Verifying laboratory performance and quality control in the context of micronutrient testing of fortified food

A GUIDANCE DOCUMENT

gain
Global Alliance for
Improved Nutrition

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Navigation Guide

Below is a description of the navigation aids used to make your reading of this report as simple as possible:

1. Throughout, Chapter/Sections are referenced, a simple click on the blue Chapter/Sections will guide you to the reference:

   the customer (7.8.3). More details about measurement uncertainty are given in Chapter 3.4: Verification and validation of analytical methods.

2. To continue your reading, simply click the relevant grey page number, see below:

   ### 3.4   Verification and validation of analytical methods

   According to ISO/IEC 17025:2017 (Chapter 7.2.1.5)[2], before any use, analytical methods published either in international or national standards or by technical organizations or by scientific publications are verified by the laboratory.The **verification** process in the laboratory

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Table of Acronyms

AAS	Atomic Absorption Spectroscopy
CAB	Conformity Assessment Body
CRM	Certified Reference Material
CV	Coefficient of Variance
EAC	East African Community
EPTIS	European Proficiency Testing Information System
GAIN	Global Alliance for Improved Nutrition
GUM	Guide to the Expression of Uncertainty in Measurement
HPLC	High Performance Liquid Chromatography
IAF	International Accreditation Forum
ILAC	International Laboratory Accreditation Cooperation
IEC	International Electrotechnical Commission
ILC	Interlaboratory Comparison
ISO	International Organization for Standardization
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
LGC	Laboratory of the Government Chemist
LOD	Limit of Detection
LOQ	Limit of Quantification
LSFF	Large Scale Food Fortification
NGO	Non-Governmental Organization
PT	Proficiency Test
QA/QC	Quality Assurance/Quality Control
QM	Quality Management
QMS	Quality Management System
RSD	Relative Standard Deviation
SD	Standard Deviation
SOP	Standard Operating Procedure
UV	Ultraviolet

1   Introduction

Large-scale food fortification (LSFF) of staple foods is a cost-effective and sustainable strategy for substantially reducing micronutrient malnutrition.

This is particularly true for various segments of the population living in low-and middle-income countries where diets do not provide enough nutritional value to reach the recommended daily intake of micronutrients[1].

The success and sustainability of large-scale food fortification programs are assessed by well-designed, well-managed monitoring, and evaluation systems. These monitoring systems can identify potential problems such as inadequate availability, awareness, and access of fortified foods by the target population so that corrective actions can be taken in the future.

Figure 1 below provides one such schematic representation of the monitoring and evaluating system for food fortification.

This plan distinguishes two main categories of monitoring: **regulatory monitoring and household and individual monitoring.**

Figure 1: Monitoring and evaluation system for food fortification schemes[1]
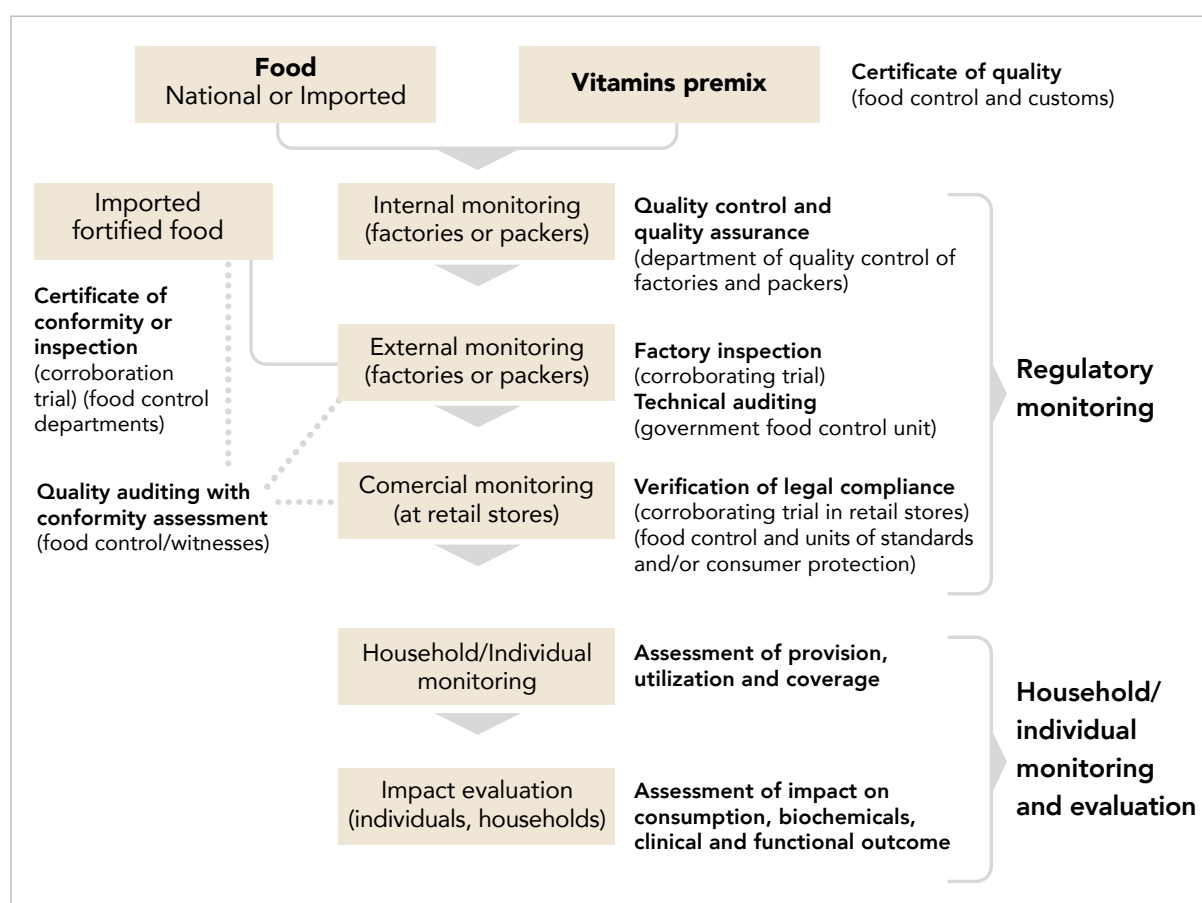
Regulatory monitoring or food control includes monitoring activities at the production level (external monitoring) and tracking and inspection at borders or customs warehouses (import monitoring) and retail stores (commercial monitoring). The primary aim of such activities is to ensure that fortified foods meet the national nutrient standards and food legislation.

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1   Allen L, de Benoist B, Dary O, Hurrell RF, eds., Guidelines on Food Fortification with Micronutrients. Geneva: World Health Organization and Food and Agriculture Organization; 2006. https://www.who.int/nutrition/publications/guide_food_fortification_micronutrients.pdf

External, import, and commercial monitoring is carried out by the relevant regulatory authorities at these locations. Internal monitoring is monitoring by the producers themselves as part of their quality control and assurance programs.

Besides regulatory monitoring, there are food consumption surveys and trend studies to monitor fortified foods' coverage and intake and to estimatenutritional impact of food fortification at the household and individual level.

Laboratory analysis plays an important role in monitoring fortified food quality throughout the supply chain. Only reliable quantitative results of qualified public or private laboratories can serve as a sound basis for official enforcement. However, merely laboratories with an effective and comprehensive Quality Management System (QMS) have the competency to deliver valid analytical data.

2   Objectives of the Guidance Document

This Guidance Document describes the various elements of quality management (QM) and quality control (QC) required in a laboratory setting.

Many qualitative elements are required to analyse the various chemical parameters in food samples, particularly for micronutrient testing and for testing food safety and food quality substances.

Based on these classified QM/QC elements, several benchmark standards are provided to authorities, NGOs, and other organizations to help them select reliable laboratories for micronutrient testing and verification of their results.

The main objectives of this Guidance Document are to provide guidelines on the:

- selection of laboratories based on quantitative testing of micronutrients in fortified foods
- verification of laboratory results for reliability

The guidance document includes the essential elements of laboratory QM/QC and the information should be laid in perspective for the above-defined objectives to be met.

Finally, general recommendations are given to stakeholders (such as laboratories, authorities, standard organisations, and NGOs) summarizing the laboratory assessments conducted by experts in numerous African and Asian countries.

2.1   What does not apply to this guidance document

The intention of this guidance document is not to provide analytical methods for micronutrient testing in foods.

The document doesn't provide statistical methods and procedures to determine method performance characteristics, such as the limit of detection (LOD) and quantification (LOQ), measurement uncertainty, etc. However, this issue supports several references to published reviews and guidelines for further in-depth study.

3 Main Elements of Laboratory Quality Management and Laboratory Quality Control

The **ISO/IEC 17025:2017** standard enables laboratories to demonstrate competent operation, validity, and confidence in their results[2].

The ISO 17025:2017 accreditation is recognized as a global standard. This standard is applicable to all organizations performing laboratory activities, regardless of the number of personneland can be used for accreditation purposes, self-assessment of laboratories, and second-party assessments.

The ISO standard provides confidence in a laboratory's operations through an effective management system guided by rigorous requirements of competency, technical operation, and reporting of results. This can further be used as the basis for continuous improvement of the laboratory's systems.

