and approved by the Official Methods Board (OMB), will evaluate candidate methods (resulting from calls for methods and literature searches) against SMPRs developed by stakeholder panels and their working groups. These methods will already have good data to support their claims. Stakeholder panels are thoroughly vetted by the OMB, and like ERPs, all stakeholder panels and working groups are managed by AOAC Headquarters.

ERPs consist of a minimum of seven members representing a balance of key stakeholders, and their meetings are open and transparent. Methods are carefully scrutinized by ERPs in a scientifically unbiased manner and measured against SMPRs (developed by stakeholders and working groups). Methods with available data adequately meeting SMPRs will be granted Official First Action status by ERPs.

Methods approved under the alternative pathway will be published by AOAC INTERNATIONAL and remain First Action for a period of about 2-3 years. During this time, methods will be used in laboratories, generating additional data.

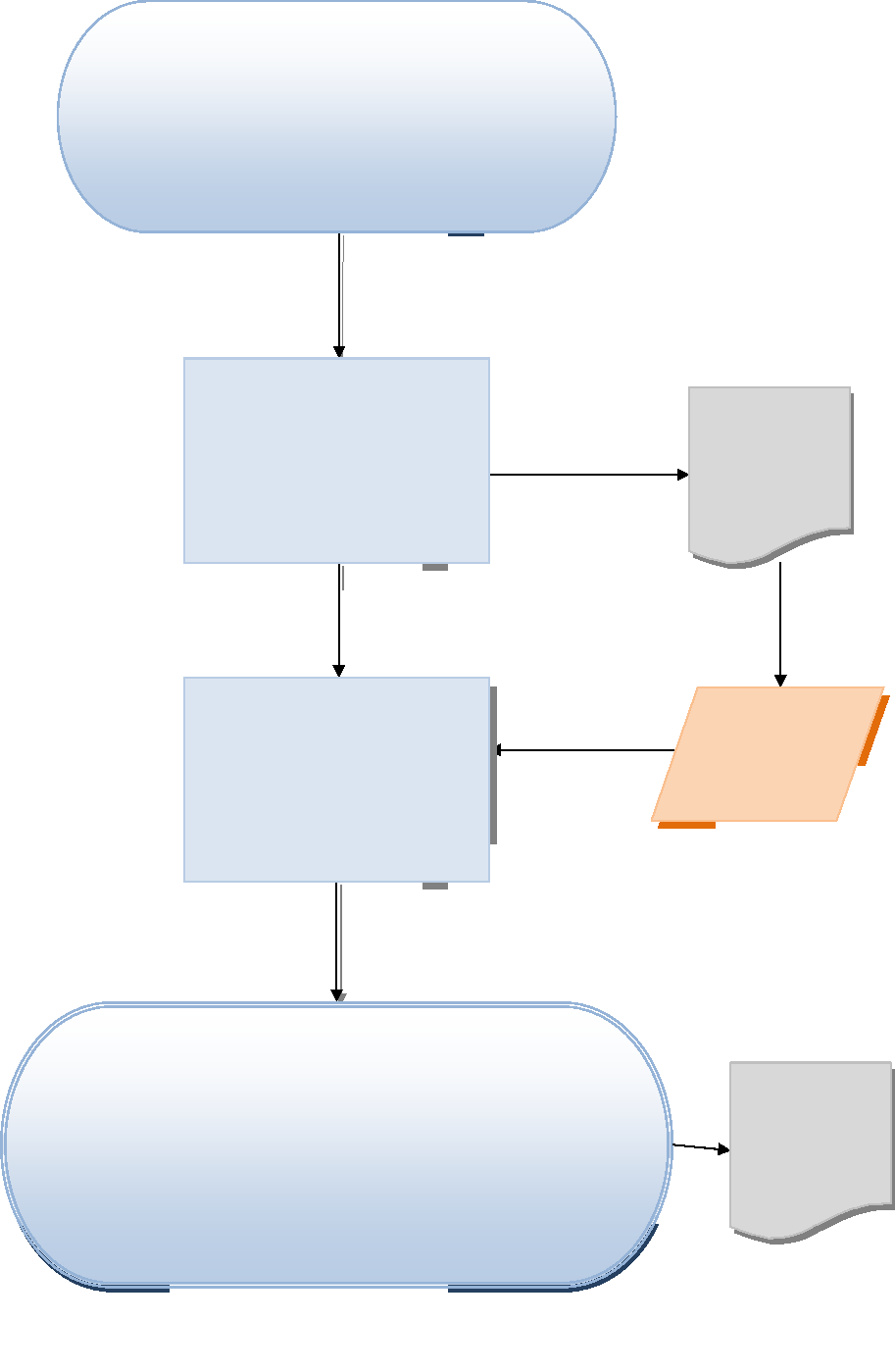
In addition, ERPs will remain in force as long as a method is in First Action status. ERPs will monitor the performance of methods, and after 2-3 years, the ERPs will determine whether the method should be recommended to the OMB for Final Action. If the method is satisfactory, Final Action status is granted by the OMB.

Alternately, ERPs can determine if methods need additional data to show reproducibility, which could be provided through proficiency testing or other data collection, for example.

At the end of the 2-3 year period, methods will be retired if they do not perform satisfactorily, if no evidence of method use is available, or if data are not indicative of adequate method reproducibility.

For more information about the guidance documents, as approved by the AOAC Board of Directors, contact Alicia Meiklejohn, AOAC executive office, at [ameiklejohn@aoac.org](mailto:ameiklejohn@aoac.org). For the process flowchart, CLICK HERE; for the process guidelines, CLICK HERE. (these 2 documents follow on).

**Alternate Pathway to Official First Action Method Status**



**Funded Stakeholder Panel**

* Managed by AOAC HQ
* Properly vetted by OMB
* Carefully documented and transparent

Adopted 2011-5-25

Revised 2011-6-27

**Working Groups**

**Expert Review Panels**

* ***Managed by AOAC HQ***
* ***Carefully documented and transparent***
* ***Managed by AOAC HQ***
* ***Properly vetted by OMB***
* ***Carefully documented and transparent***

Standard Method Performance Requirements

Call for Methods & Literature Search

JAOAC OMA Web ILM

**Official First Action Method**

* ERPs continue to monitor for two years, until method is either advanced or removed from system (period is extendable for active data collection)
* ERP recommends Final Action to OMB
* OMB grants Final Action status

**REQUIREMENTS Expert Review Panels**

-Must be supported by relevant stakeholders.

-Constituted solely for the ERP purpose, not for Standard Method Performance Requirements (SMPR) purposes or as an extension of an SMPR.

-Consist of a minimum of seven members representing balance of key stakeholders.

-ERP constituency must be approved by the Official Methods Board (OMB).

