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Manual for Food Testing Laboratories

Ensuring the Validity of Results

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Manual for Food Testing Laboratories

Ensuring the Validity of Results

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The publication considers the Projects activities from cooperating with 10 private and public food laboratories from Georgia, Armenia and Azerbaijan. It includes Project's learnings, results and experiences gained by executing tailored training programmes of ISO 17025 requirements implementation towards laboratory accreditation. The trainings organised in the region and abroad, as well as consultancy, and coaching ranged from establishing a microbiological laboratory to laboratory business planning. A variety of areas, topics and principles as to laboratory best practices were addressed that are also covered by this Manual, such as equipment maintenance; High-performance liquid chromatography (HPLC) troubleshooting training; validation/verification; calibration; tests relevant for food trade, e.g. aflatoxins and pesticides multi residue methods; EU microbiological criteria and support with reference materials; method selection, and establishing of a genetically modified organism (GMO) testing training programme to name a few. In addition, joint participations in Proficiency Testing (PT) in the area of microbiology and chemistry and support with certified reference materials (CRMs) were organised together with the trainings about quality control charts and statistics to ensure the validity of the laboratories test results. Selected laboratory staff participated in international conferences with poster presentations, attended Eurachem meetings and other conferences dealing with food testing. The Project organised annual two-week technical training programmes on trade related methods in Germany. The first food testing conference was organised in Georgia in which the laboratories presented their results, on e.g., validated test methods. Contact was made to German institutions related to food safety and testing including risk assessment during a study tour that was organised in Germany.

The contribution of laboratories to the Projects, their cooperation and enhanced use of validated test methods is highly acknowledged. A special thanks goes to the laboratory management of the 10 laboratories for their cooperation in the Projects and the constant endeavours in implementing the ISO 17025 requirements for accreditation. The participating laboratories gradually enhanced the laboratories accreditation scopes using validated test methods for testing food for national and international markets. The strengthened laboratory services attracted new clients.

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Foreword

It is of tremendous importance to the food sectors of the South Caucasus countries that their food testing laboratories offer demand oriented, accredited, and internationally recognised testing services. Accreditation is key in facilitating the acceptance of generated test results and obligatory for laboratories that test food products for international trade, e.g., as third country. Test results are the basis for legal actions in trade, therefore must be accurate and valid to avoid destruction or rejection of consignments with excessive cost implications. In addition, laboratory personnel should be competent in method selection and validation as required by their customers.

This Manual reflects the broad requirements for laboratory accreditation in line with the ISO 17025[1] standard. A structured approach to food analysis for both chemical and microbiological testing with technical and quality aspects of any food testing laboratory for complying with ISO 17025 requirements are presented. The publication has a strong emphasis on good quality assurance (QA) practice. Chapters are organised addressing requirements and procedures for ensuring the validity of test results in the areas of e.g., laboratory design and facilities, environment, equipment, reagents, personnel, calibration, sampling, traceability, validation/verification, appropriate test methods, handling of test items and adequate quality assurance measures. By covering these aspects and requirements that are interrelated, laboratories demonstrate that they are capable of providing consistently valid results that the international community can trust. Moreover, proving that all accredited measurement results can be traced back to the International System of Units (SI) or appropriate references guarantees that results are accepted between countries. In addition, risk-based thinking for cost-effective operations and evidence-based decision-making is part of the publication to know and control major risks related to tests.

For better understanding, the Manual establishes context, provides definitions and has particular emphasis on those areas where interpretation for microbiological and chemical testing of foodstuffs is required. It fosters principles and follows, where possible, the terminology defined in ISO/IEC 17000, ISO 9000, ISO 17025 (see Annex 2 for references) and the third edition of the International Vocabulary of Metrology (VIM), ISO/IEC Guide 99[2]. Relevant guidance documents in the ISO 17025 sphere are used by this publication, for instance Eurachem Guides. In addition, international standards and legal requirements in food quality and safety testing are discussed and referenced for further reading. Organisations and their publications that deal with chemical and microbiological testing are cited for consultation when implementing international (EU) requirements in food safety testing. The reader is kindly reminded to update themselves on the validity of ISO standards at the ISO website occasionally as some of the referenced ISO standards were under revision at the time of writing the Manual.

The Manual is addressed to both management and laboratory analysts for achieving accreditation or other compliances in food testing. The guidance might be helpful for laboratories that wish to establish a quality

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1 *ISO/IEC 17025:2007, General requirements for the competence of testing and calibration laboratories*

2 *ISO/IEC GUIDE 99:2007, International vocabulary of metrology - Basic and general concepts and associated terms (VIM) [also Joint Committee for Guides in Metrology 200:2012, International vocabulary of metrology - Basic and general concepts and associated terms (VIM); V J Barwick and E Prichard (Eds), Eurachem Guide: Terminology in Analytical Measurement - Introduction to VIM 3 (2011), see Annex 2.*

management system without seeking formal recognition and to implement quality assurance aspects at their laboratories. As a guide and learning material, it will also assist vocational institutions and universities in the teaching process and will provide benefit to students in deepening their knowledge related to food safety testing and laboratory management. The provided information can be a useful training aid for new staff or for staff that should take on broader duties. Finally, the guidance might be particularly suitable for state laboratories that submit analytical data to regulatory agencies in support of food safety initiatives and routine enforcement.

Overall, strengthening of laboratory services throughout the region contributes to improved health and well-being of national populations, and increased international trade in foodstuffs. At the same time, capacities are built at the national and regional levels to ensure food safety through accurate and adequate food testing.

Contents

List of Tables

Abbreviations

AAS	Atomic Absorption Spectrometer
AOAC	Association of Official Analytical Collaboration (AOAC International)
ATCC	American Type Culture Collection
AQL	Acceptance Quality Limit
BAM	The Federal Institute for Materials Research and Testing, Germany
BIPM	International Bureau of Weights and Measures
BSC	Biosafety Cabinet
BSL	Biosafety Level
CEN	European Committee for Standardization
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
DNA	Deoxyribonucleic Acid
EA	European co-operation for Accreditation
ELISA	Enzyme-linked immunosorbent Assay
EPTIS	PT database operated by BAM
EU	European Union
EQA	External Quality Assessment
Eurachem	Network of organisations in Europe; a focus for Analytical Chemistry in Europe
FDA	Food and Drug Administration
FPM	Feet's Per Minute
GC	Gas Chromatography
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GUM	Guide to the Expression of Uncertainty in Measurement
HEPA	High Efficiency Particulate Air
HPLC	High Performance Liquid Chromatography
IEC	International Electrotechnical Commission
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
IQC	Internal Quality Control
LOD	Limit of Detection
MicroVal	European certification organisation for the validation and approval of alternative methods for the microbiological analysis of food and beverages

MPN	Most Probable Number
MRL	Maximum Residue Level
MS	Mass Spectrometry
NMKL	Nordic Committee on Food Analysis
NordVal	Independent third-party, reviewing alternative methods
NTC	No Template Control
OIML	International Organization of Legal Metrology
PCR	Polymerase Chain Reaction
pH	Quantitative measure of the acidity or basicity of aqueous or other solutions
PTB	National Metrology Institute of Germany
PT	Proficiency Testing
RM	Reference Material
QA	Quality Assurance
SDS	Safety Data Sheet
SI	International System of Units
IUPAC	International Union of Pure and Applied Chemistry
UPS	Uninterrupted Power Supply
VIM	International Vocabulary of Metrology
WDCM	World Data Centre for Microorganisms

1. FOOD ANALYSIS AND ANALYTICAL REQUIREMENTS

1.1. Reasons for food analysis

Food analysis provides information about different characteristics of foodstuffs related to its composition, physicochemical properties, sensory attributes, content of contaminants and residues. This information is critical for sectors of the food industry to produce safe, nutritious and desirable foodstuffs and for consumers to make informed choices about their diet.

Various organizations or parties, including laboratories of food manufacturers or ingredient suppliers, analytical service laboratories, government laboratories, and university research laboratories analyse foodstuffs. They do this for different purposes, e.g., to fulfil Government regulations and recommendations, e.g., related to public health, for elimination of economic fraud, for quality control and to provide information to consumers about the nutritional composition of foods.

Food safety has become an important public health issue in which food-testing laboratories play a significant role in keeping consumers safe from health hazards. Risks to food safety could stem from residues of agrochemicals (e.g. residues of pesticides) and veterinary drugs, from environmental sources, fungi and cross-contamination or formation of contaminants during food processing and storage (e.g. mycotoxins). Harmful trace contaminates and residues typically have a toxicological profile and can cause adverse health impacts, if consumed in significant quantities. Thus, from the standpoint of consumers and manufacturers it is important to analyse that food does not contain harmful microorganisms, such as e.g., Listeria or Salmonella; toxic chemicals, e.g., pesticides, mycotoxins, or extraneous matter, such as e.g., glass, wood, metal, insect matter etc. and its safety is ensured. The analysis is carried out along the farm to fork continuum (e.g., regulated by the EU) to ensure that risks in food are within prescribed legal limits. Food contamination can also have economic impacts and might adversely affect international trade.

The detection of foodborne pathogens and their toxins is an important health concern worldwide, also due to changes in production systems and an increased global exchange. Pathogenic prokaryotes (Escherichia coli species, the genus Salmonella, some Bacilli, and Campylobacter) are major concerns in fresh products. Listeria (L.) monocytogenes could affect processed products quality, while the eukaryotic food pathogens such as Aspergillus spp., Fusarium sp., Penicillium spp., Alternaria spp., etc. might produce highly toxic metabolites and toxins for humans. Therefore, microbiological risks for relevant foodborne bacteria, their toxins and metabolites (e.g. Salmonella, L. monocytogenes, Enterobacter sakazakii, Staphylococcal enterotoxins and histamine in specific foods) are regulated, e.g., by food safety and hygiene criteria by the European Commission[3]. These regulations include, amongst others, the use of test methods, information of sampling plans, sample sizes, sample preparation, and interpretation of test results.

[3] *Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs*

For managing risks within the (HACCP) system, stages in the food manufacturing processes are defined to analyse properties of the food and to ensure that safety and quality are maintained, and to specify the appropriate action to take, if a problem is identified. Analyses are carried out before, during and/or after the manufacturing process. This is to ensure that the final product meets the desired standard requirements and that it consistently has the same overall properties, e.g. appearance, texture, flavour and shelf life. It is an increasing tendency in the food industry to use analytical techniques that are capable of rapidly measuring the properties of foods, e.g. on-line, without any need to remove a sample from the process. These techniques allow problems to be determined more quickly, e.g. related to food safety.

For characterization of the final product in the food manufacturing sector, the properties of the product are analysed and checked to ensure that it meets the appropriate legal and labelling requirements, and that it is safe, of high quality and retains its desirable properties until consumption. Government regulations usually require specifying the concentration of certain food components on the nutritional label of most food products, so that consumers could make informed choices about their diet. The vast majority of pre-packed foods, in the EU for instance, must bear a nutrition declaration that provide the energy value and the amounts of fat, saturated fats, carbohydrate, sugars, protein and salt of the food and to be presented in a legible tabular format on the packaging.

Other reasons for analysing foodstuffs relate to authenticity. Public awareness concerning food quality and safety, and the growth of the international trade has put authenticity of food in the spotlight. Food is considered authentic or genuine, if the product or its contents correspond to the original condition and the information on the label. Authentic foods are free from adulteration, especially with regard to composition, nature and varietal purity, geographical origin and manufacturing method. Food products that are of high value and undergo a number of processing steps are often target of fraudulent labelling, which is a major concern of producers, regulators, and consumers globally. Food fraud is considered as a type of food crime, when the scale and possible consequences of the activity are serious, e.g. because a risk is posed to public safety or potential financial losses to businesses or consumers arise. Food authentication, the analytical process to verify that a food product complies with its label description and techniques are thus gaining popularity. There is a growing need for reliable and analytical techniques to provide a decisive answer about the authenticity of foodstuffs, to detect false claims and to evaluate the safety of consuming food products along the food chain.

Because of the great importance of food products for human health, sectors of the food industry are controlled by government agencies that are responsible for publishing information, regulations and recommendations pertaining to foodstuffs. Food safety programmes have become important in addressing substantial changes in the way the food is produced and processed, capturing the use of fertilizers and pesticides, antibiotic application to animals to prevent diseases that reach food for human consumption, and changing food habits that led to the introduction of new and novel foods to name some. Government agencies have specified a number of voluntary and mandatory standards concerning the composition, quality, inspection, and labelling of specific food products. Many governments have regulated Maximum Residue Levels (MRL) for residues and contaminates in foodstuffs in a view to protecting public health to keep them at levels that are toxicologically acceptable or to ensure that ingredients and additives used in novel foods are standardized and within the recommended levels. In addition, the food

industry uses private accredited laboratories for their tests, especially in export activities.

Details related to the food safety legislation and test requirements of the countries of the South Caucasus are provided at websites of the ministries and competent authorities responsible for food safety and quality of the individual countries.

The EU food law (Sanitary and Phytosanitary requirements) and its regulations are available at the EU website[4], e.g., for third countries gaining information related to import requirements for their agro food products into the European Union. The EC Access2Markets portal[5] provides detailed information related to product requirements including testing for imports to EU. The webpage can be consulted free of charge.

1.2. Food analytical techniques

To control the fulfilment of regulations and recommendations, both government agencies and food manufacturers need analytical techniques that enables them to provide the appropriate information about food properties. As to test methods, the regulatory laboratory uses fit-for-purpose methods or methods otherwise identified as suitable by a regulatory/ enforcement agency.

Most laboratories use standard methods whenever possible. If these are not available, the laboratory either might use a non-standard method or modify a method for use with the concurrence of a regulatory agency. The laboratories have procedures and records for method validation (Chapter 9) that, at a minimum, meet the requirements and use statistical procedures and data presentation as required by regulatory agencies.

Overall, food analysis is a complex multilevel activity, which consists of several steps, such as:

- Sampling
- Sample preparation
- Analysis
- Data collection and calculation
- Reporting
- Result interpretation (if requested).

Test methods used in food analysis are divided into chemical methods and microbiological methods. The laboratory analysis for determination of physicochemical and microbiological characteristics of food products is often dissected into:

- Chemical-physical analysis
- Residues and contaminants analysis
- Sensory testing
- Molecular biological analysis
- Microbiological analysis
- Identification of foreign bodies.

Chemical methods are applied to separate, identify, and quantify matter/substance with the help of different techniques and instrumentation.

Wet chemistry techniques are used for qualitative chemical measurements, such as changes in colour (colorimetry), but often involves more quantitative chemical measurements, using methods such as gravimetry and titrimetry.

For nutritional labelling of foodstuffs, standard tests include determination of: Calories, protein, carbohydrates, dietary fiber, sugars, total fat, saturated fat, trans fat, sodium, cholesterol, Vitamin A, Vitamin C, calcium, and iron. The label may also contain information about

[4] *https://ec.europa.eu/food/horizontal-topics/general-food-law_en*

[5] *https://trade.ec.europa.eu/access-to-markets/en/content/welcome-access2markets-trade-helpdesk-users*

nutrient content claims. Official methods for nutrition labelling of food allow for automated instrumentation for instance, as in the case of the Dumas method that is in many cases replacing the use of the Kjeldahl method[6] for protein quantitation.

Sensory testing involves the objective evaluation of food products by trained human senses. Sensory attributes/ properties of food products can be defined as the human physiological/psychological perception of a number of physical and other properties of food and their interactions, subdivided into tactile and textural properties, colour and appearance, taste, odour and sound. Trained individuals measure these properties by picking up product characteristics by the sensory organs (eyes, nose, mouth, skin and ears) and analyse them according to various schemes and scientific methods as part of the product quality control for instance.

Nowadays, together with traditional "wet-chemical" techniques, advanced approaches such as infrared spectroscopy, gas and liquid chromatography coupled with mass spectrometry (MS) are widely used in food safety and quality testing. These techniques permit the detection of low concentrations of analytes in highly complex food matrices in a comparatively short time.

The reliable detection of residues and contaminants in food places the highest demands on laboratory analysis and requires state-of-the-art equipment for analysing, examining raw materials and products using multi residues methods or individual methods. Typical residue and contaminant analysis provides detection to trace residue levels. The techniques used include liquid or gas chromatography coupled tandem mass spectrometry (LC-MS/MS or GC-MS/MS); post-column UV derivatization followed by fluorescence detection (e.g. for aflatoxins); inductively coupled plasma tandem mass spectrometry (ICP-MS); capillary electro migration; enzyme-linked immunosorbent assay (ELISA), and other modern technologies respectively instruments. Mass spectrometry-based metabolomics has found applications in the study of chemical safety of food in the light of international trade, e.g. by addressing the screening of over 100 veterinary medicines in meat, or over 250 pesticides and veterinary drugs in animal feed. However, methods in the field of food contaminants are still to be improved, especially for structural identification and matrix effects that might interfere in the identification and/or quantification procedures, addressing questions such as robustness and repeatability of the methods[7] (see also Annex 3).

The increase of the number of potentially marketable species require reliable and rapid methods to verify the authenticity of the products and their origin, also to avoid false claims by manufactures about their products and to ensure that consumers are not the victims of economic fraud. For the detection, identification, and authentication of species numerous genetic methods are currently applied. These methods have advantages over the morphological characters or protein based methods that is their high robustness, reliability, efficiency, specificity and sensitivity and are therefore used.

Microbiological methods cover the use of biological, biochemical or chemical methods for the detection, identification or enumeration of microorganisms, often applied to disease causing and spoilage microorganisms. Conventional microbiological testing of food typically

[6] *For instance, protein content for nutritional labelling is determined via nitrogen determination by either the Kjeldahl method or the nitrogen combustion (Dumas) method. Both methods are official methods for nutrition labelling of food. The Dumas technique is measuring the crude protein concentration of food samples rapidly. It has replaced the Kjeldahl method as the standard method of analysis for nutritional labelling of protein content of foods (except in high fat content foods where the Kjeldahl method is still preferred due to fire risks).*

[7] *Da-Wen Sun (Ed.): Modern Techniques for Food Authentication; 2013, ISBN 78-0-12-814264-6*

encompasses culture-based methods with selective agars, or other biochemical assay procedures. New techniques enable laboratories to reduce the time needed to obtain results and offer the advantages of real-time, multi-pathogen detection. Some of the advances include enzyme-linked immunosorbent assay (ELISA) methods and diagnostic test kits for bacterial pathogens and microbial toxins; biosensor-based techniques; DNA (Deoxyribonucleic Acid) based fingerprinting techniques, and Polymerase chain reaction (PCR)-based technologies. PCR-based methods are most commonly used for the typing of bacteria and in the detection of unwanted components in foods.

Protein-based techniques, for instance ELISA tests for Halal[8] authentication are widely used by food laboratories, as well as real-time quantitative Polymerase Chain Reaction (PCR) analysis (e.g., specific meat test kits for the identification of animal species of origin in meat and meat products) to determine a contamination degree in agricultural or food industry. A multiplex real time PCR method is used e.g., for preliminary screening the possible adulteration of mozzarella cheese with cow's milk before using the official ISO 17678[9] procedure by the global food industry. In addition, there are other methods such as stable isotope analysis, origin analysis and also fingerprinting / profiling and comparison of the sample profile/spectrum with the reference material. Foodomics[10] is a useful tool to trace transformations applied to raw materials and to screen safety of the food products. However, analytical authentication can be difficult, especially for processed products and some questions cannot be answered at all or only in combination with other measures. Still proteomics of processed foods is a great challenge due to the increase of protein complexity.

1.3. Information on the analytical procedure / test methods

Different analytical techniques are used to obtain information about food products' properties, depending on the property to be measured, the type of the food to be analysed and the reason for carrying out the analysis. Information on the analytical procedure/ test methods are available from a number of different sources, such as:

Books: Food analysis books may provide a general overview of the various analytical procedures used to analyse food properties or they may deal with specific food components or physicochemical characteristics. Consulting a general textbook on food analysis is usually the best place to begin to obtain information about the types of analytical procedures available.

Tabulated Official Methods of Analysis: A number of scientific organizations have been setup to establish certain techniques/procedures as official methods, e.g., AOAC (Association of Official Analytical Collaboration), ISO (International Organization for Standardization) and so on. Usually, the method published by these organizations had undergone rigorous

[8] *Permissible (Arabic); Used to refer to meat from an animal killed in the way demanded by Islamic law*

[9] *ISO 17678:2019 [IDF 202:2019], Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides*

[10] *A discipline that studies the Food and Nutrition domains through the application and integration of advanced -omics technologies to improve consumer's well-being, health, and knowledge. Foodomics requires the combination of food chemistry, biological sciences, and data analysis.*

checking and tests by a number of independent laboratories until it becomes an official method. These organizations publish volumes that contain the officially recognized test methods for a variety of different food components and foodstuffs.

European Union Reference Laboratories (EURLs) for food and feed[11]: The EU has established a series of EURLs within the legislative framework on food safety. Their tasks are for instance to develop and validate test methods in the specific area of expertise, e.g., related to pesticides, mycotoxins, or microbiology[12] amongst others.

Journals: Often, analytical methods developed by scientists are reported in scientific journals, e.g., Journal of Food Science, Journal of Agriculture and Food Chemistry, or the Journal of the American Oil Chemists Society, Analytical Chemistry. They could be obtained by searching computer databases of scientific publications available at libraries or on the Internet (e.g., Web of Science, Medline). In case the laboratory uses such a method, they should conduct a validation to ensure that the method is fit for purpose (Chapter 9).

Equipment and Reagent Suppliers: Many companies that manufacture equipment and reagents for analysis of foodstuff advertise their products in scientific journals, trade journals, trade directories, and the internet. Various application notes are available online[13].

Internet: The Internet is an excellent source of information on the various analytical procedures available for analysing food properties. University lecturers, book suppliers, scientific organizations, scientific journals, computer databases, and equipment and reagent suppliers post information about food analysis techniques on the internet.

When selecting the test method/ procedure some of the following criteria should be taken into account:

- Precision[14]: A measure of the ability to reproduce an answer between determinations performed by the same analyst (or group of analysts) using the same equipment and experimental approach
- Reproducibility: A measure of the ability to reproduce an answer by analysts using the same experimental approach but in different laboratories using different equipment
- Accuracy: A measure of how close one can actually measure the true value of the measurand
- Simplicity of operation: A measure of the ease with which relatively unskilled workers may carry out the analysis
- Cost: The total cost of the analysis, including the reagents, instrumentation and salary of personnel required to carry it out
- Speed: The time needed to complete the analysis of a single sample or the number of samples that can be analysed in a given time
- Detection limits: The lowest concentration of a component that can be detected by a given procedure
- Specificity: A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components, e.g., fructose in the presence of sucrose or glucose

[11] *https://ec.europa.eu/food/horizontal-topics/european-union-reference-laboratories_en#food_and_feed*

[12] *For more information on the published validated test methods per EU Reference Laboratory, see https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=761&LabID=100&Lang=EN (example)*

[13] *E.g. as example "Simultaneous determination of 16 mycotoxins in cereals using a Triple Quadrupole LC/MS system and e-Method. Agilent Application Note Food Testing and Agriculture; Authors: Ye Jin, Wu Yu, Wang Songxue, Academy of State Administration of Grain, China Guo Qilei, Lu Meiling, Wu Cuiling,Chen Yuhong, Agilent Technologies; https://www.agilent.com/cs/library/applications/5991-7862EN.pdf*

[14] *For more detailed information on performance characteristics (precision, repeatability and accuracy) see Annex 3 of this Manual*

- Safety: Many reagents and procedures used in food analysis are potentially hazardous e.g., strong acids or bases, toxic chemicals or flammable materials
- Destructive/non-destructive: In some analytical methods, the sample is destroyed during the analysis, whereas in others it remains intact
- For food industry: On-line/Off-line: Some analytical methods can be used to measure the properties of a food during processing, whereas others can only be used after the sample has been taken from the production line
- Robustness: The evaluation of an analytical method wherein the results obtained are found to be reliable even when performed in a slightly varied condition. It is the ability of a method to remain unaffected when slight variations are applied.

If there are a number of alternative methods available for measuring a certain property of a food, the choice of a particular method will depend on which of the above criteria is most important. For example, accuracy and use of an official method might be the most important criteria in a government laboratory that tests for the validity of compositional or nutritional claims on food products. However, speed and the ability to make non-destructive measurements may be more important for routine quality control in a factory where a large number of samples have to be analysed rapidly.

Despite the fact of introducing advanced techniques in food analysis, a number of challenges exist, such as new environmental pollutants, new chemical residues, bacteria and viruses that can cause foodborne illnesses. Therefore, effective chemical and microbiological testing is the first line of defence in the overall efforts to ensure the safety of food and its products.

2. ORGANISATION AND MANAGEMENT OF LABORATORIES

ISO 17025[1], sections 3, 5 and 8.

2.1. Structural requirements: Organisation, responsibilities and management

For accreditation in line with ISO 17025, the laboratory must detail its basic organizational components, its range of activities, commitment to an effective management system, legal accountability, and set management's responsibilities to customers, regulatory authorities, and organizations that provide recognition. The laboratories must define the basic requirements for personnel and the authority and resources given to them to carry out their duties.

Any accredited food testing laboratories must be a legal entity or part of a legal entity. A legal entity has legal capacity to enter into agreements or contracts, assume obligations, incur and pay debts, sue and be sued in its own right, and to be held responsible for its actions. The ownership status of laboratories comprise e.g., private laboratories, state-owned laboratories, laboratories of government institutions, and laboratories that carry out tests to support the activities of their parent organizations and do not provide services to third parties. State-owned enterprises are business entities whose capital investment is entire or largely owned by the state through direct participation from separate state assets and can be in the form of limited liability companies or public companies. Both company types are legal entities and in the event of a dispute, the state government or the managing ministry is responsible. A governmental laboratory is deemed a legal entity on the basis of its governmental status. For laboratories that work within an organisation and not for third parties, the responsibility lies in the parent organization. Considered legal entities are associations, corporations, partnerships, proprietorships, trust, or individual with legal standing in the eyes of the law. In case a foundation owns a private laboratory, the responsibility for laboratory activities is with the foundation.

The laboratory must clearly identify the management that has overall responsibility for the laboratory, its policies, decision making, and allocating resources. Based on the size of the organisation, this can be e.g., a two-tier board structure consisting of a management board and a supervisory board, or an equivalent body with financial control.

The range of laboratory activities for which the laboratory conforms to ISO 17025 must be described and documented and conformity is claimed for that range only. Documentation of the developed and issued scope of accreditation might be sufficient in that case. For any scope expansion, the laboratory should define additional ranges prior to requesting conformity and proceeding with the (re)accreditation assessment. In general, laboratory activities for accreditation in line with ISO 17025 must be carried out in a way as to meet the requirements of the ISO 17025 standard, of the laboratory's customers, of regulatory authorities and organizations that provide recognition.

Any accredited laboratory must define its organization and management structure, its place in any parent organization, and the relationships between management, technical operations and support services. Organisational charts, although not explicit required for accreditation, produce objective evidence for how laboratories safeguard impartiality and how the laboratory forms part of a wider organisation and its relationships within this organisation and reporting structure, for instance, when submitting test results to a certain competent authority of a ministry.

Within the management structure of a laboratory, a distinct laboratory manager is expected. The ISO 17025 standard specifies functions for quality management or technical management, however, does not require identifying them (as personnel). With the help of an organisational chart, the relationships between technical and management staff can be visualised. Larger organisation can have several laboratory managements with specific technical mandates for their work area, but require clear specifications regarding the laboratory management and its range of responsibility.

The description of the management structure includes how supervision arrangements work. When defining the organisation, each function should be defined properly, including the reporting structure both ways upwards and downwards. Responsibilities and authority should match each other at all levels. The structural organisation of the laboratory must ensure that the laboratory never fails to act because of unclear or missing authorisation.

The laboratory must specify the responsibility, authority and interrelationship of all personnel who manage, perform or verify work affecting the results of laboratory activities. This includes typically laboratory management and technical management. Professional staff and technical management are expected to advise the laboratory management on quality issues and should have the authority and resources to carry out these functions. Some laboratories have distinct quality assurance units.

Typical laboratory structures consist of technical and professional staff with different responsibilities. Professional staff has most often responsibility for test method selection and development of new test methods, interpretation of data and the validity of results including validation/verification. Supervising technical staff in that respect is also part of their job description. At the technical level, there should be a competent technical manager or laboratory manager with responsibility to oversee all of the tests performed, to provide training as required and to certify the competence of the staff conducting the tests. The technical staff conduct the actual work at the bench and on instruments with involvement of professional staff, if needed.