Figure 2: ISO/IEC 17025:2017 specifies the general requirements for the competence, impartiality, and consistent operation of laboratories[2]
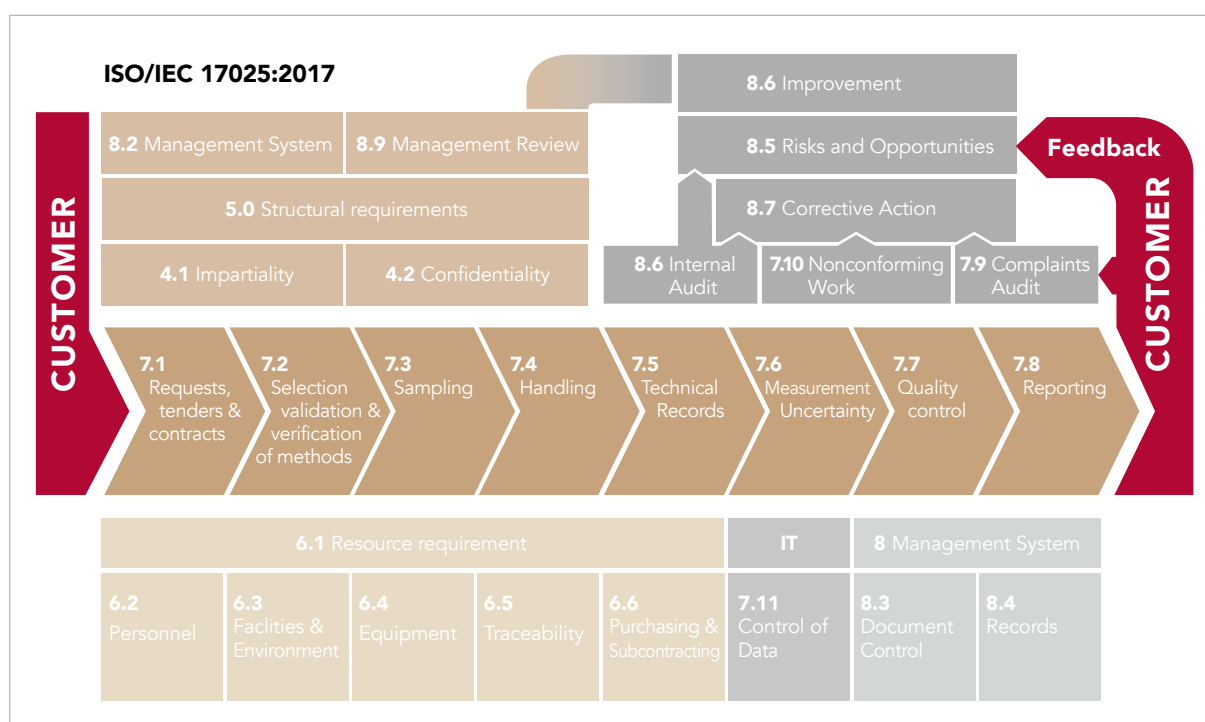
The ISO/IEC standard is structured in eight chapters (see **Figure 2**):

Chapter 1-4 comprises the 'Scope', 'Normative references', 'Terms and definitions', and 'General requirements.'

Chapter 5 'Structural requirements' covers the nature and legal status of the organization accredited.

Chapter 6 'Resource requirements' cites those issues related to the personnel, facilities, equipment, and external organizations used by the laboratory to produce technically valid results.

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2   ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories, ISO, Geneva 2017

Chapter 7   'Process requirements' describes all the relevant activities in the laboratory that ensures results are based on accepted science and aimed at technical validity.

Chapter 8   'Management system requirements' describes the steps taken by the organization to give itself the QMS tools needed to support the laboratory to produce technically valid results.

In the following section, the ISO 127025:2017 standard elements relevant for this guidance document are described in more detail.

Note: The order of this section is not according to any priority but refers to the source in the ISO standard The numbers in the brackets are the chapter numbers in the ISO standard.

- 'Quality Manager' (5.6). This term is not mentioned in the new 2017 version of the standard, even though the functions are still described. The quality manager has the authority and resources to implement, maintain, and improve the laboratory QMS, reporting directly to the laboratory management.

- Laboratory personnel (6.2). All laboratory personnel are competent and act impartially. The competence requirements are documented, and include education, qualification, training, technical knowledge, skills, and experience. In addition, procedures and records are expected for selection, training, supervision, authorization, and monitoring of competence of personnel.

- Facilities and environmental conditions (6.3). The facilities and environmental conditions is suitable for the laboratory activities and should not adversely affect the reults' validity. The laboratory monitors, controls, and records environmental conditions.

- Equipment (6.4). The laboratory has access to all equipment including reference materials, reagents, and consumables that are required for the correct performance of laboratory activities and to influence results.

- Selection, verification, and validation of methods (7.2). The laboratory uses appropriate analytical methods and procedures for all activities. Analytical methods used includes the latest valid versions of methods published in international or national standards, or relevant scientific texts or journals. The laboratory verifies that it can properly perform the selected methods.

  Deviations from these methods shall occur only if the deviation has been documented. Non-standard methods and laboratory-developed methods (so-called in-house methods) and modified standard methods is validated. Various examples of method validation procedures and performance characteristics of validated methods are given in notes to 7.2.2.1 and 7.2.2.3. Detailed records of validation shall be retained. More details about the verification and validation of analytical methods are described in the Chapter 3.4: Verification and validation of analytical methods.

- Evaluation of measurement uncertainty (7.6). Testing laboratories shall evaluate the measurement uncertainty of the analytical method. This is reported if requested by the customer (7.8.3). More details about measurement uncertainty are given in Chapter 3.4: Verification and validation of analytical methods.

- Ensuring the validity of results (7.7.1). The laboratory has procedures for regular monitoring of the validity of results, including: the use of reference materials or QC materials, QC samples with QC charts, replicate tests, retesting, intra-laboratory

comparisons, and others. The resulting data is recorded to detect trends and statistical assessments. More details about these procedures is included in Chapter 3.5: Internal quality control procedures.
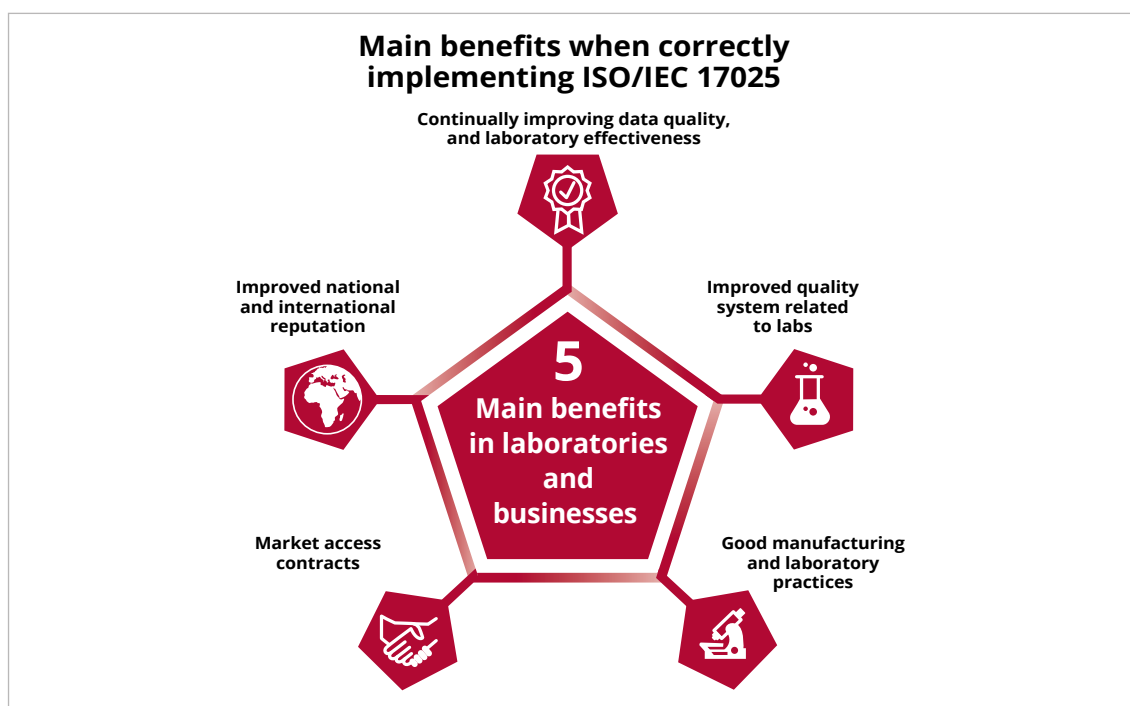
- Participation in proficiency tests (PTs) or interlaboratory comparisons (ILCs) (7.7.2). The laboratory monitors its performance by comparing its results with other laboratories (by PTs or ILCs), where applicable. Results of PTs or ILCs are analyzed and in case of unsatisfactory results, appropriate actions are taken and recorded. More details about PTs/ILCs is provided in Chapter 3.6: External quality control procedures.

- Reporting of test results (7.8). Laboratory results is reported for each sample. The standard sets details common and specific requirements for the information given in test reports.

- Control of records and documents (7.5, 8.2, 8.3, 8.4). Technical records, laboratory management documentation, and all internal and external documents are controlled and retained for a given period.

- Internal audits (8.8). The laboratory shall conduct internal audits at regular intervals to confirm that the QMS is effectively implemented and maintained. Details about the audit program is given. The results of the audit is recorded, and appropriate corrective actions are implemented.

3.1 Accreditation bodies

An accreditation body is an organization that provides accreditation services, which is a formal, third party recognition of competence to perform specific tasks.

In this context, it means that laboratories seeking accreditation can demonstrate to their customers that they are successful at meeting the ISO/IEC 17025:2017[2] requirements (see **Figure 3**).

Figure 3: Benefits of ISO/IEC 17025:2017[1, 30]

Main benefits when correctly implementing ISO/IEC 17025

Continually improving data quality, and laboratory effectiveness

Improved national and international reputation

Improved quality system related to labs

5 Main benefits in laboratories and businesses

Market access contracts

Good manufacturing and laboratory practices

2   ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories, ISO, Geneva 2017

30   Minimizing Barriers and Maximizing Profits of Lab Services with ISO/IEC 17025.

ISO/IEC 17011:2017[3] specifies requirements for the competence, consistent operation, and impartiality of accreditation bodies assessing and accrediting conformity assessment bodies (CABs), such as laboratories.

In general, laboratories can apply to national or international (foreign) bodies for accreditation according to the ISO/IE 17025:2017.