-Holds transparent public meetings only.

-Remains in force as long as method in First Action Status.

**Official First Action Method Status decision**

-Must be made by an ERP constituted or reinstated post 2011-03-28 for Official First Action Status Method Approval (OFASMA).

-Must be made by an ERP vetted for OFASMA purposes by OMB post 2011-03-28.

-Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders.

-Method must be adopted by unanimous decision of ERP on first ballot, If not unanimous, negative votes must delineate scientific reasons.

-Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration -Method becomes Official First Action on date when ERP decision is made.

-Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.

-Report of OFAMS decision complete with ERP report regarding decision including scientific background (references etc) to be published concurrently with method in traditional AOAC publication venues.

**Method in First Action Status and Transitioning to Final Action Status**

-Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

-Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).

-Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

-Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

-ERP to recommend Method to Official Final Action Status to the OMB. -OMB decision on First to Final Action Status

Board of Directors Adopted May 25, 2011 Revised June 27, 2011

**APPENDIX III: MODULAR VALIDATION**

Validating methods are resource-demanding. Many methods have similar procedural steps. Subdividing the analytical process into separate parts called “modules”, and independently validate these are cost-effective as the validated modules can be applied in various analytical methods. Each step in the procedure can then be seen as an “analytical system” and can thus be validated separately and combined later on with other “modules” in a flexible way. Modular validation is thus a stepwise validation of a whole procedure, taking into consideration all possible difficulties or uncertainty factors at each level in the procedure1. For a method to be considered fully validated, all the modules included have to be validated. The modular validation approach was first described by Arne Holst-Jensen and Knut G. Berdal in 2004 in the paper the Modular Analytical Procedure and Validation Approach and the Units of Measurement for Genetically Modified Materials in Foods and Feeds2.

The stepwise validation was however not new in 2004. When estimating measurement uncertainty (MU) according to GUM3, the Euroachem Citac Guide4 or the NMKL Procedure No. 55, a stepwise approach has been used as the combined MU is calculated by adding up the relative uncertainties from the different sources of uncertainties, or modules. As laboratories often need to carry out a single laboratory validation, i.e. if not a collaboratively validated method is taken into use, when establishing an estimate for the measurement uncertainty, the validations are carried out in modules for the uncertainty budget. The first step would then be to identify the sources of uncertainties, the different modules.

In an analytical method the different modules could be:

1. Homogenisation
2. Sample preparation
3. Extraction / Clean up
4. Determination / Calibration of the instrumentation

The different modules should then be validated. An example on how to carry out the validation and which method performance characteristics to calculate is being developed.

References:

1. Holst-Jensen, A., Berdal, K. G, ( 2004) J. AOAC Int. Vol. 87, No 4, (927-936)

2. Guide to the Expression of Uncertainty in Measurement, ISO, 1 ed. 1993, ISBN 92-67-10188-9.

3. Quantifying Uncertainty in Analytical Measurement, 2nd Edition EURACHEM/CITAC Guide, QUAM: 2000.P1, 2000,

4. Estimation and Expression of Measurement Uncertainty in Chemical Analysis, NMKL Procedure No 5, 2nd. Ed., 2003

5. Kagli, D. M, et.al: Application of the Modular Approach to an In-House Validation Study of Real-Time PCR Method for the Detection and Serogroup Determination of Verocytotoxigenic *Eschericia coli*.

Note: This approach will also link in with the Accreditation Agency’s “flexible scope” requirements. Needs development.

**APPENDIX IV: TERMINOLOGY: GUIDELINES ON ANALYTICAL TERMINOLOGY ADOPTED BY THE CODEX ALIMENTARIUS COMMISSION, JULY 2009**

**INTRODUCTION**

The Codex Committee on Methods of Analysis and Sampling has agreed on Analytical Terminology for Codex Alimentarius and government use. A number of these terms were previously included in the Codex Procedural Manual. In most cases terms used in the Procedural Manual were adopted over time with an underlying hierarchy and can be traced verbatim to specific editions of ISO 3534, the GUM, the VIM, the IUPAC Orange Book or other international standards already adopted by Codex. Definitions of terms that have changed with newer editions of the international standards from which they were originally adopted have been updated preserving the original hierarchy found in the Procedural Manual. In cases where terms have been added in addition to those originally found in the procedural manual an effort has been made to preserve the conceptual continuity and relationship of the newer terms with extant ones. These terms, together with the terms which are included in specific International Protocols/Guidelines already adopted by Codex by reference are given below.

**ANALYTICAL TERMS**

The following analytical terms are now defined by Codex for Codex purposes:

* Accuracy
* Analyte
* Applicability
* Bias
* Calibration
* Certified reference material
* Conventional quantity value
* Critical value
* Defining (Empirical) method of analysis
* Error
* Expanded measurement uncertainty
* Fitness for purpose
* HorRat
* Inter-laboratory study
* Laboratory performance (Proficiency) study
* Limit of detection
* Limit of quantification
* Linearity
* Material certification study
* Measurand
* Measurement method
* Measurement procedure
* Measurement uncertainty
* Method-performance study
* Metrological Traceability
* Outlier
* Precision
* Quality assurance
* Rational method of analysis
* Recovery/recovery factors
* Reference material
* Reference value
* Repeatability (Reproducibility)
* Repeatability conditions
* Repeatability (Reproducibility) limit
* Repeatability (Reproducibility) standard deviation
* Repeatability (Reproducibility relative standard deviation
* Reproducibility conditions
* Result
* Robustness (ruggedness)
* Selectivity
* Sensitivity
* Surrogate
* Systematic error
* Trueness
* True value
* Validated range
* Validated Test Method
* Validation Verification

**DEFINITIONS OF ANALYTICAL TERMS**

***Accuracy:*** The closeness of agreement between a test result or measurement result and a reference value.

Notes:

The term “accuracy,” when applied to a set of test results or measurement results, involves a combination of random components and a common systematic error or bias component.

When applied to a test method, the term accuracy refers to a combination of trueness and precision.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

***Analyte***: The chemical substance sought or determined in a sample.

Note:

This definition does not apply to molecular biological analytical methods.

Reference:

Codex Guidelines on Good Laboratory Practice in Residue Analysis (CAC/GL 40-1993)

***Applicability:*** the analytes, matrices, and concentrations for which a method of analysis may be used satisfactorily.

Note:

In addition to a statement of the range of capability of satisfactory performance for each factor, the statement of applicability (scope) may also include warnings as to known interference by other analytes, or inapplicability to certain matrices and situations.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Bias:*** The difference between the expectation of the test result or measurement result and the true value. In practice conventional quantity value (VIM, 2007) can be substituted for true value.

Notes:

Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.