A possible structure to manage, perform or verify work affecting the results of laboratory activities and interaction and supervision could be for instance:

1. Director of Analytical Services: Interacts with the upper management and laboratory staff regarding the laboratory's shortcomings, direction, new instrumentation, technical issues and customer complaints. He has a leadership/supervisory role, also for support staff.
2. Laboratory Manager/Technical Manager: Oversees the day-to-day laboratory operations, monitors flow of samples in and out of the laboratory and oversees the quality control system. He coordinates with other directors, e.g., other Technical Directors, Director of Analytical Services, and the Customer Services Director and informs them of any unexpected delays to ensure customers are notified. He attends meetings regularly and gives input, if appropriate.
3. Laboratory Supervisor: Monitors the proficiency of the laboratory chemists, microbiologist, and technicians, laboratory assistant's day-to-day operations to ensure samples are moving through the laboratory including statistics to track laboratory capacity at any given time. Along with the Laboratory Manager, the Laboratory Supervisor is responsible for the reporting of results.

The key positions of a laboratory structure can be described in the management system documentation overall as positions, e.g., "Laboratory Manager" with its defined responsibilities to provide insides in the laboratory structure and how work is organised in the laboratory, including supervision functions. At the laboratory level, individual job descriptions should be available for each level of management, including responsibilities, given authorities, functions and the supervisory role and responsibilities (Chapter 4.2).

It is not required, but recommended to assign deputies for all key functions in the laboratory to cover their functions in their absence. In small organisations, it might not be practicable to have designated deputies for all functions. However, the allocated responsibilities, especially with authorising activities, should be carefully analysed and arrangements made to cover in their absence by showing where the responsibility is re-allocated.

Accredited laboratories can use external personnel for functions requiring a certain separation, such as for internal audits or for handling complaints.

The laboratory is required to document its procedures to the extent necessary to ensure the consistent application of activities and for the validity of their test results. This means to have access to procedures, e.g., international standards, and its uniform implementation and use as needed. Procedures could cover for example, instructions how to meet quality control criteria and how to check data for recording in the final report or they could detail on a process that leads to a decision for validating a new test method. Supervision of the technical staff can involve instructions for bench workers and explaining how to return and to check data.

The laboratory must have personnel who have the authority and resources needed to carry out their duties. It includes personnel for implementation, maintaining and improving of the management system and for reporting on its performance and need for improvement to the management level. It further includes activities to identify deviations from the management system or from the procedures for performing laboratory activities and initiation of actions to prevent or minimize such deviations and for ensuring the effectiveness of laboratory activities. These all are tasks of quality management including controlling and managing documents (Chapter 2.3) and for organising and managing the management review and internal audits. Assigned personnel for these functions are responsible for the effective enforcement of the management system's objectives. Since not all laboratories can afford a fulltime working quality manager, these functions can be covered by representatives within the organisation with technical expertise or by personnel with the necessary technical background from the laboratory management level. In small organisations, it might be difficult to separate the responsibility for the management system and technical management functions completely. Some laboratory managers can function as their own quality managers, but responsibilities must be clearly defined.

The Laboratory management must ensure communication regarding the effectiveness and integrity of the management system, also during planned and implemented changes, and the importance of meeting customers' and other requirements. This is an output requirement of the Management review (Chapter 14.3.; ISO 17025). The outputs from the management review must record all decisions and actions related to at least the effectiveness of the management system and its processes.

2.2. Management system

A management system, also called quality management system or quality system, is a set of policies, processes and procedures used by laboratories to ensure that it can fulfil the tasks required to achieve its objectives. These objectives, by covering aspects of the organization's operations, could include for instance financial success, safe operation, product quality, customer relationships, legislative and regulatory conformance and worker management.

The principles of quality management is formalized in a number of published guidelines and standards. The most widely recognized standard used by laboratories for developing a management system for administrative, quality and technical operations/processes is ISO/IEC 17025 (Chapter 14). It addresses the technical competence of laboratories to carry out specific tests and is used by accreditation bodies worldwide as the core requirement for the recognition of a laboratory's competence.

The standard requires the laboratory to implement its policies and objectives for fulfilling the standard at all levels and explicitly to the competence, impartiality and consistent operation of the laboratory. There has to be evidence of the laboratory's management commitment in this regard. Furthermore, all information relevant to the fulfilment of this standard, such as documentation, processes, procedures, systems and records, must be linked to the management system. Corresponding to their respective responsibilities, personnel need access to the documentation of the management system (Chapter 2.3).

The ISO 9001 standard is a management system standard that relates primarily to the quality management for organisation carrying out production, or providing services. It promotes the adoption of a process approach when developing, implementing and improving the effectiveness of a quality management system. A simplification of the main aspects of a management system is the four (4)-step "Plan-do-check-act" approach that carries out the change in a process, product, or service. It is an iterative design and management method used in business for the control and continuous improvement of processes, products, services. Moreover, this model is a project-planning tool. Both standards, ISO 17025 and ISO 9001, place emphasis on continual improvement of the effectiveness of its quality management system through activities such as setting quality objectives, reviewing audit results, and management reviews.

Management system documentation (ISO 17025) serves the purpose of maintaining and, where necessary, improving quality and that (quality) management is applied comprehensively, appropriately and consistently. It proves evidence that if something goes wrong, the error can be tracked and modifications to the system can be made in order to reduce the likelihood of its recurrence, e.g., by implementing corrective action, which addresses the root cause of the problem.

The laboratory will select a standard according to its needs. However, central is that at the technical level, good practice in quality management is independent of the formal management system adopted. There are two distinct options for establishing a management system as provided by the ISO 17025 standard for accreditation of the laboratory (see Chapter 14.1 for more details). Laboratories need to conform to only one of the options (not both). The intention is to achieve the same results with either of them; both require that the management system is capable of supporting and demonstrating the consistent achievement of the requirements of ISO/ IEC 17025 sections 4 to 7 (Chapter 14.3, Annex 4) and assuring the

quality of the laboratory results. If laboratories have already implemented ISO 9001, they can use the management system they already have implemented as the overarching system (Option B, for more see Chapter 14.1).

For details related to the aspects of the management system reference is provided to ISO 17025 (for contents of the standard see Annex 4), and related guidance documents (see Annex 2 for references).

2.3. Documentation and document control

Documentation is an important means of communication within the laboratory so that all personnel know their responsibilities and the procedures that apply.

The term "document" in its broadest meaning covers information in all forms, including computer files, software and other electronic or digital information. Documents provide information or instructions for use in technical processes, such as masters and templates used for record keeping, publications, notices, calibration tables, drawings and plans to name some. Documents are also used in management processes and cover e.g., policy statements and management procedures. They can be prepared by the laboratory or are published material or other externally provided information.

The management system documentation consists of the laboratory's policies and objectives regarding the quality of the laboratories work, including the respective commitment of the highest management level and applicable regulatory documents. It covers the management structure that defines how responsibility and authority for dealing with quality are allocated in the laboratory, and the procedures, which constitute the working management system (e.g., control of documents and records or dealing with nonconforming work).

Accredited laboratories must control the documents where documentation is obligatory and as to their extent in use. A controlled document is a policy or procedure related to the documented management system. It is subject to controls to ensure that the same version of the document is available to all personnel to whom it is applicable. The control of documents ensures that documents are approved for adequacy, are reviewed and updated with changes, that revisions are identified and that their unintended use is prevented.

All management system documentation, either electronically or as hard copy, must be compiled in a consistent and comprehensive manner. A system for that could make use of references to subsidiary documents (e.g., procedures or equipment logs), but has to contain all necessary information or clearly explain, where such information is to be found. As such it is the basis for demonstrating the fulfilment of ISO/IEC 17025 (e.g., by accreditation) when the laboratory will be assessed against the requirements of the standard and its own management system documentation.

The document control system must be aware of every copy of a document in circulation in order to ensure its review and update when necessary. Recording of all issued copies of documents is required, so that if documents need to be reviewed, withdrawn or amended, all copies can be subjected to the same procedure.

It is recommended to keep copies of all versions of each controlled document to trace back to the content at any point in its history.

Some laboratories retain copies of older versions of documents too, for instance when customers wish to use the previous version of a standard method. Documents, which are obsolete for general use, but are retained for specific purposes, must be suitably marked. The marking should either specify the scope of use of the document or simply warn that it is not for general use and refer the reader to an authority (e.g., senior laboratory personnel) who can provide information on when it is to be used.

There should be a procedure to ensure that controlled documents are reviewed from time to time. Some published documents (e.g., ISO or national standards describing technical methods) are subject to revision by the issuing body and the laboratory will need a mechanism for ensuring that revisions of published documents are noted and that the laboratory's copies of the documents are replaced with the updated versions. The simplest way is to have a list of all the documents in this category, to check on a regular basis for updates, and to record these checks.

Documents that provide information or instructions for use in technical and management processes must be controlled, regardless if prepared by the laboratory, published, or externally provided. The laboratory's management should be aware of and approve the documents used by personnel to guide them in their work. The established documentation system should consider that documents might need to be issued, and amended quickly and by the most appropriately qualified person. In case of cross department agreement, document revision should not be excessively bureaucratic. Minimum identification of documents must be agreed.

The issuing and amendment of each controlled document is an assigned responsibility to individuals with the relevant knowledge to evaluate the document, irrespective of the line of management.

New or altered text should be identified in amended or revised documents or in attachments as agreed. It serves to communicate the amendments, to identify easily the gravity of changes and if changes affect the way to carry out a procedure. Electronic available documentation, e.g., files must be read-only for users and are amended by authorised persons only. They are prevented from printed without authorisation and recording (the document revision and last modification date in the electronic system should be the same).

This Manual keeps focus on the technical and quality aspects of any food testing laboratory to satisfy accreditation requirements. It is not covering details and requirements related to complaints, nonconformities, of externally provided resources, on contracts, for reviewing requests and tenders, and management aspects, e.g., related to internal audits, management reviews or non-conformities etc. For further information on these aspects, the ISO 17025 standard and guidance documents could be consulted, e.g., the EUROLAB Cook Book or the Eurachem guidance documents (see Annex 2 for more information).

Laboratories must have the following procedures in place for accreditation in line with ISO 17025, describing:

- Personnel, see Chapter 4 for more information on the requirements to determine the competence requirements, for selection of personnel, training, supervision, authorisation, and monitoring the competence of personnel
- Equipment, see Chapter 5 for more information and on aspects of handling, transport, storage, use, planned maintenance and intermediate checks of equipment
- Externally provided resources, such as defining, reviewing and approving resources, evaluation, selection, monitoring of performance of provided resources, ensuring that externally provided resources conform

to the applicable requirements, and taking corresponding actions
- Contracts and reviewing requests and tenders
- Laboratory activities, describing methods, procedures, and validation of methods, as described in Chapter 9
- Test items and transportation, receipt, handling, protection, storage, retention, and disposal or return, as described in Chapter 6
- Quality control and monitoring the validity of results, for more information see Chapter 12
- Complaints and receiving, evaluating and making decisions
- Nonconformities and defining, managing immediate actions, evaluating significance and follow-ups.

2.4. Risk based thinking

Accredited laboratories should plan and implement actions to address risks and opportunities to increase the effectiveness of the management system, to achieving reliable results and to prevent negative effects. The main objective is not only to minimize risks, but also to optimize the opportunity profile defined in the laboratory's strategy. For definitions of risks and opportunities, see Annex 1.

The objective of risk management activities is to recognize, assess, and manage risks early on and to implement appropriate measures to minimize them. If a laboratory knows its risks, it has the capability to assess and prioritize them and is aware of its consequences. It will be easier to plan how to handle risks and their effects. Mistakes or nonconformities detected at an earlier stage allow laboratories to react early and financial penalties or other heavy losses might be averted.

The process of risk management includes steps to get an overview of specific risks and to identify, analyse, evaluate risks in order to reduce the uncertainties and to increase the likelihood of achieving the objectives, improving the identification of opportunities, and to effectively allocate and use resources for risk treatment.

The laboratory is responsible for deciding which risks and opportunities associated with its policies and procedures they address. This applies to:

- Risks to the laboratory's impartiality
- Risks caused by invalid methods
- Risks of false accept or false reject when providing statements of conformity
- Risks caused by nonconforming work and such becoming apparent during corrective actions
- Risks to the effectiveness of the management system and risks of potential failure of the laboratory activities
- Risks identified and subjected to management reviews.

The laboratory should consider both, the internal and external context of the organization, including risks related to the customers, the supplier, and other stakeholders. The Accreditation Body will then assess whether the laboratory has established appropriate actions for dealing with risks and opportunities.

There is no requirement for formal methods for risk management or a documented risk management process. Risk identification methods range from common sense and brainstorming, via the use of pre-established lists for each subject area, to the use of standards setting good practices. Laboratories are free to decide to develop a more extensive risk management methodology, e.g., in line with requirements of ISO 31000 and related

standards[15] or to use a minimum of formalism that allows to exploit on the approach and to motivate more effectively the deployment of provisions, sometimes perceived as constraints only.

Helpful parameters when assessing risks are likelihood (What is the probability of a harmful event?) and significance (If something happens, how serious the event is). In addition, it can be useful to establish certain risk categories. For example:

i. No risk – no action required
ii. Remote risk, serious harm very unlikely – random monitoring advised
iii. Some risk, serious harm possible – monitoring required
iv. High risk, serious harm probable – action required
v. Maximum risk, serious harm virtually certain – stop work.

Decisions and operations of the laboratory should be guided by the potential influence on the intended effect. Many options are possible and can be combined for addressing risks, such as avoiding the risk, taking the risk to seize an opportunity, eliminating the source of the risk, changing the likelihood of occurrence or consequences, risk sharing or accepting the risk, and informing about it. An acceptable risk should be classified as such.

Risk management should be embedded in organizational practices and processes. It is for instance traditionally applied in connection with the validation of methods and the introduction of the concept of uncertainty of measurement (Chapters 9 and 13).

Risk register could be used to document and record risk management processes for identified risks and to cover the significant risks faced by the organization. Recorded are results of the risk assessment related to the processes, operations, locations, business units or projects under consideration.

Risks identified are subject to management reviews covering the risk analysis and the adequateness of the resulting actions.

For further reading reference is provided to the EUROLAB Handbook[16], EUROLAB Cook Book No18: An introduction to risk consideration, Cook Book No8: Determination of Conformance, and EUROLAB Cook Book No7: Management Reviews (see Annex 2 for references).

[15] *ISO 31000:2018, Risk management- Guidelines; ISO Guide 73: 2009, Risk management – Vocabulary; ISO/IEC 31010:2019, Risk management – Risk assessment technique.*
[16] *EUROLAB Handbook ISO/IEC 17025:2017, www.eurolab.org;*

3. LABORATORY FACILITIES AND ENVIRONMENTAL CONDITIONS

ISO/IEC 17025[1], section 6.3; see also ISO 7218[17].

Facilities and environmental conditions must be suitable to realize the laboratory activities; they should not adversely affect the validity of results or the safety of laboratory staff.

3.1. Laboratory layout

Both a single discipline-testing laboratory and a complex of laboratories must be carefully designed to ensure efficient and secure operation. This is important both for the conduct of testing and for the purpose of accreditation, where the accommodation must be well suited for its designated purpose.

Facilities consist of laboratories, office areas, storage rooms, and special purpose areas. Laboratory design and layout reflects the different operations involved in the testing programme. A well-conceived working space is one of the most important aspects to successful laboratory operations. Planning the flow of the test materials through the laboratory and interaction of different tests is crucial.

There is no single correct laboratory design and avoiding many problems is a matter of combining engineering and test experience with the construction experience of builders and architects. For the most efficient design, all related services should be located in close proximity. For optimizing laboratory delineation of laboratory activities considered are: Grouping either related activities in a single room, or to clearly delineate bench space for specific activities, taking measures to prevent cross-contamination of samples. Service rooms to accommodate autoclaves, sinks for cleaning glassware, preparation and sterilization of culture media should be located in a central area to minimize distances and facilitate circulation paths of materials, samples and goods.

Laboratory design and layout reflects the different operations involved in the testing programme. It can be a major factor in its success. A well-designed laboratory can be more efficient, quieter, more attractive and cheaper than one with design-related problems. The accommodation layout must be designed to facilitate all elements of the testing operation from sample receipt to the issue of the final report, also considering steps in the testing process that must be separated from other activities. The laboratory should be arranged to minimise risks of cross-contamination, where these are significant to the type of test being performed, for example by:

- Constructing the laboratory according to the 'no way back' layout principle (microbiology)
- Carrying out procedures in a sequential manner using appropriate precautions to ensure test and sample integrity (e.g., use of sealed containers)
- Separating and maintaining areas for incompatible activities, and taking measures to prevent cross-contamination (with

[17] *ISO 7218:2007+A1:2013, Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

different requirements for microbiology laboratories and for different types of chemistry laboratories, as discussed later). Provisions made for effective separation could be in terms of space (e.g., by carrying out the activities in different laboratory areas) or time (e.g., by scheduling work so that the incompatible activities happen sequentially with adequate cleaning procedures between the two). For microbiological and PCR laboratories appropriate biosafety and biosecurity levels must be reflected (for more see e.g., WHO: Laboratory Biosafety Manual in Annex 2; and Chapters 3.5.2. and 3.7).

Examples of good practice and separate locations, or clearly designated areas are:

- Conducting sample receiving and storage in designated areas, which are separate from the main part of the laboratory
- To separate laboratory areas from other sections in the building, such as administrative services, lunchroom and conference room facilities for instance
- Separating areas of specialized testing from general work areas
- Where feasible, separating volatile organic operations physically from solvent and sample handling areas and vented
- Separating chemicals, standards, reference materials and cultures from samples
- Separating microbiology media preparation and sterilization areas from work areas to prevent contamination of clean media.

Advisably, the head of laboratory participates in the layout/design and planning stages of new laboratory facilities, assessing all potential risks and for applying basic concepts of organization in order to provide a proper and safe environment for conducting laboratory activities. Likewise, when developing new activities or new diagnostic techniques in the laboratory, the head of a laboratory is required to consider the organization of the laboratory. Health and safety of laboratory personnel is of prime importance and thus considered as a major factor in the development of a laboratory including specific environmental requirements for the testing facilities. Further, an experienced architect and a good builder can be very helpful in laying out the facility, but experienced engineers and technicians are indispensable.

The facility and environmental requirements necessary for the performance of the laboratory activities should be documented and records of the ongoing monitoring and periodic review maintained, including measures taken (Chapter 3.6).

3.2. Access to laboratories and security

Irrespective of size, laboratories must observe certain principles to maintain the conditions of security and restricted access that is required by customers and any Accreditation Body. A policy on access to the laboratory should be guided by the two principles of ensuring confidentiality (quality of work) and considering impact on data (validity of results).

The public area of the organisation should be evidently separated from the laboratory. All access points must be locked or manned to ensure that only authorized personnel are granted entry and that visitors are registered on entry, escorted at all times and registered as leaving. The introduction of a door lock system whereby staff can enter using a magnetic card or by entering a code will allow entry for authorized personnel only. Authorisation of access should differentiate between visitors and supporting services, such as cleaning staff or maintenance engineers.

It may be necessary to restrict access to particular areas or rooms of a laboratory because of security, safety, or sensitivity to contamination or interferences and the nature of the work carried out there.

Restrictions to authorized persons, usually laboratory technical staff and maintenance staff, is typically for areas of laboratories carrying out microbiological and/or polymerase chain reaction techniques and trace analysis, or working with radioactive materials or carcinogens, or where hazardous chemicals or other materials are stored. They are described in procedures and might be accomplished using signs on doors and locks, when appropriate, and by staff identification badges. Where such restrictions are in force, personnel should be made aware of the:

- Intended use of a particular area
- Restrictions imposed on working within such an area
- Reasons for imposing such restrictions
- Procedures to follow when such restrictions are breached.

3.3. Laboratory design

The typical laboratory comprises testing facilities or areas, where specific testing and associated activities are carried out, and of additional areas, such as administration blocks, storage rooms, archives, corridors, entrances, cloakroom and toilets.

Laboratories are sectioned by testing compatibility and design capabilities. Each type of laboratory has some common activities with additional or specialized rooms, depending on the nature of the testing operation. Common laboratory activities include:

- Sample reception
- Sample storage
- Washing up or decontamination
- Weighing activities
- Sample preparation
- Sample processing
- Test and data processing areas
- Office and data storage areas.

Laboratory facilities and laboratory workspace for testing, including, but not limited to energy sources, lighting and environmental conditions, should be as such as to facilitate the correct performance of the tests without compromising the quality of the work and the safety of the laboratory staff.

The following explains more about the activity areas and services/facilities required.

3.3.1. Sample reception

For any testing activity, samples are delivered to the laboratory. A key provision for accreditation is to ensure sample and/or testing data security and confidentiality. The laboratories usually have a sample receipt area. It is a secure area to collect and register sample details with limited access to authorized staff only. The responsible person in the sample receipt office receives the samples, completes the sample registration procedure and notifies the relevant laboratory for storage/testing of the sample. Each sample is given a unique registration number/code to ensure that samples cannot be confused or mixed up. Nowadays, samples are registered using an electronic database (e.g., a Laboratory Information Management System, LIMS) and a sample coding system to maintain the confidentiality and the sample. Samples entering the testing area should be anonymous and the identity of the sample supplier is known only to the sample registration and reporting functions containing the test results (Chapter 8.2).

3.3.2. Sample storage area

It is vital that all samples are labelled correctly and that samples are stored in safe and approved locations. Samples should be stored in such a way that cross-contamination is not possible. They should be separately secured, ideally in locked storage at least, and be tidied away into drawers or cupboards.

The laboratory should guard against sample deterioration, contamination and loss of identity, taking into account any specific requirements stated by the supplier or specified in the method.

Storage arrangements (temporary or permanent) must ensure that the samples do not deteriorate in any way and are kept in the same condition as on receipt. Depending on the tests to be conducted, storage of foodstuff samples requires a varying degree of access to refrigerator and freezer space. Freezer storage is generally applicable for samples for chemical testing (pre- and post analysis; for more see Chapter 8.2).

Refrigerators and freezers should ideally be in a room separate from the laboratory area, but if space is limited, they could be placed in the sample preparation room, as long as other activities are not impeded. They could also be kept in corridors adjacent to the sample processing room considering they do not restrict access or compromise fire regulations. All cabinets (refrigerators or freezers) containing samples must be in an area preventing unauthorized access and be fitted with a lock.

3.3.3. Washing-up room/decontamination area

To have a separate washing-up room for the cleaning/decontamination of glassware is desirable. In a small chemistry laboratory, this activity could be confined to a sink area in one of the operational areas. A chemical laboratory with a number of testing activities often has a common washing-up room.

It is good practice to wash glassware in batches from the different sources to minimize any possible cross-contamination. The use of an automatic washing machine is recommended as the most effective and consistent form of glassware cleaning.

For a microbiological laboratory a separate „dirty room“ must be maintained and the above conditions apply. In addition, there must be an autoclave for the decontamination of used materials.

3.3.4. Balance room and weighing

Weighing activities can be divided by virtue of the type of balance and weighing range. Analytical balances are used for measuring weights ranging from 0.1 mg to 200 g. Analytical balances weighing to an accuracy of ± 0.1 mg must be used whenever four or more significant figure accuracy is required. This will be the case for weighing out samples, primary standards or when taking crucibles to constant weight. Analytical balance weighing accuracy is a measure of how close the displayed weight is to the actual weight of samples on the weighing pan.

In general, balances are located on a firm, level surface in an area with minimal exposure to changes in temperature, humidity, air movement and vibration. It is also important to avoid draughts; that is why analytical balances are enclosed with shields. Analytical balances weighting a few milligrams to several grams should be maintained within a purpose-built balance room, partitioned off from other activities and with vibration-resistant benching.

A suggested balance room should be 3 × 2 m with a single vibration resistant bench along one side. A second bench is useful for the placement of glassware or materials to be weighed. In a warm environment, the room should be air-conditioned, but without a direct draught on to the balances.

A small laboratory could use a discrete area of the main laboratory for this purpose, but this is not advisable unless strict controls are put in place. There can be a risk of laboratory contamination distorting the true analytical result. Where analytical balances are located in the main laboratory area, vibration resistant benching is essential.

Top-loading balances will weigh to an accuracy of ± 1 mg and are suitable for most weighing of amounts that are specified to only two or three significant figures. In chemistry laboratories, top pan balances are sited either in a separate balance room or in the laboratory area. They are used for weighing quantities of general reagent or sample material. A microbiology laboratory requires top pan balances of differing accuracy. It is unlikely that these will be located in a separate room, but balances are required in the media preparation and sample preparation areas. Balances for such purposes must be on vibration-resistant mountings.

Where possible, separate balances are used for reagents and samples, but this is not essential. Good practice with regard to cleanliness of operation is critical. However, when weighing powders operators should either wear facemasks or weigh out powders under an extraction hood. If using a hood, checks are made to ensure that airflow or movement is not interfering with the accuracy of measurements.

Balances must be calibrated once in place (Chapter 5).

3.3.5. Sample preparation room

The sample preparation room is where received samples are defrosted (if frozen), macerated and homogenized and subsamples are taken for analysis. Extraction can also take place in this area should space permit. Depending on the type of sample, this room can become wet and dirty necessitating a design that is easy to clean. Floor drainage is ideal as it permits spillage to be washed away.

The room should be a minimum of 3 × 3 m with a sink with taps for hot and cold running water. The benches are sealed against the wall to permit effective cleaning and the floor should be of good quality and sealed around the edges. The room should be air-conditioned providing for an optimum temperature range of 20–22°C and designed in such a way that the air flow from the unit is not directly on to the area where the samples are prepared or on to any sensitive equipment (e.g., balances if present).

Samples for microbiological test purposes generally require less manipulation than samples for chemical testing, where the received sample may be significantly larger, and can be prepared directly in the sample processing room.

3.3.6. Sample processing room(s)

The samples, whether chemical or microbiological, are prepared for analysis in sample processing room(s). The space of this(se) rooms(s) depend(s) on the testing programme; they might contain much of the day-to-day apparatus (equipment and glassware) and chemicals, reagents and reference standards used for the analysis. Such a room will also be used for sample testing where more general procedures are used (in chemistry, for example, the physio-chemical tests for water turbidity, conductivity, nitrite content, etc.), which do not involve the use of more sophisticated equipment.

The space requirements for such a room are approximately 10 × 5 m with benching installed to maximize the working space available, whilst holding room for the requirements for free-standing equipment (e.g., refrigerators, freezers, etc.). A room of this size requires four sinks. However, the space should be as required by national regulations, when such exists and sufficient to allow work areas to be kept clean and tidy. As always, the space required should

be commensurate with the volume of analyses handled and the overall internal organization of the laboratory (ISO 7218[17]).

Larger laboratories with up-to-date mechanical and engineering systems in place might provide piped vacuum to taps within the laboratory, although in most cases, portable electric vacuum pumps will be adequate. The room should also be equipped with fume extraction facilities for use when working with hazardous reagents or volatile organic solvents.

Bench units against the wall should be sealed to allow for easier cleaning and to prevent the build-up of dirt and contaminants. The floor should similarly be of good quality (linoleum or similar) and sealed against the walls. The room should be air-conditioned.

3.3.7. Test rooms

Test rooms include all those where the determinative step of an analysis are conducted, from chemical testing to the examination of plates of cultures of microbiological test samples.

Chemistry instrument rooms

For chemical analysis, the cross-contamination between samples and possible environmental contamination of samples must be avoided. Chemical testing often requires standards, pure samples or concentrated solutions of materials, for testing at trace levels, that requires explicit consideration to not cross contaminate.

Good practice in chemical testing should generally observe:

- Segregated areas with own glassware for the storage of standards and the preparation of concentrated solutions
- Operating rules to ensure that only much diluted solutions of standards necessary for calibration of equipment are ever introduced into areas where samples are being handled and processed
- Precautions to avoid spillage of standards, for example by carrying them inside double containers
- Where samples are handled containing high levels and low levels of the same targets, for example, pesticide formulations and samples for residues analysis, here, the sample preparation work and, where possible, the instrumental analysis should be carried out in well-separated rooms with their own glassware. Where possible, providing segregated washing up facilities for glassware with segregated uses are preferred. If this is not possible, a management regime should be established for ensuring not to interchange glassware, e.g., by the use of clearly labelled baskets to deliver it to and collect it from the washroom.

Normally, separate instrument rooms are in use to house equipment such as the gas chromatograph (GC), a high performance liquid chromatograph (HPLC) or atomic absorption spectrometer (AAS). Each of these systems have different requirements and require special facilities or are best sited away from other operations, especially heavy metals analyses requires a dust free area.

GC systems require adequate benching for the instrument itself, for any associated detectors such as a mass selective detector and its computer control and data handling system with printer (approximately 2–2.5 m of benching for each system and four power outlets). For the gas supply, the number and range depends on the specification of each GC system and the requirement of three to four gases is common. It generally necessitates the use of gas cylinders (ideally situated outside the laboratory with the gas piped into the room).