It is also possible (and in some cases reasonable) that laboratories have parallel accreditations from varying accreditation bodies. It is recommended that accreditation bodies are members of the International Accreditation Forum (IAF) and International Laboratory Accreditation Cooperation (ILAC) because both organisations provide international recognition to accreditation bodies.

There are many internationally-recognized accreditation bodies approved by IAF and ILAC (see list: https://ilac.org/signatory-search/).

3.2 Accreditation process

Figure 4: The accreditation process
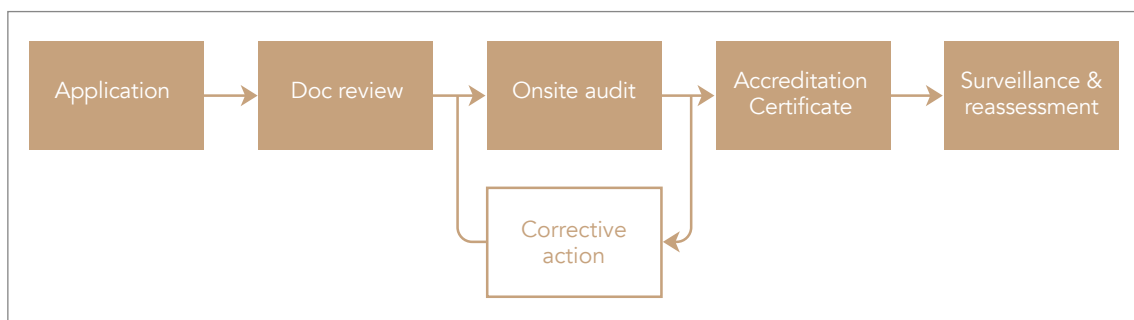
The accreditation process consists of several phases (**Figure 4**).

Application: The accreditation process begins with the submission of an accreditation application, which includes submitting all relevant documents to the accreditation body for review.

Document Review: A document review is undertaken by the accreditation body.

On-site Audit and Assessment: After a successful review, an on-site audit follows. A committee evaluates the assessment results and decides about the grant of accreditation. If not, corrective actions needs to be taken by the laboratory.

Corrective Action: The laboratory takes a corrective action/s where necessary.

Follow-up Visit: A follow-up visit is undertaken to review corrective actions taken.

Accreditation Certificate: The accreditation certificate is issued, and the information is included in the database and added on the webpage of the accreditation body.

Surveillance and Reasssement: Accreditations are valid for a few years. The exact period depends upon the decision of the respective body. During this period, the accreditation body may conduct surveillance audits. Thus, the accreditation cycle ends with the expiry of the accreditation date or the re-accreditation date if the laboratory was reassessed.

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3   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

3.3 Scope of accreditation

The scope of accreditation of a laboratory is the formal and precise statement of the activities, and in this context, the micronutrient testing methods the laboratory is accredited for[4].

The assessment of the scope of accreditation represents the core of the accreditation process and can have a documented list of

- testing fields
- parameters
- products tested
- and methods

The scope document is attached to the accreditation certificate and is incomplete without it. The accreditation of a laboratory without a scope of accreditation is not feasible, the accreditation process needs to include in some type of analytical methods.

However, only the testing methods listed in the scope document may be offered as accredited tests. Also, the report of laboratory results indicates clearly whether an accredited or non-accredited method was used for the said type of testing.

According to ISO/IEC 17011:2017[3], the accreditation body publishes the current status of accreditation of the laboratories (on its webpage), including the ac-creditation certificate and the updated scope of accreditation.

3.4 Verification and validation of analytical methods

According to ISO/IEC 17025:2017 (Chapter 7.2.1.5)[2], before any use, analytical methods published either in international or national standards or by technical organizations or by scientific publications are verified by the laboratory.

The **verification** process in the laboratory ensures that the previously validated "standard" method achieves the required and described performance parameters, with its staff, equipment, environmental conditions, etc.

Verification confirms that the laboratory adequately operates standard methods and delivers accurate and reliable results. Verification also accounts for any important change in the laboratory, such as a new, but similar instrument, relocation of equipment, new software, etc.

Verification procedures and outcomes are recorded and documented.

The laboratory shall validate non-standard, laboratory-developed, and standard methods outside their intended scope (ISO/IEC 17025:2017[2], Chapter 7.2.2).

The **validation** of an analytical method confirms the method under consideration has capabilities consistent with what the application requires and is fit for purpose.

The validation process's ultimate objective is to provide evidence that the method is ready to obtain reliable results during future analyses.

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2   ISO/IEC 17011:2017: Conformity assessment — Requirements for accreditation bodies accrediting conformity assessment
    bodies, ISO, Geneva 2017

Method validation is closely tied to method development and is performed before the method is used for routine analysis. The laboratory has a standard operation procedure (SOP) about the general validation procedure and a validation protocol/scheme. Validation details and results, including all laboratory raw data (e.g. chromatograms) are documented and retained.

Many international guidelines and publications about method validation are published in literature: A recent review[5] summarized and evaluated 37 documents. The two common documents, the Eurachem Guide[6] and the IUPAC Guidelines[7] are helpful for understanding and implementing the method validation process.

The ISO/IEC 17025:2017[2] Chapter 7.2.2.3 provides an exemplary list of performance characteristics for validation and verification. This list is given in **Table 1** below.

Table 1: General performance characteristics for method validation and verification

Performance Characteristic	Validation	Verification
Selectivity	YES	NO
Limit of Detection (LOD)	YES	YES
Limit of Quantification (LOQ)	YES	YES
Linearity	YES	NO
Analytical Range	YES	NO
Accuracy	YES	YES
Trueness	YES	YES
Precision	YES	YES
Repeatability	YES	YES
Reproducibility	YES	YES
Robustness	YES	NO
Measurement Uncertainty	YES	YES

The standard characteristics for method validation and verifcation include:

- **Selectivity** (also defined as "specificity") is the extent of the testing method used to determine particular analytes in mixtures or matrices without interference from other components of similar behavior.

  The food matrix, in general, is a complex mixture of many natural substances and components. These food substances may behave similarly to the analyte and may disturb the analysis.

  The test procedures for selectivity depend upon the detection system (e.g., AAS) or chromatographic separation (e.g., HPLC). A typical first procedure to test the selectivity of a method is to analyze a matrix blank (e.g. unfortified flour or oil for vitamin A) to demonstrate the absence of any interferences (i.e. no reading for vitamin A). If substances interfere with the analysis, measures have to be taken such

2   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

5   Raposo, F. and Ibelli-Bianco, C., 2020. Performance parameters for analytical method validation: Controversies and discrepancies among numerous guidelines. TrAC Trends in Analytical Chemistry, 129, pp.115913.

6   Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (2nd ed. 2014) https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf

7   Thompson, M., Ellison, S.L. and Wood, R., 2002. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure and Applied Chemistry, 74(5), pp.835-855. https://www.degruyter.com/view/journals/pac/74/5/article-p835.xml

as, changing the conditions of the detection system, chromatography, or improving the clean-up procedure of the sample. As a second step, the same blank can be spiked with potential interferents with an appropriate concentration.

Thus, selectivity is the degree to which a method can quantify the analyte accurately in the presence of interferences.

- **Limit of Detection (LOD)** (see **Figure 5**) is the smallest concentration of the analyte in the test sample that can be reliably detected and distinguished from zero. The estimation of LOD is especially important when trace and ultra-trace quantities of analyte are determined, and therefore this parameter is not relevant for micronutrient testing.

- **Limit of Quantification (LOQ)** is the lowest amount of analyte in a sample, which can be quantitatively determined with accuracy. There are several (statistical) methods to estimate LOQ from simple to complex approaches such as signal-to-noise ratio, standard deviation of blank samples, and calibration curve.

Figure 5: LOD and LOQ[31]
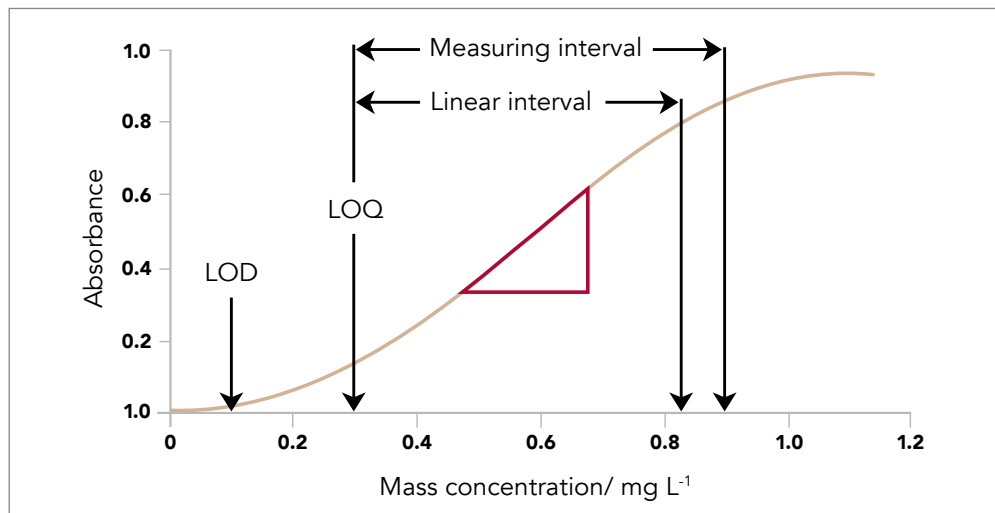
However, these estimated values, obtained by theoretical calculation, should be checked to get reliable values. Therefore, it is required to verify estimated LOQ values by analysing independent samples around the LOQ (by diluting and analysing samples down to the LOQ).

The LOQ must be significantly below the fortification level. Those concentrations below the LOQ which cannot be quantified should be reported as "below the LOQ (< LOQ)," with numbering the quantitative LOQ concentration. In quantitative analysis, a "zero result" does not exist.

- **Linearity of the calibration** The analytical calibration represents the relationship between known concentration of the analyte in the sample or the calibration standards and the instrument's response.