The bias of a measuring instrument is normally estimated by averaging the error of indication over the appropriate number of repeated measurements. The error of indication is the: “indication of a measuring instrument minus a true value of the corresponding input quantity”.

Expectation is the expected value of a random variable, e.g. assigned value or long term average {ISO 5725- 1}

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

***Calibration:*** Operation that, under specified conditions, in a first step, establishes a relation between the values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and in a second step uses this information to establish a relation for obtaining a measurement result from an indication.

Notes:

A calibration may be expressed by a statement, calibration function, calibration diagram, calibration curve, or calibration table. In some cases it may consist of an additive or multiplicative correction of the indication with associated measurement uncertainty.

Calibration should not be confused with adjustment of a measuring system often mistakenly called “self calibration,” or with verification of calibration.

Often the first step alone in the above definition is perceived as being calibration.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Certified reference material (CRM):*** Reference material accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures

Notes:

Documentation is given in the form of a “certificate” (see ISO guide 30:1992).

Procedures for the production and certification of certified reference materials are given, e.g. in ISO Guide 34 and ISO Guide 35.

In this definition, “uncertainty” covers both measurement uncertainty and uncertainty associated with the value of the nominal property, such as for identity and sequence.

Traceability covers both metrological traceability of a value and traceability of a nominal property value.

Specified values of certified reference materials require metrological traceability with associated measurement uncertainty {Accred. Qual. Assur., 2006}

ISO/REMCO has an analogous definition {Accred. Qual. Assur., 2006} but uses the modifiers metrological and metrologically to refer to both quantity and nominal properties.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

New definitions on reference materials, Accreditation and Quality Assurance, 10:576-578, 2006

***Conventional quantity value:*** quantity value attributed by agreement to a quantity for a given purpose.

Notes:

The term “conventional true quantity value” is sometimes used for this concept, but its use is discouraged. Sometimes a conventional quantity value is an estimate of a true quantity value.

A conventional quantity value is generally accepted as being associated with a suitably small measurement uncertainty, which might be zero.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Critical value (LC):*** The value of the net concentration or amount the exceeding of which leads, for a given error probability a, to the decision that the concentration or amount of the analyte in the analyzed material is larger than that in the blank material. It is defined as:

Pr ( >LC | L=0) ≤ a

_Pic71

Where is the estimated value, L is the expectation or true value and LC is the critical value.

_Pic73

Notes:

The definition of critical value is important for defining the Limit of Detection (LOD). The critical value Lc is estimated by

LC = t1-avso,

Where t1-av is Student's-t, based on v degrees of freedom for a one-sided confidence interval of 1-a and so is the sample standard deviation.

If L is normally distributed with known variance, i.e. v = ∞ with the default a of 0.05, LC = 1.645so.

A result falling below the LC triggering the decision “not detected” should not be construed as demonstrating analyte absence. Reporting such a result as “zero” or as < LOD is not recommended. The estimated value and its uncertainty should always be reported.

References:

ISO Standard 11843: Capability of Detection-1, ISO, Geneva,

1997 Nomenclature in evaluation of analytical methods, IUPAC, 1995

***Defining (empirical/conventional) method of analysis:*** A method in which the quantity measured is defined by the result found on following the stated procedure.

Notes:

Empirical methods are used for purposes that cannot be covered by rational methods. Bias in empirical methods is conventionally zero.

Reference:

Harmonised guidelines for single-laboratory validation of methods of analysis, 2002

***Error***: Measured quantity value minus a reference quantity value.

Note:

The concept of measurement ‘error’ can be used both: when there is a single reference value to refer to, which occurs if a calibration is made by means of a measurement standard with a measured value having a negligible measurement uncertainty or if a conventional value is given, in which case the measurement error is not known and if a measurand is supposed to be represented by a unique true value or a set of true values of negligible range, in which case the measurement error is not known.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Expanded measurement uncertainty:*** product of a combined standard measurement uncertainty and a factor larger than the number one

Notes:

The factor depends upon the type of probability distribution of the output quantity in a measurement model and on the selected coverage probability.

The term factor in this definition refers to a coverage factor.

Expanded measurement uncertainty is also termed expanded uncertainty.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Fitness for purpose:*** Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.

Reference:

Eurachem Guide: The fitness for purpose of analytical methods: A laboratory guide to method validation and related topics, 1998

***HorRat:*** The ratio of the reproducibility relative standard deviation to that calculated from the Horwitz equation,

Predicted relative standard deviation (PRSD)R =2C-0.15:

HorRat(R) = RSDR/PRSDR ,

HorRat(r) = RSDr/PRSDR

Where C is concentration expressed as a mass fraction (both numerator and denominator expressed in the same units).

Notes:

The HorRat is indicative of method performance for a large majority of methods in chemistry.

Normal values lie between 0.5 and 2. (To check proper calculation of PRSDR, a C of 10-6 should give a PRSDR of 16 %.)

If applied to within-laboratory studies, the normal range of HorRat(r) is 0.3-1.3.

For concentrations less than 0.12 mg/kg the predicted relative standard deviation developed by Thompson (The Analyst, 2000), 22% should be used.

References:

A simple method for evaluating data from an inter-laboratory study, J AOAC, 81(6): 1257-1265, 1998

Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, The Analyst, 125:385-386, 2000

***Inter-laboratory study:*** A study in which several laboratories measure a quantity in one or more “identical” portions of homogeneous, stable materials under documented conditions, the results of which are compiled into a single document.

Notes:

The larger the number of participating laboratories, the greater the confidence that can be placed in the resulting estimates of the statistical parameters. The IUPAC-1987 protocol (Pure & Appl. Chem., 66, 1903- 1911(1994)) requires a minimum of eight laboratories for method-performance studies.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Laboratory-performance (proficiency) study:*** An inter-laboratory study that consists of one or more measurements by a group of laboratories on one or more homogeneous, stable, test samples by the method selected or used by each laboratory. The reported results are compared with those from other laboratories or with the known or assigned reference value, usually with the objective of improving laboratory performance.

Notes:

Laboratory-performance studies can be used to support laboratory accreditation of laboratories or to audit performance. If a study is conducted by an organization with some type of management control over the participating laboratories: organizational, accreditation, regulatory or contractual, the method may be specified or the selection may be limited to a list of approved or equivalent methods. In such situations, a single test sample is insufficient to judge performance.

A laboratory-performance study may be used to select a method of analysis that will be used in a method- performance study. If all laboratories, or a sufficiently large subgroup, of laboratories, use the same method, the study may also be interpreted as a method-performance study, provided that the test samples cover the range of concentration of the analyte.