The gas piping takes up space, as do the gas filtration systems necessary to purify the bottled gases. Alternatively, laboratories can use gas produced from generators, e.g., for nitrogen, compressed air and hydrogen, while no such systems exist for oxygen, argon or helium. These generator systems are effective although they

generate heat, but require a constant power supply and the use of filtration/purification systems. The use of generators further increases the number of required power outlets.

The provision of gas outlets for gases appropriate to the tests undertaken (e.g., compressed air, nitrogen, natural gas or a similar combustible gas) should be ideally external to the laboratory and piped into the room. Where gas cylinders are sited externally, they should be kept in a locked cage, shielded from rain and not be exposed to direct sunlight. Where this is not possible, freestanding cylinders, appropriately strapped to a support to ensure stability, are used.

HPLC systems only require bench space for the unit itself and for its control system and data handling. A usual HPLC „footprint" is of the order of 1.5 m length. Bench space for the samples being loaded and injected and for data review is also required. Provision should be made for four power outlets for each HPLC system.

AAS systems are large and require an extraction hood to remove the fumes from the combustion system. The instrument occupies approximately 1.5 m but with associated space for a hydride generator and working space, a minimum of 2.5–3 m should be reserved. Gas supplies (acetylene and air) are needed and these should ideally be piped into the room. Provision should be made for up to six power outlets for each system to cover the main instrument, a hydride generator if attached, a computer control and data handling station with printer and spare capacity for test equipment used by a service engineer.

Microbiology suite of rooms

The microbiology laboratory is designed to prevent or reduce risks of cross-contamination. All operations in the laboratory should be linked together smoothly without samples crossing and the scope for contamination minimized. Separate rooms and/or separate areas and/or specific enclosures should be provided for the following:

- Sample receipt and sample storage. The reception desk with incoming register is located as close as possible to the entry door.
- Preparation of samples, particularly in the case of raw materials (e.g., powdered products containing a high number of microorganisms). The sample processing area should be separated from, but nearby, the testing areas.
- Manipulation of pathogens (e.g., Salmonella, Listeria monocytogenes)
- Preparation and sterilization of culture media and equipment
- Cleaning of glassware and of other equipment, as well as the decontamination of equipment and contaminated culture media
- Sterility assessment of foodstuff.

Separation of the following areas should also be considered:

- The areas used for the preparation of culture media, and the room used for sterilization of culture media and of the equipment, and
- The decontamination area and washing area.

The suite should contain toilet and changing room facilities immediately upon entrance and separated from the main laboratory and operational areas. Laboratory coats worn within the microbiological suite must not leave that suite to minimize the risk of contamination.

If possible, circulation pathways of clean and dirty laboratory materials should never cross, and circulation pathways of contaminated waste should be isolated. Laboratory equipment should not be moved routinely between areas to avoid accidental cross-contamination.

Incubators, refrigerators and freezers can be placed in specific adapted rooms. Incubators and incubator rooms must be properly constructed and controlled (see Tables 2 and 3). Incubator rooms must be well insulated and conditioned as incubators should be placed in rooms where temperatures are within the

range 16–27 °C. The rooms should be supplied with stainless steel shelves suitable for holding Petri dishes, flasks, and other items (wooden tables are not adequate).

The ambient temperature and air quality must be compatible with carrying out tests in the microbiology. A filter ventilation system for incoming air is recommended for this purpose. In practice, microbiology laboratories will need to be air conditioned with split type units and all windows should be sealed to prevent opening. Entry to the laboratory should always be double doored with a vestibule and changing/washing area. When tests are to be conducted in a low-contamination atmosphere, the room should be specially equipped with a clean air laminar flow cabinet and/or a safety cabinet.

In areas where microbiology procedures are performed, workbenches should be separated according to the different types of samples or pathogens that are analysed. This is to minimize risks of cross-contamination and for reasons of personal protection.

In PCR analysis, the main source of the feedback contamination are the amplicons (tools for designing PCR primers) generated by the previous PCR products. Activities in molecular biology must therefore consider to have at least two rooms to separate the source of the amplicons, namely the post-PCR activities from the pre-PCR activities. If the preparation of DNA extracts is not in the same room as the subsequent steps (preparation of reagent mixes and DNA amplification), the potential for contamination is significantly reduced. In addition, dedicated pipettes, tips, centrifuges, tubes, adequate protective clothing, vials, heating blocks etc. should be located in each work area (e.g., low-medium-high DNA working environments). A change of the laboratory coat may suffice when moving between areas.

For more information, covering fittings of microbiology laboratories and suggested laboratory premises, laboratory areas etc., reference is provided to the international standard ISO 7218[17].

3.3.8. Office and data storage

For post-examination pathways, after the analysis of the samples, results must be accurately recorded, properly filed, and delivered on time to the right person. The sample receiving area is in most cases also in charge for communicating the test results. Communication systems appropriate to the size and complexity of the laboratory, including the efficient and reliable transfer of messages, should be part of the laboratory design.

3.4. Physical aspects of premises

Laboratory facilities for testing should be designed to provide space, engineering controls, and proper environmental conditions for optimal sample storage, sample handling, and analysis, in accordance with general laboratory practices, safety, and applicable national and local regulations.

Any laboratory must be designed so that it can easily be kept clean and that any spillages can be contained and thoroughly cleaned up. The test premises should be fitted out in the following ways, or applying the following principles in order to reduce the risks of contamination:

- Smooth surfaces on walls, ceilings, floors and benches (smoothness is judged on how easily it may be cleaned). In case of suspended ceilings and hanging lights, the laboratory should have documented evidence that they control any risks to hygiene and have effective means of overcoming them, e.g., a surface cleaning and inspection programme
- Microbiology laboratories are designed to minimize areas with cracks or fibers that could serve to accumulate debris and serve as an area for growth of microorganisms
- A precisely installed laboratory floor must deny the penetration of dirt, liquid or any other type of contaminate that could potentially damage or ruin the subfloor
- Use of continuous sealed surfaces constructed from a material that is resistant to most chemical spills and can easily be cleaned and disinfected. Impervious bench tops with good seals against walls and floor and around fittings such as sinks. Tiles are not recommended as bench covering material
- Use of concave joints between the floor, walls and ceiling
- Sunshades are placed on the outside. If they cannot fit outside, easy access for cleaning of internal sun shades is required
- Fluid conveying pipes must not pass above work surfaces unless placed in hermetically sealed casings
- A dust-filtered air inlet for the ventilation system
- Separate hand-washing arrangements, preferably non-manually controlled (especially for the microbiology area)
- Cupboards are up to the ceiling
- No use of rough and bare wood. Wooden surfaces of fixtures and fittings are adequately sealed
- Workbench spaces must be sufficient to perform operations and to prevent clutter
- Presenting separate storage areas of sufficient size in the laboratory to ensure that glassware, portable instrumentation, microbiological media, supplies, reagents, solvents, chemicals, hazardous or regulated wastes and reference standards and materials are properly stored. Storage is relevant to prevent contamination or degradation, to ensure that the laboratory complies with regulatory authorities, to meet security needs and to assure personnel safety, as well as to minimize clutter.

This list is not exhaustive and not all examples will apply in every situation.

Further, when selecting designated areas for new work, account must be taken of the previous use of the area. Thus, before use, checks must ensure that the area is free of contamination. Decontamination procedures may be appropriate where the environment or equipment is subject to change of use or where accidental contamination has occurred.

All technical requirements for accommodation and environmental conditions that can affect the results of the tests must be considered and documented, see chapter 3.6. for more information. Where it is critical to the quality of its work, the laboratory must maintain documented procedures and records relating to cleaning processes (3.5.6.)

3.5. Laboratory maintenance and inspection

Laboratory maintenance is important to ensure efficient operation and the health and safety of the staff. It covers the basic operations, such as:

- Key services of power, water and drainage, fume cupboard and air circulation/air-conditioning systems
- Repair of the working environment (e.g., benches, floors, etc.)
- Cleaning
- Pest control, where appropriate and
- Waste disposal and management.

Laboratories must put procedures in place to deal with each of these operations, including designating responsible personnel to ensure full compliance.

3.5.1. Power, water and drainage; laboratory water quality

It is important to ensure that the laboratory has adequate outlets for power and water and effective drainage is significant to minimize/eliminate the use of extension leads and trailing cables, and long lengths of tubing for water for instance. It is equally important to ensure that power, water and drainage services are effectively maintained and in good working condition for operational reasons and for health and safety purposes and that reported problems are resolved with minimum delay.

There is a need for a stable power supply for sensitive equipment and a backup power supply or emergency generator for times when the laboratory's primary power source is down. A fluctuation of electric voltage in the laboratory is one of the most important reasons, which reduces the longevity of the equipment and sometimes damages it.

Automatic voltage regulation is important for instrumentation to maintain a stable, drift-free operation. Recommended parameters for electrical power include voltage regulation to within 5 to 10% of nominal with minimum line transients and a grounding system. The laboratory ensures that voltage-sensitive equipment is provided with voltage protection devices, such as stabilizers, servo stabilizers or constant voltage transformers as per the recommendations of the manufacturers of the equipment. Constant-voltage transformers are in place to regulate voltage where line fluctuations occur.

Computers, balances and some sophisticated equipment should be connected through uninterrupted power supply (UPS), as any breakdown in the electric power supply during their operation could severely damage some of their sensitive components or data. The laboratory should have a high capacity generator to supply electric current to the whole laboratory in case of power failure. Power failure not only brings the activities of the laboratory to a standstill, it also brings about undesirable irreversible changes in the samples stored in the deep-fridges and refrigerators.

All instruments and equipment should be grounded. Ground fault interrupters are used in wet areas where there is a shock hazard.

Purified water has a very wide range of uses in chemical and microbiological laboratories, from glassware washing to autoclave filling up to HPLC mobile phase preparation. It provides a more consistent, less contaminated reagent than potable water leading to improved reproducibility.

Both, the ASTM International (American Society for Testing and Materials)[18] and the the International Organization for Standardization (ISO)[19] have set standards for defining and

[18] *ASTM D1193 – 06 (2018) Standard Specification for Reagent Water*
[19] *ISO 3696:1987 - Water for analytical laboratory use - Specification and test methods*

categorizing water purity in laboratory-grade water. The specifications cover requirements for water suitable for use in methods of chemical analysis and physical testing. While ASTM International defines four grades of water purity ranging from Types I through IV, the ISO instead uses a scale containing Grades 1 to 3 with grade 1 being the highest quality, most pure water (also referred as ultrapure water), that is more expensive to produce than grade 2 or grade 3 water. These standards could be consulted for the use of the right water quality (parameters) for analyses by the laboratories and for the adequate selection of a water purification application systems and required water quality types.

ISO 3696[19] specifies the use of right water quality for a specific application, while limiting laboratory operating costs. Laboratory Grade 1 water is essentially free from dissolved or colloidal ionic and organic contaminants and suitable for the most stringent analytical requirements and advanced analytical procedures. It is used in laboratory applications, such as HPLC mobile phase preparation, blanks[20] and sample dilution in analysis using a HPLC, AAS, ICP-MS (inductively coupled plasma mass spectrometry) and other advanced analytical techniques; for preparation of reagents for molecular biology applications (DNA sequencing, PCR); and in preparation of solutions for electrophoresis, fingerprinting and blotting. It should be produced by further treatment of grade 2 water (for example reverse osmosis or deionization followed by filtration through a 0.22µm membrane filter of a certain pore to remove particular matter or re-distillation from a fused silica apparatus). Using grade 1 water for grade 2 water applications is a common laboratory practice in order to decrease the risk of artefact generation during experimental procedures.

Grade 2 water is very low in inorganic, organic or colloidal contaminants and suitable for sensitive analytical purposes, and inorganic analytical applications. It is used in general laboratory applications for preparation of reagents for chemical analysis, such as buffers, pH solutions and microbiological culture media preparation and as feed to grade 1 water systems, cell culture incubators and weathering test chamber. It should be produced, for example, by multiple distillation, or by deionization or reverse osmosis followed by distillation.

Grade 3 water quality has the lowest laboratory water grade, and is recommended for glassware rinsing, for filling heating baths and autoclaves. It is suitable for most laboratory wet chemistry work and preparation of reagents solutions, unless otherwise specified. The water quality should be produced, for example, by single distillation, by deionization, or by reverse osmosis.

The provided water quality specifications (parameters and limits are shown by the standards to be checked) are guidelines only. Some specific laboratory applications might require a quality superior to the quality indicated by the standards. For instance, several molecular biology applications require Type 1 (Grade 1) water that is both RNase-free and DNase-free. For elemental trace analysis at sub part per trillion levels water of a higher purity than regular Type 1 (Grade 1) water is required, and glassware washing might require pyrogen-free water for some experiments.

Laboratories should ensure that their water is fit for use. Distilled or de-ionized water systems are monitored at least monthly to ensure the water meets method specific quality attributes (Chapter 3.5.4), e.g., by measuring its electrical conductivity.

[20] *A blank solution is a solution containing little to no analyte of interest, usually used to calibrate instruments. See also: Blanks in method validation, A Supplement to the Eurachem Guide "The Fitness for Purpose of Analytical Methods", 2019, https://eurachem.org/index.php/publications/guides/blanks-in-method-validation*

3.5.2. Fume cupboards, Biosafety Cabinets

The laboratories are equipped with chemical hoods to capture hazardous or odorous materials used or produced in the analyses and to protect employees from hazardous concentrations of airborne toxic substances.

Fixed fume cupboards and portable fume extraction units and chambers require regular maintenance to ensure that they function effectively. They should be periodically tested on the efficiency of airflow (the face velocity) for each unit. National requirements might apply for face velocities for chemical hoods. The draw from a hood in good working repair should be within 80 to 120 FPM (feet per minute) with minimal distortion of air movement (cross-drafts) through the face of the hood from activities or sources in the room. If face velocities on a fume hood are too high, air turbulence will occur between the fume hood's face and a worker. As a minimum, airflow velocities in hoods are measured annually, and whenever there is a fluctuation in performance. Where filters are fitted to the systems, they should be checked at a frequency as defined by the manufacturer/supplier. Contaminated filters should be disposed of in the appropriate manner.

A Class II Biosafety Cabinet (BSC) must be used for working with infectious agents or with tissue culture, for biohazardous analysis and sterility work. A Class II BSC is a ventilated cabinet, which provides personnel, product and environmental protection. It has an open front with inward airflow for personnel protection, a downward High Efficiency Particulate Air (HEPA) filtered laminar airflow over the work surface for product protection, and a HEPA filtered exhausted air for environmental protection. BSCs usually have a magnehelic gauge that monitors the static pressure on the HEPA filter to determine when the filter needs changing. The down flow velocity for EN (European standard) certified class II laminar flow cabinets[21] must not be less than 0.25m/s and for NSF[22] certified cabinets not less than 0.51m/s. In addition, BSCs are monitored for particulates whenever there is a concern. The certification of BSCs is required before operation. Chemical hoods and Biosafety Cabinets are visually checked for proper operation before each use and both are ideally certified annually.

The operation sterility of a BSC can be tested by a classical microbiological sedimentation test with an exposition of the plate for 1 min under operational conditions. Overloading the BSC is not recommended; flame burners and heavy equipment are not allowed to be placed in BSCs.

For more information on classes of Biosafety Cabinets, reference is provided for instance to the Laboratory Biosafety Manual of the World Health Organisation[23] and to "A Guide to Biosafety & Biological Safety Cabinet by Esco" (see Annex 2 for the reference and, also Chapter 3.7).

3.5.3. Air circulation and air-conditioning systems

Laboratories are equipped with climate and ventilation control designed to ensure proper ventilation at a suitable temperature throughout, with an active ventilation system and adequate space for circulation of people, laboratory carts and trolleys. Air circulation and air-conditioning systems maintain the environmental conditions of the laboratory and provide a comfortable working environment for

[21] *Class II BSC is specified in the European Standard (EN 12469) and the Class II, Type A2 BSC is specified in NSF/ANSI 49, YY 0569*

[22] *NSF International (National Sanitation Foundation International) is an American product testing, inspection and certification organization*

[23] *WHO, Laboratory Biosafety Manual, 4th edition https://www.who.int/publications/i/item/9789240011311*

the staff. These systems respect the environmental requirements of the test method and of the equipment used in the testing. Natural ventilation is not recommended in clean rooms or workrooms handling pathogens.

The ventilation systems and their filter should be regularly maintained and filters changed when necessary. Where air conditioners are used, filters should be appropriate, inspected, maintained and replaced according to the type of work being carried out. An overload of particulate matter in/around the filter can cause breakdowns or result in damage to the filter and dirt blown into the laboratory area, with the consequent risks of contamination or damage to sensitive items of equipment.

Regular monitoring of the temperature for work areas will indicate if a problem is developing with the efficiency of the systems (Chapter 3.6).

3.5.4. Work environment

The laboratory environment, services and facilities should be sufficiently uncrowded, clean and tidy to ensure the quality of the work carried out and not to compromise it (Chapter 3.4). Measures should be taken to avoid accumulation of dust, e.g., by the provision of sufficient storage space and minimal paperwork in the laboratory and by prohibiting plants and personal possessions in the laboratory work area. Stored items and equipment are arranged to facilitate easy cleaning. No furniture, documents or other items, other than those strictly necessary for testing activities, are to be found in the laboratory.

Floors are clean, dry, and in sound condition so there are no tripping hazards. The floors, walls, ceilings, laboratory bench tops and furniture should be subject to regular maintenance and repair in order to avoid cracks where dirt might particularly accumulate and thus cause a source of contamination. All cases of damaged bench surfaces and floors should be reported and they should not be used until repaired. Any temporary repair must not introduce any further safety issues.

In the microbiological suites, samples/reference materials should be handled in laminar flow cabinets under a filtered, clean air supply.

Work and storage areas and restrooms should be free of noxious odours. It is recommended to maintain exhaust ventilation for 24 hours per day in any area where chemicals are stored or used.

The laboratory should maintain sufficient illumination to perform certain procedure. The noise levels should conform to national Occupational Health and Safety guidelines. Specialized lighting might be required in areas where direct sunlight can be deleterious to samples, reagents and media or can interfere with instrumentation or analysis. Direct sunlight is not allowed in microbiological laboratories.

3.5.5. Cleaning and housekeeping

To facilitate the efficiency of laboratory operations, laboratories apply Good housekeeping to maintain laboratory areas sufficiently clean and orderly to prevent contamination of samples with a minimum of the following:

- Sweeping or mopping floors, including walk-in refrigerators
- Cleaning up spills immediately. The laboratory has a system for reporting and recording all spillages and where foreseeable, has a documented procedure for dealing with specific types of spillages.
- Adequately decontaminating and cleansing glassware
- Cleaning contaminated equipment, removing all chemicals upon completion of analysis, removing all contaminants when the equipment is placed in surplus;
- Disposing of chemical and biological wastes, hazardous and infectious wastes as well as

universal wastes properly (in line with procedures, guidelines and legislation that applies)
- Controlling pests; the use of aerosol agents to eliminate pests is discouraged as they can cause cross-contamination to samples for several chemistry analysis, such as pesticides
- Emptying trash cans
- Cleaning restrooms and
- Monitoring storage areas to ensure storage conditions are clean and dry, there is no leakage of product, timely disposition of materials, and proper containment of offensive materials (e.g. any inflammable, dangerous, noxious, or deleterious substance, material).

Regular cleaning and disinfection is required in order to keep the premises in a condition suitable for conducting the tests. It is important that all areas of the laboratory are cleaned and maintained on a regular basis. Laboratories use documented cleaning programmes for laboratory fixtures, equipment and surfaces, taken into account the results of environmental monitoring and the possibility of cross-contamination. Cleaning and disinfection of laboratory areas are recorded, including the date and name of the person performing the maintenance. Routinely, microbiological laboratories use a series of antimicrobial cleansers at the end of an analysis, at the end of the day, or in the event of a spill to minimize any potential microbial contamination.

Examples of areas that are daily cleaned and disinfected:

- Technical staff performing tests clean and disinfect benchtops after completing examinations and after any spills of samples or reagents. All workbenches are wiped each working day using the materials defined in the cleaning policy for the type of laboratory. All other laboratory surfaces (e.g., door handles, windows, cupboards, etc.) are wiped weekly.
- Floors are usually cleaned by cleaning personnel, unless restricted access allows only technical staff to disinfect the floors at the end of the day. Sweeping must be carried out carefully to minimize the amount of dust in the air that can settle on, and contaminate, workbenches. Floors should normally be washed with a detergent solution twice each working week.

Other areas of the laboratory should be scheduled for cleaning on a weekly or monthly basis, depending on laboratory conditions. For example, ceilings and walls may require a weekly cleaning, whereas items such as refrigerators and storage areas might be scheduled for a monthly cleaning.

3.5.6. Waste disposal and management

Procedures for waste disposal are important to ensure that laboratories are kept free from unwanted and used materials to minimize the risk of contamination.

Laboratory waste management is a critical issue. Quantities of hazardous materials (chemical and biological) should be kept to a minimum, and disposed of with due regard to public and environmental health. The correct disposal of reagents and samples is a matter of good laboratory practice complying with national environmental, health and safety regulations. Laboratories should be sure to consider how liquid wastes will be handled in the first place in order to prevent contamination of community sewage systems with pathogens or toxic chemicals and to comply with local and national requirements for liquid waste disposal.

Laboratories should use separate waste containers depending on the nature of the waste (e.g., contaminated materials, waste solvent, etc.), clearly identified and adequately labelled, for instance by a colour code. Such containers, (e.g., contaminated waste, waste solvent etc.). are kept separate from reactive substances. Specific attention should be given

to the management of potentially harmful contaminated waste such as sharps, needles or broken glassware. Sharps containers must be available on workbenches so they are conveniently accessible to staff.

Each working day, all waste bins should be emptied in a manner following the defined procedures of the organization for the level of hazard associated with the waste. Items that have come in contact with microbial samples (e.g., pipettes and pipette tips) are usually discarded in jars containing disinfectant. Periodically, the contents must be emptied and decontaminated in an autoclave.

All waste must be kept in a locked and ventilated store whilst awaiting disposal. For disposal of hazardous waste national legislation should be considered and adequate services rendered, if available. Disposal companies might advise on how they want the waste to be segregated and packaged.

3.6. Environmental monitoring

Accredited laboratories must monitor, control, record and periodically review environmental conditions, where they influence the validity of the results and as required by the relevant specifications, methods, and procedures they use to prevent contamination, interference or adverse influences on laboratory activities.

Influences that can adversely affect the validity of results (and are subject to measures and control) comprise, but are not limited to microbial contamination, dust, electromagnetic disturbances, radiation, humidity, electrical supply, temperature, sound and vibration. For microbiological laboratories, in particular ensuring protection against excess temperature, dust, humidity, steam, vibration, and exposure to direct sunlight is crucial.

Laboratory temperature and humidity are two factors in creating ideal laboratory conditions. However, work areas of laboratories should be free of temperature extremes that are hazardous to health or that interfere with safe operations. The right temperature helps to get accurate results, while improper climate control might lead to growth of microbes and bacteria. Silicone stoppers, for example, might contaminate if the climate is too dry due to enhanced static build up on the stoppers.

In laboratory rooms where these might affect the analytical results, temperature and humidity are monitored and recorded. Many organizations have outlined laboratory temperature and humidity requirements to prevent sample contamination[24]. A comfortable working environment is considered 20 – 25°C with relative humidity of 35 – 50 %, depending on the geographical area[25]. Monitoring temperature of chemistry laboratories is necessary for reference materials, chemicals reagents and in cases when data will be affected, once a specific range of temperature is not observed, e.g., in heavy metal analysis. Many temperature and humidity controller are available on the market, while the use of a (wireless) temperature data logger will support identification and tracking fluctuations in temperature 24/7.

[24] *E.g., The World Health Organization; The International Organization for Standardization; The Food and Drug Administration Office of Regulatory Affairs*

[25] *Food and Drug Administration Office of Regulatory Affairs, ORA Laboratory Manual Volume I; https://www.fda.gov/media/73912/download (example)*

Other critical environmental conditions are observed by monitoring systems or by quality control results produced during the particular tests. Microbiological laboratories keep appropriate environmental monitoring programmes, including, for example, frequent use of air settlement plates for bacterial and fungal contaminants as well as periodic surface swabbing (bench surfaces, hoods) for a variety of relevant microorganisms, especially pathogens. Acceptable background counts are defined and documented procedures are available for dealing with situations when limits are exceeded. Overall, microbiological laboratories apply the following principles to address prevention of cross-contamination:

- Clear segregation of samples, references and media storage (and checks)
- Dedicated laboratory coats and footwear with a changing area where staff can wash
- A planned cleaning regime for the laboratory, covering benches, floors, windows, light fittings, ventilation grills, air conditioners, water baths and autoclaves (Chapter 3.5.5). For the frequency and actual scope of these activities guidance exist, e.g., by producers of the cleaning and disinfections preparations and accreditation bodies will normally have technical guidance documents specifying their particular expectations
- Regular monitoring of the environment with surface swabs and air plates. A weekly regime is typical (see above)
- Documented procedures for dealing with spillages and records of spillages and action taken
- Monitoring of temperature and humidity: limits need not be stringent, but humidity above 50% and temperature above 25°C can lead to problems of mould growth. Contractors that perform a portion of the housekeeping responsibilities are not allowed in certain laboratories without escort and guidance.

In the chemical laboratories, air monitoring is conducted whenever there is a complaint of odours or other suspect indications of the presence of a chemical. In laboratories that perform metal analysis, benches, hoods and glassware are monitored periodically for metal contamination.

In case of molecular techniques, monitoring for DNA contaminants should be undertaken by employing a "No Template Control" (NTC)[26]. Routine insertion of analytical and media blanks with sample analysis could detect laboratory environmental contamination and any cross-contamination.

Water used for microbiology analyses is verified monthly for acceptable levels of chlorine and aerobic plate count. Additional tests are performed on the water systems as defined by the laboratories.

Analyses of samples are not performed and be stopped, when the monitoring reveals that required environmental conditions are not met, jeopardizing the results of the tests. Requirements for facilities and environmental conditions necessary for the performance of the laboratory activities must be documented.

Monitoring equipment must be adequately maintained, verified and/or calibrated (Chapter 5.6).

Analysis of data from monitoring and quality control should enable the detection of trends in levels of contamination. The impact of such failures could be assessed as part of ruggedness testing during method validation (Chapter 9, Annex 3) and be followed up as nonconformity by the management system. Accredited laboratories maintain records of the ongoing monitoring and periodic review with respect to the facility and environmental requirements.

[26] *Non-template control omits any DNA or RNA template from a reaction and serves as a general control for extraneous nucleic acid contamination*

3.7. Safety

Laboratories should have developed a complete description of basic safety rules and its organization, ensuring that personnel are trained in their specific duties when new activities or techniques are introduced into the laboratory.

Each member of the laboratory staff must be familiar with all potential hazards. The Safety Data Sheet (SDS) supplied with each chemical should be available for immediate reference. Components of the globally Harmonized System of Classification and Labelling of Chemicals (GHS) including pictograms, signal words, hazard statements, hazard categories (ranking), and precautionary statements are explained and understood by the laboratory personnel.

Procedures must be put into place for dealing with all potential hazards to minimize any risks associated with their use. They should include provision for all hazardous and volatile reagents to be handled only in an enclosed fume cupboard. The provision of personal protective equipment should not be an alternative to the introduction of safe working procedures, but should complement those procedures.

Chemicals and solvents are stored compatibly and in accordance with the manufacturer's guidance in the in the Safety Data Sheet (SDS) and the fire code. Shelving units in storage areas are braced to prevent collapsing of the shelves. All stored hazardous chemicals should carry correct labelling to indicate hazards[27]. Visual inspection of the chemical and its container should be carried out on a regular basis and a procedure should be in place for dealing with any issues that may be identified, such as damage to the container, illegible labels, etc. An appropriate chemical inventory management system, which as a minimum includes an inventory list, would assist in monitoring laboratory chemicals on a regular basis. Safety Data Sheets (SDS) must be readily available for all hazardous chemicals stored, and these should be referred to for advice on storage, accidental release measures and incompatibilities.

As a rule, diagnostic laboratories working with pathogens in food safety should be designed and organized for biosafety level 2 (BSL-2) safety cabinets (also named class 2 safety cabinets, cleanroom safety cabinets or sterile safety cabinets, see also Chapter 3.5.2). BSL-2 is suitable for experiments involving agents of moderate potential hazard to personnel and the environment. The organisms that require BSL-2 in laboratories include e.g., the pathogenic strains of E. coli, Staphylococcus, or Salmonella. The control of potential biohazards at the BSL-2 level is provided by use of standard microbiological practices with the addition of personnel protective equipment (laboratory coat and gloves).