In general, the simple model of linearity describes the concentration – signal relationship. As an example, for the construction of a calibration curve, several calibration standards (including the blank) are prepared and analyzed, evenly spaced over the concentration range of interest (below and above the fortification level).

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31   Limit of detection, limit of quantification and limit of blank. https://www.eflm.eu/files/efcc/Zagreb-Theodorsson_2.pdf

Besides, the calibration should be run at least twice. A commonly used indicator for the goodness-of-fit of the calibration curve is the Pearson correlation coefficient r or the square of the correlation coefficient R^2. A R^2 close to 1 indicates a high degree of linearity (see **Figure 6**). For routine analysis, reduce the number of calibration standards (about 3 standards).

Figure 6: An example of a linear calibration curve


y=0.002x + 0.0218
R^2=0.9961
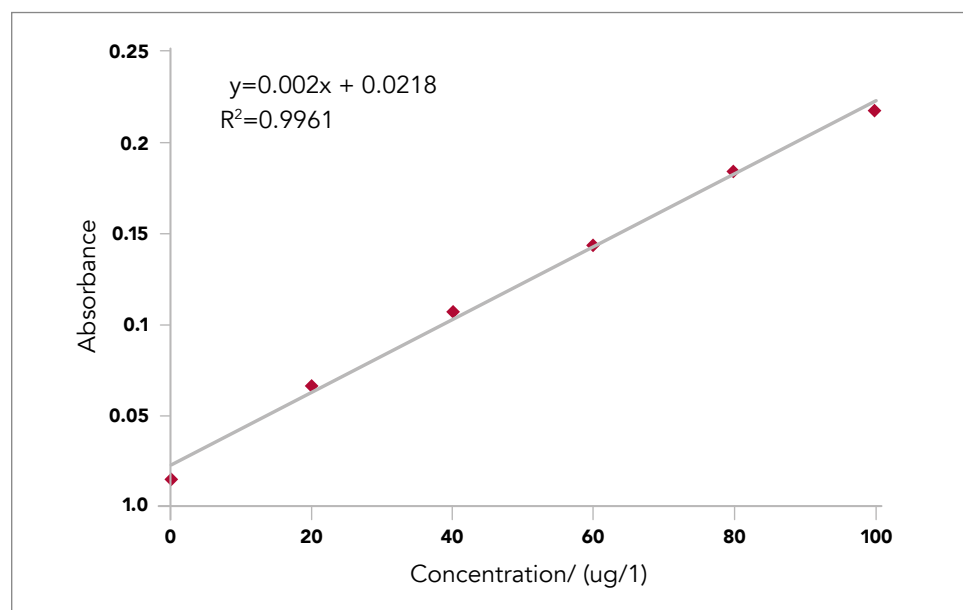
- **Analytical Range** is the interval over which the method provides results with an acceptable uncertainty. The LOQ bounds the lower end of the operating range. The upper end of the working range is defined by concentrations at which significant anomalies in the calibration curve are observed (non-linearity).

  An example of this is the plateauing effect at high absorbance values in UV spec-troscopy. The analytical range for micronutrient testing methods should be large enough to cover concentrations sufficiently below and above the fortification level.

- **Accuracy** is defined here as the combined performance of both trueness (closeness of the measurements to the expected value), and precision (closeness of the measurements to each other) (see **Figure 7**).

Figure 7: An example of type of errors[32]
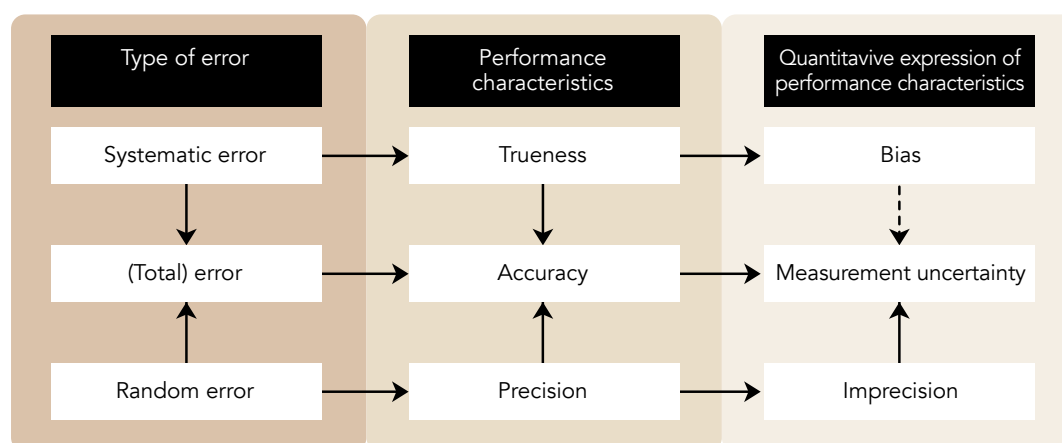
- Trueness relates to the systematic error of a measurement system. In other words, it refers to the closeness of agreement between a test result and the true value of the measured quantity. Trueness can be determined by analyzing a certified reference material (CRM) and comparing the measured value to the true value (more detail in Chapter 3.5: Internal quality control procedures).

  In the absence of suitable CRMs, the trueness can be investigated by spiking and recovery. In this case a known amount of analyte is spiked into the sample matrix. The recovery term (in %) is the ratio of the concentration measured versus the added (true) concentration (more detail in Chapter 3.5: Internal quality control procedures).

- Precision characterizes the closeness of agreement between the measured values obtained by replicate measurements on the same or similar samples under specified conditions.

  Precision is a description of random errors, a measure of statistical variability that is assessed by repeated analysis of validation samples and expressed in the form of "imprecision" such as absolute standard deviation (s or SD), relative standard deviation (RSD), or coefficient of variation (CV).

  Although the precision of a method is often constant over most of the working range, take into consideration that experimental precision shows a large variability, mainly decreasing at the extreme levels. Therefore, assess the precision not only at the fortification level, but also at 0.5 and 1.5 of the fortification level.

  When reporting the standard deviation, for example, as or RSD, the corresponding concentration level should also be documented. The materials for testing the precision should be representative of test samples in terms of matrix and analyte concentration, homogeneity, and stability.

  It is important to use samples of routine application (which would have a concentration of usually assessed samples) and not standard solutions of the analyte to evaluate the precision of the entire method. Also, spiked samples (blank material fortified with standard) can be applied for precision studies.

  ISO 5725-2:2019[8] provides a detailed practical description of the basic method for routine use in estimating the precision of measurement methods.

  Precision is evaluated at two main levels: r**epeatability and (intra-laboratory) reproducibility.**

  - Repeatability measures the variability in results using the same sample (or subsamples of the same sample), same method, same operators, same instrumentation and reagents, same operating conditions, and same location over a short period of time (mostly within a day). These conditions are called repeatability conditions. Such repeatability gives the smallest possible variation in results and is expressed in standard deviations.

  - (Intra-laboratory) Reproducibility is the precision obtained within a single laboratory over a longer period of time taking into account more changes than repeatability, in particular: different analysts, calibrants, batches of reagents, instruments, etc. As more effects are estimated by this procedure, its value, expressed as a standard deviation, is larger than the repeatability standard deviation.

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8   ISO 5725-2:2019: 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 2019

The above two basic concepts of trueness and precision are independent of each other, so a particular set of data is either true, or precise, or both (accurate), or neither. A classic way of demonstrating the difference between precision, trueness, and accuracy is with a dartboard (**Figure 8**). The center ("bullseye") symbolizes the true value. The closer the results are to the bullseye, the more accurate they are.

Figure 8: Dartboards showing different accuracy and precision scenarios[33]
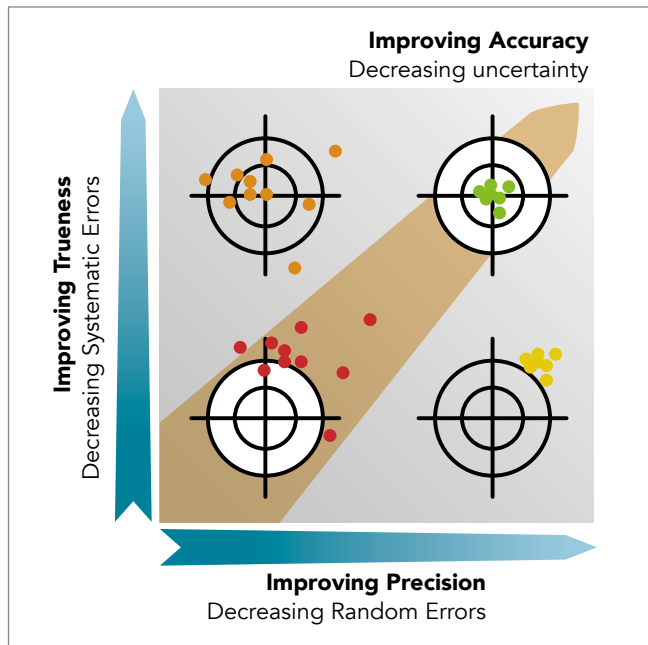
If the values are neither close to the bullseye, nor close to each other, there is no trueness or precision, as they are inaccurate (**Figure 8-1**). If all of the values are very close together, but far from the bullseye and the true value, there is precision, but not trueness (**Figure 8-2**).

If the values are all about an equal distance from and spaced equally around the bullseye, there is mathematical trueness because the average of the darts is in the bullseye. This scenario represents data that is true, but not precise (**Figure 8-3**). If the values are close to the bullseye and close to-gether, there is both trueness and precision, and they are accurate (**Figure 8-4**).

- **Measurement uncertainty** is a "parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand[9]."

  To explain further: No measurement can be absolutely exact. When a quantity is measured, the outcome depends on many components, such as the measuring system and the procedure, the skill of the operator, the environment, etc.

  Even if the quantity is measured several times, in the same way, under the same circumstances, a different measured value is obtained, assuming the measuring system has sufficient resolution to distinguish between the values.