Laboratories of a single organization with independent facilities, instruments, and calibration materials, are treated as different laboratories.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Limit of Detection (LOD):*** The true net concentration or amount of the analyte in the material to be analyzed which will lead, with probability (1-13), to the conclusion that the concentration or amount of the analyte in the analyzed material is larger than that in the blank material. It is defined as:

Pr ( ≤LC | L=LOD) = 13

Where is the estimated value, L is the expectation or true value and LC is the critical value.

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Notes:

The limit of detection LOD is estimated by,

LOD zi 2t1-avao [where a = 13i,

Where t1-áv is Student's-t, based on v degrees of freedom for a one-sided confidence interval of 1-a and ao is the standard deviation of the true value (expectation).

LOD = 3.29 ao, when the uncertainty in the mean (expected) value of the blank is negligible, a = 13 = 0.05 and L is normally distributed with known constant variance. However, LOD is not defined simply as a fixed coefficient (e.g. 3, 6, etc.) times the standard deviation of a pure solution background. To do so can be extremely misleading. The correct estimation of LOD must take into account degrees of freedom, a and 13, and the distribution of L as influenced by factors such as analyte concentration, matrix effects and interference.

This definition provides a basis for taking into account exceptions to simple case that is described, i.e. involving non-normal distributions and heteroscedasticity (e.g. “counting” (Poisson) processes as those used for real time PCR).

It is essential to specify the measurement process under consideration, since distributions, a’s and blanks can be dramatically different for different measurement processes.

At the limit of detection, a positive identification can be achieved with reasonable and/or previously determined confidence in a defined matrix using a specific analytical method.

References:

ISO Standard 11843: Capability of Detection-1, ISO, Geneva, 1997

Nomenclature in evaluation of analytical methods, IUPAC, 1995

Guidance document on pesticide residue analytical methods, Organization for Economic Cooperation and Development, 2007

***Limit of Quantification (LOQ):*** A method performance characteristic generally expressed in terms of the signal or measurement (true) value that will produce estimates having a specified relative standard deviation (RSD), commonly 10% (or 6%). LOQ is estimated by:

LOQ = kQ aQ, kQ = 1/RSDQ

Where LOQ is the limit of quantification, aQ is the standard deviation at that point and kQ is the multiplier whose reciprocal equals the selected RSD. (The approximate RSD of an estimated a, based on v-degrees of freedom is 1/ Al2v.)

Notes:

If a is known and constant, then aQ = ao, since the standard deviation of the estimated quantity is independent of concentration. Substituting 10% in for kQ gives:

LOQ = (10 \* aQ) = 10 ao

In this case, the LOQ is just 3.04 times the limit of detection, given normality and a = 13 = 0.05

At the LOQ, a positive identification can be achieved with reasonable and/or previously determined confidence in a defined matrix using a specific analytical method.

This definition provides a basis for taking into account exceptions to the simple case that is described, i.e. involving non-normal distributions and heteroscedasticity (e.g. “counting” (Poisson) processes as those used for real time PCR).

Nomenclature in evaluation of analytical methods, IUPAC, 1995

Guidance document on pesticide residue analytical methods, Organization for Economic Co-operation and Development, 2007

***Linearity:*** The ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quantity of analyte to be determined in the laboratory sample. This proportionality is expressed by an *a priori* defined mathematical expression. The linearity limits are the experimental limits of concentrations between which a linear calibration model can be applied with an acceptable uncertainty.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Material-Certification Study:*** An inter-laboratory study that assigns a reference value (“true value”) to a quantity (concentration or property) in the test material, usually with a stated uncertainty.

Note:

A material-certification study often utilizes selected reference laboratories to analyse a candidate reference material by a method(s) judged most likely to provide the least-biased estimates of concentration (or of a characteristic property) and the smallest associated uncertainty.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Measurand:*** Quantity intended to be measured.

Notes:

The specification of a measurand requires knowledge of the kind of quantity, description of the state of the substance carrying the quantity, including any relevant component and the chemical entities involved.

In chemistry, ‘analyte’ or the name of a substance or compound are terms sometime used for measurand. This usage is erroneous because these terms do not refer to quantities.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Measurement method:*** Generic description of a logical organization of operations used in a measurement.

Note:

Measurement methods may be qualified in various ways such as: substitution measurement method, differential measurement method, and null measurement method; or direct measurement method, and indirect measurement method.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Measurement procedure:*** Detailed description of a measurement according to one or more measurement principles and to a given measurement method, based on a measurement model and including any calculation to obtain a result.

Notes:

A measurement procedure is usually documented in sufficient detail to enable an operator to perform a measurement.

A measurement procedure can include a statement concerning a target measurement uncertainty. A measurement procedure is sometimes called a standard operating procedure (SOP).

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Measurement uncertainty:*** Non-negative parameter characterizing the dispersion of the values being attributed to a measurand, based on the information used.

Notes:

Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned values of measurement standards, as well as the definitional uncertainty. Sometimes estimated systematic effects are not corrected for but, instead associated measurement uncertainty components are incorporated.

The parameter may be, for example, a standard deviation called standard measurement uncertainty (or a given multiple of it), or the half-width of interval having a stated coverage probability.

Measurement uncertainty comprises, in general many components. Some of these components may be evaluated by Type A evaluation of measurement uncertainty from the statistical distribution of the values from a series of measurements and can be characterized by experimental standard deviations. The other components which may be evaluated by Type B evaluation of measurement uncertainty can also be characterized by standard deviations, evaluated from assumed probability distributions based on experience or other information.

In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quality value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Method-Performance Study:*** An inter-laboratory study in which all laboratories follow the same written protocol and use the same test method to measure a quantity in sets of identical test samples. The reported results are used to estimate the performance characteristics of the method. Usually these characteristics are within-laboratory and among-laboratories precision, and when necessary and possible, other pertinent characteristics such as systematic error, recovery, internal quality control parameters, sensitivity, limit of quantification, and applicability.

Notes:

The materials used in such a study of analytical quantities are usually representative of materials to be analyzed in actual practice with respect to matrices, amount of test component (concentration), and interfering components and effects. Usually the analyst is not aware of the actual composition of the test samples but is aware of the matrix.

The number of laboratories, number of test samples, number of determinations, and other details of the study are specified in the study protocol. Part of the study protocol is the procedure which provides the written directions for performing the analysis.

The main distinguishing feature of this type of study is the necessity to follow the same written protocol and test method exactly.

Several methods may be compared using the same test materials. If all laboratories use the same set of directions for each method and if the statistical analysis is conducted separately for each method, the study is a set of method-performance studies. Such a study may also be designated as a method-comparison study.

Reference:

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Metrological Traceability:*** Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the stated measurement uncertainty.

Notes:

A reference can be a definition of a measurement unit through its practical realization, or a measurement procedure including the measurement unit for a non-ordinal quantity, or a measurement standard.

Metrological traceability requires an established calibration hierarchy.