Basic Laboratory Safety covers:

- Stating the general rules for working safely in the laboratory
- Describing the possible routes of exposure for a hazardous material
- Explaining the reasons food and drinks are not permitted in the laboratory
- Listing the general considerations for appropriate waste disposal
- Stating the general hazards associated with mercury, mercury compounds, and pyrophoric compounds etc.
- Identifying potential unusual situations or unplanned events in the laboratory (e.g., chemical spills, odours)

[27] *For instance, according to CLP (Classification, Labelling and Packaging) Regulation (EC) No. 1272/2008. It handles the classification, labelling and packaging of chemical substances and mixtures released on the EU market*

- Explaining the reasons of long hair that is not tied back, neckties, jewellery, and loose articles of clothing are considered hazards
- Stating the purpose of regulatory agencies
- Stating the purpose of the Chemical Hygiene Plan[28] and components
- Using hazard information to prepare labels, as per GHS, for secondary containers and information from the SDS
- Identifying common safety concerns upon casual examination of a laboratory
- Adequate hand washing facilities should be available and a policy regarding appropriate glove use should be in place to avoid the spreading of microorganisms in the microbiological laboratory.

In the area of personal hygiene and personal protective equipment, precautions must be taken to avoid contamination of the samples and culture media, but also to avoid risk of infection for personnel. The following is advised in a microbiological laboratory:

- Wearing laboratory clothing, clean and in good conditions, texture inflammable; not wearing this clothing outside the work areas and possibly cloakrooms
- If necessary protection for hair, beard, hands, shoes, etc.
- Washing hands thoroughly
- When inoculating avoiding speaking
- Taking precautions when persons have infections, not to invalidate results
- Not storing food for personal consumption in the laboratories' refrigerators.

Sometimes a list of do's and don'ts can be a helpful notice of safety issues to the laboratory staff with reminder trainings.

[28] *The plan outlines policies, processes, protective equipment, etc. to safeguard employees from hazardous chemical substances in the laboratory setting*

4. Personnel

ISO/IEC 17025[1], sections 5.5; 5.6; 6.2

Personnel are the most important laboratory resource. The provision of effective laboratory services requires a combination of good management, well-trained staff and effective technical staff supervision. Recruiting and retaining qualified staff is essential to laboratory quality.

4.1. Staff requirement

Management of personnel is critical to the success of a quality management programme. Several elements are important in this management process. Job descriptions should reflect all skills needed and accurately describe tasks, roles, and authorities.

The laboratory specifies the responsibilities, authorities and inter-relationships of personnel and their particular duties, including implementing, maintaining and improving the management system, as required for accreditation.

As a head of laboratory, it is important to hire an appropriate number of staff to cover the workload and to train each employee in their specific duties, to provide orientation for new employees and opportunities for continuing education, new techniques or updates for existing methods. Annual employee performance appraisals should be conducted.

The laboratory management normally defines the minimum levels of qualification and experience necessary for the key posts within the laboratory. These should include for chemical/microbiological laboratories:

- Technical manager/laboratory manager
- Chemists (Food Chemists)
- Microbiologists
- Laboratory Technicians and support staff.

Chemical analysis must be carried out by, or under the supervision of qualified, experienced and competent analysts. In the chemistry section, the technical manager/laboratory manager and chemists should be at graduate level with experience of analytical chemistry. Other senior laboratory staff will normally possess similar competencies. Lower formal qualifications may be acceptable, when staff has extensive relevant experience and/or the scope of activities is limited. Staff qualified to degree level will normally have at least two years relevant work experience before being considered as experienced analysts. Staff undergoing training or with no relevant qualifications may undertake analyses, if they have demonstrably received an adequate level of training and are adequately supervised. In certain circumstances, the minimum requirements for qualifications and experience for staff carrying out particular types of analysis might be specified in regulations.

Microbiological testing should either be performed or supervised by an experienced person qualified to a degree level in microbiology or equivalent. Alternatively, qualifications might meet requirements where a member of staff has extensive relevant experience to perform work covered by the scope of accreditation without supervision, or before being considered as experienced for supervision of accredited work. Specific national regulations may override this. If the background in food microbiology, e.g., by microbiology

graduates is not strong enough, it could be augmented by training, either through appropriate courses or on-the-job mentoring by a suitably experienced colleague.

The personnel in charge of performing tests should have a good knowledge and sufficient practical experience with microbiological techniques and the microorganism sought. They should be able to interpret the accuracy and precision required to yield acceptable results. For that, they can take part in Proficiency Testing (Chapter 12.3), use reference materials (Chapter 11) or achieve self-assessment tests for enumeration of microorganisms for instance (Chapter 4.2).

Staff numbers will obviously reflect the volume of work that the laboratory has to undertake. In most cases, additional support will be at the technician and support grade level. In exceptional cases, an additional chemist or microbiologist may be required. Technicians could be graduates, but this is not so critical, provided they have some basic chemistry/microbiology qualifications (A level, diploma or equivalent) and receive appropriate on-the-job training.

4.2. Staff competence

Personnel performing specific tasks in laboratories must be qualified based on appropriate education, training, and experience and/or demonstrated skills, as required.

The laboratory management ensures the competence of all who operate specific equipment, perform tests, evaluate results, give statements of conformity and opinions/interpretations, report and sign test reports. The competence requirements of staff is based on education, experience, demonstrated skills, and training. Importantly, accredited laboratories authorize specific personnel to develop, modify, and validate methods and to analyse results. If opinions and interpretations of test results are part of reports, these must be executed by authorised personnel with suitable experience and relevant knowledge of the specific application.

The laboratory should maintain records of the relevant authorization(s), competence, educational and professional qualifications, training, skills and experience of all technical personnel, including contracted personnel. This information should be readily available including the date on which authorization and/or competence is confirmed. Access to these training records will be necessary in the course of everyday work. Access to other staff records, usually held centrally by the laboratory and listing personal details, might be restricted by national legislation on data protection.

The process of assessing the qualification of personnel is organised as follows: The laboratory formulates the necessary requirements (e.g., higher-level functional or individual job descriptions) covering required expertise and experience of the personnel. This might include a technical degree, certificate, diploma; involvement in publications; required qualifications and training programmes, e.g., records of the involvement in testing operations and assessment of the participation; records of involvement in internal or external comparisons; record of involvement in research partnership/research networks; record of involvement in standardization work; records of specific evaluation. For non-frequent activities (>12 month), records of the performance of reference tests might be necessary. Overall other requirements cover human behaviour, language skills etc. The laboratory management then evaluates the qualification, the

correspondence between job description and staff knowledge by reviewing the adequacy of education, training, experience and/or demonstrated skills. If evidence is available to demonstrate that a person meets all the criteria, he/she is considered qualified. If not, training measures must be taken including the evaluation of the new competence and its recording.

Accredited laboratories should supervise personnel before authorisation and monitor them after authorisation (Chapter 4.3). Skills of personnel are based upon demonstration of competence. Personnel may only perform tests on samples, if they are recognised as competent to do so, or if not, are under adequate supervision. The required demonstration should be completed successfully before laboratory personnel generate data independently. For instance, the interpretation of test results for identification and verification of microorganisms is strongly connected to the experience of the performing analyst and therefore should be monitored for each analyst on a regular basis.

The assessment of the competence of staff has to be fit for purpose by determining that the staff is capable of generating technically valid results. The better the competence specifications are defined, the easier it will be to demonstrate the fulfilment of them. It is the laboratories' responsibility to find a good balance between competence assessment of the staff and other quality requirements with impact on the test results. The assessment and the competence of staff should attribute to continuous improvement for the benefits of the laboratories and their clients. Competency requirements for each function influencing the results of laboratory activities are documented in the laboratory training documents.

The use of a database can improve the laboratory's capability to identify the right person for a particular job quickly.

4.3. Staff training and monitoring

The laboratory management should formulate the goals with respect to the education, training and skills of their laboratory personnel. It should have a policy and procedures for identifying training needs and for providing training to personnel with a training programme relevant to the present and anticipated tasks of the laboratory as well as procedure for evaluation of training effectiveness.

The laboratory management ensures that all personnel have received adequate training for the competent performance of tests and for the operation of equipment. In general, each member of staff must be trained in all aspects of their duties, whether it is in the use of specific items of equipment or full analytical procedures. For a microbiologist this should include e.g., training in basic techniques such as plate pouring, counting of colonies, aseptic technique, etc., with acceptability determined using objective criteria. Where appropriate, training in the principles and theory behind particular techniques is required. All personnel should receive relevant updated information as necessary in hygiene and laboratory safety matters.

Continuing education is vital to personnel competency, but does not need to be expensive. New testing methodologies and instruments are constantly introduced to the marketplace. Employees need to update their knowledge and skills. Although the laboratory management is responsible for ensuring adequate trainings, particularly amongst more experienced analysts self-training is relevant and is a strong element of competent laboratories.

The training programme should address what is relevant for initial training and what for further training. Where a method or technique is not in regular use, verification of personnel performance before testing might be necessary and periodically re-training. The critical interval between performances of tests should be established and documented. This may require a re-evaluation of competence. Training and qualification programs can be outputs of research and technical development activities of a laboratory. For repetitive, but not frequent activities, a very detailed testing procedure can be used to reduce the training programme and to verify that staff understands of the procedure before use.

Usually laboratories have a monitoring plan for personnel. Both specifications and qualifications of personnel are regularly reviewed, taking into account the current and future needs of the laboratory and its customers. Skills of personnel, based upon demonstration of competence, are evaluated at the time of hiring and monitored on a regular, recurring basis. Where possible, the laboratory should use objective measures to assess the fulfilment of competence during training, based on procedures, with provision for retraining, where necessary. It is recommended to record these activities. The most frequently used supervision/monitoring methods cover:

- Measuring known samples: Reference standards, inter-comparison samples etc.
- Measuring blind samples
- Participating in intra- and/or interlaboratory comparisons and proficiency testing schemes
- Exams (for intellectual knowledge).

The laboratory should maintain up-to-date records of the training proving that individual members of staff have been adequately trained and that their competence to carry out particular tests has been assessed. The records should typically include:

- Academic qualifications
- External and internal courses attended
- Relevant on-the-job training (and retraining as necessary)
- Possibly also: participation in quality control (QC) and/or proficiency testing schemes, with associated data (Chapter 12)
- Technical papers published and presentations given at conferences.

The competence of personnel to perform tests should be documented in relation to the results of internal and external quality control. In some cases, it may be relevant to state any particular limitations to evidence about competences.

The effectiveness of the competence process (Chapter 4.2) and training actions taken must be evaluated and documented, e.g., at management reviews, internal audits, by external assessments, proficiency testing, and performance evaluations to verify that the operations continue to comply with the requirements of the management system and ISO/IEC 17025. The effectiveness of the training programme, as well as the identification of further training needs, should also be evaluated based on results from competence monitoring and witness audits.

For further reading related to assessing the competence of personnel reference is provided to EUROLAB Cook Book No 6[29] and Cook Book No 11[30]. The later covers introducing new staff members and its purpose, objective, content and duration of induction training, documentation and records, and effectiveness. A well-organised induction training targeted to the needs of the new jobholder is a pro-active effort that is worth spending.

[29] *EUROLAB Cook Book 6 - How to assess the competence of Staff. https://www.eurolab.org/CookBooks/6*
[30] *EUROLAB Cook Book 11 - Induction of new staff members. https://www.eurolab.org/CookBooks/11*

5. EQUIPMENT

ISO/IEC 17025[1], section 6.4.; ISO 7218[17], ILAC P10:07[31]; ISO 10012[32]; Eurachem/CITAC Guide to Quality in Analytical Chemistry[33]; Eurachem Guide: Accreditation for Microbiological Laboratories[34]; EURAMET calibration guide[35]; ISO 8655 standards[36]; ISO 4787[37].

5.1. Equipment management and qualification

Accredited laboratories must document the procedures and requirements for handling, transport, storage, use and maintenance of equipment. The term equipment includes, but is not limited to measuring instruments, software, measurement standards, reference material, reagents, consumables or auxiliary apparatus (ISO 17025). Reference material, measurement standards, reagents and consumables are specifically addressed in Chapters 7 and 11 of this Manual to which the addressed principles of this chapter apply.

In general, laboratories should have access to equipment that is required for the correct performance of laboratory activities. Equipment used for measurements must achieve the required measurement accuracy and/or measurement uncertainty to provide a valid result. Laboratories further ensure that their equipment meets documented specifications to confirm proper functioning and to prevent equipment contamination or deterioration.

Functional and reliable equipment contributes to the technical ability of a laboratory. A proper equipment management at laboratories supports to maintain a high level of laboratory performance. It ensures accurate, reliable and timely testing, due to:

- Reducing variation in test results, and improving the technologist's, personnel's confidence in the accuracy of testing results
- Lowering repair costs, as fewer repairs will be needed for a well-maintained instrument
- Lengthening instrument life
- Reducing interruption of services due to breakdowns and failures
- Increasing safety for workers and
- Producing greater customer satisfaction.

When putting an equipment management programme in place, the laboratory and the responsible person must consider:

- What criteria should be used to select new equipment?

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[31] *ILAC P10:07:2020, ILAC Policy on the Traceability of Measurement Results; https://ilac.org/publications-and-resources/ilac-policy-series/*

[32] *ISO 10012:2003, Measurement management systems — Requirements for measurement processes and measuring equipment*

[33] *V. Barwick (Ed), Eurachem/CITAC Guide: Guide to Quality in Analytical Chemistry: An Aid to Accreditation (3rd ed. 2016). ISBN 978-0-948926-32-7. Available from www.eurachem.org.*

[34] *M. Eleftheriadou and K. C. Tsimillis (Eds), Eurachem guide: Accreditation for Microbiological Laboratories, Second edition (2013), ISBN: 978-91-87017-92-6. Available from www.eurachem.org.*

[35] *Guidelines on the Calibration of Non-Automatic Weighing Instruments, EURAMET Calibration Guide No. 18 Version 4.0 (11/2015)*

[36] *ISO 8655-1:2022, Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations; ISO 8655-2:2022, Part 2: Pipettes; ISO 8655-3:2022, Part 3: Burettes; ISO 8655-4:2022, Part 4: Dilutors etc. (for more see Annex 2)*

[37] *ISO 4787:2021, Laboratory glass and plastic ware — Volumetric instruments — Methods for testing of capacity and for use*

- What are the installation requirements for new equipment and who will install the new instrument?
- Calibration and performance evaluation: What is needed to calibrate the equipment and to validate it for its correct operation? How will these procedures be conducted for both old and new instruments?
- What maintenance schedule is recommended by the manufacturer? Are additional preventive maintenance procedures required? Are current maintenance procedures conducted properly?
- Is there a clear procedure for troubleshooting for each instrument?
- What are the costs for service and repair? Is there necessary service and repair in the geographical area?
- What must be done to dispose of old equipment when it needs to be replaced?

Equipment management covers the process of equipment qualification that ensures that equipment performance is appropriate for its intended use. It comprises usually four (4) levels, each dealing with different aspects of the equipment history:

➢ Level I (Design Qualification, DQ) – Selection of an instrument and supplier

Key functions and levels of performance are defined, also requirements for other services, such as calibration, maintenance and training, according to the needs and the intended use of the instrument and the laboratory's capabilities.

➢ Level II (Installation Qualification, IQ) – Installation and release for use

The level is covering operations to be performed and documented when the equipment is received and installed, before it can be released for routine use. It usually includes checks that the equipment is received in good condition as ordered, and provides an assessment of its full functionality in the selected environment. It covers the start-up checks undertaken by the instrument supplier, followed by a full check of the equipment's key performance parameters, irrespective of any analytical method. Whenever required, calibration (5.4.) is performed as part of this stage. A documented release for use should be authorised by the person responsible for the instrument.

➢ Level III (Operational Qualification, OQ) – Periodic and motivated instrument checks

The checks performed before release form the basis for periodic assessments of the instrument's functionality (Level III), performed at intervals, which will depend on the frequency of use and knowledge of the stability of the instrument in the conditions of use. Checks should be performed, if the instrument is moved to a new environment, or undergoes significant repair or maintenance operations. For measuring equipment, a process of metrological confirmation must be devised to ensure that relevant metrological characteristics are kept under control (see below). Acceptance criteria for the tested parameters should take into account the specification from the manufacturer of the instrument as well as the requirements for the intended use of the equipment.

➢ Level IV (Performance Qualification, PQ) – In use instrument checks

Checks of the equipment performance during routine use must be planned, to confirm, that the same quality level is achieved on a day-to-day basis. They are usually built into the analytical methods themselves, as analytical response for blanks and calibration standards as well as quality control and acceptance criteria given by methods or legislation. Control charts (Chapter 12.4) of such responses (response blank, quality control sample) of the conducted analytical methods allow for the recording and monitoring of the equipment's performance over time. Further guidance and practical examples for instance for the

qualification of spectrophotometers, mass spectrometers, and HPLCs are provided by Official Medicine Control Laboratories (OMCL)[38]. For more information, see also Chapter 5.6.

ISO 10012[32] provides guidance related to managing measurement processes and the metrological confirmation of measuring equipment that typically includes:

- Calibration and checks of the calibration status
- Maintenance and/or repair, followed by recalibration as necessary
- A comparison with the metrological requirements for the intended use
- Sealing and/or labelling as required.

Typical examples of characteristics for which metrological requirements should be established are: measuring interval, resolution, repeatability and trueness (see also Chapter 9.2. and Annex 3).

After checks of the correct functioning to ensure conformity with the specified design, dimensions and performance requirements (level II), the following has to be addressed for new installed equipment:

- Assigning responsibility for performing the maintenance and operation programmes
- Developing a system for recording the use of parts and supplies
- Implementing a written plan for calibration (Chapter 5.4), performance verification (Chapters 5.6 and 5.7) and proper operation of the equipment
- Establishing a scheduled maintenance programme that includes daily, weekly and monthly maintenance tasks (Chapters 5.2. and 5.3.)
- Providing training for all operators in operation and maintenance, and only personnel trained specifically to properly use the equipment should be authorized as operators.

The laboratory should ensure that the operation of equipment is only conducted by competent and authorised personnel including disposition of equipment (e.g., in case of damaged or broken seals), for instance by clear instructions. It is the responsibility of the head of laboratory or the Technical Manager to oversee all the equipment management systems in the laboratory, and to ensure the training programme for operators including an understanding how to both properly operate the instrument and to perform all necessary routine maintenance procedures.

The oversight of an equipment management programme covers monitoring the equipment management activities, including reviewing all equipment records routinely, updating maintenance procedures, as necessary, and ensuring that all procedures are followed. These responsibilities might be assigned to personnel in the laboratory with good skills in equipment maintenance and troubleshooting. Daily maintenance should be the responsibility of the technical operator, while everyone who uses the equipment must be trained in calibration and daily maintenance. In larger laboratories, individual responsibilities for each piece or category of equipment (plus deputy) are appointed.

Accredited laboratories might no longer keep a master list of all equipment, while it is good practice to keep an equipment log for each equipment item. It should start with details of the checks and calibrations carried out before the equipment is placed in service and continue with a detailed record of all calibrations, repairs, and routine maintenance and performance checks. Any supporting documentation, such as service reports, calibration certificates and output from performance checks, should be

[38] *PA/PH/OMCL (08) 73 2R - OMCL (Official Medicine Control Laboratories) Network of the Council of Europe, Guideline on Qualification of Equipment – Core document, 1st July 2011 (see at https://european-accreditation.org)*

attached, so that this record becomes a complete history of the equipment and its state of calibration and performance can be demonstrated at any point in time.

It could be useful to have a copy of operating procedures for the equipment as part of the equipment log. In cases where equipment operation is described adequately in the methods documentation, there is no need to repeat this information in the equipment log. For smaller items of equipment, a composite log (e.g., covering all of the laboratory's thermometers) would be appropriate. Historical information from an inventory of existing equipment (e.g., copies of service reports, calibration history, commissioning reports) could be included in the equipment log.

Each piece of equipment has a record of:

- The identity of the equipment, including software and firmware version
- The manufacturer's name and contact information, identification (make and model number), and serial number or other unique identification so that any problems can be discussed with the manufacturer
- Date the equipment was purchased, and whether it was purchased new, used or reconditioned
- Presence or absence of documentation
- Spare parts and maintenance contract
- Warranty expiration dates
- Specific inventory number indicating the year of acquisition
- Evidence of verification that equipment conforms with specific requirements
- The current location
- Calibration dates, results of calibrations, adjustments, acceptance criteria, and the calibration interval
- Documentation of reference materials, results, acceptance criteria, relevant dates and the period of validity
- The maintenance plan and maintenance carried out to date, where relevant to the performance of the equipment
- Details of any damage, malfunction, modification to, or repair of the equipment.

There are also rules and best practices to be followed in order not to invalidate results that relate to equipment (ISO 17025), such as for instance:

- Equipment, hardware and software must be safeguarded from unauthorised changes and adjustments that could invalidate results. The laboratory must take adequate practicable measures for that, e.g., by use of password protected software and sealing access to adjustable devices on equipment so that tampering is clearly apparent. The laboratory must retain records to prevent unintended adjustments of equipment from invalidating results, where applicable.
- Equipment that has been subject to overloading or mishandling, giving questionable results, or has been shown to be defective or outside specific requirement must be taken out of service by either isolating it to prevent its use or by clearly labelling or marking it as being out of service, until it has been verified to perform correctly. The laboratory must examine the effect of the defect or deviation from specified requirements and should initiate the management of a non-conforming work procedure.
- Equipment undergoing checks (e.g., during calibration or after giving suspicious results) must either be segregated or clearly labelled, so that there is no possibility of it being inadvertently used for routine work, until it is formally accepted.

5.2. Equipment maintenance, verification and inspection

All equipment used in laboratories (including any associated software) must be of a specification sufficient for the intended purpose, and kept in a state of maintenance and metrological control consistent with its use[34]. Maintenance of equipment is essential to maximize equipment's operational life, to ensure that it functions to an acceptable standard, also to minimize the risk of malfunctioning causing delays in testing of samples that have been submitted.

Routine or preventive maintenance is a procedure that laboratories apply to minimize the likelihood of instrument malfunction that can range from inconsistencies in the results obtained to a complete breakdown. Maintenance operates at two levels, conducted by laboratory staff and maintenance that necessitates the visit of an external engineer. Daily maintenance should be under the responsibility of the technical operator trained in calibration and daily maintenance.

In the case of new equipment, and particularly with sophisticated analytical equipment, it is important to ensure that the installation engineer delivers a course in routine maintenance to laboratory personnel, covering issues that the laboratory itself can undertake. This normally comprises the replacement or cleaning of certain easy to access parts, since frequent attention to such parts can reduce the risk of instrument malfunction and the identification and maintenance of stock of replacement parts. The manufacturers could assist already in identifying such parts and its provision, e.g., as part of the equipment procurement process.

With additional training and experience, tasks that are more complex could be carried out by the trained operator including, for example, replacing detector units in a gas chromatograph or cleaning the source in a mass selective detector to avoid the risk of causing further damage or affecting the calibration of the instrument.

There are typically four (4) categories of equipment used by food testing laboratories. These categories have specific requirements related to maintenance by cleaning and servicing, inspection for damage, and in the verification of equipment suitability that are briefly presented as follows:

1. General service equipment that is not used for conducting measurements or with minimal influence on measurements (e.g., hotplates, stirrers, non-volumetric glassware and glassware used for approximate volume measurements such as measuring cylinders) and laboratory heating or ventilation systems.

It will typically be maintained by cleaning and safety checks as necessary. Calibrations or performance checks become necessary where the setting can significantly affect the test or analytical result (e.g., the temperature of a muffle furnace or constant temperature bath) and such checks must be documented. For microbiological laboratories cross-contamination must be avoided arising from equipment, for example disposable equipment should be clean and sterile when appropriate and re-used glassware should be properly cleaned and sterilised. Ideally, laboratories should have a separate autoclave for decontamination. If precautions are taken to separate decontamination and sterilisation loads, one autoclave is acceptable, provided that an adequate and documented cleaning programme is in place to address both the internal and external environment of the autoclave.

2. Measuring instruments (used to measure the unit value of a quantity) including volume measuring instruments equipment (e.g., flasks, pipettes, pycnometers, burettes etc.) and other measuring instruments (e.g., hydrometers, U-tube viscometers, thermometers, timers, spectrometers, chromatographs, electrochemical meters, balances etc.).

The correct use of this equipment is critical to analytical measurements, including maintenance and calibration in line with environmental considerations. The performance of some volumetric glassware depends on certain factors that could be affected by cleaning methods for instance. Thus strict procedures for maintenance are required and possibly more regular calibration, depending on the use of the volumetric glassware (see below). Pycnometers, U-tube viscometers, pipettes, and burettes depend in their performance on "wetting" and surface tension characteristics. Therefore, cleaning procedures must be chosen not to compromise these properties. In addition, attention should be paid to the possibility of contamination arising either from the fabric of the equipment itself, which may not be inert, or from cross-contamination from previous use. In the case of volumetric glassware, cleaning procedures, storage, and segregation of volumetric equipment might be critical, particularly for trace analyses where leaching and adsorption can be significant. Particularly for volumetric equipment, measuring the volume of liquids near 20°C is recommended, since the liquid volume highly depends on the temperature.

The correct use of instruments such as chromatographs combined with periodic servicing, cleaning and calibration will not necessarily ensure their adequate performance. Thus, where appropriate, periodic performance checks should be carried out (e.g., to check the response, stability and linearity of sources, sensors and detectors, the separating efficiency of chromatographic systems, the resolution, alignment and wavelength accuracy of spectrometers, etc. (Chapter 5.7). The frequency of such performance checks can be specified in manuals or operating procedures or if not, be determined by experience and based on need, type and previous performance of the equipment. Intervals between checks should be shorter than the time the equipment has been found, in practice, to take to drift outside acceptable limits. It is possible to build performance checks and system suitability checks into test methods (e.g., based on the levels of expected detector or sensor response to reference materials, the resolution of component mixtures by separation systems, the spectral characteristics of measurement standards, etc.). These checks must be satisfactorily completed before the equipment is used.

In some cases, a test and its performance is actually defined in terms of a particular piece of equipment and checks are necessary to confirm that the equipment conforms to the relevant specification. For example, flashpoint values for a particular flammable sample are dependent of the dimensions and geometry of the apparatus used in the testing.

3. Physical measurement standards (reference weight sets, reference thermometers).

Where physical parameters are critical to the correct performance of a particular test, the laboratory should have access to the relevant measurement standard for calibration. Checks on the calibration status should be performed at regular intervals and laboratories should establish acceptance criteria for the results of their metrological control. Any provided storage advice by documentation supplied with the measurement standard should be importantly followed. Certificates and other relevant documentation should be stored in such a way as to be readily available as long as it deems necessary to document the metrological traceability of the measurements linked to them.

4. Computers and data processors (See Chapter 6.9. for more details).

In Table 1 examples of maintenance and its frequency of equipment for food testing laboratories are provided for guidance purposes only. The actual frequency of maintenance will be based on the need, type and previous performance of the equipment (and defined by the laboratory, see below).

Table 1: Guidance on maintenance of laboratory equipment[33];[34]

Type of equipment	Requirement		Suggested frequency	
Incubators,fridges,freezers, Ovens	Clean and disinfect internal surfaces		Monthly when required (e.g., every three months); When required (e.g., annually).	
Water baths	Empty, clean, disinfect and refill		Monthly or every six months if biocide is used	
Centrifuges	(a)	Service	(a)	Annually
	(b)	Clean and disinfect	(b)	Each use
Autoclaves	(a)	Make visual checks of gasket, clean/drain chamber	(a)	Regularly, as recommended by manufacturer
	(b)	Full service	(b)	Annually or as recommended by the manufacturer
	(c)	Safety check of pressure vessel	(c)	Annually
Safety cabinets Laminar flow cabinets	Full service and mechanical check		Annually or as recommended by manufacturer	
Microscopes	Full maintenance service		Annually	
pH meters	Clean electrode		Each use	
Balances, gravimetric diluters	(a)	Clean	(a)	Each use
	(b)	Service	(b)	Annually
Stills	Clean and de-scale		As required (e.g., every three months)	
De-ionisers, reverse osmosis units	Replace cartridge/membrane		As recommended by manufacturer	
Anaerobic jars	Clean/disinfect		After each use	
Media dispensers, volumetric equipment, pipettes, and general service equipment	Decontaminate, clean and sterilise as appropriate		Each use	
Spiral platers	(a)	Service	(a)	Annually
	(b)	Decontaminate, clean and sterilise	(b)	Each use
Laboratory	(a)	Clean and disinfect working surfaces	(a)	Daily, and during use
	(b)	Clean floors, disinfect sinks and basins	(b)	Weekly or more frequently if required
	(c)	Clean and disinfect other surfaces	(c)	Every 3–12 months depending on type of laboratory work

The laboratory in turn should consider each item and develop a procedure for its use and the maintenance including frequency and how to conduct such operations. The description can be developed by the laboratory or reference is provided to the specific instructions of the service manual of the equipment (or it can be copied and attached to the procedures). Detailed records of the maintenance of essential equipment should be kept.