  Thus, the measurement uncertainty defines the range of values and is given as a (confidence) interval.

  Measurement uncertainty does not imply doubt about the validity of a measurement; on the contrary, it implies increased confidence in the validity of a measurement result.

9   JCGM 100:2008 (GUM 1995 with minor corrections), Evaluation of measurement data - Guide to the expression of uncertainty in measurement, JCGM 2008 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf

33   Lab Report 4: Defining Accuracy, Precision and Trueness. https://www.artel.co/learning_center/defining-accuracy-precision-and-trueness/

The "Guide to the Expression of Uncertainty in Measurement" (commonly known as the GUM)[9-10] is the basic document on this subject. There are several mathematical models, approaches, and practical procedures to evaluate the measurement uncertainty, described in various guidelines[6, 11-16].

A commonly used laboratory procedure quantifying measurement uncertainty is the use of QC charts or data from CRMs, recovery studies, or PT results (see Chapter 3.5: Internal quality control procedures).

When data is used from a QC chart during an in-house validation study, the observed standard deviation over an extended time period equals the standard uncertainty u.

Measurement uncertainty is normally expressed as **U=k*u**, the *expanded measurement uncertainty*, using a coverage factor k = 2

- K reflects the number of standard deviations used when calculating a confidence level
- 2 corresponds to 95.5% confidence level
- and u is the standard uncertainty.

U provides a confidence level of 95.5 % that the true content is within the range defined by U around the reported result. Therefore, results (x) should be stated together with U: x ±U (units). The evaluation of measurement un-certainty is also a requirement of ISO/IEC 17025:2017 (see Chapter: ISO/IEC 17025:2017).

Measurement uncertainty helps decide whether an analytical result is com-pliant or non-compliant with regulatory levels/limits (**Figure 9**).

Figure 9: Measurement uncertainty and compliance limits[34]
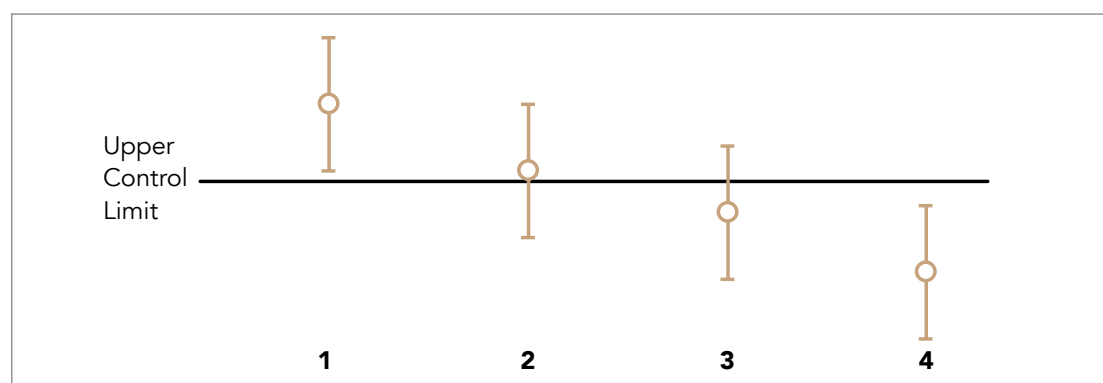
6   Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (2nd ed. 2014) https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf

9   JCGM 100:2008 (GUM 1995 with minor corrections), Evaluation of measurement data - Guide to the expression of uncertainty in measurement, JCGM 2008 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf

10   ISO/IEC Guide 98-3:2008: Uncertainty of measurement - Part 3: Guide to the expression of uncertainty in measurement (GUM:1995), ISO, Geneva, 2008

11   EA-4/16 Guidance Document: EA guidelines on the expression of uncertainty in quantitative testing, EA (European Accreditation), Paris, 2003 https://european-accreditation.org/wp-content/uploads/2018/10/ea-4-16-g-rev00-december-2003-rev.pdf

12   EURACHEM/CITAC Guide CG4: Quantifying Uncertainty in Analytical Measurement, 3rd Edition, Eurachem, LGC/UK, 2012 https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf

13   EUROLAB Technical Report No. 1/2002: Measurement Uncertainty in Testing. A short introduction on how to characterize accuracy and reliability of results including a list of useful references, EUROLAB, Paris, 2002 https://drive.google.com/file/d/1PKAXNnnkpt0elN-U5AUyrjnsRUjyawbJ/view

14   EUROLAB Technical Report No. 1/2007: Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation, EUROLAB, Paris, 2017 https://drive.google.com/file/d/1b_bKTxrJ-a9fYfxxfMqlj-CrTNnvOXNZ/view

15   Codex Alimentarius Guideline CAC/GL 54-2004: Guidelines on Measurement Uncertainty, Codex Alimentarius, 2011 http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B54-2004%252FCXG_054e.pdf

16   ISO 21748:2017: Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty evaluation, ISO, Geneva, 2017

34   Estimation of measurement uncertainty by Jana Snoj Tratnik, 1st HBM4EU Training School 2018. https://www.hbm4eu.eu/?mdocs-file=3741

The result 1, including the expanded measurement uncertainty interval U is clearly above the upper limit. The result 2 is above the limit, however con-sidering the U interval, the true content may be above or below the limit. The result 3 is below the limit, but the U interval is both, above and below the limit. Result 4, including U interval is clearly below the limit. The compliance of results 2 and 3 is debatable, therefore well-defined decision rules should be used establishing acceptance or rejection criteria[17].

- **Robustness** (also called ruggedness) of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters.

  In any method, certain parameters, if not carried out carefully, will have a significant effect on the method performance and may even result in the method not working at all.

  These parameters are identified, usually as part of method development, and if possible, their influence on method performance is evaluated using a robustness test. This test involves making deliberate changes to method parameters and investigating the subsequent effect on the performance.

  Thus, robustness provides an indication of the method's reliability during normal usage and insensitivity against changes in the test conditions.

3.5 Internal quality control procedures

A laboratory performs the verification or validation of an analytical method before the accreditation, or the routine analysis happens. However, this doesn't guarantee that the method will always deliver accurate results going forward.

Internal QC procedures are undertaken by the laboratory, which involves daily monitoring of operations and measurement of results to decide whether the re-sults are reliable to be released.

Internal QC applies several routine and practical procedures on a day-to-day basis to verify that the method remains in control. Thus, internal QC is an essential de-terminant of the continuous quality of analytical data ensuring its validity.

Several internal QC procedureslaid down in ISO/IEC 17025:2017[2] in Chapter 7.7.1 are described in detail in the IUPAC Guidelines[18]. The laboratory describes and summarizes the details and frequencies of internal QC procedures in an SOP. Some procedures for internal QC are explained in the following sections.

- Quality control charts and quality control samples. **Quality control charts** are a powerful and simple tool for daily QC of routine analytical work. The laboratory runs quality control samples together with the routine/ordered samples in each analytical run/batch. The test results of these control samples are then put into a control chart (**Figure 10**).

  The control chart has two quality limits: the warning or control limit at ±2s (s=standard deviation) around the mean value and the action limit at ±3s.

  As long as the values of the QC samples remain in the control region and are acceptable, the results are reliable.

2   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

17   EURACHEM/CITAC Guide: Use of uncertainty information in compliance assessment, 1st Edition, Eurachem, LGC/UK, 2007 https://www.eurachem.org/images/stories/Guides/pdf/Interpretation_with_expanded_uncertainty_2007_v1.pdf

18   Thompson, M. and Wood, R., 1995. Harmonized guidelines for internal quality control in analytical chemistry laboratories (Technical Report). Pure and Applied Chemistry, 67(4), pp.649-666. http://publications.iupac.org/pac/pdf/1995/pdf/6704x0649pdf

Figure 10: Principles of a control chart [19]
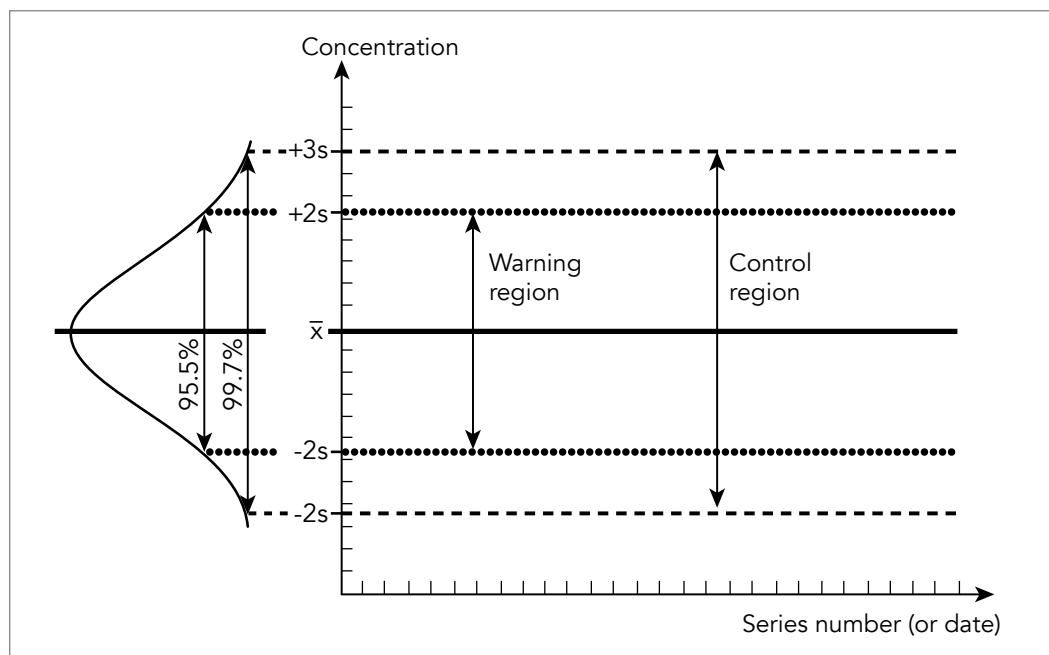
Similarly, results outside the action limits ±3s indicate an "out-of-control" situation and requires immediate investigation and remedial action before the continuation of analysis.