Specification of the reference must include the time at which this reference was used in establishing the calibration hierarchy, along with any other relevant metrological information about the reference, such as when the first calibration in the calibration hierarchy was performed.

For measurements with more than one input quantity each of the input values should itself be traceable and the calibration hierarchy involved may form a branched structure or network. The effort involved in establishing the metrological traceability for each input value should be commensurate with its relative contribution to the measurement result.

Metrological traceability of a measurement result does not ensure that the measurement uncertainty is adequate for a given purpose or that there is an absence of mistakes.

A comparison between two measurement standards may be viewed as a calibration if the comparison is used to check and if necessary correct the value and measurement uncertainty of the measurement standards.

The ILAC considers the elements for confirming metrological to be an unbroken metrological traceability chain to an international measurement standard or a national measurement standard, a documented procedure, accredited technical competence, metrological to the SI and calibration intervals (see ILAC P­10:2002)

The abbreviated term ‘traceability’ is sometimes used to mean ‘metrological traceability’ as well as other concepts, such as sample traceability or document traceability or instrument traceability or material traceability, where history (trace) is meant. Therefore the full term of metrological traceability is preferred if there is any risk of confusion.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

Harmonized guidelines for internal quality control in analytical chemistry laboratories, 1995

ILAC P-10, 2002

***Outlier:*** A member of a set of values which is inconsistent with other members of that set

Note:

The following practice is recommended for dealing with outliers.

1. Tests such as Cochran’s or Grubb’s tests are applied to identify stragglers or outliers:

- if the test statistic is less than or equal to its 5 % critical value, the item tested is accepted as correct;

- if the test statistic is greater than its 5 % critical value and less than or equal to its 1 % critical value, the item tested is called a straggler and is indicated by a single asterisk;

- if the test statistic is greater than its 1 % critical value, the item is called a statistical outlier and is indicated by a double asterisk.

1. It is next investigated whether the stragglers and/or statistical outliers can be explained by some technical error, for example:

- a slip in performing the measurement,

- an error in computation,

- a simple clerical error in transcribing a test result,

- analysis of the wrong sample.

Where the error was one of the computation or transcription type, the suspect result should be replaced by the correct value; where the error was from analyzing a wrong sample, the result should be placed in its correct cell. After such correction has been made, the examination for stragglers or outliers should be repeated. If the explanation of the technical error is such that it proves impossible to replace the suspect test result, then it should be discarded as a “genuine” outlier that does not belong to the experiment proper.

c) When any stragglers and/or statistical outliers remain that have not been explained or rejected as belonging to an outlying laboratory, the stragglers are retained as correct items and the statistical outliers are discarded unless the statistician for good reason decides to retain them.

References:

ISO Standard 5725-1: Accuracy (trueness and precision) of measurement methods and results Part 1: General principles and definitions, ISO, Geneva, 1994

ISO Standard 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, ISO, Geneva, 1994

***Precision:*** The closeness of agreement between independent test/measurement results obtained under stipulated conditions.

Notes:

Precision depends only on the distribution of random errors and does not relate to the true value or to the specified value.

The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation.

Quantitative measures of precision depend critically on the stipulated conditions.

Repeatability and reproducibility conditions are particular sets of extreme conditions.

Intermediate conditions between these two extreme conditions are also conceivable, when one or more factors within a laboratory (intra-laboratory e.g. the operator, the equipment used, the calibration of the equipment used, the environment, the batch of reagent and the elapsed time between measurements) are allowed to vary and are useful in specified circumstances.

Precision is normally expressed in terms of standard deviation.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

ISO Standard 5725-3: Accuracy (trueness and precision) of measurement methods and results Part 3: Intermediate measures of the precision of a standard measurement method, ISO, Geneva, 1994

***Quality assurance:*** All those planned and systematic actions necessary to provide adequate confidence that analytical results will satisfy given requirements for quality.

Reference:

Harmonized guidelines for internal quality control in analytical chemistry laboratories, 1995

***Rational method of analysis*:** A method that determines an identifiable chemical(s) or analytes(s) for which there may be several equivalent methods of analysis available.

Reference:

Harmonized guidelines for the use of recovery information in analytical measurement, 1998

ISO/IEC Guide 17025:2005: General requirements for the competence of calibration and testing laboratories, ISO, Geneva, 2005

***Recovery/recovery factors:*** Proportion of the amount of analyte, present in, added to or present in and added to the analytical portion of the test material, which is presented for measurement.

Notes:

Recovery is assessed by the ratio ***R* = *Cobs* / *C ref*** of the observed concentration or amount ***Cobs*** obtained by the application of an analytical procedure to a material containing analyte at a reference level ***Cref .***

***Cref*** will be: (a) a reference material certified value, (b) measured by an alternative definitive method, (c) defined by a spike addition or (d) marginal recovery.

Recovery is primarily intended for use in methods that rely on transferring the analyte from a complex matrix into a simpler solution, during which loss of analyte can be anticipated.

Reference:

Harmonized guidelines for the use of recovery information in analytical measurement, 1998 Use of the terms “recovery” and “apparent recovery” in analytical procedures, 2002

***Reference material:*** Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process or in examination of nominal properties.

Notes:

Examination of a nominal property provides a nominal property value and associated uncertainty. This uncertainty is not a measurement uncertainty.

Reference materials with or without assigned values can be used for measurement precision control whereas only reference materials with assigned values can be used for calibration and measurement trueness control.

Some reference materials have assigned values that are metrologically traceable to a measurement unit outside a system of units. In a given measurement, a given reference material can only be used for either calibration or quality assurance.

The specification of a reference material should include its material traceability, indicating its origin and processing. {Accred. Qual. Assur., 2006}

ISO/REMCO has an analogous definition that uses the term measurement process to mean examination which covers both measurement of a quantity and examination of a nominal property.

References:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

New definitions on reference materials, Accred. Qual. Assur., 10:576-578, 2006

***Reference value:*** Quantity value used as a basis of comparison with values of quantity of the same kind.

Notes:

A reference quantity value can be a true quantity value of a measurand, in which case it is unknown, or a conventional quantity value in which case it is known.

A reference quantity value with an associated measurement uncertainty is usually provided with reference to

1. a material, e.g. a certified reference material
2. a reference measurement procedure
3. a comparison of measurement standards.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Repeatability (Reproducibility):*** Precision under repeatability (reproducibility) conditions.

Reference:

ISO 3534-1 Statistics, vocabulary and symbols-Part 1: Probability and general statistical terms, ISO, 1993 ISO Standard 78-2: Chemistry – Layouts for Standards – Part 2: Methods of Chemical Analysis, 1999) Codex Alimentarius Commission, Procedural Manual, 17th Edition, 2007

AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis, 2002.