5.3. Preventive maintenance requiring a service engineer

Preventive maintenance includes measures such as systematic and routine cleaning, adjustment and replacement of equipment parts at scheduled intervals.

Manufacturers generally recommend a set of equipment maintenance tasks performed at regular intervals: daily, weekly, monthly or yearly to ensure that the equipment performs at maximum efficiency and it will increase the lifespan of the equipment. It will also help to prevent inaccurate test results due to equipment failure, delays in reporting results, low productivity and large repair costs.

Trained service engineers are able to replace most parts of the instrument and to check that critical components are functioning according to their specification. They will dismantle elements of the instrument and check a wider range of functions including essential components for calibration (e.g., gas flows, spectral wavelengths, temperature functions, etc.) and replace components that do not function to their specification.

Preventive maintenance is usually undertaken at intervals of 6 months. The laboratory should have written policies and procedures for maintaining equipment, including routine maintenance plans for each piece of equipment, the frequency of performing all maintenance tasks, record formats and staff training on the use and maintenance of the equipment. A maintenance plan usually includes preventive maintenance procedures as well as provision for inventory, troubleshooting and repair of equipment and assigned responsibility for providing oversight.

Most laboratories attach a label to the instrument indicating when the next maintenance or service should be performed.

5.4. Calibration programme/type of calibration required

Accredited laboratories must have an overall calibration programme in place as well as procedures for the calibration of their measuring equipment (including performance verification of equipment). This is to ensure that all measurements that have a significant effect on test results are traceable to a measurement standard. The calibration programme must be reviewed and adjusted as necessary to maintain confidence in the status of calibration. Ultimately, the reason for calibration is that tests conducted at a laboratory are comparable with those of any other laboratory or tests performed in standardised conditions.

Per definition, calibration is an operation that, under specified conditions, in a first step, establishes a relation between the quantity

values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties. In a second step, it uses this information to establish a relation for obtaining a measurement result from an indication (International Vocabulary of Metrology VIM 2.39, Annex 2).

At best, every device used in a calibration measurement is traced back to the international standard units through a well-defined calibration process. Calibration services build measurement standards of several different levels of accuracy to enable comparison of devices to measurement standards, also referred to as a calibration "traceable to the SI" (see also Chapters 10 and 11).

Measuring equipment used in the laboratory must be capable of achieving the measurement accuracy and/or measurement uncertainty required in order to provide a valid result. Accredited laboratories must calibrate measurement equipment, when:

i. The measurement accuracy or measurement uncertainty affects the validity of the reported results and/or
ii. Calibration of the equipment is required to establish the metrological traceability of the reported results.

Equipment used for tests (sampling) including equipment for subsidiary measurements (e.g., for environmental conditions) with a significant effect on the accuracy or validity of the result of the test (or sampling) must be calibrated before being placed into service. Some pieces of equipment (e.g., balances) must be calibrated in situ, so even if these are shipped with a factory calibration certificate, calibration after installation and before use will be essential. This should include checks against the manufacturer's specifications and checks to confirm that the equipment gives satisfactory results when used to make the measurements for which it is intended.

The approach to calibration is to ensure proper and reliable functioning of the equipment. It should be conservative in order to pick up any calibration problems before they affect the validity of results. Individual internal calibration programmes are established depending on the specific requirements of the analysis. It might be necessary to check instrument calibration after any shutdown, whether deliberate or otherwise, to be followed by service work or conducting other substantial maintenance. Reference is provided to the related national Accreditation Body guidelines for additional information.

Newly acquired equipment must be checked by the laboratory before use to ensure conformity with specified design, performance and dimension requirements.

When performing the initial calibration of the instrument, the manufacturer's directions should be followed carefully. It might be beneficial to use calibrators provided by or purchased from the manufacturer. Procedures for performing calibrations should be adequately documented, either as part of specific analytical methods or as a general calibration document. It should indicate how to perform the calibration, how often calibration is necessary, and the action to be taken in the event of calibration failure.

Laboratories establish individual calibration programmes depending on the specific requirements of the analysis. Analytical tests could be sub-divided into general classes depending on the type of calibration required:

- For analytical tests that depend critically on the measurement of physical properties, such as weight measurement in gravimetry and volume measurement in titrimetry, a suitable calibration programme for these quantities is essential, due to their significant effect on the test results. Requirements and methods for the calibration and control of balances are described by EURAMET (European Association of National Metrology

Institutes)[35]. Procedures for the calibration of volumetric devices, such as piston pipettes and burettes are described in ISO 8655[35]. In addition, the calibration of measuring devices used to establish the purity or concentration of chemical standards need to be considered.

- For tests that measure an empirical property of a sample, e.g., flashpoint, equipment is most often defined in a national or international standard method. In this case, reference materials (RMs) should be used for calibration, where available.

Instruments, which require calibration as part of their normal operation, such as spectrometers or such used for chromatography should be calibrated using reference materials of known composition (usually solutions of pure chemicals). The RM might be either a synthetic mixture prepared in the laboratory from materials of known (and preferably certified) purity, or a purchased certified matrix RM. Where formally designated measurement standards are not available, the laboratory should prepare or select a material with suitable properties and stability as a laboratory measurement standard that is characterised by repeat testing, preferably by more than one laboratory and using a variety of validated methods (see also ISO Guide 35[39]). More information on the use of RMs is provided in Chapter 11.

Calibration in analytical chemistry is mainly performed at the measurement stage (e.g., in GC-based analysis) and by use of synthetic solutions of the analyte investigated at various concentrations, while possible contamination or losses during the sample preparation and extraction or derivatisation stages are not considered. This must be addressed during validation of the entire measurement process for which a close match between the test sample and the matrix RM (nature of the matrix and the concentration of the analyte) must be assured and where appropriate and feasible, CRMs should be used. The measurement output from a sample must be compared with the output produced by a suitable RM that has been subjected to the same full analytical process as the sample. The day-to-day calibration procedure and quality control checks must be designed accordingly (see Chapters 9 and 10).

The calibration of volumetric glassware is performed indirectly by mass determination of a specific volume of water of known density at a given temperature[37]. If the glassware is later used with liquids and properties very different from water (wetting characteristics, surface tension etc.), the uncertainty in the measured volume is expected to increase. This is particularly pertinent for volumetric glassware calibrated to deliver a certain volume. Therefore, it is recommended to determine the volume indirectly through mass and density of the particular liquid(s) for methods obtaining a result with a low uncertainty.

The process of calibration involves the direct comparison of the item to be calibrated against a reference. It is, therefore, the reference itself, which provides the guarantee of accuracy, and so it is critical that the reference itself is maintained and checked regularly. Often this will only be possible by sending the reference for calibration to an accredited calibration laboratory. In most cases, the calibration laboratory can work with a hierarchy of standards, whereby a reference standard is maintained and used only for occasional checks on working standards (Chapter 10).

[39] *ISO Guide 35:2017, Reference materials — Guidance for characterization and assessment of homogeneity and stability. The guidance provided supports the implementation of ISO 17034.*

5.5. Calibration frequency (calibration cycle)

Laboratories are obliged to justify their need for calibration. The level and frequency of calibrations (and performance verification) is determined by documented experience, based on the need, type and previous performance of the equipment and should be at least those that are recommended by the manufacturer.

Calibration and alignment are performed on a time cycle also called calibration interval. The most common cycle for calibration is one year. However, calibration will not inform at what point during that yearly cycle the device became out of calibration and every measurement taken with that device since its last calibration is now suspect. For example, if a sensor measuring temperature in a freezer is out of tolerance, everything that was stored in that freezer may have to be discarded or recalled. Thus, the sooner the laboratory knows when a device or sensor has become out of tolerance, the lower the risk and costs for the business will be.

For each instrument or group of instruments, an estimate should be made as to the length of time the instrument is likely to remain within the maximum permissible error after calibration. An initial calibration interval should be set based on the manufacturer's recommendations, the frequency of use of the instrument, the accuracy required, the perceived risk of a loss of calibration and the magnitude of the impact, the stability of the measurement system and the local experience of similar instruments. The calibration is then checked at the end of this interval and, if it is still correct, the interval is confirmed as adequate. Alternatively, the interval is reduced if the check shows that recalibration is required. Records should be kept so that the laboratory can justify the interval chosen. Intervals between calibration and verification should be shorter than the time the equipment has been found to take to drift outside acceptable limits. In some cases, calibration laboratories can suggest intervals for particular instruments. In addition, it may be necessary to check instrument calibration after any shutdown, whether deliberate or otherwise, and following service or other substantial maintenance.

The decision on calibration intervals should be made by a person(s) with general experience of measurements, or of the particular instruments to be calibrated, and preferably with knowledge of the intervals used by other laboratories.

The frequency of calibration will depend on the:

- Required level of uncertainty and the criticality of the work
- Expected extent and severity of use
- Frequency of use of the instrument
- Influence of the environment
- Maximum permissible errors (for instance by legal metrology authorities)
- Adjustment of (or change in) the individual instrument
- Influence of the measured quantity (e.g., high temperature effect on thermocouples)
- Pooled or published data about the same or similar devices.

The laboratory must also have a programme and procedure for the calibration of its reference standards, calibrated by a body that can provide traceability (Chapter 10). These should be used for calibration only and be calibrated before and after any adjustment. When calibration and reference material include reference values or correction factors, the laboratory should ensure these are updated and implemented, as appropriate, to meet specific requirements.

The calibration status of identified equipment must be indicated. Equipment that requires calibration or has a defined period of validity must be labelled, coded or otherwise identified to allow the user of the equipment to readily identify the status of calibration or the period of validity. Some equipment is difficult to label

in the conventional sense. In these cases, the calibration status could be indicated by means of a colour code or other marking. The personnel of the laboratory should be instructed that they must not use any equipment where the label shows that it is overdue for a check or calibration. In addition, ideally, equipment carries a label showing that it is not calibrated and hence not to be used for measurements where traceability is required. Equipment undergoing checks (e.g., during calibration or after giving suspicious results) must either be segregated or clearly labelled as not to be used, so that there is no possibility of it being inadvertently used for routine work until it is formally accepted. The calibration programme can be a combination of service from the supplier and in-house checks and calibrations by the laboratory. The approach has to ensure proper and reliable functioning of the equipment. ILAC G24[40] provides guidance to laboratories, particularly while setting up their calibration system, on how to determine calibration intervals. The guide identifies and describes methods that are available for the evaluation of calibration intervals (Chapter 12.4) based on:

1. Automatic adjustment or "staircase" (calendar-time)
2. Quality Control chart
3. "In-use" time
4. In service checking, or "black-box" testing and,
5. Statistical approach

The laboratory might implement any of these methods based on its individual needs and assessed risks, but will need to evaluate the effectiveness of the chosen method and decisions taken. Many Accreditation Bodies provide recommendations to calibration intervals (recommended calibration and performance check) of equipment commonly used in chemical & biological testing laboratories available from their websites.

Where equipment replaces or duplicates existing equipment, the checks should include a comparison of the results from each unit to establish the variations that might result.

If a calibration is not a dominant factor in the testing result, the laboratory should have quantitative evidence to demonstrate that the associated contribution of a calibration contributes little (insignificantly) to the measurement result and the measurement uncertainty and thus traceability does not need to be demonstrated.

It is the responsibility of the laboratory to evaluate the effectiveness of the method chosen and its consequences.

Table 2 below, as a guidance, presents examples of calibration intervals and typical checks for various types of laboratory instruments commonly used in analytical chemical and microbiological laboratories and on which the calibration of other instruments might be dependent. The frequency will be based on the need, type and previous performance of the equipment. More comprehensive advice is available in the literature that can be consulted (Annex 2) and in equipment manuals. For equipment verification, see also Chapter 4.6.

[40] *ILAC-G24:2022 / OIML D 10:2022 Guidelines for the determination of recalibration intervals of measuring equipment. https://ilac.org/publications-and-resources/ilac-guidance-series/*

Table 2: Guidance on calibration and calibration checks of laboratory equipment[33];[34]

Type of equipment	Requirement	Suggested frequency
Balances	Full traceable calibration	Annually in the first three (3) years, followed by less frequence, based on satisfactory performance
Calibration weights	Full traceable calibration	Every five (5) years
Check weight(s)	Check against calibrated weight or check on balance immediately following traceable calibration	Every two (2) years
Volumetric glassware	Gravimetric calibration to required tolerance	Annually
Pipettors/pipettes	Full traceable calibration	Annually
Hygrometer (working)	One point calibration versus reference hydrometer	Annually
Hygrometer (reference)	One point calibration using measurements standard of known specific gravity	Five (5) years
Barometers	One point	Five (5) years
Reference thermometers(liquid-in-glass)	Full traceable re-calibration Single point (e.g., ice-point check)	Every five (5) years Annually
Reference thermocouples	Full traceable re-calibration Check against reference thermometer	Every three (3) years Annually
Working thermometers & Working thermocouples	Check against reference thermometer at ice-point and/or working temperature range	Annually

Note: Some instruments will normally be calibrated in an accredited calibration laboratory, and should at least provide results traceable to national measurement standards.

5.6. Verification and validation of equipment

Intermediate checks or verifications of equipment are measurements of equipment in smaller increments of time than the calibration cycle. This activity is often overlooked, but is of vital importance for maintaining accurate measurements by verifying that the equipment is still within the limits of acceptable performance. The process can catch errors in sensors and measurement devices for instance, before they become serious problems.

Verification is a process of "confirming" that a given specification is fulfilled[41]. It is not a comparison to a higher standard, but a simple check to confirm the correct operation of equipment or a process according to its stated

[41] *Provision of objective evidence that a given item fulfils specified requirements (ISO 17025, 3.1 plus examples; Source ISO/IEC Guide 99)*

operating specifications and to monitor instrument parameters.

The laboratory must verify performance prior to putting the measuring instrument in place. It ensures also that re-verification is performed subsequently, depending on the type of the measuring instrument. Instruments must be re-verified, if sealing breaks, in case of structural changes to the measuring instrument and if the measuring instrument no longer complies with the applied tolerances. If a device e.g., performs outside of its published specifications, adjustment are performed to eliminate the errors by using information obtained during the calibration, so that the device will measure much closer to its nominal value.

Accredited laboratories implement a written plan for performance verification (that is part of proper operation of the equipment information). When intermediate checks are necessary to maintain confidence in the performance of the equipment, these checks should be carried out according to procedure.

Instrument validation[42] is a series of processes through which the system is tested to verify the performance specifications published by the manufacturer of the instrument. It ensures the acceptability of the implemented measurement process and is the combined effect of calibration and verification, but the result is in the final output of the process.

If the equipment and associated techniques are new, validation processes are important. Prior to testing, the performance of new equipment is evaluated to ensure it is working correctly with respect to accuracy and precision and by use of checks against the manufacturer's specifications and checks to confirm that the equipment gives satisfactory results. An instrument validation could be carried out by running samples in parallel using both old and new equipment and methods for a period of time to determine that the expected results can be obtained. These validation procedures must be completely recorded. For relevant equipment (that could influence laboratory activities and results), records must be retained including, where applicable, evidence that the equipment conforms to the specified requirements. Revalidation should be considered following changes in premises or instrumentation.

The Table 3 provides guidance on equipment validation and performance verification. However, the actual frequency will be based on the need, type and previous performance of the equipment.

Table 3: Guidance on equipment validation and verification of performance (Source[33];[34])

Type of equipment	Requirement		Suggested frequency	
Temperature controlled equipment (incubators, baths, fridges, freezers)	(a)	Establish stability and uniformity of temperature	(a)	Initially, periodically, at documented frequency, and after repair/ modification
	(b)	Monitor temperature	(b)	Daily, each use
Sterilising ovens	(a)	Establish stability and uniformity of temperature	(a)	Initially, periodically, at documented frequency, and after repair/ modification
	(b)	Monitor temperature	(b)	Daily/each use
Autoclaves	(a)	Establish characteristics for loads/cycles	(a)	Initially, periodically, at documented frequency, and after repair/ modification
	(b)	Monitor temperature/time	(b)	Daily/each use

[42] *Validation: Where the specified requirements are adequate for an intended use (ISO 17025, 3.9 and example; Source ISO/IEC Guide 99)*

Type of equipment	Requirement		Suggested frequency	
Safety cabinets	(a)	Establish performance	(a)	Initially, every year and after repair/ modification
	(b)	Microbiological monitoring	(b)	Weekly
	(c)	Air flow monitoring	(c)	Daily/each use
Laminar air flow cabinets	(a)	Establish performance	(a)	Initially, and after repair/modification
	(b)	Check with sterility plates	(b)	Weekly
Timers	Check against national time signal		Annually	
Miscoscopes	Check alignment		Daily/each use	
pH meters	Adjust using at least two buffers of suitable quality		Daily/each use	
Balances	Check zero, and reading against check (calibration) weight		Daily/each use	
De-ionisers and reverse osmosis units	Check conductivity		Weekly	
	Check microbial contamination		Monthly	
Gravimetric diluters	(a)	Check weight of volume dispensed	(a)	Daily/each use
	(b)	Check dilution ratio	(b)	Daily/each use
Media dispensers	Check volume dispensed		Each adjustment or replacement	
Pipettors/pipettes	Check accuracy and precision of volume dispensed by gravimetric method		Regularly (to be defined by taking account of the frequency and nature of use)	
Spiral platers	(a)	Establish performance against conventional method	(a)	Initially and annually
	(b)	Check stylus condition and the start and end points	(b)	Daily/each use
	(c)	Check volume dispensed	(c)	Monthly
Colony counters	Check against number counted manually		Annually	
Centrifuges	Check speed against a calibrated and independent tachometer		Annually	
Anaerobic jars/incubators	Check with anaerobic indicator		Daily/each use	
Laboratory environment	Monitor for airborne and surface microbial contamination using, e.g., air samplers, settle plates, contact plates or swabs		Weekly for total count and moulds; Biannually for pathogens or as otherwise decided by the laboratory based on activities and historical trends and results	
Volumetric Glassware	Accuracy, Precision (pipettes/burettes)		Depends on Use	

5.7. Performance verification and calibration (examples)

Listed below are examples for:

Temperature measuring devices

Where temperature has a direct influence on the result of an analysis or is critical for the correct performance of equipment, temperature-measuring devices, e.g., thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves, must be of an appropriate quality to achieve the accuracy required. For health and safety reasons, mercury and toluene liquid-in-glass thermometers are not used in the laboratory.

Calibration of these devices must be traceable to national or international standards for temperature. However, if accuracy requirements permit, measurement devices that can be demonstrated to conform to an appropriate nationally or internationally accepted manufacturing specification may also be used, for example for monitoring storage fridges, freezers, incubators and water baths, where acceptable tolerance around the target temperature permits. Verification of the performance of such devices is necessary.

Incubators, water baths, ovens

The stability of temperature, uniformity of temperature distribution and time required to achieve equilibrium conditions in incubators, water baths, ovens and temperature-controlled rooms must be established initially, then periodically checked at a documented frequency, in particular with respect to typical usage (for example: position, space between, and height of stacks of Petri dishes in microbiology). The operating temperature of this equipment should be monitored daily, or according to usage and records are to be retained.

Autoclaves, including media preparators

Autoclaves should be capable of meeting specified time and temperature tolerances. Pressure cookers fitted with a pressure gauge are not acceptable. Sensors used for controlling or monitoring operating cycles require calibration and the performance of timers should be verified.

Initial validation of autoclaves should include performance studies (spatial temperature distribution surveys) for each operating cycle and for each load configuration used in practice. This process must be repeated after significant repair or modification (e.g., replacement of thermo-regulator probe, modification of loading arrangements) or where indicated by the results of quality control checks on media. Sufficient temperature sensors should be positioned within the load (e.g., in containers filled with liquid/medium) to enable demonstration of location differences. In the case of media preparators, the use of two sensors, one adjacent to the control probe and one remote, is considered appropriate, where uniform heating cannot be demonstrated by other means. Validation and re-validation should consider the suitability of come-up and come-down times and time at the sterilisation temperature.

Clear operating instructions based on the heating profiles determined for typical uses during validation/re-validation should be provided and acceptance/rejection criteria established. In addition, records of autoclave operations, including temperature and time are to be maintained for every cycle.

Monitoring might be achieved by one of the following: Using a thermocouple and recorder to produce a chart or printout or by direct observation and recording of maximum temperature achieved and time at that temperature. In addition to directly monitoring the temperature of an autoclave, the effectiveness of its operation during each cycle might be checked by the use of chemical or biological indicators for sterilisation / decontamination purposes. Autoclave tape or indicator strips shows that a load has been processed.

Weights and balances

Weights and balances should be calibrated traceably at regular intervals (according to their intended use, see Chapter 6.7 for more information). In general, balances (all types including micro balances) are checked when used at level of balance and zero point (taring). Monthly calibrations relate to accuracy by use of reference weights for a one point check. The calibration procedures should be documented.

Volumetric equipment

Volumetric equipment such as automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable pipettes should undergo an initial verification as volumetric equipment, followed by regular checks to ensure that the equipment is performing within the required specification. Verification is not necessary for glassware, which has been certified to a specific tolerance. Equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments) and the precision of the repeat deliveries should be measured.

For 'single-use' disposable volumetric equipment, laboratories should obtain supplies from companies with a recognised and relevant quality system. After initial validation of the suitability of the equipment, it is recommended to carry out random checks on accuracy. If the supplier has no recognised quality system, laboratories should check each batch of equipment for suitability.

Thermal cyclers

The laboratories should carry out the verification of temperature, ramp rate, overshoots / undershoots, and the hold time.

Other equipment

Regular verification of conductivity meters, oxygen meters, pH meters and other similar instruments are to be carried out before each use. The buffers used for verification purposes should be stored in appropriate conditions and marked with an expiry date and any evaporation is to be avoided (tight closing of the cap).

Where humidity is important to the outcome of the test, hygrometers should be calibrated traceable to national or international standards. Timers, including the autoclave timer, should be verified by use of a calibrated timer or national time signal.

For centrifuges used in test procedures, an assessment should be made of the criticality of the centrifugal force. Where it is critical, the centrifuge will require calibration.

The following aspects of the instruments listed below, may need to be checked, depending on the method:

Chromatographic equipment

- Overall system checks, precision of repeat sample injections, carry-over
- Column performance (capacity, resolution, retention)
- Detector performance (output, response, noise, drift, selectivity, linearity)
- System heating/thermostating (trueness, precision, stability, ramping characteristics)
- Autosampler (trueness and precision of time routines).

Liquid and ion chromatographs

- Composition of mobile phase
- Mobile phase delivery system (pressure, precision, trueness, pulse-free).

Electrode/meter systems, including conductivity, pH and ion-selective

- Electrode drift or reduced response
- Fixed point and slope checks using chemical measurement standards.

Heating/cooling apparatus, including freeze dryers, freezers, furnaces, hot air sterilisers, incubators, melting and boiling point apparatus, oil baths, ovens, steam sterilisers and water baths:

- Periodic calibration of temperature sensing system using the appropriate calibrated thermometer or pyroprobe

- Thermal stability
- Heating/cooling rates and cycles
- Temperature gradients in ovens and furnaces
- Ability to achieve and sustain pressure or vacuum.

Spectrometers and spectrophotometers, including atomic absorption, fluorimetric, inductively coupled plasma-optical emission, infrared, luminescence, mass, nuclear magnetic resonance, ultraviolet/visible and X-ray fluorescence

- Selected wavelength trueness, precision, stability
- Source stability
- Detector performance (resolution, selectivity, stability, linearity, trueness, precision)
- Signal to noise ratio
- Detector calibration (mass, wavelength, frequency, absorbance, transmittance bandwidth, intensity etc.)
- Internal temperature controllers and indicators where applicable
- Atomic Absorption Spectrophotometer (flame, graphite furnace) are checked on absorption intensity by a standard solution of a specific element (certified reference) for instance.

Microscopes

- Resolving power
- Performance under various lighting conditions (fluorescence, polarisation, etc.)
- Graticule calibration (for length measurement).

Autosamplers

- Trueness and precision of timing systems
- Reliability of sequencing programmes
- Trueness and precision of sample delivery systems.

5.8. Software and computer verification and validation

Especially in chemical testing laboratories, computers have a wide variety of uses, including:

- Control of critical environmental conditions
- Monitoring and control of inventories
- Calibration and maintenance schedules
- Stock control of reagents and measurement standards
- Statistical analysis of data
- Scheduling of samples and monitoring of work throughput
- Control chart generation
- Monitoring of test procedures
- Control of automated instrumentation
- Capture, storage, retrieval, processing of data, manually or automatically
- Data transfer
- On-board instrumental data processing
- Matching of sample and library data (e.g., comparing mass spectra)
- Sample tracking;
- Generation of test reports
- Word processing
- Communication
- Laboratory Information Management System (LIMS).

For the operation of computers and storage of computer media in laboratories care should be taken to avoid damage due to chemical, microbiological or dust contamination, heat, damp, and magnetic fields.

Computer use in laboratory must be controlled and any electronic system that generates and manages documents/records must meet requirements of control of documents and records by ISO 17025[1] (2.3). Each computer should have a log indicating the hardware and the installed software, so that the recreation of the previous versions of any software is possible, in case an error or query arises to determine whether the software was responsible. Computer networks ease control of software since work areas can be established with restricted access and often with different levels of access. Care must be taken where the workstation machines have local drives.

Before use, computers are subject to checks for correct functioning. This applies to all hardware and software and especially to software written in-house or applications developed by personnel (e.g., spreadsheets). Initial checking should verify as many aspects of a computer's operation as possible. Similar checks should be carried out if the computer's use is changed, or after maintenance or revision of software.

The laboratory will need to have a policy on software usage and permission. Software checks and controls should be conducted related to accessibility, security and, in particular controls to prevent unauthorised modification, and retrieval and accessibility of documents/records after future hardware/software upgrades. The requirements apply to new software and any updates or modifications as well as to applications such as spreadsheets. The use of Word processing packages must be controlled sufficiently to prevent the production of unauthorised reports or other documents. In cases, where the computer acts as little more than an electronic typewriter, validation by manually checking and approving hard or soft copies is enough. More sophisticated systems that read and process data automatically in predetermined report formats will require additional checks. A person for authorising software for use in the laboratory should be assigned, who is e.g. checking that the software does not corrupt data.

Spreadsheet packages are in use in laboratories to store, collate, summarise and present data, to calculate measurement results from instrument outputs, to plot charts and to carry out statistical analysis. Wherever possible, spreadsheets must be protected from alteration by using passwords reserved to the responsible and authorised personnel. Where this is not possible, a set of sample data should be available, which can be loaded before the spreadsheet is used, to check that the calculated values are determined correctly. A validation is required, if spreadsheets have inbuilt functions (particularly statistical analysis) to confirm that used equations/in-built functions return the correct value and to establish that the correct input data are being referenced. It can be done by using a test dataset and comparing the results with manual calculations. After the spreadsheet has been validated, procedures should be put in place to minimise the risk of incorrect data entry/transfer and to ensure that any calculations cannot be edited (either intentionally or accidentally).

For computers used to process data associated with chemical testing, validation of that function is usually sufficient, if the computer produces the expected answers. In chemical testing, suitable checks on the data gathering and handling functions could be made by using a CRM for the initial validation. Usually the whole system is validated in one go, by using chemical measurement standards. Such validation is normally acceptable and documented by the validation procedure of a particular system, followed by regular checks using quality control samples with results recorded. It may be difficult to validate these systems in isolation from the analytical instrument producing the original signal (Chapter 5.5).

Computer programs performing calculations could be validated by comparison with manually generated results; records of validation should be kept. It is necessary to ensure that the dataset used for validation provides all the variables that might occur during the expected use. At least three sets of data are necessary for the validation. In all cases, the software must be verified before use and a correct functioning should be recorded. Commercial off the-shelf software used within its designated application range could be considered as sufficiently validated and the validation can be replaced by the certification provided by the manufacturer.

Microprocessor controlled instruments have normally a self-checking routine activated, when switched on, including the recognition and checking of all peripheral equipment. Often the software is not accessible and under most circumstances validation can be performed by testing the various aspects of instrument function using known parameters, e.g., by testing RMs, physical or chemical measurement standards or quality control samples. The output from measuring instruments will usually be converted into digitised data and translated

into a recognisable signal (numbers, peaks, spectra according to the system) by the software algorithm. The algorithm for a number of factors provides programmed instructions, e.g., deciding where peaks start and finish, whether a number should be rounded up or down. The algorithm is a common source of unexpected performance and validation should test the logic behind the decisions made by the algorithm.