These action procedures taken by the laboratory are laid down in a SOP. The results of a QC chart can be used to calculate the long-term intra-laboratory reproducibility and the measurement uncertainty.

More details about QC charts are given in ISO standard 7870 [20].

QC samples should be very similar to test samples and should remain stable over time. There needs to be a sufficient number of samples for a longer period and a suitable analyte concentration.

Certified reference materials (CRMs) are ideal control materials because they not only prove precision but also trueness of the testing, thus both, random and systematic errors.

An alternative to this is the preparation and use of *in-house QC samples*. These samples are natural and stable, often taken from retail or production. Before routine usage the assigned value of this QC material has to be de-termined by careful and independent analyses or ILCs and recorded.

Also, *materials validated in PTs* comprise a valuable source for QC samples. Such materials have been analyzed by many laboratories using a variety of analytical methods. Several international PT providers offer such validated and "certified" materials from former PT rounds.

- Certified reference materials (CRMs). CRMs certificate indicates the characteristic values and the uncertainty in a complex determination process. CRMs are ideal control materials to check the trueness of testing and are supplied by various companies and international organisations.

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19 Funk, W, Dammann V, and Donnevert, G, eds., Quality Assurance in Analytical Chemistry, WILEY-VCH Verlag, Germany 2007, ISBN 978-3-527-31114-9

20 ISO 7870: Control charts, several parts, ISO, Geneva

However, for micronutrient testing, closely matching and suitable CRMs are rarely available. In addition, they are expensive, and thus, not applicable for daily internal QC, only at longer intervals.

For the analysis of iron and zinc in wheat flour, there is a suitable CRM on the market (NIST 1567b) which can be also utilised to analyse other relevant elements in flour. More details about the selection and usage of reference materials are given in[22, 23].

Another option is recovery checks of *spiked samples*.

- Recovery of spiked samples. A portion of the sample is spiked (also called fortified) with a known amount of the analyte and analyzed alongside the original test material. The spike and recovery testing approach is widely applied in internal QC and QC charts.

  The recovery of the added analyte is the difference between the two measurements divided by the amount of spike that is added. Usually, the recovery is expressed as a percentage, and a total recovery is given as 100% recovery. In routine analysis the recovery is in the range of 80–120%, however this depends on the concentration of the spike.

  A poor recovery result is always indicative of inconsistencies in the analytical procedure and should trigger actions of the laboratory.

- Blanks determination. Blanks are an important tool and an essential part of the analytical and internal QC process. The simplest form is the **reagent blank** (also called a procedural blank) which executes the entire analytical procedure without a matrix/food sample.

  Reagent blank tests the purity of the used reagents and any (cross-)contamination in the analytical system or the laboratory environment.

  Another form of blanks are **sample blanks** which uses sample matrices without analyte (e.g. flour or edible oil without added vitamin A). In pre-tests, checks must be performed to confirm the blank is analyte-free.

  Sample blanks cover in addition to reagent blanks also any interferences from the food matrix. Interferences from the food matrix are not always constant therefore blanks should be checked regularly. Any inconsistent results of blanks in the internal QC should trigger actions. More details about the use of blanks are found in[21].

- Replicate tests. There are different types of replicates in analytical methods. Two most common types are: preparation replicates and measurement replicates.

  Preparation replicates are identical samples prepared from the beginning to end of the procedure in the same way, but separately. In internal QC, preparation replicates control the entire analytical process.

  Measurement replicate is a single sample measured more than once (an example would be multiple injections in the case of HPLC or flame AAS).

  Replicate analysis of routine test samples provides a means to check precision changes in an analytical method.

21 H. Cantwell (ed.) Blanks in Method Validation - Supplement to Eurachem Guide The Fitness for Purpose of Analytical Methods, (1st ed. 2019), LGC/UK, 2019 https://www.eurachem.org/images/stories/Guides/pdf/MV_Guide_Blanks_supplement_EN.pdf

22 EA-4/14 Document: The Selection and Use of Reference Materials, EA (European Accreditation), Paris, 2003. https://european-accreditation.org/wp-content/uploads/2018/10/ea-4-14-inf-rev00-february-2003-rev.pdf

23 ISO GUIDE 33:2015: Reference materials — Good practice in using reference materials; ISO, Geneva 2015

In summary, for internal QC, each analytical run/batch of routine samples should first run calibration standards, a blank, a QC sample (for the QC chart), a recovery sample.

Ideally, after analyses of 5-10 samples a calibration standard should be used to control the calibration. Replicates and CRMs are optional, but it is recommended to use replicates in addition, in particular, when starting a new analytical method. CRMs if available should be applied from time to time to check the trueness of the method.

QC samples before every lab sample batch:

- **3-5 Calibration standards** (solvent standards)

- **1 Blank sample**
  - Sample blank or
  - Reagent blank

- **1 Precision check (for QC chart):**
  - Inhouse QC sample or
  - PT material or
  - CRM

Optional:
- **1 Trueness (Recovery) check:**
  - Spiked sample (sample or blank spiked with analyte) or
  - CRM

- **1 Replicate**
  - Measurement replicate or
  - Preparation replicate

3.6   External quality control procedures

Audits are systematic and independent evaluations conducted by independent auditors who check for compliance and quality standards with the described procedures.

Audits may be performed internally or externally.

- Internal audits are a requirement of ISO/IEC 17025:2017[2]. They are carried out regularly by competent and independent members of the staff or external consultants. The aim of an internal audit is the assessment of the current QA/QC system and the proposal of necessary corrective measures.

- External audits ("the view from outside") are performed by accreditation bodies for accreditation, re-accreditation, or surveillance. There are also external audits conducted by customers or potential future customers. Official audits can be performed by government or regulatory agencies.

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2   ISO/IEC 17011:2017: Conformity assessment — Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

For internal or external audits there are different strategies, e.g. process-, documentation- and system-orientation. ISO 19011:2018 [24] provides guidance on all aspects of auditing.

Proficiency tests. Regularly participating in proficiency tests (PTs) demonstrates the competence of the participating laboratory, the variation be-tween laboratories (inter-laboratory reproducibility), and systematic errors (accu-racy) during an external QC.

The ISO/IEC 17025:2017 [2] requires laboratories to participate in PTs. Many ac-creditation bodies, too, specify PTs as a requirement to receive accreditation of a new analytical method.

ISO/IEC 17043:2010 [25] specifies general requirements for the competence of PT providers and the development and operation of PT schemes.

Several detailed guidelines and protocols about PTs are provided in [26-28]. Besides, the term "proficiency test" also known as "ring test/trial", "interlaboratory comparison" or "round-robin test" are often used to describe similar issues.

At the beginning of a PT, the sample material is produced which must be identical for all participants. The concentration of the analytes in the samples should lie within a range which can be realistically expected in routine analysis.

Randomly selected sample portions are checked to verify whether the analytes are distributed homogeneously. Only homogenous materials enable a sound evaluation of the PT.

In addition, by means of appropriate tests the material is proven stable, at least for the duration of the study. The samples are distributed to the participating laboratories in such a way to avoid any changes to the material during shipment.

Then the laboratories analyse the samples with their own routine test methods. and results are submitted within a prescribed time.

During the performance evaluation, there are two basic elements established by the PT provider or organizer: **the assigned value and the standard deviation for proficiency assessment.**

The *assigned value* is obtained by using different methods (a working estimate of the true value), according to ISO 13528 [29]. Often, the assigned value is derived from one expert laboratory or a group of expert laboratories.

These expert laboratories may be participants in the PT round or selected external laboratories.

Another approach is to calculate the robust average based on the results from all participants. This method is only applicable when the number of laboratories is high enough and the participating laboratories have comparable proficiency levels.

2   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

24 ISO 19011:2018: Guidelines for auditing management systems, ISO, Geneva 2018

25 ISO/IEC 17043:2010: Conformity assessment – General requirements for proficiency testing; ISO, Geneva 2010

26 EURACHEM Document: Selection, Use and Interpretation of Proficiency Testing (PT) Schemes, 2nd Edition 22011, Eurachem LGC/UK, 2011 https://www.eurachem.org/images/stories/Guides/pdf/Eurachem_PT_Guide_2011.pdf

27 Fera Science Ltd (Fera), Protocol for Proficiency Testing Schemes, Version 6, April 2017, Fera Science, York/UK, 2017 https://fapas.com/sites/default/files/2017-05/FeraPTSprotocol_pt1_common.pdf

28 Thompson, M., Ellison, S.L. and Wood, R., 2006. The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). Pure and Applied Chemistry, 78(1), pp.145-196. https://www.degruyter.com/view/journals/pac/78/1/article-p145.xml

29 ISO 13528:2015: Statistical methods for use in proficiency testing by interlaboratory comparison, ISO, Geneva 2015

The *standard deviation* for proficiency assessment [29], also has different approaches i.e. the acceptable range of participant results. Standard deviation's prescribed value can be obtained from expert opinion, statistical models, or data received in the PT round.

The latter approach is not applicable when the number of participating laboratories is small (below 10). The standard deviation by expert opinion takes several factors into account: e.g. the complexity of the analytical method used, the concentration level (lower concentrations result in higher standard deviations), the overall performance of the participants.

The most common and very often used approach to evaluate the performance of the participating laboratories is the z-score.

$$z = \frac{(x - X)}{\hat{\sigma}}$$

where:

x = result reported by participant

X = assigned value

$\hat{\sigma}$ = standard deviation for proficiency assessment

The following judgement is commonly made for z-score:

- z-scores in the range ±2.0 indicate 'satisfactory' performance and no actions are required.
- z scores between 2.0 < z-score < 3.0 indicate 'questionable' performance requiring consideration and appropriate action.
- z-scores above ±3 indicate 'unsatisfactory' performance and should trigger root cause investigation and corrective actions which shall be recorded.