***Repeatability conditions:*** Observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time.

Note:

Repeatability conditions include: the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

***Repeatability (Reproducibility) limit:*** The value less than or equal to which the absolute difference between final values, each of them representing a series of test results or measurement results obtained under repeatability (reproducibility) conditions may be expected to be with a probability of 95%.

Notes:

The symbol used is r [R]. {ISO 3534-2}

When examining two single test results obtained under repeatability (reproducibility) conditions, the comparison should be made with the repeatability (reproducibility) limit, r [R] = 2.8σr[R]. {ISO 5725-6, 4.1.4}

When groups of measurements are used as the basis for the calculation of the repeatability (reproducibility) limits (now called the critical difference), more complicated formulae are required that are given in ISO 5725-6: 1994, 4.2.1 and 4.2.2.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

ISO 5725-6 “Accuracy (trueness and precision) of a measurement methods and results—Part 6:

Use in practice of accuracy value”, ISO, 1994

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Repeatability (reproducibility) standard deviation*:** Standard deviation of test results or measurement results obtained under repeatability (reproducibility) conditions.

Notes:

It is a measure of the dispersion of the distribution of the test or measurement results under repeatability (reproducibility) conditions.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

***Repeatability (reproducibility) relative standard deviation (coefficient of variation):*** Repeatability (reproducibility) standard deviation divided by the mean.

RSDr[R] is computed by dividing the repeatability (reproducibility) standard deviation by the mean. Notes:

Relative standard deviation (RSD) is a useful measure of precision in quantitative studies.

This is done so that one can compare variability of sets with different means. RSD values are independent of the amount of analyte over a reasonable range and facilitate comparison of variabilities at different concentrations.

The result of a collaborative test may be summarized by giving the RSD for repeatability (RSDr) and RSD for reproducibility (RSDR).

The RSD is also known as coefficient variation.

Reference:

ISO Standard 3 534-2: Vocabulary and Symbols Part 1: General statistical terms used in probability, ISO, Geneva, 2006

AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis, 2002.

***Reproducibility conditions*:** Observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment.

Reference:

ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, ISO, Geneva, 2006

***Result:*** Set of values being attributed to a measurand together with any other available relevant information

Notes:

A result of measurement generally contains ‘relevant information’ about the set of values, such that some may be more representative of the measurand than others. This may be expressed in the form of a probability density function.

A result of measurement is generally expressed as a single measured value and a measurement uncertainty. If the measurement uncertainty is considered to be negligible for some purpose, the measurement result may be expressed as a single measured value. In many fields, this is the common way of expressing a measurement result.

In the traditional literature and in the previous edition of the VIM, result was defined as a value attributed to a measurand and explained to mean an indication or an uncorrected result or a corrected result according to the context.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Robustness (ruggedness*):** A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage

Reference:

ICH Topic Q2 Validation of Analytical Methods, the European Agency for the Evaluation of Medicinal Products: ICH Topic Q 2 A - Definitions and Terminology (CPMP/ICH/381/95), 1995

Harmonized guidelines for single laboratory validation of methods of analysis, Pure and Appl. Chem., 2002

***Selectivity:*** Selectivity is the extent to which a method can determine particular analyte(s) in a mixture(s) or matrice(s) without interferences from other components of similar behaviour.

Note:

Selectivity is the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence other components. Selectivity can be graded. The use of the term specificity for the same concept is to be discouraged as this often leads to confusion.

Reference:

Selectivity in analytical chemistry, IUPAC, Pure Appl Chem, 2001 Codex Alimentarius Commission, Alinorm 04/27/23, 2004

Codex Alimentarius Commission, Procedural Manual, 1 7th Edition, 2007

***Sensitivity:*** Quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured.

Notes:

The sensitivity can depend on the value of the quantity being measured

The change considered in the value of the quantity being measured must be large compared with the resolution of the measurement system.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Surrogate:*** Pure compound or element added to the test material, the chemical and physical behaviour of which is taken to be representative of the native analyte.

Reference:

Harmonized guidelines for the use of recovery information in analytical measurement, 1998

***Systematic error:*** Component of measurement error that in replicate measurements remains constant or varies in a predictable manner.

Notes:

A reference value for a systematic error is a true quantity value, or a measured value of a measurement standard of negligible measurement uncertainty, or a conventional value.

Sytematic error and its causes can be known or unknown. A correction can be applied to compensate for a known systematic error.

Systematic error equals measurement error minus random measurement error.

Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Trueness:*** The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Note 1: Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725.

Note 2: Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error.

Note 3: Measurement accuracy should not be used for 'measurement trueness' and vice versa. Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***True value:*** Quantity value consistent with the definition of a quantity.

Notes:

In the error approach to describing measurement, a true quantity value is considered unique and in practice unknowable. The uncertainty approach is to recognize that, owing to the inherently incomplete amount of detail in the definition of quantity, there is not a single true quantity value, but rather a set of quantity values consistent with the definition of a quantity. However, this set of values is, in principle and in practice unknowable. Other approaches dispense altogether with the concept of true quantity value and rely on the concept of metrological compatibility of measurement results for assessing their validity.

When the definitional uncertainty associated with the measurand is considered to be negligible compared to the other components of the measurement uncertainty the measurand may be considered to have an essentially “unique” true value.

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Validation:*** Verification, where the specified requirements are adequate for an intended use. Reference:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

***Validated Test Method:*** An accepted test method for which validation studies have been completed to determine the accuracy and reliability of this method for a specific purpose.

Reference:

ICCVAM Guidelines for the nomination and submission of new, revised and alternative test methods, 2003

***Validated range:*** That part of the concentration range of an analytical method which has been subjected to validation.

Reference:

Harmonized guidelines for single-laboratory validation of methods of analysis, 2002

***Verification:*** Provision of objective evidence that a given item fulfils specified requirements.

Notes:

When applicable method uncertainty should be taken into consideration.

The item may be e.g. a process, measuring procedure, material, compound or measuring system. The specified requirement may be that a manufacturer’s specifications are met.

Verification in legal metrology, as defined in VIM and in conformity assessment in general pertains to the examination and marketing and/or issuing of a verification certificate for a measuring system.

Verification should not be confused with calibration. Not every verification is a validation.

In chemistry, verification of the identity of the entity involved or of the activity, requires a description of the structure and properties of that entity or activity.

References:

VIM, International Vocabulary of Metrology – Basic and general concepts and associated terms, 3rd edition, JCGM 200: 2008

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**APPENDIX V: PROPRIETARY METHODS OF ANALYSIS**

**PROVISIONS ON THE USE OF PROPRIETARY METHODS IN CODEX STANDARDS  
(To be added to the Codex Procedural Manual)**

***Definition of a Proprietary Method of Analysis***

For Codex purposes a proprietary method of analysis is one that contains protected intellectual property preventing full disclosure of information about the method and/or where the intellectual property owner restricts the use or distribution of the method or materials for its performance such that no alternative source of these would be available. It does not extend to a method which is subject only to copyright.