Computer controlled automated system are validated by checking for satisfactory operation (also under extreme circumstances) and the reliability of the system before it is allowed to run unattended. It covers validation of individual components and the overall check on the dialogue between individual components and the controlling computer, assessing also the likely causes of system malfunction. Computer, interfaces and connecting cabling must have sufficient capacity, not to cause data loss that could have serious consequences where the operations include time-sequenced routines. Where possible the controlling software should be tailored to identify and highlight any such malfunctions and tag associated data. The use of quality control samples and standards run at intervals in the sample batches should then be sufficient to monitor correct performance on a day-to-day basis.

Calculation routines are checked by testing with known parameter values. To ensure that no corruption has occurred during transmission of electronic data e.g., checks are conducted via the use of verification files but, wherever practical, the transmission should be backed-up by a hard copy of the data.

Laboratory Information Management Systems (LIMS) are widely used for managing laboratory activities, including electronic collation, calculation and dissemination of data, often received directly from analytical instruments. The various input operations into a Laboratory Information Management Systems (LIMS) are bearing risks of data corruption, where data cross from one system to another in case of system incompatibility or the need to reformat the information. Particular validation requirements include management of access to the various functions, and audit trails to catalogue alterations and file management. Where data are transmitted electronically, it will be necessary to build in safety checks to guard against data corruption and unauthorised access.

For further reading and guidance related to the management of computers and software in laboratories in the context of ISO/IEC 17025 accreditation, reference is provided to a EUROLAB publication[43] and to the EUROLAB Cook Book Document on the use of EXCEL for data handling in laboratories[44].

All computers connected with measuring devices and those used for data collection and interpretation of results must have appropriate backup systems to prevent any loss of data or software. The backup system or local server can be located within the laboratory or outside of it. In case another organization is used for data storage, the laboratory must file a contract of confidentiality with this organization or use encrypted data protection.

[43] *EUROLAB Technical Report No. 2/2006, Guidance for the management of computers and software in laboratories with reference to ISO/IEC 17025/2005, Eurolab (2006). Available from www.eurolab.org.*

[44] *EUROLAB Cook Book 12, Use of Excel Data Handling in Laboratories, 01/10/2018; Resource Requirements; https://www.eurolab.org/CookBooks/12*

6. HANDLING OF TEST ITEMS

ISO/IEC 17025[1], section 7.4, ISO 6887[45].

Food testing laboratories must ensure sample integrity, meaning that nothing the laboratory does has the effect of making the material non-representative. Aspects to ensure sample integrity include sample storage, handling and transport in the laboratory, maintenance of proper storage temperature, and opening sample containers in the appropriate level of controlled environment. Laboratory waste disposal, workflow layout, cross-contamination, etc. could also affect the sample integrity. Further to that, intentional adulteration or substitution of the laboratory sample must be prevented, ensuring that the material collected remains representative of the product. The latter is of utmost importance when used as evidence in court (keeping the samples analytical validity).

Laboratories take precautions to avoid deterioration, contamination, loss or damage to the test item. They must have procedures in place for the transportation, receipt, protection, storage, retention and/or disposal of test items, including all necessary provisions necessary to protect the integrity of the test item, and to protect the interests of the laboratory and the customer. Records are kept as part of the procedures, for example related to sample storage this might be refrigerator temperatures or microbiology laboratory environmental monitoring.

The sampling technique should not modify the microbiota and alter the quantity of microorganism present for microbiological analysis. Sample handling procedures and the transport as such must not affect the microbiological quality of samples. Samples must be kept under conditions that maintain their integrity (e.g., chilled or frozen where appropriate) with conditions monitored and records kept. Means of fastest transport are preferred, especially for samples for microbiological examination that must be delivered to the laboratory promptly with the original storage conditions maintained as nearly as possible. When collecting liquid samples, an additional sample (control) should be taken as a temperature control and its temperature is to be checked at the time of collection and on receipt at the laboratory.

Water samples from natural sources, tap water or ground water should be analysed less than 5 hour after sample taking. Where appropriate, the responsibility for transport and storage between sampling and arrival at the testing laboratory must be documented. Non perishable dry or canned foods collected at ambient temperatures must not be refrigerated. Frozen samples should be collected in pre-chilled containers that were placed in a freezer long enough to chill them thoroughly and must be kept solidly frozen at all times. Frozen or refrigerated products should be transported in approved insulated containers of rigid construction so that they will arrive at the laboratory unchanged. Cool refrigerated samples, are kept in ice at 0-4°C, transported in a sample chest with suitable refrigerant capable of maintaining the sample at 0-4°C until arrival at the laboratory.

Upon receipt of the sample, abnormalities or departures from normal or specified conditions,

[45] *ISO 6887: Microbiology of the Food Chain Package. It includes ISO 6887-1:2017 ISO 6887-2:2017. ISO 6887-1:2017, Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions; Part 2: Specific rules for the preparation of meat and meat products*

as described in the test method, must be checked and recorded, e.g., for microbial flora sensitive to factors such as temperature or duration of storage and transport.

If there is insufficient sample or the sample is in poor condition, due to physical deterioration, incorrect temperature, damaged packaging or deficient labelling, the laboratory should consult with the customer before deciding whether to test or refuse the sample. In any case, records should be maintained with the condition of the sample indicated on the test report. If there are any problems, action must be taken to ensure that no work is done before the problems are resolved with the customer and a record of any communication with the customer is kept, since such communications involve amendment to the contract review (Chapter 11).

Testing of the samples should be performed as soon as possible after sampling, conforming to relevant standards and/or national, international regulations (Chapter 2). Samples, tests items awaiting tests must be stored or conditioned under specified environmental conditions, e.g., at an appropriate temperature and in such a manner so that there is no risk to laboratory personnel and that the integrity of the samples is preserved. Extremes of environmental conditions (e.g., temperature, humidity), which might change the composition of the sample should be avoided, as this can lead to loss of analyte through degradation or adsorption, or an increase in analyte concentration (e.g., mycotoxin level to give an example). If necessary, environmental monitoring should be used and recorded (Chapter 3.6).

The following examples illustrate the laboratories attention to examination deadlines for certain food product and storage conditions and storage temperature, such as:

- Analysing stable products as early as possible and before the storage limit date
- Keeping grounded samples in glass or plastic containers with air and watertight covers. Samples not analysed immediately, should be left in cold storage to minimise spoilage and other chemical reactions. Samples for lipid analysis are to be stored under nitrogen at low temperature to prevent oxidation of unsaturated lipids
- Analysing fresh and refrigerated products within 24 hours after receipt. If longer storage period cannot be avoided, freezing the sample ASAP at a temperature below -18°C is advised, indicated in the test report, since in certain products freezing modifies the composition of the microbial flora
- Samples received in a chilled state can be kept in this condition, but should normally be tested within 24 h. Samples of tissue or feedstuffs for chemical testing should be frozen, if testing cannot be conducted within this time
- Water for quality tests on chemical parameters could be held in a chilled condition for up to 48 or 72 hours
- Refrigerated products must not be frozen, unless otherwise specified
- Refrigerated samples should not be analysed more than 36 h after collection
- Analyse pasteurized or similar products as early as possible and before the storage limit date
- Analysing spoiled stable units as soon as possible and in less than 48 hours.

Sub-sampling by the laboratory immediately prior to testing is considered part of the test method, to be performed according to national or international standards, where they exist, or by validated in-house methods. Sub-sampling procedures should be designed to take account of uneven distribution of microorganisms (general guidance is provided by ISO 6887[45]).

Storage areas for samples are to accommodate retention of samples for the times in conditions to protect their integrity. Storage areas should be kept clean and organised so that there is no risk of contamination or cross-contamination, or packaging and any related seals damaging. An appropriate level of security should be exercised to restrict unauthorised access to the samples. Samples should be stored until the test results

are obtained and longer, if required and applicable, e.g., based on legislative requirements or by customer request. Laboratory sample portions that are known to be highly contaminated should be decontaminated prior to being discarded.

Physical accountability of a sample ensures that the laboratory samples, test samples, test portions, test solutions, etc., are traceable. The life of the laboratory sample should be documented until final disposal, including all test samples and test portions to support regulatory action for instance (Chapter 8). The laboratory should have a documented policy for the retention and disposal of samples. Regulatory guidance on disposal of test items might vary with laboratories.

All staff concerned with administration of the sample handling system must be properly trained. Where a test item or a portion of an item must be held secure, the laboratory should have arrangements for storage and security that protect the condition and integrity of the secured items or portions concerned.

7. REAGENTS AND CULTURE MEDIA

ISO/IEC 17025[1], section 6; ISO 11133[46], ISO 3696[19].

7.1. Reagents and consumables

Laboratories must ensure that the quality of reagents they use is appropriate for the tests concerned. Attention is required regarding the selection, purchase, reception and storage of chemical reagents that play an essential role in the accuracy of a chemical experiment. Reagents and laboratory chemicals include substances of sufficient purity for use in chemical analysis, chemical reactions or physical testing.

Accredited laboratories must evaluate and approve suppliers of critical reagents and consumables and maintain relevant documentation and records to prevent possible deviations from the expected quality of the results, that may arise from failure of any critical supply to meet the requirements. The laboratories should select certified/accredited supplier of reagents, tests and chemicals[1], which helps to ensure to receive the highest quality product offered. The process of selecting accredited suppliers should be based on a risk assessment (Chapter 2.4) for the reagents and materials supplied with the following possible key questions addressed:

- What may happen and why, should a given product fail to match the relevant specifications
- What would be the consequences for the laboratory work
- What is the chance of such a failure occurring
- Are there any factors that may reduce either the probability of the failure or its consequences
- Is the level of risk acceptable?

Documents referring to the purchase of reagents and other items affecting the quality of laboratory operations must contain an adequate description of the order, including a specification and the purpose for which the reagent is purchased. Prior to release, the documents should be reviewed and approved as appropriate.

The grade of any critical reagent used (including water) is normally stated in the method description, together with guidance on any particular precautions to be observed in its preparation, storage and use. Precautions to observe relate to toxicity, flammability, stability to heat, air and light, to reactivity to other chemicals and to particular containers and to other hazards.

Reagents received into the laboratory must be labelled with the dates of receipt, opening and expiry dates, plus the name of the person opening the reagent. The laboratory must ensure compliance with the expiry dates of reagents. For this purpose, the rule of FIFO (First In-First Out) or of FEFO (First Expired-First Out) could be applied.

In general, laboratories should ensure that all reagents (including stock solutions), media, diluents, and other suspending fluids are adequately labelled to indicate, as appropriate, identity, concentration, storage conditions, date of opening, preparation date, validated expiry date and/or recommended storage periods. Reagents and reference materials prepared in the laboratory should be labelled to identify the

[46] *ISO 11133:2014/Amd 1:2018 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media — Amendment 1*

substance, concentration, solvent (where not water), any special precautions or hazards, restrictions of use, and date of preparation and/or expiry. The person responsible for preparations of media, solutions or others should be identifiable from records. The appropriate information can be found in the Safety Data Sheet (SDS) supplied with chemicals and reagents.

Where the quality of a reagent is critical to a test, the quality of a new batch should be verified against the outgoing batch before use, if the outgoing batch is known to be still serviceable. Records are kept that also inform about the methodology applied. However, in all cases, the reagents and other consumables must be inspected and verified as complying with set specifications.

The correct disposal of reagents is a matter of good laboratory practice. It should comply with national environmental or health and safety regulations (Chapter 4.5).

7.2. Culture media

A growth or culture media is a special medium composed of different nutrients used in microbiological laboratories. Culture media are formulations of substances, in liquid, semi-solid or solid form, which contain natural and/or synthetic constituents, intended to support the multiplication (with or without inhibition of certain microorganisms), identification or preservation of viability of microorganisms. Selective media and differential media are two types of growth media, the first allowing the growth of a specific type of microorganisms and inhibiting the rest of the other microorganisms; the second distinguishes the microorganisms by allowing them to produce visible growth pattern or different characteristics on the media. A range of biochemical reagents, known for the identification of specific metabolisms and to differentiate between serotypes of bacteria, is also used.

The accurate preparation of culture media is one of the fundamental steps in microbiological analysis and the water quality used is important. For media preparation, distilled deionised, or reverse osmosis produced water, free from bactericidal inhibitory or interfering substances is used (3.4), unless the test method specifies otherwise. Distilled water must be stored in containers made from inert material. In case, chlorinated water is the base to prepare distilled water, the chlorine needs to be neutralized prior to the distillation. A good quality distilled water has an electrical conductivity of <2 µS/cm, at around 0.1µS/cm[23]. More information related to water quality and media preparation is provided by ISO 7218[17].

Culture media can be prepared either from basic ingredients dehydrated or from dehydrated complex media. Raw materials (both commercial dehydrated formulations and individual constituents) are stored under appropriate conditions, e.g., cool, dry and dark and away from light and at a temperature as stated by the manufacturer. All containers, especially those for dehydrated media, must be tightly sealed, and quickly and carefully closed after use.

Media should not be used beyond shelf life. A dehydrated media that shows signs of caking, colour change or is solidifying is unusable, due to the water uptake. The shelf life of prepared media under defined storage conditions must be determined and verified. For all new batches of nutrient media the below mentioned parameters need to be checked.

- Selectivity (of culture media). It refers to the degree to which culture media (used for enrichment and detection in qualitative

analysis) allow only the growth of the specific microorganisms being detected for.
- Sensitivity (of culture media). It refers to the degree to which culture media (used for enrichment and detection in qualitative analysis) allow for the detection of a target microorganism.
- Specificity. It demonstrates, under defined conditions, that non-target microorganisms do not show the same visual characteristics as target microorganisms.

For the differentiation or chromogenic nutrient media the productivity ratio and the selectivity factor must be determined, for more information reference is provided to ISO 11133[46].

The laboratory should always allow media to equilibrate to room temperature before use, to minimize the potential of thermal shock to the organisms. Culture media should not freeze or overheat unless specifically indicated in the technical insert or the product-specific "Instructions for Use". It must not be incubated prior to inoculation. The storage temperature of media must be carefully monitored on a daily basis.

Culture media dispensed in tubes or bottle and reagents not used immediately must be protected against light and desiccations. For instance, by refrigerating for a maximum period of 3 months or between 18-23°C for a maximum of one month under conditions that prevent their composition being modified, if not otherwise specified in international standards. It is generally recommended not to exceed two to four weeks of storage for plates and three to six months for sealed bottles and tubes, in refrigerated conditions, unless otherwise specified in specific standards or by results of the laboratory shelf-life evaluation indicating a longer shelf-life. The expiry date for stored media should be established by checking media after defined storage times for their physical, chemical and microbiological performance characteristics. The laboratory should specify the frequency of verification.

All media, including diluents and other suspension fluids procured ready-to-use or partially complete, require performance evaluation before use in line with criteria laid down in ISO 11133, an international standard that all accredited laboratories must apply when performing microbiological food and water testing using culture media. It defines the preparation and quality control of all types of culture media (from dehydrated to ready-to-use media for classical to alternative microbiological testing methods) and specifies requirements for the preparation, production, storage, and performance testing of culture media and conditions. They must resemble the intended sample testing conditions as closely as possible for the most accurate and meaningful results.

ISO 11133 provides systematic instructions and flowcharts for performing and evaluating performance tests as well as comprehensive tables for the specifications for most culture media in food and water testing. They are covering: the medium's target microorganism and the relevant ISO standard, each function to be tested (productivity, selectivity, specificity), the appropriate control strains for these functions, including their World Data Centre for Microorganisms (WDCM) numbers, and the test criteria and/or characteristic reactions and other practical information.

Accredited microbiological laboratories must verify the suitability of each batch of reagents critical for the test, initially and during its shelf life by using positive and negative control organisms that are traceable to recognised national or international culture collections. The suitable performance of culture media, diluents and other suspension fluids prepared in-house must be checked, where relevant, with regard to:

- Recovery or survival maintenance of target organisms
- Inhibition or suppression of non-target organisms
- Biochemical (differential and diagnostic) properties

- Physical properties (e.g., pH, volume and sterility).

Attributes (e.g., physical and biochemical properties) should be evaluated using objective criteria. The evaluation of performance in recovery or survival of target organisms, and the inhibition or suppression of non-target organisms should be quantitative.

As part of this performance evaluation, the user laboratory needs to have adequate knowledge of the manufacturer's quality system and the product specifications, which include at least the following:

- Name of the media and list of components, including any supplements
- Shelf-life and the acceptability criteria applied
- It is necessary to comply with the manufactures instructions: expiry date, storage temperature and conditions, conditions for use (pH, etc.) and efficiency control.

Many laboratories source their culture media from suppliers to simplify their workflows. To ensure high quality and batch-to-batch consistency of the purchased ready-to-use media they rely on the performance tests conducted by the manufacturer in line with ISO 11133 as indicated by a supporting quality control certificate. It informs about the test organisms used for the acceptance criteria of the performance tests and about the test results. Therefore, checks by the user laboratory might involve only initial checks for every new manufacturer, a review for acceptability and indirect checks through internal quality control procedures. In case of preparing media in-house, laboratories must conduct quality control according to ISO 11133 on every batch of media received based on the strains named in the standard (see above). The performance testing conditions must resemble the intended sample testing conditions as closely as possible for more accurate and meaningful results.

8. SAMPLING AND SAMPLE PREPARATION

ISO/IEC 17025[1], section 7.3; ISO 7218[17]; ISO 6887[45], ISO 19458[47], ISO 7002[48]; ISO/TS 17728[49]; ISO 3951-1[50]; ISO 2859-2:2020[51]; NMKL Procedure No. 12[52]; CAC/GL 50-2004[53]; Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling[54]; IUPAC Rec. Nomenclature for sampling in analytical chemistry[55].

8.1. Sample taking and transport

The process of sampling is an important factor that determines a result of an analyte, and therefore ISO 17025 uses it as one of the criteria for laboratory accreditation.

In many cases, testing laboratories are not responsible for primary sampling of obtained test items. Where they are responsible, it is strongly recommended, that this sampling is covered by quality assurance and ideally by accreditation. National accreditation bodies have their own procedures for the accreditation of sampling and can accredit sampling as a stand-alone activity.

The sampling procedure is used to draw and constitute a sample (ISO 7002). It covers and describes the allocation, withdrawal and preparation of samples (e.g., from a matrix, or a batch of products) and must, whenever reasonable, be based on appropriate statistical methods. The way samples are taken will depend on the reason for the analysis (ISO 7002) and of the laboratory analysis through which samples will undergo, and characteristic of the ingredients and the finished products. The objectives and sampling purposes for developing the sampling procedures must be clear. The importance of the sampling stage for subsequent testing cannot be overemphasised. The adequacy and condition of the sample or specimen received for examination are of primary importance. If samples are improperly collected and mishandled or are not representative of the sampled lot, the laboratory results will be meaningless.

Because interpretations about a large consignment of food are based on a relatively small sample of the lot, the established

[47] *ISO 19458:2006, Water quality — Sampling for microbiological analysis*

[48] *ISO 7002:1986, Agricultural food products—Layout for a standard method of sampling from a lot*

[49] *ISO/TS 17728:2015, Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples*

[50] *ISO 3951-1:2022, Sampling procedures for inspection by variables — Part 1: Specification for single sampling plans indexed by acceptance quality limit (AQL) for lot-by-lot inspection for a single quality characteristic and a single AQL*

[51] *ISO 2859-2:2020, Sampling procedures for inspection by attributes — Part 2: Sampling plans indexed by limiting quality (LQ) for isolated lot inspection*

[52] *NMKL (Nordic Committee on Food Analysis) Procedure No. 12: Guide on Sampling for Analysis of Foods. www.nmkl.org.*

[53] *CAC/GL 50-2004, General guidelines on sampling, www.fao.org*

[54] *M. H. Ramsey and S. L. R. Ellison (eds.), Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches, Eurachem (2007). ISBN 978-0-948926-26-6. Available from www.eurachem.org.*

[55] *W. Horwitz, Nomenclature for sampling in analytical chemistry (IUPAC Recommendations 1990), Pure Appl. Chem., 62(6), 1193-1208 (1990)*

sampling procedures must be applied uniformly. A representative sample is essential when pathogens or toxins are sparsely distributed within the food or when disposal of a food shipment depends on the demonstrated bacterial content in relation to a legal standard.

Before starting sampling, the minimum quantity required for analysis and any instructions on pooling sub-samples on site must be agreed with the customer coupled with other details, to ensure correct interpretation of the results of analysis. For example, it is important to decide on:

- Type of product and batches to be sampled
- Sampling techniques (e.g., for microbiological analysis)
- Purpose of the analysis of the product
- Legal requirements
- The dress-code of samplers (according to local rules in the factory for instance)
- Usage of sterile or non-sterile tools etc.
- All necessary safety and health precautions for the sampler and environment.

Accredited laboratories operate by a sampling plan and a sampling method, when carrying out sampling for subsequent testing. Both documents must be available at the location where sampling is undertaken.

Sampling plans are designed in such a way that the resulting data will be representative of the parameters of interest, allowing the answer to all questions as stated in the analytical requirement. These sampling plans could be random, systematic or sequential, and carried out to obtain quantitative or qualitative information, or to determine conformance or non-conformance with a specification. In the case of bulk products, locations for sub-sampling (and the sampling techniques) should be included in the sampling plan. All interested parties must agree upon the sampling plan in use.

The sampling method (e.g., Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs to mention an example) describes the process of sampling and specifies the factors to be controlled in order to ensure the validity of results (e.g., to avoid contamination during sampling or distortion during transport of samples). It includes descriptions of the selection of samples or sites, the sampling plan and the preparation and treatment of sample(s) from a substance, material or product to yield the required item for the subsequent testing. It specifies the number and size of the portions to be taken from the bulk material, and describes how to obtain the laboratory sample. In the case of sampling bulk or packaged goods, the sampling procedure reduces the original consignment through lots or batches, increments, primary or gross samples, composite or aggregate samples, subsamples or secondary samples to a laboratory sample. Where the customer requires deviation, additions or exclusion from the documented sampling procedure, these must be recorded in detail with appropriate sampling data and included in all documents containing the test results.

The laboratory sample, if heterogeneous, might be further prepared to produce the test sample, and is considered as the end of the sampling procedure.

When designing, adapting, or following a sampling plan the following rules should be applied:

- When adapting the sampling strategy to test requirements and conditions, it is recommended to consider the average analyte concentration in the material, the analyte profile across the material, suspected product contamination by a particular analyte, heterogeneously distributed contaminants and other non-analytical factors, including the nature of the area under examination.
- Always ensuring that the material is homogeneous. Portions of the material that are non homogeneous should be sampled

separately and should not make a composite as it can mask quality problems.
- In solids, there might be a considerable variation in analyte concentration, if the particle size distribution of the main material varies significantly, and over time, the material might settle. Before sampling, it might be appropriate thus, if practical, to mix the material to ensure a representative particle size distribution.
- Taking properties of the analyte(s) of interest into account, e.g., volatility, sensitivity to light, thermal stability and chemical reactivity, as important considerations for designing the sampling plan and in choosing equipment, packaging and storage conditions.

Equipment used for sampling, subsampling, sample handling, sample preparation and sample extraction must be selected and be appropriate to avoid unintended changes to the nature of the sample which may influence the results. Several ISO standards are dealing with sampling equipment and its use (e.g., ISO 24333[56]). All sampling equipment, tools and auxiliary materials should be inert, and in a clean condition before and after their use. For any critical equipment in use, the significance of gravimetric or volumetric errors during sampling and the calibration state must be considered. Adding chemicals such as acids, or antioxidants to stabilise the sample might be appropriate. This is of particular importance in trace element analysis where there is a danger of adsorption of the analyte onto the storage vessel. Sampling for microbiological examination should be carried out aseptically using sterile equipment. One-piece stainless steel spoons, forceps, spatulas, and scissors using in microbiological sampling are sterilized in an autoclave or dry-heat oven.

Whenever possible, samples are submitted to the laboratory in the original unopened containers or representative portions are transferred to sterile containers under aseptic conditions. The laboratory should use containers that are clean, dry, leak-proof, wide-mouthed, sterile, and of a size suitable for samples of the product. Light sensitivity of samples must be addressed. Containers such as plastic jars or metal cans that are leak-proof might be hermetically sealed. Glass containers, which might break and contaminate the food products, should be avoided, whenever possible. Sterile metal boxes, cans, bags, or packets with suitable closures are useful for sampling dry materials. Sterile plastic bags (for dry, unfrozen materials only) or plastic bottles are suitable containers for line samples[57].

Bags should not be overfilled or permit puncture by wire closure. The sample containers used for the packaging of volatile liquid samples should be filled to approximately 90 % of their total holding capacity. The outer surface of packages must be clean and dry. The sample containers must be checked for leaks. The closure of the packaging should be adequate to ensure there is no leakage of sample from the container, and that the sample itself cannot be contaminated. If leaks occur, caps and stoppers should be reinforced or replaced.

Each sample unit (defined later) must be identified with a properly marked strip of masking tape. A felt pen should not be used on plastic because the ink might penetrate the container.

As to sampling sizes, information is provided by standard methods or by legislation, by e.g., EU legislation related to sampling and analysis of certain contaminants in food[58]. For

[56] *ISO 24333:2009 - Cereals and cereal products - Sampling*

[57] *A method of sampling in a geographical area.*

[58] *Laying down methods of sampling and analysis for the control of (maximum) levels of (official controls) in foodstuffs: Mycotoxins: Commission Regulation (EC) 401/2006. Lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene: Commission Regulation (EC) No 333/2007. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs: Commission Regulation (EU) 2017/644. Levels of nitrates: Commission Regulation (EC) 1882/2006 https://food.ec.europa.eu/safety/chemical-safety/contaminants_en*

microbiological examinations, a rule of thumb is, whenever possible, to obtain at least 100 g for each sample unit, while the analytical unit is most often 25 g, sometimes 10 g for most pathogen detection (absence in 25g respectively 10 g)[3]. The submission of open and closed controls of sterile containers with the sample is considered a quality control measure.

Analysts should also refer to national or sectoral standards as appropriate related to sampling and analysis. Where specific methods are not available, the analyst should rely on experience or adapt methods from similar applications. When in doubt, the material of interest, and any samples taken from it, should always be treated as heterogeneous.

International methods of sampling should be used to ensure that valid sampling procedures are applied when foodstuff is being tested for compliance to a particular standard or objective. The Food and Agricultural Organisation, FAO[59] provides a good overview on international standards used in sampling of food and feed. Official control laboratories receive samples by the competent authority based on their sampling strategy including procedures for objective sampling, selective sampling and suspect sampling. Sampling methods used in food safety EU official controls must comply with relevant Community rules or, if no such rules exist, with internationally recognized rules or protocols (e.g., with the European Committee for Standardization - CEN) or with other methods fit for purpose, e.g., standards of ISO, the Nordic Committee on Food Analysis (NMKL) and guidelines of Codex Alimentarius.

A sampling procedure should stipulate the conditions based on which a lot should be inspected and classified. For testing of agro food products, ISO 7002[48] provides the layout for a standard method of sampling from a lot and rule-based guidance on drafting a sampling method of agricultural food products for intended users. Except for milk products that is covered by ISO 707:2008[60]. The typical examples of the standards regulated by this document are:

- ISO 3100-1:1991 Meat and meat products—Sampling and preparation of test samples—Part 1: Sampling
- ISO 6670:2002 Instant coffee—Sampling method for bulk units with liners
- ISO 13690:1999 Cereals, pulses and milled products—Sampling of static batches (bulk grain with a depth of 3 m) and ISO 24333:2009 (bulk grain with a depth between 3–12 m).

Sampling always contributes to the measurement uncertainty[54] (uncertainty of sampling Chapters 9.5 and 13). As analytical methodology improves and methods allow or require the use of smaller test portions, the uncertainties associated with sampling become increasingly important. They can increase the total uncertainty associated with the measurement result. The measurement uncertainty associated with sub-sampling should always be included in the test results measurement uncertainty, but the measurement uncertainty associated with the basic sampling process is commonly treated separately (since carried out prior to submission of a sample to the laboratory, and is often outside of its control). Activities undertaken after the stage of the laboratory test sample are 'analytical operations' that do not contribute to the uncertainty associated with sampling.