There are several international commercial PT providers which regularly organize PTs for food testing.

The EPTIS database (European Proficiency Testing Information System) (https://www.eptis.org/) helps to find PTs for specific analysis, and this database is not limited to Europe.

However, for micronutrient testing in fortified foods, there are rarely any PTs available. Thus, PTs for the analysis of micronutrient has to be organized on a national basis or by a group of countries, like the PT scheme of the East African Community (EAC).

The EAC PT scheme, covering micronutrient parameters is organized by the National Bureaus of Standards of Kenya, Uganda, Tanzania, and Rwanda.

For the performance of a PT for micronutrients, suitable fortified foods from well-known brands can be taken from the retail and used as PT materials. These materials have typically sufficient homogeneity and stability. However, several studies indicated that foods from the retail/market are often not or only inadequately fortified. It is not very useful or meaningful to use such unfortified or minor fortified material for PT. Therefore, in a kind of selection and screening process market samples have to be tested to ensure they are fortified by an expert laboratory before using them for a PT. Another possibility is the preparation of tailor-made reference material with well-defined micronutrient levels by a specialized company. For the evaluation of the PT assigned values are needed. Due to the

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29   ISO 13528:2015: Statistical methods for use in proficiency testing by interlaboratory comparison, ISO, Geneva 2015

usually small number of PT participants, the assigned values cannot be calculated as average of the results from the laboratories. Therefore, it is recommendable to commission one or more expert laboratories for this task, it can be combined with the above-described pretesting of market samples. The standard deviation for proficiency assessment for the calculation of z-scores cannot be calculated from the data received from the too-small number of participants but should be obtained from expert opinion by perception. In this context, it should be emphasized that unsatisfactory PT results need in-depth root cause analysis by the laboratory and afterwards well-documented corrective actions. Also, trainings and mentoring programs might be initiated after PT rounds to improve the laboratory performance.

4   Criteria to Select Accredited Laboratories for Micronutrients Testing

This chapter discusses relevant criteria required to select a potential laboratory to quantitatively analyze micronutrients in fortified foods.

The chapter names given in brackets are cross-references to chapters in this Guidance Document for more details and explanation).

Customers, authorities, and NGOs can verify the below mentioned criteria either by a document check or an on-site audit/assessment in the laboratory. In addition, interlaboratory comparison exercises can be organized between the laboratory in question and other (expert) laboratories to check technical competency.

- **Accreditation status** (Chapter 3.1: Accreditation bodies)

  According to ISO/IEC 17025:2017[2] the laboratory is accredited by a national or international accreditation body. These accreditation bodies must be full members of the ILAC and IAF.

  The accreditation must be valid and not expired. The scope of accreditation should include the specific analytical method needed for micronutrient testing.

  This method should involve accreditation for the same combination of the matrix and parameter because it doesn't suffice if the parameter (e.g. iron) is accredited for another matrix such as water or soil.

  If the analytical method in question is not included the scope of the accredited laboratory, then reasons for non-accreditation should be clarified and the following requirements should be applied.

- **Analytical methods** (Chapter 3.4: Verification and validation of analytical methods))

  The analytical methods used should be included in detail in the standard operating procedure (SOP) manual, also indicating whether an official or (national/international) standard or published method is in place.

  Also, non-standard, laboratory-developed, or standard methods outside the intended scope (so-called 'in-house methods') can be used but shall be validated thoroughly.

  Each method is described with all details in a SOP owned by the laboratory, which is registered by the QMS and has a specific template/format. Only photocopies of standards or literature methods are not acceptable as SOPs in the laboratory.

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2   ISO/IEC 17011:2017: Conformity assessment — Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

- **Verification or validation of analytical methods** (Chapter 3.4: Verification and validation of analytical methods)

  Before usage, standard or literature methods is verified by the laboratory to ensure that the methods can achieve the required and described performance in the laboratory, with all its staff, equipment, reagents, environmental conditions, and more.

  Thus, this verification process confirms that the laboratory can deliver reliable results. The verification procedure and outcome must be documented and presented on request.

  Also, validation is necessary when using in-house methods requiring more comprehensive and stringent actions than for the verification process.

  Method validation is part of the method development and is performed before the method is used in routine analysis. Validation details and results are documented and retained. In summary, verification or validation files must be in place and forwarded on request.

- **Testing infrastructure**

  Before analyses, a clarification is required on the necessary needs such as: equipment/instrument, operations, maintenance, or repair.

  Any non-operational equipment can extend analysis time or make the analysis virtually impossible to undertake. The same is true for the necessary reagents/test kits/chemicals. They need to be not-expired, well-labeled, and from a traceable source.

- **Testing experience**

  The number of samples tested over time with a specific analytical method is a robust indicator for the laboratory's experience. A recommended minimum of 10-20 samples analyzed regularly per quarter and kept on record is a good indicator for experience.

- **PT results** (Chapter 3.6: External quality control procedures)

  ISO/IEC 17025:2017[2] requires participating in PTs, therefore PT results of accredited methods, or at least the interlaboratory comparison with another (expert) laboratory should be available.

  In case of unsatisfactory PT results, corrective actions should be documented.

  Largely, the PT results of micronutrient testing in fortified food should be evaluated, however results of PT rounds on analyses of food quality and food safety parameters can also be helpful to judge the QMS of the laboratory.

  Overall, there are not many PTs available for the specific analysis of micronutrients in fortified food. Therefore, it is proposed to organize such PTs on a regional or national level by authorities, NGOs, or customers.

- **Documentation of internal QC** (Chapter 3.5: Internal quality control procedures)

  Internal QC procedures guarantee that methods deliver valid results on a day-to-day basis. Therefore, it should be checked whether control charts are available for this analytical method, and recovery of spiked samples and blanks are in use.

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2   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017

The following questions can be used as a checklist to evaluate the selection criteria:

- ❓ Is the laboratory accredited according to ISO 17025:2017?

- ❓ What accreditation body has accredited the laboratory?

- ❓ Is the accreditation of the laboratory currently valid?

- ❓ Is the relevant analytical method included in the scope of accreditation?

- ❓ If the method is not in the scope of accreditation: What are the reasons that currently this method is not included in the scope of accreditation?

- ❓ Is the analytical method in use an official or (national/international) standard or published method? Please send in detailed information about the method.

- ❓ Is a detailed and updated SOP available in the laboratory, authorized by the quality manager and the management?

- ❓ Is a verification (or validation) report of this analytical method available? Please send in details about the major verification (validation) parameters.

- ❓ Are the necessary chemicals/reagents and equipment/instruments in place and operation?

- ❓ How many samples were tested by this analytical method this year and last year?

- ❓ Did the laboratory participate in PTs with this method and in relevant food matrices? Please list the results and the PT provider/details.

- ❓ In case of unsatisfactory PT results: did the laboratory introduce root cause analysis and corrective actions? Please report details.

- ❓ Are internal QC procedures (control charts, recovery of spiked samples, blanks) in general and particular for this analytical method in place? Please report details.

5 Criteria to Select Non-accredited Laboratories for Micronutrients Testing

The accreditation of a laboratory demonstrates its technical competency with a QMS and well-managed documentation system in place.

The accreditation also provides confidence and acceptance in the reliability of the laboratory results. Therefore, it is a substantial selection criterion.

If the laboratory is not accredited for any reason, the said reasons for non-accreditation should be evaluated carefully. In some cases the ISO/IEC 17025/2017 requirements are partially or fully implemented, and that the laboratory may be on its path to accreditation.

In any case, evalutate the basic requirements of ISO/IEC 17025/2017, as given in Chapter 3: Main Elements of Laboratory Quality Management and Laboratory Quality Control. And Chapter 5: Criteria to Select Accredited Laboratories for Micronutrients Testing must be included in this evaluation as well.

The following questions can be used as a checklist to evaluate the selection criteria:

- ❓ What are the reasons for the non-accreditation of the laboratory?

- ❓ Is an accreditation of the laboratory in preparation? If yes: please send details of the action plan and the time schedule.

- ❓ What requirements of ISO/IEC 17025/2017 are already partially or fully implemented?

In addition, the questions in the Chapter 5:Criteria to Select Accredited Laboratories for Micronutrients Testing summary should also be included, barring the first 5 questions.

6 Verification of the Reliability of Laboratory Results

In general, laboratory results can be verified on a day-to-day basis by components of the internal and external QC to ensure that the method remains in control and delivers accurate and precise results.

The customer can ask for the corresponding documentation to verify the reliability and validity of the laboratory results.

This is in particular when different laboratories deliver conflicting results of the same sample. In this context, the following QC data are significant:

- **Quality control charts and quality control samples** (Chapter 3.5: Internal quality control procedures)

  There are different types of material that can be used as QC sample, as described in detail in Chapter 3.5: Internal quality control procedures. At the beginning of each analytical run/batch (after the calibration standard), one QC sample should run together with the routine samples.

  The corresponding QC chart indicates clearly whether the analytical procedure is under control or out-of-control. As long as the values of the QC sample are in the control region of the QC chart and are acceptable, it is likely that the results from samples in the same batch as the QC sample can be taken as reliable.

  Thus, QC charts are a powerful tool to verify whether the laboratory results are reliable, primarily precision, and trueness as well if CRM or PT samples are used.

- **Recovery of spiked samples** (Chapter 3.5: Internal quality control procedures)

  For this purpose, a portion of the sample is spiked with a known amount of a solution of the respective micronutrient and analyzed alongside the original test material.

  The recovery of the added micronutrient is the difference between the two measurements divided by the amount of spike that is added. In routine analysis the recovery should be in the range of 80 – 120%, however this range depends on the concentration of the spike.

  A poor recovery (or sometimes too high recovery) indicates of inconsistencies in the analytical procedure and should trigger remedial actions of the laboratory. Recovery data can be used to prove the reliability of the laboratory results.