***Requirements***

Codex Committees may occasionally submit methods of analysis which are proprietary, or are based on proprietary aspects, to the Codex Committee on Methods of Analysis and Sampling for endorsement. CCMAS encourages the method sponsors to provide data for CCMAS assessment.

1. A proprietary method should not be endorsed if there is available a suitable non-proprietary method of analysis which has been or could be endorsed and which has similar or better performance characteristics. This should ensure that no approach is taken such that it appears as if a proprietary method is endorsed by Codex to the detriment of other potential methods; if possible preference should be given to adopting appropriate method criteria rather than endorsing a specific proprietary method of analysis.
2. Preference should be given to endorsing those methods of analysis where the reagents and/or apparatus are described in the method to the degree that either laboratories or other manufacturers could produce them themselves.
3. Method performance criteria established for proprietary methods are the same as those for non­-proprietary methods. Performance criteria should be those stipulated above. If appropriate, information on the effect of manufacturing variability of the proprietary method on the method performance should be provided.
4. After endorsing, any changes that influence performance characteristics must be reported to CCMAS for consideration.
5. A proprietary method should be either fully collaboratively validated or validated and reviewed by an independent third party according to internationally recognised protocols. The results of such studies should be made available for CCMAS. If a proprietary method has not been validated by a full collaborative trial, it may be eligible for adoption into the Codex system as a Codex Type IV method, but not as a Type I, II or III method.
6. Whilst respecting the necessity for reasonable protection of intellectual property, sufficient information should be available to enable reliable use of the method by analysts and to enable evaluation of the performance of the method by CCMAS. In any particular case this may extend beyond performance data, for example to include details of operating principle, at the sole discretion of CCMAS.
7. The supplier or submitter of a proprietary method should demonstrate to CCMAS’s satisfaction that the method will be readily available to all interested parties.
8. CCMAS may decline to endorse a proprietary method if restrictions by intellectual property unduly restrict research into determining the method properties, scope of claim and validity or development of improvements to the technology.
9. If suitable non-proprietary methods become available and endorsed, the status of the previously endorsed proprietary method should be reviewed and may be revised.

**ANNEX V: ROUNDING RULES**

**Directives for expression of analytical results**

The final analytical results expressed on analytical certificates must be rounded as given below.

1) The analytical result can be expressed as a figure or as a figure with confidence limits.

2) If the analytical result is expressed as a figure, it must have as many significant figures, so that the next to the last of these is certain. For the decision, which is the first uncertain figure, the standard deviation (sd) is used, it falls on the last figure of the analytical result. See examples in Table 1. Rounding is to be done as given in 4).

3) If the analytical result is expressed with confidence limits (X ), then the middle of the interval (X) must have as many significant figures as given for the result in 2). The term 2\*sd (half of the interval ±2\*sd) must have the same number of significant figures as the middle of the interval (X).

4) The results are rounded by increasing the last significant figure by 1, if the first insignificant figure, which must be discarded together with other insignificant figures, is 5 or larger. The last significant figure is left the same, if the first insignificant figure is 4 or less.

Analytical results from single determinations must have one more significant figure than indicated above for final analytical results to avoid rounding errors when calculating mean values.

**Table 1.** Deciding the number of significant figures

|  |  |  |
| --- | --- | --- |
| Result | sd | Expressed result |
| 478 | 12 | 480 |
| 478 | 8,0 | 478 |
| 478 | 23 | 480 |
| 478 | 124 | 500 |
| 5,4 | 1,2 | 5 |
| 4,73 | 0,61 | 4,7 |

\_ indicates the first uncertain figure

If rounding is specified by legal regulations, rules given there have to be applied.

**ANNEX VI: METHOD VERIFICATION**

**Scope**

This document is intended as guidance to end users for the implementation of standardised methods in laboratories and should provide support to those laboratories that need to fulfil requirements resulting from ISO/IEC 17025:2005 [] related to the accreditation of standardised methods. The document relates specifically to chemical methods of analysis.

**Need for method verification**

National legislation as well as international agreements refer to documentary standards (product as well as procedural standards) to enable international trade as well as to support the well being of consumers. Many of the standardized methods available nowadays have been validated by a collaborative study organized and evaluated according to internationally agreed protocols. The application of validated methods by testing laboratories is key to the generation of reliable and comparable test results. Laboratories accredited to ISO/IEC 17025 need to validate laboratory-developed or non-standard methods as extensively as is necessary to meet the needs of the given application (clause 5.4.5.2). Methods that have been performance tested in a collaborative study are highly appreciated by users and are a prerequisite for formal standardisation by standardisation bodies. Although a standardised method is regarded as fully validated, a laboratory intending to implement it is required to demonstrate its ability to apply the method correctly. It has to provide evidence that the method performs, under the described conditions of use, within the limits of the criteria established in the original method validation study. Clause 5.4.2 ISO/IEC 17025 formulates this requirement as 'The laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations'. In other words, the laboratory has to demonstrate that a validated procedure to be used for the first time with a particular product will yield acceptable results using the laboratory's equipment, personnel, and reagents. While guidance on method validation is abundant in the chemical literature, only little information on what needs to be done to verify the correctness of implementation of a standardised testing method in a laboratory is available. The Analytical Laboratory Accreditation Criteria Committee (ALACC) of AOAC International prepared a guide to define the activities that are required to fulfil method verification based on the performance characteristics of a standardised method of analysis []. Likewise, US Pharmacopeia (USP) has introduced General Chapter <1226> "Verification of Compendial Procedures" to provide guidance about the verification process for testing drug substances and drug products [].

**Validation and verification of methods of analysis**

Several more or less general definitions for the two terms exist:

The International Organization for Standardization (ISO) as well as the Joint Committee for Guides in Metrology (JCGM) define *validation* as:

ISO 9000:2005 []: confirmation, through the provision of objective evidence, that the requirements for a specific intended use of application have been fulfilled;

JCGM 200:2008 (VIM-3) []: verification, where the specific requirements are adequate for an intended use;

ISO 17025:2005 []: confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (clause 5.4.5.1);

whereas more specific definitions exist to relate the validation process to laboratory operations, such as

Eurachem Guide – the fitness for purpose of analytical methods []: method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires;

IUPAC Harmonized guidelines for single laboratory validation of methods of analysis []: method validation makes use of a set of tests that both test any assumptions on which the analytical method is based and establish and document the performance characteristics of a method, thereby demonstrating whether the method is fit for a particular analytical purpose;

AOAC Guidelines for single laboratory validation of chemical methods for dietary

supplements and botanicals []: validation is the process of demonstrating or confirming the performance characteristics of a method of analysis;

ICH Harmonised tripartite guideline – Validation of analytical procedures: text and methodology []: the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose;

USP chapter <1225> []: validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements of the intended analytical application.