It is important to cause minimum disruption at the sampling site and to follow any security

[59] *The Food and Agriculture Organization (FAO) and the International Feed Industry Federation (IFIF) (2010): Good practices for the feed industry – Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding. FAO Animal Production and Health Manual No. 9. Rom; Section 5 Methods of sampling and analysis; www.fao.org/3/i1379e/i1379e05.pdf*

[60] *ISO 707:2008, Milk and milk products — Guidance on sampling*

instructions in the interest of the properties of the analyte(s). Only trained personnel should perform sampling. Whenever the laboratory is responsible for sampling, the involved personnel should be authorised for sampling and be trained on the applicable procedure. For microbiological examination sampling should only be performed by personnel trained and experienced in aseptic sampling techniques, in the types of products sampled and the requirement to minimize change in the normal microflora of the products[53].

When documenting a sampling procedure, all terms used must be clearly defined, so that the procedure will be clear to other users. For sampling terminology, reference is provided to recommendations published by e.g., IUPAC[55], Eurachem[54] and ISO/TS 17728[49]. The Eurachem Guide is dealing with measurement uncertainty arising from sampling, guiding methods and approaches.

It is of vital importance that the samplers keep a clear record of the procedures followed in order to be able to repeat the sampling process exactly. Retained records of sampling data that forms part of the testing undertaken include, where relevant:

- Reference to the sampling method and sampling procedure used
- Date and time of sampling
- Data to identify and describe the sample
- The identification of the sampler
- Environmental or transport conditions (e.g., air contamination, temperature etc.)
- Diagrams or other equivalent means to identify the sampling location as necessary and, if appropriate
- Deviations, additions to, or exclusions from the sampling method and sampling plan.

Environmental conditions, such as air contamination and temperature must be monitored and recorded at the sampling site.

If the laboratory has conducted or directed the sampling stage, it should report the procedures used and comment on any consequent limitations imposed on the results. If not, the laboratory should state in the report that the samples were analysed as received.

To fully evaluate an analytical result for conformity assessment, or for other purposes, it is important to have knowledge of the sampling plan and its statistical basis. Sampling procedures for inspection by variables (ISO 3951-1[50]) assume that the inspected characteristic is measurable and follows the normal distribution. In contrast, sampling for inspection by attributes (ISO 2859-2[51]) is a method whereby the unit of product is classified either as conforming or nonconforming, or the number of nonconformities in the unit of products is counted with respect to a given set of requirements. In inspection by attributes, the Acceptable Quality Limit (AQL) and the Rejectable Quality Limit, defined using appropriate statistical techniques, predetermine the risks associated with acceptance/rejection of nonconformities. The AQL is a quality control concept. It is the maximum number of faults acceptable in a sample of a manufactured product for the entire batch of the product to be accepted. If the number of faults is higher than the AQL, then the entire batch is rejected.

The laboratory should have procedures in place for cleaning of all items used in sampling, including flasks and auxiliary equipment. Records of cleaning processes should be maintained.

8.2. Sample reception, labelling and traceability

Samples must be handled and labelled to guarantee both their legal and analytical validity (Chapter 6). Labels must be firmly attached to the sample packaging and, where appropriate, be resistant to fading, autoclaving, sample or reagent spillage, and to reasonable changes in temperature and humidity. Generally, product labelling includes the name, details of the sample content, an expiry date, contact details and batch identification (for other characteristic see footnote[61]).

The sample label is important for documentation. It should unambiguously identify the sample to related plans or notes. Labelling of samples is particularly important later in the analytical process, when the sample is divided, subsampled, or modified in some way. In such circumstances, additional information might be appropriate, such as references to the main sample, and to any processes used to extract or subsample the sample (8.2). Labels might be required to identify all those who have been involved with the sample, including the person taking the sample and the analysts involved in the testing. Receipts might support this, to testify that one signatory (as identified on the label) has handed the sample to the next signatory to prove that sample continuity was maintained.

Some samples, e.g., those involved in litigation for example, might have special labelling and documentation requirements. Samples taken for legal purposes might be sealed so that access to the sample is only possible by breaking the seal. Confirmation of the satisfactory condition of the seals will normally form part of the analytical report.

The packaging and labels from samples might be highly contaminated and thus should be handled and stored with care to avoid any spread of contamination (relevant for microbiological laboratories).

The documentation of the sample or item receipt should specify the personnel authorised to receive and record items. The person receiving the items should also be responsible for examining them to ensure that they are suitable for the intended test. All relevant information and records of sampling are retained and contain:

- Date and, where relevant, the time of receipt
- Condition of the sample on receipt and, when necessary, temperature
- Characteristics of the sampling operation (sampling date, sampling conditions, etc.)
- Identification of the person making the register entry.

It is vital, that samples entering the testing area are anonymous. The identity of the sample supplier is only known to the sample registration staff and the staff in charge to prepare the report containing the test results. The laboratory must define a unique identification of samples, e.g., a unique registration number or code and labelling requirements to ensure the traceability of laboratory samples, test items, test portions and that items cannot be confused physically or when referred to in records or other documents. The identification must be retained throughout the life of the test item in the laboratory including a sub-division of groups of items and the transfer of items within and from the laboratory. Sufficient information must be recorded in the sampling report, to give full traceability of the samples and to allow interpretation of the results of analysis.

Often, laboratories identify samples by means of barcodes linked to a Laboratory Information Management System (LIMS), particular those laboratories that are handling high sample

[61] *OECD Series on Principles of Good Laboratory Practice (GLP) and Compliance Monitoring No. 19: Management, Characterisation and Use of Test Items, ENV/JM/MONO(2018)6*

numbers. Barcode readers scan and record the necessary information. Its routine use simplifies many time consuming tasks, e.g., the recording of sample information. It further contributes to reduce possible human error, thus improving the overall traceability of supplies and samples. The LIMS has also the advantage, that it could collect relevant sample and reagent information and by this allowing flagging any incorrect or expired supplies by analysts and technicians.

8.3. Sample preparation

Foodstuff analysis requires sample preparation steps, because of the complex matrix structure of the foodstuffs and the diluted analytes.

Sample preparation refers to a family of solid/liquid handling techniques to extract or to enrich analytes from sample matrices into the final analyte solution. Many sample preparation techniques are well documented, while often more elaborate sample preparation for complex sample matrices is needed, e.g., newer technologies such as solid-phase extraction (SPE). Non-robust sample preparation procedures, poor techniques, or incomplete extraction is the major causes of out-of-trend and out-of-specification results.

Once received into the laboratory, the laboratory sample(s) might require further treatment such as removal of extraneous material, subdivision and/or milling and grinding to make it suitable for analysis. The Table 4 provides an overview on the necessary sample preparation steps for common instrumental methods in chemical analysis.

Wherever possible, replicate samples should be prepared to readily repeat analysis in cases of accidental sample loss or where the determined values require checking.

Sample preparation is the critical step in microbiological examinations, aiming ideally to divide the processed sample rapidly in a small volume with the highest concentration of analyte possible, free of substances interfering with the applied detection method. Additionally, the applied sample processing procedures should not result in any loss of the bacterial analyte, thereby enabling quantitative measurements[62].

In chemical analysis, depending on the applied instrument technique, sample preparation covers extraction, concertation, clean up, derivatisation and digestion. Methods of sample preparation are detailed either in standards or by legislation. Many standard methods contain a section that details the preparation of the laboratory sample prior to the removal of the test portion for analysis.

The analytical operations begin with the removal of a known amount (test portion) from the laboratory sample or the test sample that then proceed through various operations to the final measurement. The test portion refers to the actual material weighed or measured for the analysis and unless otherwise specified, the test portion taken for analysis must be representative of the laboratory sample.

To ensure that the test portion is homogeneous, it might be necessary to reduce the particle size by grinding or milling. The particle size reduction step are either performed manually (mortar and pestle) or mechanically using crushers or mills. During the processes care is

[62] *Jan W. Kretzer et al.: Sample Preparation – An Essential Prerequisite for High-Quality Bacteria Detection; Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems, 2008*

taken to avoid cross-contamination of samples, to ensure that the equipment does not contaminate the sample (e.g., the metals) and that the composition of the sample is not altered (e.g., loss of moisture).

In case of a large laboratory sample, it could be necessary to subdivide it first. There are variety of techniques for that, including coning and quartering, riffling, or by means of a rotating or a centrifugal sample divider. In some cases, it will be necessary to crush or coarsely grind the sample prior to subdivision into test samples.

Both, sample packaging and instruments used in sample preparation should guarantee that all surfaces in contact with the sample are essentially inert. Particular attention must be paid to possible contamination of samples by metals or plasticisers leaching from the container or its stopper into the sample. The packaging should also ensure that the sample could be handled without causing a chemical, microbiological, or other hazard.

For sample preparation in microbiological testing, in order to avoid contamination of the environment and of the test portion, it is recommended to work in special premises or in a safety cabinet (Chapter 3.5.2), otherwise on clean and disinfected areas.

To achieve good food sample homogeneity[63] in pesticides analysis powerful devices mill/homogenisers are used. Comminution (Annex 1) at room temperature might lead to major losses for several sensitive pesticides and an insufficient degree of reduction hindering the extractability of residues enclosed in the remaining particles. Thus, the use of dry ice in sample preparation is highly recommended to minimize pesticide losses. Furthermore, the degree of homogeneity achieved by cryogenic processing leads to greater sub-sampling variations. If the necessary degree of comminution cannot be achieved with the common equipment, the use of larger sample amounts for analysis (scaling up) and/or the use of Ultra-Turrax[64] during the first extraction step might help to overcome these problems[65]. In addition, using more than one internal standard[66] and quality control standards to enable recognition of errors due to miss pipetting or discrimination during partitioning or clean-up is highly recommended (Chapter 11).

The laboratory must be aware that sample preparation might be related to the edible portion of each unit collected (e.g., regulated in heavy metal analysis in food by the EU[63]).

Other steps in sample preparation are thawing and mixing. Thawing of frozen samples before analysis is not recommended. If tempering of a frozen sample is required to obtain an analytical portion, thawing in the original container (container in which it was received in the laboratory) is recommended at 2-5°C within 18 h. In case of required rapid thawing, thawing the sample at less than 45°C for not more than 15 min is the procedure of choice. When thawing a sample at elevated temperatures, the sample should be continuously agitated in a thermostatically controlled water bath.

Mixing of the sample is an important step in sample preparation, e.g., in microbiological testing, since various degrees of non-uniform

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[63] *EU Reference Laboratory –Single Residue Methods: Quick Method for the Analysis of Numerous Highly Polar Pesticides in Food. Involving Extraction with Acidified Methanol and LC-MS/MS Measurement I. Food of Plant Origin (QuPPe-PO (Quick Polar Pesticides) Method) and recommending e.g. Stephan UM 5, Retsch Grindomix GM 300 or Vorwerk-Thermomix TM31-1 (see the websites of the producers Retsch, Vorwerk etc. for more information)*

[64] *Ultra Turrax is a high-performance dispersing instrument for volumes from 1 – 2000 ml*

[65] *QuEChERS A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products, EN 15662 short (see the EU Reference Laboratory for Pesticides Requiring Single Residue Methods https://www.eurl-pesticides.eu/docs/public/home.asp?LabID=200&Lang=EN)*

[66] *For instance, deuterated internal standards for gas chromatographic-mass spectrometric analysis*

distribution of microorganisms are to be expected in any food sample. Liquid samples must be shacked thoroughly and, if practical, dried samples are mixed with sterile spoons or other utensils before withdrawing the analytical unit from a sample of 100 g or greater.

Usually, 50 g of liquid or dry foodstuffs are used to determine aerobic plate count values and most probable number of coliforms. For Salmonella detection, the EU, for instance, regulates the use of 25g of sample[63]. If contents of package are obviously not homogeneous (e.g., a frozen dinner), the entire contents of package is macerated for withdrawing the analytical unit, or, preferably, each different food portion is analysed separately, depending on the purpose of the test. Related to weighing, blending and diluting of samples for enumeration of microorganisms reference is provided to ISO 7218[21] for more information.

For specifying analytical units in chemical analysis, reference is provided to standard methods and EU regulations related to sampling and analysis[63], where such information is available.

Table 4: Common Instrumental Methods and the necessary sample preparation steps prior to analysis – Recommendations[67]

Analytes	Sample Preparation	Instrument
Organics	Extraction, concentration, clean-up, derivatisation	GC, HPLC, GC/MS, LC/MS
Volatile organics	Transfer to vapour phase, concentration	GC, GC-MS
Metals	Extraction, concentration, speciation	AA, GFAA, ICP, ICP/MS
Metals	Extraction, derivatisation, concentration, speciation	UV-Vis molecular absorption, spectrophotometry, ion chromatography
Ions	Extraction, concentration, derivatisation	IC, UV-VIS
DNA/RNA	Cell lysis, extraction, PCR	Electrophoresis, UV-VIS, fluorescence
Amino acids, fats, carbohydrates,	Extraction, clean up	GC, HPLC, electrophoresis
Microstructures	Etching, polishing, reactive ion technique, ion bombardments, etc.	Microscopy, surface spectroscopy

GC: Gas chromatography; HPLC: High performance liquid chromatography; MS: Mass spectroscopy; AA: Atomic absorption; GFAA: graphite furnace atomic absorption; ICP: inductively coupled plasma; UV-VIS: Ultraviolet-visible molecular absorption spectroscopy; IC, ion chromatography.

[67] *Source: Sample preparation techniques in analytical chemistry, edited by Somenath Mitra. P. (Chemical analysis; v. 162) ISBN 0-471-32845-6*

9. SELECTION, VERIFICATION AND VALIDATION OF METHODS

ISO/IEC 17025[1], sections 3.8, 3.9 and 7.2; ISO 7218[17], ISO 13843[68], ISO 16140 series of standards[69]; ISO 5725-2[70], Eurachem Guide: The Fitness for Purpose of Analytical Methods and Supplements[71]; Eurachem/CITAC Guide CG4[72]; ILAC-P14:09[73]; ILAC G17:01[74]; ISO 19036[75]; ISO 29201[76]; IUPAC/CITAC Guide: Investigating out of-specification test results of chemical composition based on metrological concepts[77]; ISO 21748[78]; ISO/IEC Guide 98-3[79].

9.1. Selection and verification of methods

Laboratories must use appropriate methods and procedures for all their laboratory activities and, where appropriate, for evaluation of the measurement uncertainty as well as for statistical techniques for analysis of data. The most important considerations are that the

[68] *ISO 13843:2017, Water quality — Requirements for establishing performance characteristics of quantitative microbiological methods*

[69] *The ISO 16140 series of standards, ISO/TC 34, Food products, subcommittee SC 9, Microbiology. ISO 16140-1:2016, Microbiology of the food chain — Method validation — Part 1: Vocabulary. Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. ISO 16140-3:2020: …Part 3, Protocol for the verification of reference methods and validated alternative methods in a single laboratory. ISO 16140-4:2020: … Part 4, Protocol for method validation in a single laboratory. ISO 16140-5:2020: ….Part 5, Protocol for factorial interlaboratory validation for non-proprietary methods. ISO 16140-6:2019: …Part 6, Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures*

[70] *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*

[71] *B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (2nd ed. 2014). ISBN 978- 91-87461-59-0. V. Barwick (ed.), Planning and Reporting Method Validation Studies – Supplement to Eurachem Guide on the Fitness for Purpose of Analytical Methods (1st ed. 2019). 3. H. Cantwell (ed.) Blanks in Method Validation – Supplement to Eurachem Guide on The Fitness for Purpose of Analytical Methods, (1st ed. 2019). Available from www.eurachem.org*

[72] *L. R. Ellison, A. Williams (eds.), Eurachem/CITAC Guide CG4: Quantifying uncertainty in analytical measurement, (3rd ed. 2012). ISBN 978-0-948926-30-3. Available from www.eurachem.org*

[73] *ILAC-P14:09/2020: ILAC Policy for Measurement Uncertainty in Calibration; https://ilac.org/publications-and-resources/ilac-policy-series/*

[74] *ILAC G17:01/2021 ILAC Guidelines for Measurement Uncertainty in Testing; Available from https://ilac.org/publications-and-resources/ilac-guidance-series/*

[75] *ISO 19036:2019, Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations*

[76] *ISO 29201:2012, Water quality – The variability of test results and the uncertainty of measurement of microbiological enumeration methods*

[77] *I. Kuselman, F. Pennecchi, C. Burns, A. Fajgelj, P. de Zorzi, IUPAC/CITAC Guide: Investigating out of-specification test results of chemical composition based on metrological concepts (IUPAC Technical Report), Pure Appl. Chem., 84(9), 1939-1971 (2012)*

[78] *ISO 21748:2017, Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty evaluation*

[79] *ISO/IEC Guide 98-3:2008, Uncertainty of measurement — Part 3, Guide to the expression of uncertainty in measurement (GUM:1995)*

methods used should be suitable for the purpose intended, adequately validated and documented, and that results are provided that are traceable to stated references with an appropriate level of uncertainty.

The ISO 17025 standard[1] uses the terms method and measurements synonymous[2].

Accredited laboratories must validate or verify each new method prior to the implementation. Both validation and verification of methods are confirmations of declared information provided as claims. Their aims are either plausible about the intended future use (validation, see Chapter 9.2) or truthfully stated (verification). For definitions of these terms, see Annex 1 and 8.4.

The laboratory is responsible to use methods that are appropriate for the required application. It might use its own judgement or select a method in consultation with the customer, or the method is specified in a regulation or by the customer. If a customer is not specifying the use of a method, the laboratory selects an appropriate method for the required application and informs the customer on the method chosen (see also Chapter 2). Non-standard methods (not covered by standard methods) must be agreed with the customer. In general, all selected methods have to be communicated to the customer and the customer acceptance is usually given in written form, for instance as part of a contract. When a deviation from the method occurs, the deviation must be notified to the customer unless a specific statement has been already included as part of the contract. It is also necessary to ensure that customers are aware of the variation and that they accept the resulting data as still being suitable for their purposes. Deviations from methods for all laboratory activities should only take place if it is technically justified, authorized, and accepted by the customer.

It is recommended that the laboratories use methods published either in international, regional or national standards, or by reputable (technical) organizations, or in relevant scientific texts or journals. Methods specified by manufacturers could be used too as well as laboratory-developed or modified methods. Food standards often favour the use of standard or collaboratively tested methods. They are required in situations where a method is widely used or defined in regulation.

In practice, methods used by laboratories fall into one of three categories requiring a different degree of validation:

a) Standard methods that are published as standard specifications, e.g., by the International Organization for Standardization[80], Association of Official Analytical Collaboration, AOAC[81] (Official Methods of Analysis, with many countries and organizations contributing their expertise to standards development and method validation. It is a comprehensive and reliable collection of chemical and microbiological methods available in the world and are contained in many of the Codex food standards). Many "Official Methods" have been adopted as harmonized international reference methods by the International Organization for Standardization (ISO), International Dairy

[80] *ISO has over 1600 standards for the food production sector that work to improve agricultural methods and distribution and promote sustainable production, while also enhancing food safety and nutrition. Food safety testing standards from ISO, address the general foundations for a wide range of tests as well as provide standards dealing with the specific methods used in individual tests. For example, 67.050 General methods of tests and analysis for food products (under ICS 76: Food Technology; also Food microbiology, see 07.100.30 or Sensory analysis, see 67.240; https://www.iso.org/standards-catalogue/browse-by-ics.html*

[81] *Official Methods of Analysis (OMA) is the most comprehensive and reliable collection of chemical and microbiological methods and consensus standards available. Many Official Methods have been adopted as harmonized international reference methods by the International Organization for Standardization (ISO), International Dairy Federation (IDF), International Union of Pure and Applied Chemistry (IUPAC), and the Codex Alimentarius Commission. Official Methods of Analysis, 21st Edition (2019)*

Federation (IDF), International Union of Pure and Applied Chemistry (IUPAC), and the Codex Alimentarius Commission[82] or of other standardisation bodies, or are published in the scientific literature. Methods in national or international standards are regarded as validated, but the laboratory must verify that all conditions (performance criteria) are fulfilled in the laboratory's application including the stated measurement uncertainty.

If laboratories claim standard methods in their scope of accreditation, these must be followed precisely without variation from the published specification. By this, the laboratory must not carry out a method validation, but showing by acquired data that it can achieve the level of performance that the standard specification claims for the method. If the measurement uncertainty of the result is not mentioned or stated in the national or international standard some reflection about this should be made by the laboratory using it.

b) Documented in-house methods that are the laboratory's own methods. They are subject to a high level of validation that the method is technically sound, suitable for the purpose claimed and acceptable to customers. Production of test methods developed by the laboratory for its own use should be a planned activity and assigned to qualified personnel equipped with adequate resources and validated against standard methods.

c) Documented in-house methods based on standard specifications. They make up a major part of many laboratories' scopes. They reduce the amount of validation on the extent of the departure from the standard specification. When reporting data from such methods, the variation from the standard specification must be recognized. It is also necessary to ensure that customers are aware of the variation and accept the resulting data as still being suitable for their purposes.

For a laboratory, it is always preferable to use standard published methods that are validated with most factors investigated and specified as part of the method documentation. Verification of the performance of a standard method requires substantially less work than validation of a method developed in-house. It presents a simplified validation process to check or verify a test method's performance characteristics by evaluation of a subset of parameters.

The laboratory must always test its own capability directly. There is no guarantee that the laboratory skills and instrument performance is of the same standard as those used to generate the standard validation data. Thus, verification is necessary to provide objective evidence that the laboratory has the ability to achieve acceptable results for a given test method, to prove that an externally validated test method is acceptable for its intended use. It is the demonstration that the laboratory is capable of replicating, with an acceptable level of performance, a standard method during time between revalidation.

The laboratory must verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance and for that, the laboratory should:

- Develop a clear, detailed verification procedure that defines the parameters to be evaluated
- Define and approve the acceptance criteria (Annex 1) to be used in analysing the results
- Compare experimental results to the previously established performance characteristics
- Based on the results, accept or reject the test methods
- Summarize the data collected from the verification study in a final report (see Chapter 9.3. for more information and below).

[82] *http://www.fao.org/fao-who-codexalimentarius/en/*

Under conditions of use, verification is demonstrated by meeting system suitability specifications established for the method, as well as a demonstration of accuracy and precision or other method parameters for the type of method (JCGM 200:2012[2], §2.44 for additional details and examples). Guidance related to verifying the performance of a standard method is provided by ISO 5725-2:2019[70].

Table 5: Verification parameters (for their definition, see Annex 1)

Characteristics analysed	Types of experiments performed
Accuracy	Comparison of methods to estimate inaccuracy or bias. Bias and linearity
Precision	Replication experiment to estimate imprecision. Repeatability and Reproducibility
Reported range of test results for the test method	Linearity type experiment to estimate imprecision and to determine reportable range

The verification must be documented and records of the verification retained to provide evidence that the laboratory is capable of achieving the required performance characteristics of the method. In line with the ISO 17025 standard[1], this can include:

- Estimation of repeatability and/or reproducibility (see above)
- Characteristics of instruments
- Operator qualification (training, experience, competences, etc.)
- Environmental conditions
- Materials or reagents
- Any other characteristics that could influence the result.

The following aspects also apply:

For occasionally used methods, a reasoning should be made related to the personal competence or the fitness of the equipment, considering e.g., the experience and education of the personnel in areas close to the method in question or the straightforwardness of the method.

If an issuing body is revising a standard method, the laboratory must repeat the verification to the extent necessary. As a rule, customers of the laboratory requesting a test to a particular standard specification are entitled to assume that the laboratory will use the current version. If the laboratory is using an older version, the laboratory must inform the customer about the differences and whether to proceed with the old version becomes the customer's decision. If the customer specifies an older version, the laboratory must respect the customer wishes, subject to the requirement to draw the customer attention to any limitations introduced by this choice.

Test methods using test kits or laboratory instruments should be evaluated for their ability to detect sensitivity, specificity, positive and negative predictive values and to determine normal and reportable ranges. Manufacturers of test kits or instruments provide information related to performance evaluation for testing methods in the package inserts or in the operator's manuals. The laboratories need to verify the manufacturer's performance claims, and demonstrate that they can get the same results using the kits or equipment in their laboratory, with their personnel. Some of the steps that should be followed to verify the performance will include testing samples with known values and

comparing the results to the expected or certified value and if the equipment is temperature controlled, establishing the stability and uniformity of the temperature.

9.2. Validation of test methods and performance criteria

Accredited laboratories must validate non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified as extensive as it is necessary to meet the needs of the given application or field of application. Test methods should be validated, when a new test method is developed, an established test method is modified, and quality control indicates that an established test method is changing with time and by demonstrating the equivalence between two methods.

Method validation is defined as the confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled (ISO 17025[1], Annex 1). Validation of a method establishes, by systematic laboratory studies, that the method's performance characteristics are capable of producing results in line with the needs of the analytical problem. The aim is to establish the operational limits and performance characteristics (Annex 3) to a new, modified or inadequately characterised test method, to provide evidence that the results are accurate and reliable and to demonstrate that the test method is fit for purpose. If a modified version of a method is required to meet the same specification as the original method, then the modified method must be validated for those parameters that are likely to be affected by the revision.

Validation is a relative concept and its extent and how much validation is needed for a method depends on the requirement to be "adequate for intended use". If the laboratory has developed the method itself, then appropriate validation can be a very complex process requiring a demonstration of the scope of applicability of the method in terms of samples and numerical range, selectivity, robustness in use, accuracy, precision, bias, linearity, detection limit, and any other relevant characteristics (Annex 3). Method validation is the process by which the laboratory demonstrates whether a method is "fit for purpose" (Fig. 1) and if tests carried out are appropriate with respect to uncertainty, cost, and time etc. The laboratory must show that the method as applied is suitable for the purpose claimed or demanded by customers and that the laboratory personnel can achieve the stated performance criteria.

Techniques used for method validation can be one or a combination of the following (source ISO 17025, note in 7.2.2.1 of the standard):

- Calibration and evaluation of bias and precision using reference standards or reference materials
- Systematic assessment of the factors influencing the result
- Testing method robustness through variation of controlled parameters, such as incubator temperature, volume dispensed
- Comparison of results achieved with other validated methods
- Interlaboratory comparisons
- Evaluation of measurement uncertainty of the results based on an understanding of the theoretical principles of the method and practical experience of the performance of the sampling or test method.

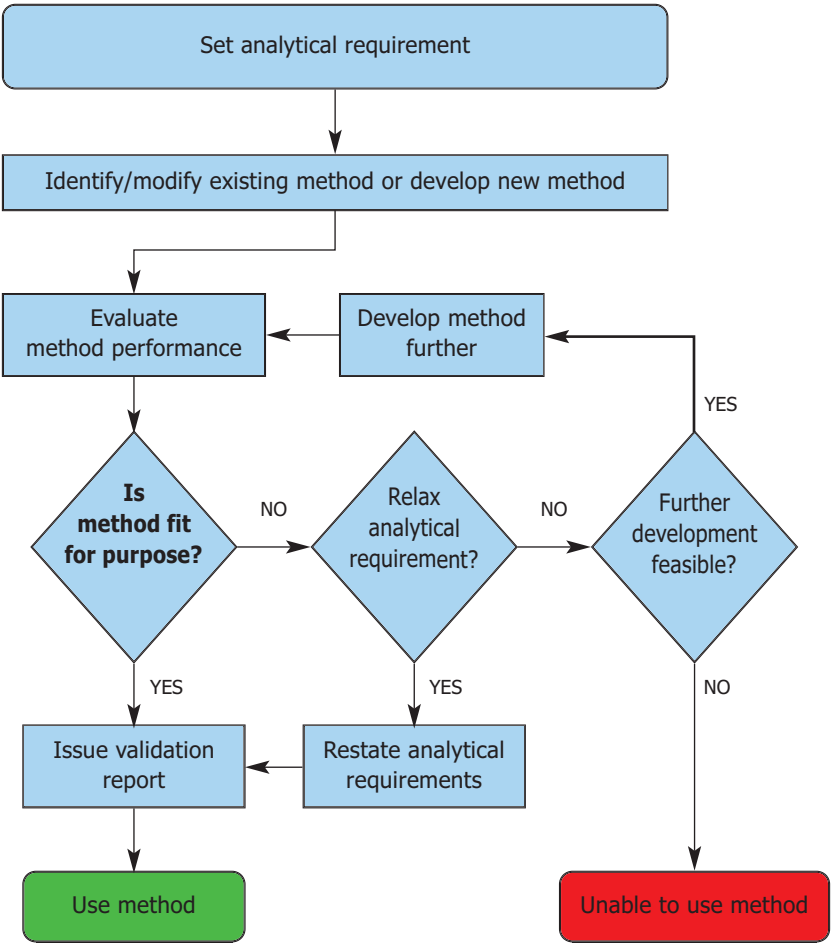

Figure 1: Method validation process, source Eurachem[83]

Validation could include procedures for sampling, handling and transportation of test items. If sampling and subsampling are part of the measurement/testing procedure they must be included in validation.

When validating methods by one or more alternative techniques, the apparent differences can be analysed statistically to confirm their significance. Experimental design and analysis of results must be statistically valid.