- **Certified reference materials, CRMs** (Chapter 3.5: Internal quality control procedures)

  CRMs are ideal control materials to check the trueness of testing. However, for micronutrient testing they aren't many matching or suitable CRMs available – and in addition, they are expensive to use.

  As an alternative, materials which were validated in PTs can be used as a kind of "certified" reference material. Such materials have been analyzed by many laboratories often using a variety of analytical methods.

  Several international PT providers offer validated and "certified" materials from former PT rounds. Data from reference materials which are co-analyzed with samples are well suited to verify the reliability of laboratory results.

- **Proficiency test (PT) results** (Chapter 3.6: External quality control procedures and Chapter 4: Criteria to Select Accredited Laboratories for Micronutrients Testing)

  The participation in the PTs is the basic element of external QC, demonstrating the competence of the participating laboratory. However, there are marginal or no PTs available from international professional PT providers for the specific analysis of micronutrients in fortified food. Therefore, it is proposed to organize such PTs on a regional or national level by authorities, NGOs or customers before or during a micronutrient testing program/project.

The requirements for test reports are described in detail in ISO/IEC 17025:2017[2] Chapter 7.8. The details are given in the following:

- ◆ A title e.g. 'Test Report'.

- ◆ The name and address of the laboratory (if the laboratory is accredited: together with the symbol of the accreditation body and the accreditation number).

- ◆ The name and contact information of the customer.

- ◆ Identification of the analytical method used (if the laboratory is accredited: information whether the method is included in the accreditation scope).

- ◆ Description, unambiguous identification (e.g. sample identification number of the customer and/or batch number, laboratory number of the sample 'sample code') and the condition of the sample (e.g. frozen).

- ◆ The date of sample receipt.

- ◆ The date of performance the analysis in the laboratory.

- ◆ The date of issue of the test report.

- ◆ A statement to the effect that the results relate only to the samples tested.

- ◆ A statement specifying that the report shall not be reproduced except in full without approval of the laboratory can provide assurance that parts of a report are not taken out of contex.

- ◆ The results with the units of measurement.

- ◆ Identification of the person authorizing the report.

- ◆ Clear identification when results are from external providers.

In addition, specific additional requirements (agreed with or instructed by the customer) could be included in the test report:

◆ A statement of conformity with requirements or specifications (e.g. fortification levels). The decision rule employed should be documented.

◆ The measurement uncertainty presented in the same unit as the analyte or as percentage. This is relevant e.g. when the measurement uncertainty affects the conformity to a specification limit.

The following questions can be used as a checklist to evaluate the verification criteria:

❓ What kind of internal QC procedures were used during the analysis of the ordered samples?

❓ Was a QC sample/material in use?

❓ Was a QC chart prepared and used? If yes, please send in the QC chart.

❓ Were "out-of-control" situations were observed while testing the QC material? If yes: what kind of actions were taken?

❓ Were suitable spiked samples regularly used to test the recovery? If yes, please send the recovery data and details of the spiking procedure.

❓ Were suitable CRMs used during testing the samples? If yes, please report the results and details of the used CRM.

❓ Did the laboratory participate in suitable PTs before or during testing the samples? If yes, please report the PT results and details of the PT.

7 Recommendations

7.1 General recommendations to laboratories and laboratory management

• Ensure training and mentoring activities for laboratory personnel to maintain or improve their competency and expertise. In particular, hands-on training about internal and external QC control secures the reliability of analytical data handled.

• Train thoroughly new laboratory staff and document the competency.

• Ensure an annual budget for the maintenance of equipment and instruments, to guarantee their readiness for testing and continuous working.

• Ensure an annual budget for PTs and reference materials relevant for external quality control.

• Use chemicals (e.g. reagents, standards) before their expiry date and from well-defined sources.

• Ensure a minimum number of 10-20 fortified food samples are tested per quarter. Governmental laboratories should analyze samples from external and commercial monitoring including imported fortified food which are sampled by health/food inspectors; private laboratories samples from internal monitoring (QC/QA samples from mills/factories/packers). Laboratories should indicate the minimum number and advocate for a regular sample reception from their main clients.

- Follow-up on unsatisfactory PT results and to document corrective actions taken. Also, the results above the action limits of QC charts must trigger an immediate investigation and remedial actions which have to be recorded.

- Calculate and report micronutrient results in the units according to national standards/ regulations, e.g. vitamin A concentration in a food matrix should be reported in the same unit as given in the corresponding national standard (IU or mg retinol/kg or mg retinyl palmitate/kg). This kind of reporting facilitates the reading for the customer and decision-maker.

7.2 General recommendations to authorities and standard organizations

- Enforce national fortification through process monitoring at the production site, verification of fortification specifications of food imports mandated to be fortified and regular sampling and testing system for fortified foods.

- Consider the laboratories' routine and capacity in terms of sample number and frequency when defining food sampling plans to ensure laboratories get regular experience in the different micronutrient analyses, plan procurement and use of consumables efficiently and thereby maintain result reliability.

- Follow-up on unsatisfactory results by industry and authorities of internal and external monitoring samples, respectively by e.g. resampling, on-site investigations, increment of controls and sampling.

- Define fortification levels in national standards/regulations on factory and market-level (due to the depletion of some fortificants in some food matrices) and in addition as an interval.

- Define exactly compliance and non-compliance of analytical results against national fortification levels. This requirement indicates if a range around the desired fortification value is acceptable.

- Specify the fortification levels in well-defined units (e.g. vitamin A as IU or mg retinol/kg or mg retinyl palmitate/kg).

7.3 General recommendations to NGOs

Industry, authorities, and standard organizations can be supported in the following ways:

- Training and mentoring programs for laboratory staff.

- Organising and participating in PTs,

- Provision or enabling access to suitable reference materials for internal QC of laboratories.

- Establishing and describing well-defined fortification levels by national standard organization or Bureaus of Standards.

- Strengthening any aspects to establish a continuous national monitoring system for fortified foods, this requires inter alia setting up a required minimum of samples that remains feasible.

GLOSSARY

Accuracy is defined here as the combined performance of both trueness (closeness of the measurements to the expected value), and precision (closeness of the measurements to each other).

Analytical calibration represents the relationship between the known concentration of the analyte in the sample or of the calibration standards and the response of the instrument.

Analytical Range is the interval period of a said method that provides results with an acceptable uncertainty. The lower end of the working range is bounded by the LOQ. The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed (non-linearity).

Blank sample is a sample that is free of the analytes of interest.

Certified reference materials (CRMs) are ideal control materials to check the accuracy of testing.

Internal QC refers to procedures undertaken by the laboratory for the daily monitoring of operations and measurement results in order to decide whether results are reliable enough to be released. Internal QC comprises several routine practical procedures that are applied on a day-to-day basis to verify that the method remains in control.

Limit of Detection (LOD) is the smallest concentration of analyte in the test sample that can be reliably detected and distinguished from zero.

Limit of Quantification (LOQ) is the lowest amount of analyte in a sample, which can be quantitatively determined with precision and accuracy appropriate to analyte and matrix considered.

Measurement replicates are measurements of a single sample measured more than once.

Measurement uncertainty is a "parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand"[9].

Precision characterizes the closeness of agreement between the measured values obtained by replicate measurements on the same or similar samples under specified conditions. Precision is a description of random errors, a measure of statistical variability.

Preparation replicates are the identical samples that are prepared from the beginning to end of the procedure in the same way but separately.

Proficiency test (PT) are done to demonstrate the competence of the participating laboratories. PTs are organized on a regional or national level by authorities, NGOs, or cus-tomers before or during micronutrients testing.

Quality control chart indicates clearly whether the analytical procedure is under control or out-of-control.

Quality control (QC) samples should run together with the routine samples in each analytical run/batch.

Reagent blank (also called a procedural blank) which executes the entire analytical procedure without a matrix/food sample.

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9   JCGM 100:2008 (GUM 1995 with minor corrections), Evaluation of measurement data - Guide to the expression of uncertainty in measurement, JCGM 2008 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf

Recovery Proportion of the amount of analyte, added (spiked) to the analytical portion of the test material, which is extracted and presented for measurement.

Repeatability is a measure of the variability in results when a measurement obtained with the same sample (or subsamples of the same sample) using the same method, same op-erators, same instrumentation and reagents, same operating conditions, and same loca-tion over a short period of time (mostly within a day). These conditions are called repeatability conditions. Repeatability is expected to give the smallest possible variation in results and is expressed in standard deviations.

Intra-laboratory Reproducibility is different from repeatability and is the precision obtained within a single laboratory over a longer period taking into account more changes than repeatability, in particular: different analysts, calibrants, batches of reagents, instruments etc. Because more effects are estimated by this procedure, its value, expressed as standard deviation, is larger than the repeatability standard deviation.

Robustness (also called ruggedness) of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness provides an indication of the method's reliability during normal usage and insensitivity against changes in the test conditions.

Selectivity (sometimes also defined as "specificity") is the extent to which the testing method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior. Thus, it is the degree to which a method can quantify the analyte accurately in the presence of interferences.

Spiked samples are samples to which a known amount of the respective micronutrient is added.

Trueness relates to the systematic error of a measurement system. Rigorously defined, refers to the closeness of agreement between a test result and the true value of the measured quantity.

Verification process ensures that the previously validated "standard" method can achieve the required and described performance in the laboratory, with its staff, equipment, environmental conditions, etc. Thus, the verification confirms that the laboratory can properly operate standard methods and deliver accurate and reliable results. Verification is also required when there is an important change in the laboratory such as a new but similar instrument, relocation of equipment, new software, etc.

Validation. Non-standard, laboratory-developed, and standard methods outside their in-tended scope shall be validated (ISO/IEC 17025:2017[2], chapter 7.2.2). Validation of an analytical method shall confirm that the method under consideration has capabilities con-sistent with what the application requires and is fit for purpose. The ultimate objective of the validation process is to provide evidence that the method is ready to obtain reliable results during future analyses..

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2   ISO/IEC 17011:2017: Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies, ISO, Geneva 2017