The ISO and the JCGM definitions for *verification* are:

ISO 9000:2005 []: confirmation, through the provision of objective evidence, that specific requirements have been fulfilled

JCGM 200:2008 (VIM-3) []: provision of objective evidence that a given items fulfils specified requirements

Definitions related to analytical procedures are less frequently in use, besides the general notion that laboratories have to verify that they "can properly operate standard methods". The two most relevant explanations about the verification process are found in the ALACC Guide and USP chapter <1226>.

ALACC Guide []: Verification that a laboratory can adequately operate a standard method requires that the laboratory provide objective evidence the performance parameters specified in the test method have been met with the matrices to

which the method is being applied.

USP chapter <1226> [] Users of compendial analytical procedures are not required to validate these procedures when first used in their laboratories, but documented evidence of suitability should be established under actual conditions of use.

The IUPAC single-laboratory validation guide makes reference to the need of performance verification of collaboratively tested methods []:

*A laboratory using a collaboratively studied method, which has been found to be fit for the intended purpose, needs only to demonstrate that it can achieve the performance characteristics stated in the method*

*The laboratory should undertake precision studies, bias studies (including matrix variation studies), and possibly linearity studies*

From the definitions given above it follows that the general system properties of an item, e.g. the performance characteristics of an analytical method, have to be verified as far as needed by the user, whereas the adequacy for a special purpose, not covered by the original claims, must be validated []. Related to laboratory operations the term *validation* means the demonstration of suitability of a method or process for its intended purpose, and the term *verification* means the demonstration that the previously validated method is suitable under actual conditions of use in a given laboratory which applies it to a certain product.

**Verification of the performance characteristics of a standardised method under conditions of actual use**

In practical terms, the laboratory has to demonstrate that the method performance characteristics under the actual condition of use of the method (analyst(s), laboratory infrastructure, equipment, reagents, consumables, test items) are equivalent to those established during the validation study. Precision and trueness are usually the performance characteristics of quantitative methods which need to be verified. Verification of other characteristics, such as selectivity, limit of detection, etc. may be necessary, depending on the intended use of the method. In case that the verification study shall meet ISO 17025 requirements the planning, conduct, evaluation and interpretation of results has to be properly documented. In the planning phase acceptance criteria for the method characteristics to be verified have to be set. Usually the established performance characteristics of the validated method will form the benchmark, although other acceptance criteria can be set depending on the specific objective of the analysis. If the data produced during the verification exercise meets the acceptance criteria, the standardised method performs as intended under actual usage conditions; if the criteria are not met, root cause analysis to identify the source of the deviation and corrective action has to be initiated. In case that the deviation from the criteria still persists then it can be concluded that the procedure might not be suitable for use with the item being tested.

To carry out method verification the following will be needed:

* Work instruction of the method to be verified
* Documented verification plan inclusive acceptance criteria
* Suitable test items (samples representative for the range of products to be tested)
* Suitable (certified) reference material, if available
* Suitable blank matrices, if applicable
* Maintained and calibrated equipment
* Reagents and consumables as needed by the work instruction
* Trained personnel

The table below summarises the method characteristics that need to be verified for chemical methods of analysis:

|  |  |  |
| --- | --- | --- |
|  | Verification | |
| Method performance characteristic | Direct | Indirect1) |
| Applicability (matrix and concentration range) | Y |  |
| Calibration, linearity, working range, sensitivity | N | Y |
| Limit of detection | N |  |
| Limit of quantification | N |  |
| Precision (repeatability, reproducibility) | Y |  |
| Trueness (recovery) | Y |  |
| Selectivity / specificity | N | Y |
| Ruggedness | N |  |
| (Measurement uncertainty) | N |  |

1) Certain elements are already included in the verification of other performance characteristis (e.g. calibration related characteristics are indirectly addressed by trueness studies)

The results of a collaborative study yield performance parameters for precision (reproducibility standard deviation, sR; and repeatability standard deviation, sr), and, in some circumstances, a method bias estimate, which form a “specification” for method performance. In adopting the method for its specified purpose, a laboratory is normally expected to demonstrate that it is meeting this “specification.” In most cases, this is achieved by carrying out experiments to verify control of precision and bias. Principles outlined in ISO 21748:2010 Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation [], can serve as the basis for the verification exercise, although ISO 21748:2010 is intended as guidance for evaluation of measurement uncertainties using data obtained from studies conducted in accordance with ISO 5725-2:1994. However, the ISO 21748:2010 approach can be used for verification purposes as well:

a) Obtain estimates of the repeatability, reproducibility and trueness of the method in use from published information about the method;

b) Establish whether the laboratory bias for the measurements is within that expected on the basis of the data at a);

c) Establish whether the precision attained by current measurements is within that expected on the basis of the repeatability and reproducibility estimates obtained at a);

d) If the bias and precision estimates meet the specification, the verification process has demonstrated the proficient use of the method in the specific laboratory.

Verification of precision

To demonstrate that repeatability is consistent with the repeatability standard deviation obtained in the course of the collaborative exercise the laboratory has to carry out

* replicate analysis of one or more suitable test materials, to obtain a repeatability standard deviation si, which is
* compared, using an F-test if necessary, with the repeatability standard deviation sr obtained in the collaborative study.

If si is found to be significantly greater than sr, the laboratory should identify and correct the source of deviation.

Demonstrating control of bias

To demonstrate that bias of the method is under control the laboratory has to perform replicate measurements on a reference material under repeatability conditions and

* calculate standard deviation of measurements (sw), and
* form an estimate Δ (= laboratory mean – reference value) of bias on this material, and check whether



where

sR is the reproducibility standard deviation obtained in the collaborative study

Work flow for the study to verify adequate method precision

Produce a list of potential deviations from collaborative study conditions

Are conditions equivalent?

Evaluate/take into account/correct influence of deviating conditions

Do test items vary with respect to composition and analyte level(s)

Carry out precision study on a represen-tative test item (matrix/analyte(s) at one or several levels)

Carry out precision study on several representative test items (different matrices/analyte(s) at one or several levels)

**NO**

**YES**

**YES**

**NO**

Work flow for the study to verify absence of method bias

Suitable reference material(s) available?

Blank matrix(ces) available for spiking

Replicate analyses of reference material(s)

Replicate analyses of spiked blank matrix(ces)

Replicate analyses of spiked sample(s)

**NO**

**NO**

**YES**

**YES**

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