Any method validation study will require the laboratory to investigate several performance characteristics, which are individual characteristics required for satisfactory

[83] *Leaflet Eurachem: The importance of method validation, https://www.eurachem.org/index.php/publications/leaflets/mnu-il-mv*

performance of validated methods, and assessed for the intended use (see Annex 3 for details). These performance characteristics must be relevant to the customer needs and consistent with specified requirements. Exactly which characteristics are studied will depend on the analytical application. In addition, legislative and/or sectoral requirements must be considered too. Even if these steps are performed elsewhere, it is useful to include information about them in the validation plan and report. Guidelines related to method verification, validation and quality control, if available, should be used (e.g., the ones for pesticides analysis by the EA[84]). It could be that accreditation bodies require the laboratories to follow specific guidelines related to quality control and validation, verification in order to receive accreditation in the specific test area.

The analytical method validation contains the following (performance) characteristics[85]:

- Measurement range: The concentration interval over which acceptable accuracy, linearity and precision are obtained
- Accuracy: According to ISO 5725-1[86] it describes the closeness of a measurement to the true value. For sets of measurements of the same measurand, trueness is the closeness of the mean of a set of measurement results to the actual (true) value and precision is the closeness of agreement among a set of results
- Bias of a measuring system (method) is the systematic error of that measuring system. Bias and recovery are mostly treated as synonyms and as indicators of accuracy. The difference in recovery might be the function of analyte content and matrix mismatch (Linsinger, 2008[87])
- Precision: Closeness of agreement between indications obtained by replicate measurements on the same or similar objects under stated specified conditions. It is a general term for the variability among repeated tests under specified conditions. Two types of precision, repeatability and reproducibility, have been found necessary and, for many practical cases, are sufficient for describing the variability of a test method
- Repeatability or reproducibility: Measure of the degree of repeatability. The amount of scatter in the results obtained from multiple analysis of a homogenous sample
- Limit of detection (LOD): Lowest concentration of the analyte that can be confidently detected by the method
- Limit of quantification: Strictly, the lowest concentration that can be determined with an acceptable level of repeatability, precision, trueness and measurement uncertainty
- Linearity: Ability of the analytical method to produce test results which are proportional to the concentration of analyte in samples within a given concentration range
- Robustness against external influences or cross-sensitivity against interference from the matrix of the sample or test object: Ability of the test method to remain unaffected by small and deliberate changes, e.g., temperature
- Measurement uncertainty of the results (Chapter 9.5).

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[84] *EA-4/22 G: 2018, EA Guidance on Accreditation of Pesticide Residues Analysis in Food and Feed; Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed by the EU. SANTE/11813/2017*

[85] *For official definitions and explanations see Annex 3*

[86] *ISO 5725-1:1994, Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[87] *Thomas P J Linsinger, European Commission: Use of recovery and bias information in analytical chemistry and estimation of its uncertainty contribution; TrAC Trends in Analytical Chemistry 27(10):916-923, 2008*

The characteristics above are interrelated, and many of these contribute to the overall measurement uncertainty. The data generated could be used to evaluate the measurement uncertainty. For additional reference, an overview of most relevant performance characteristics is presented in Annex 3. Typically, method accuracy/bias and precision are always in the path of food method evaluation and associated acceptance/failure in release testing.

Good practice in method validation is described by a guide and its supplements published by EURACHEM[71] (with a vast list of useful literature resources and reference documents). The guide informs how to plan, record and report validation studies to best support the statement of "fitness for purpose". It provides key definitions and the rationale behind the experiments for assessing the various performance characteristics and has a quick reference tables for experiments and statistical calculations included for evaluation and reporting of each performance characteristic. The guidance supports the analyst on how to make the best use of method validation data for setting up an internal quality control plan.

The following aspects are also relevant in method validation:

When stating validation data, it is advisable to inform on the convention followed.

Method validation, if required, must be a planned activity, assigned to competent personnel equipped with adequate resources. Validation might be obtained by using scientific knowledge and experience to describe and demonstrate the validity of factors involved. The laboratory should authorise personnel for method development and validation/verification (Chapter 4). An applied systematic approach includes judging which factors are of most importance and deserve most attention. The validation procedure should be chosen in accordance with the actual type of method. Three main phases could be applied, such as:

- Distinguish between method of test, and of producing and processing the specimen, including sampling
- Consider the test or measurement factors (equipment and calibration, handling of specimen, testing or measurement procedure, analysis and form of results)
- Consider supplementary changing factors (environment, education and experience of operator, frequency of use of the method)

As method development proceeds, periodic reviews must be carried out to confirm that the needs of the customer are still being fulfilled.

The laboratory, if possible, uses interlaboratory comparison, proficiency tests or reference materials to show that the complete chain of testing or analysis gives the stated result, including measurement uncertainty.

The laboratory must validate in-house methods, but with consideration of a cost-benefit perspective and in agreement with the customers. Often the method is an extension or a simple combination of known methods.

Method extensions or variations of methodologies are very important for services to innovative branches of industry and laboratories. For efficient accreditation by a flexible scope approach, such validation is important.

When methods are modified, it is to be considered if the validation needs to be updated, depending on the extent and significance of the modification. The extent of revalidation will depend on the nature of the change and the level of required revalidation increases with the scale of the changes made to the method. Revalidation should also be considered following changes in premises or instrumentation.

Deviating from the documented procedure is acceptable, provided an appropriately qualified and authorised person makes the decision and that details are recorded. If relevant to the interpretation of the results, the deviation must also appear on the test report.

The influence of changes made to a validated method must be determined and where they are found to affect the original validation, a new method validation should be performed. The extent of validation must be clearly stated in the documented method so that the user can assess the suitability of the method for their particular needs.

The laboratory must retain the following records of validation (ISO 17025):

- The validation procedure used
- Specification of the requirements
- Determination of the performance characteristics of the method
- Results obtained
- A statement on the validity of the method, detailing its fitness for the intended use.

The documentation of the validations should clearly describe which factors are of significance and why and how they are treated in the validation, describing conditions and limitations. The final report should present analytical data in such a way that the customer can readily interpret it and draw appropriate conclusions for the purpose claimed or demanded by customers.

Regular (though not necessarily frequent) review of the performance is required to ensure that methods are still fit-for-purpose. Any modifications to the development plan must be approved and authorized. Even when validation is completed, the users will still need to verify, as appropriate, (e.g., when there is a change in the critical factors) that the documented performance can be met. It can be accomplished by the use of spiked samples or matrix reference materials (Chapter 13).

The validation (or quantification) of microbiological tests is not as demanding as for chemical, analytical method. An important aim of microbiological tests is to determine whether the sample to be examined has any inherent anti-microbial properties and whether the incubation and growth conditions can recover microorganism that might be present to an acceptable level.

Aspects to be considered in defining assessment criteria for a microbiological test might include for example:

- The limit of detection: What is the lowest level of microorganisms that can be detected?
- Specificity: What range of different microorganisms can be detected?
- Selectivity: Can the method determine particular microorganism in a complex mixture?
- Quantification: The counting accuracy.

These types of questions should form the basis of a microbiological method validation strategy.

Qualitative microbiological test methods in which results are expressed as detected/not detected, confirmation and identification procedures should be validated by determining, if appropriate, the specificity, sensitivity, relative trueness, positive and negative deviation, limit of detection, matrix effect, repeatability and reproducibility (Annex 3). For quantitative microbiological test methods, in addition to the above characteristics, the limit of quantification within a defined variability should be considered and quantitatively determined. The differences due to the matrices must be taken into account, when testing different types of samples. The results should be evaluated with appropriate statistical methods.

For assessing recovery in microbiology, reference materials are used (a stable bacterial suspension with a known number of colony forming units of the target or unwanted strain).

The recovery from the new batch of culture medium will be compared to the expected number of colony forming units (CFU) from the reference material (RM, CRM or internally produced RM).

Validation of microbiological test methods should reflect actual test conditions achieved by using naturally contaminated products or products spiked with a predetermined level of contaminating organisms. The analyst must be aware that the addition of contaminating organisms to a matrix only mimics the presence of the naturally occurring contaminants in a superficial way. However, it is often the best and only solution available. The extent of necessary validation will depend on the method and the application. The laboratory should validate standard methods applied to matrices not specified in the standard procedure.

For validation in microbiological food and water examinations, the following standards can assist laboratories in obtaining method validation data: ISO 13483[68], ISO 16140 series[69] and ISO 17994[88]. The ISO 7218[17] contains a generalized flow chart to assist with some of the considerations above.

The ISO 16140 series (EN ISO 16140) has been developed to provide a common reference protocol for the validation of new and alternative microbiological methods, as well as to determine general principles for their possible subsequent certification. In EU legislation, the use of alternative analytical methods is acceptable when the methods are validated against the reference method in accordance with the protocol set out by the standard or other internationally accepted similar protocols (Commission Regulation (EC) No 2073/2005[2]). Data generated will provide laboratories with performance data for a given method, enabling them to make an informed choice on the adoption of a particular (alternative) method.

ISO 16140-3 e.g., covers procedures and acceptance criteria for implementing test methods in a laboratory to help food and feed testing laboratories, test kit manufacturers, competent authorities, and food and feed business operators to implement microbiological methods in their laboratories. It includes two phases, the implementation verification study and the (food) item verification study, with separate protocols for the verification of qualitative and quantitative microbiological methods, and confirmation and typing methods. It also provides an informative protocol for the verification of reference methods not yet fully validated.

Laboratories must retain validation data on commercial test systems (kits) used in the laboratory, obtained through collaborative testing and from validation data submitted by the manufacturers, subject to third party evaluation (e.g., by AFNOR (Groupe Association Française De Normalisation); NMKL NordVal[89]; Microval[90], or AOAC). If the validation data are not available, or not fully applicable, the laboratory is responsible for completing the validation of the method.

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[88] *ISO 17994:2014, Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods*

[89] *Nordval International is an independent third party who reviews alternative methods under NMKL, the Nordic Committee on Food Analysis*

[90] *MicroVal is an international certification organisation for the validation and approval of alternative methods for the microbiological analysis of food and beverages. MicroVal facilitates validation and certification against ISO 16140-2:2016, ISO 16140-6:2019 and other validation standard*

9.3. Documentation of test methods

All methods, procedures and supporting documentation, such as instructions, standards, manuals and reference data relevant to the laboratory activities must be suitable, valid, kept up to date and available to personnel, unless it is for some reason not appropriate or possible.

Laboratories ensure the use of appropriate methods and procedures for all their laboratory activities including statistical techniques for analysis of data. The laboratory has to document its technical procedures, such as tests or methods or operating details for the instrumentation. The level of detail for these documents should be such as to enable a trained analyst to carry out tests in a proper and consistent manner. The instruction can be provided either as part of the method description or as separate descriptions of operating procedures.

Not all methods and operating procedures must be written up. Where standard methods are used, the requirement for a method description could be met by providing a copy of the standard specification to personnel. Similarly, equipment operating instructions might be made available entirely in the form of manufacturers' manuals, if these provide all of the information necessary. A combination of the two approaches is often used, with documentation prepared by the laboratory being produced to refer to, amplify and clarify standard specifications and manufacturers' manuals. In any case, the documentation has to be available, either containing all necessary technical information for carrying out the laboratory activities or making clear where the relevant information is to be found. In general, all personnel have a source of reference to enable them to work properly and consistently.

When necessary, the application of the method can be supplemented with additional details to ensure consistent application. For instance by instructions on the use of the laboratory's particular instrumentation or optional steps in the method, details on local quality control regimes and the quality control data to be collected. Often supplementary documentation is required where a standard specification involves choices of procedure, based, for example, on sample item type and for consistence use. Guidance on how to make the choice in the supplementary documentation is considered relevant, also since standard specifications are frequently less than explicit in this area.

Developments in methodology and techniques will require methods to be changed from time to time, because of investigations following poor performance in proficiency tests, or failure to meet internal quality control criteria. Method documentation is therefore subject to adequate document control and all documentation of methods are issued as controlled documents (Chapter 2). This is typically done by compiling a methods manual consisting of in-house methods documentation, any supplementary documentation for standard methods and a list of standard methods used by the laboratory. The methods manual should also inform on the locations of the standard specifications to be found in the laboratory and provide reference to the appropriate instrument manuals and instructions. Each copy of the method should show the issue number, date, issuing authority, and copy number.

It must be possible to determine which is the most up-to-date version of each method that is authorised for use from records. Any report must specify exactly which method was used and noting any deviations from the standard procedure. An out-of-date standard should be included amongst the laboratory's documentation only with care, and the document should be clearly marked with details of when it is appropriate to use, for example for work for a particular customer. The laboratory will have to demonstrate that there is no danger of the method being used in error as to the current version.

Obsolete methods should be withdrawn, but retained, archived and clearly labelled as obsolete. The difference in performance between revised and obsolete methods should be established so that it is possible to compare new and old data. The selected methods must be communicated with the customer and the laboratory must ensure that it uses the latest valid version of a method, unless it is not appropriate or possible to do so.

In-house methods will need a complete documentation. If based on a standard specification, the variations from the standard must be specified in the documentation cross-referring to the specification. The documentation of these methods should include validation data (values for key performance characteristics such as repeatability, bias and limit of detection etc., see Annex 3), information on the scope of applicability of the method and any limitations, and procedures for quality control, raw data if equipment used, calibration and document control. It presents further information on how the results should be reported, including the statement of its measurement uncertainty (9.4) along with instructions on how to deal with failures or out of specification test results. Guidance on investigating and reporting out-of-specification results is provided by IUPAC/CITAC[77].

9.4. Estimation of measurement uncertainty

Food testing laboratories should evaluate measurement uncertainty (MU) or at least estimate measurement uncertainty, identifying the contributions to measurement uncertainty for its measurements. They should have procedure in place and personnel trained to conduct estimation of uncertainty where relevant, e.g., for the validity or application of the test results; a customer's instruction so requires; or for a statement of conformity. Laboratories are required to report measurement uncertainty under specific circumstances, for example, where it is relevant to the interpretation of the test result (e.g., in heavy metals or mycotoxins analysis as required by the European Union).

Measurement uncertainty characterises the range of values within which the true value is asserted to lie, with a specified level of confidence. A statement of uncertainty is a quantitative estimate of the limits within which the value of a measurand (such as an analyte concentration) is expected to lie (see also Annex 1). The MU provides laboratories and customers with valuable information about the accuracy and reliability of test data and tells how the results represent the value of the quantity measured. It also shows, whether the result is within the acceptable limits or outside of it. It gives confidence in the comparability of results that helps to reduce barriers of trade.

While the measurement "error" describes the difference between an actual measurement result and the true value, the "uncertainty" quantifies the doubt about the result. In order to evaluate this uncertainty, it has to be established:

(i) How big the margin of doubt is and

(ii) With which certainty the true result lies within this margin (confidence level).

An indication of the associated uncertainty, e.g., the margin of doubt as well as the confidence level, is important when deciding whether the results are adequate for the intended use.

Every measurement has an uncertainty associated with it, resulting from errors arising

in the various stages of sampling and analysis and from imperfect knowledge of factors affecting the result. The exact deviation of a single measurement result from the (unknown) true value is impossible to obtain. This is because different factors vary from experiment to experiment, also the effect of each factor on the result is never known exactly. A wide variety of factors can make any analytical measurement result liable to deviate from the true value. These are for example temperature effects on volumetric equipment, reflection and stray light in spectroscopic instruments, variations in electrical supply voltages, individual analysts' interpretation of specified methods and incomplete extraction recoveries, all of them potentially influence the result. As far as reasonably possible, such errors must be minimised by external control or explicitly corrected for, e.g., by applying a suitable correction factor. Any error of unknown value, which cannot be compensated by applying correction factors, is a source of uncertainty. Similarly, the used standards, materials and equipment, the applied methods, environmental conditions or the operating personnel can contribute to the measurement uncertainty. Repeatability or reproducibility, for example, are usually not full estimates of the uncertainty, since neither takes full account of any uncertainties associated with systematic effects inherent in a method. The likely range of deviation must therefore be estimated.

For estimating the uncertainty for a particular method and analyte, it is essential to ensure that the estimate explicitly considers all the possible sources of uncertainty, evaluating significant components. The MU is evaluated by quantifying and combining a number of uncertainty components, which could be:

a) Random effects, e.g., fluctuations in temperature, humidity, air pressure, variability in performance of the measurement sampling or

b) systematic effects, e.g., offset of measuring instruments, drift in characteristics between calibrations, personal bias in reading an analogue scale of uncertainty of a reference standard value.

Typically, uncertainty contributions for analytical results might fall into four main groups:

1. Contributions from short-term random variability, typically estimated from repeatability experiments
2. Contributions such as operator effects, calibration uncertainty, scale graduation errors, equipment and laboratory effects, estimates from inter-laboratory reproducibility trials, in-house inter-comparisons, proficiency test results or professional judgement
3. Contributions outside the scope of inter-laboratory trials, such as reference material uncertainty
4. Other sources of uncertainty, such as sampling variability (inhomogeneity), matrix effects, and uncertainty about underlying assumptions (such as assumptions about completeness of derivatisation).

The primary task in assigning a value to the uncertainty of a measurement is the identification of the relevant sources of uncertainty and the assignment of a value to each significant contribution. The separate contributions must then be combined in order to give an overall value. In identifying relevant sources of uncertainty, consideration must be given to the complete sequence of events necessary to achieve the purpose of the analysis. Typically, this sequence includes sampling and sub-sampling, sample preparation, extraction, clean up, concentration or dilution, instrument calibration (including reference material preparation), instrumental analysis, raw data processing and transcription of the output result. The overall approach might be considered as a 'black box'. A record should be kept of the individual sources of uncertainty identified, the value of each contribution, and the source of the value (for example, repeat measurements,

literature reference, CRM data, etc.).

When evaluating measurement uncertainty, significant contributions are taken into account by quantifying them either by evaluation of the results of several repeated measurements or by estimation based on the data received from records, previous measurements, and knowledge of the equipment and experience of the measurement. The evaluation from repeated measurements is covered by applying a mathematical formula derived from statistical theory. The uncertainty contributions for each source must all be expressed in the same way, ideally as standard deviations or relative standard deviations. In some cases, this will entail some conversion.

The MU might be expressed as a standard deviation or a calculated multiple of the standard deviation. It is usually articulated as an expanded uncertainty and provides an interval within which the value of the meausurand is believed to lie with higher level of confidence. It is obtained by multiplying the combined standard uncertainty by a coverage factor k where k is based on the level of confidence desired. For a level of confidence of 95%, k is two (2).

The international definition of uncertainty of measurement is given in the International Vocabulary of Metrology (VIM 3) and the concept and approach to MU described in a more practical way for analytical measurements, mainly of chemical nature, by the EURACHEM/CITAC Guide "Quantifying uncertainty in analytical measurement[72]". The policy of accreditation bodies for uncertainty in calibration is provided by ILAC P14[73]. For further information, see also ISO/IEC Guide 98-3[79], ISO 21748[78], the ISO 5725-2[70] & series[91] (Annex 2); and ILAC G17[74].

Laboratories are not required to evaluate a unique measurement uncertainty every time a test is performed, provided the measurement uncertainty of the results has been established and verified and the laboratory can demonstrate that the identified critical influencing factors are under control. In those cases where a well-recognised test method specifies limits to the values of the major sources of measurement uncertainty and specifies the form of presentation of the calculated results, the laboratory is considered to have satisfied these by following the test method and reporting instructions. EU legislation for instance is specifying how to calculate required MU for result of the analysis of certain contaminants in foodstuffs[63] and in some cases a given MU is available that could be used by laboratories for a certain test method as in the case of an international multiresidue pesticides method[89].

Evaluation of measurement uncertainty is required for all calibrations, including those the laboratory performs on its own equipment, e.g., "in-house" calibrations (Chapter 5).

The Table 6 presents differences as well as advantages and disadvantages of the two broad approaches in the measurement uncertainty, (MU) evaluation processes.

The modelling approach to the MU estimation is described in detail by ISO GUM 85[92], and is interpreted in the Eurachem measurement uncertainty guide[93](for chemical analysis; see

[91] *ISO/CD 5725-3: Accuracy (trueness and precision) of measurement methods and results — Part 3: Intermediate precision and alternative designs for collaborative studies; ISO 5725-4: …Part 4: Basic methods for the determination of the trueness of a standard measurement method; ISO 5725-5:1998)… - Part 5: Alternative methods for the determination of the precision of a standard measurement method (ISO 5725-5:1998)*

[92] *For the version of JCGM 100, on which ISO/IEC Guide 98-3:2008 is based, see http://www.iso.org/sites/JCGM/JCGM-introduction.htm.*

[93] *A. Williams and B. Magnusson (eds.) Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment (2nd ed. 2021). ISBN 978-0-948926-38-9. Available from www.eurachem.org*

Annex 2). It is also called the "bottom-up" approach that means that the uncertainties of the input quantities are found, and thereafter combined into the combined standard uncertainty. The top down approach presents a systematic approach to capture contributions to MU. It considers mainly data from own statistical analysis from within-laboratory method validation and interlaboratory comparison studies. Most accreditation bodies recommend the use of either approaches for estimating measurement uncertainty in chemical analyses.

Table 6: Approaches in measurement uncertainty[94]

Approach	GUM - bottom up approach	Top down approach
Principles	Component-by-component using Gauss' error propagation law for uncorrelated errors	Component-by-component using Gauss' error propagation law for uncorrelated errors
Components	Studying uncertainty contributions in each step of test method as much as possible "Modelling approach" or "bottom up approach", based on a comprehensive mathematical model of the measurement procedure, evaluating individual uncertainty contribution as dedicated input quantities	Using repeatability, reproducibility and trueness of test method, according to basic principle: accuracy = trueness (estimates of bias) + precision (estimates of random variability) "Empirical approach" or "top up approach", based on whole method performance to comprise the effects from as many relevant uncertainty sources as possible using the method bias and precision data. Such approaches are fully in compliant with the GUM, if GUM principles are observed
	Acknowledged as the master document on the subject of measurement uncertainty	There are few alternative top down approaches, receiving greater attention by global testing community today
	GUM classifies uncertainty components according to their method of determination into type A and type B: Type A – obtained by statistical analysis Type B – obtained by means other than statistical analysis, such as transforming a given uncertainty (e.g., CRM) or past experience	Top down approaches consider mainly Type A data from own statistical analysis from within laboratory method validation and inter-laboratory comparison studies

[94] *https://consultglp.com/2017/04/27/measurement-uncertainty-comparing-gum-and-top-down-approaches/*

	GUM assumes that systematic errors are either eliminated by technical means or corrected by calculation	The top down approach strategy combines the use of existing data from validation studies with the flexibility of additional model-based evaluation of individual residual effect uncertainty contributions.
	In GUM, when calculating the combined standard uncertainty of the final test result, all uncertainty components are treated equally	
Advantages	Demanding critical assessment and full understanding of the analytical steps in a test method Consistent with other fields of measurements such as calibration The MU result generated is relevant to the particular laboratory that produces it	Readily available quality data from method validation and interlaboratory comparison studies by well-run accredited laboratory Much simpler process in MU evaluation The approach is based on statistical analysis of data generated in intra- and inter-laboratory collaborative studies on the use of a method to optimise a diversity of sample matrices
Disadvantages	1. The GUM approach process is tedious and time consuming 2. This methodology might underestimate the measurement uncertainty, partly because it is hard to include all possible uncertainty contributions 3. GUM might unrealistically assume certain errors are random (i.e. normally distributed) and independent 4. GUM provides a broad indication of the possible level of uncertainty associated with the method rather than a measurement 5. It does not take into account either matrix-associated errors or the actual day-to-day variation seen in a laboratory 6. GUM does not apply well when there is no mathematical model in the test method	1. The top down approach might not by itself identify where the major errors could be occurring in process and the results generated are the products of technical competence of the laboratory concerned 2. Interlaboratory reproducibility data, considered in certain instances, might not be fully representative for the variability of results on actual samples, unless data is standardized

9.4.1. Estimation of measurement uncertainty (MU) in microbiological analyses

It is expected that accredited microbiological testing laboratories have a good understanding of the distributions of organisms within the matrices they test. However, it is not always practical that MU is included in estimates unless the customer's needs dictate otherwise. The principal reasons for this are the uncertainty due to the distribution of organisms within the product matrix that is not a function of the laboratory's performance and might be unique to individual samples tested. The test methods should specify the sample size to be used taking into account poor homogeneity.

Microbiological tests generally fall into the category of those that exclude the rigorous, metrological and statistically valid calculation of measurement uncertainty as described in the ISO Guide to the expression of uncertainty in measurement[95].

Approaches to evaluate and express the MU in microbiological testing of food and water are either based on ISO 19036[81] (for food), or ISO 29201[76] (for water). ISO 29201 covers both colony counts and Most Probable Number (MPN) results. The standard presents two different approaches to uncertainty estimation (component approach/bottom-up and a modified global/top-down approach, see also Table 6 for general understanding).

The ISO 19036 standard provides guidance for the estimation and expression of MU associated with quantitative results in food microbiology. It is mainly applicable to the enumeration of microorganisms using a colony-count technique, but also applies to other quantitative analyses, including most probable number techniques, instrumental methods and molecular methods. It covers three uncertainty components to estimate the MU: technical uncertainty, matrix uncertainty, and distributional uncertainty. The uncertainty contribution from systematic effects (bias) is not included, since in food chain quantitative microbiology, particular with conventional microbiology techniques, assigned values or reference quantity values are usually not available, and thus bias cannot be reliably estimated. For assessing recovery in microbiology, reference materials are used (a stable bacterial suspension with a known number of colony forming units of the target or unwanted strain, see also Chapter 9.1.).

In food microbiology, the main sources of uncertainty are sampling, the laboratory sample, the matrix, equipment, growing media, growing conditions and reagents, additional random errors, the taking of subsamples, primary dilution, the analyst, time, and the systematic error. Usually, a bottom-up approach is not applicable for calculation of uncertainty in microbiological examinations due to difficulty to construct the measurement model and modelling approach and involving all possible variables of microbiological examinations and to identify all uncertainty contributions. Thus Type A uncertainty evaluation based on reproducibility standard deviation with intermediate precision are typically used for evaluation of uncertainty in microbiological examinations. It is generally appropriate to base the estimate of measurement uncertainty on repeatability and intermediate precision (within laboratory reproducibility) data. The individual uncertainty components should be identified and demonstrated to be under control and their contribution to the variability of results evaluated. Some components (e.g., pipetting, weighing, dilution effects and incubator effects) might be readily measured and easily evaluated to demonstrate a negligible contribution to the overall measurement uncertainty. Other components (e.g., sample stability and sample preparation) cannot be measured directly and

[95] *ISO/IEC GUIDE 98-1:2009 Uncertainty of measurement — Part 1: Introduction to the expression of uncertainty in measurement*

their contribution cannot be evaluated in a statistical manner but their importance to the variability of results should also be considered.

The three uncertainty components are:

1. Technical uncertainty is estimated by the reproducibility standard deviation (SD) on the final measurement result, with three (3) options in a decreasing order of priority: intra-laboratory reproducibility SD, inter-laboratory reproducibility SD from a method validation study and inter-laboratory reproducibility SD from a PT. Technical uncertainty is linked to the method as used in a given laboratory
2. Matrix uncertainty arises from heterogeneity of matrix contamination, resulting in variability of microbial levels between test portions, which can be large for solid matrices and composite food products. For each kind of matrix, matrix uncertainty is taken as a fixed value for homogeneous matrices, or estimated by a repeatability experiment, or derived from historical values
3. Distributional uncertainties derive from the random distribution of microorganisms. According to the feature of the analytical method, they are calculated mathematically in three potential cases: for colony-count techniques: Poisson uncertainty (significant at low levels) and confirmation uncertainty (when the method includes a partial confirmation step).

The standard proposes two options for estimating and reporting MU: 1. The complete option: the technical, matrix and distributional uncertainty components are estimated separately from each other, then combined to calculate MU. 2. A simplified option: a general MU value might be reported as based only on technical uncertainty, if consistent with laboratory protocols and client requirements. An Excel tool to implement the calculations of the standard are also provided.

10. METROLOGICAL TRACEABILITY

ISO/IEC 17025[1], section 6.5; ISO 17034[96]; ISO 7218[17]; ILAC P10:07[31]; EURACHEM/CITAC Guide: Metrological Traceability in Analytical measurement[97]; IUPAC Technical Report "Metrological traceability of measurement results in chemistry: Concepts and implementation[98]; SI Brochure[99]; IUPAC: Harmonised guidelines for the use of recovery information in analytical measurements[100]

10.1. Establishing metrological traceability at food testing laboratories

Establishing metrological traceability is essential for food testing laboratories. The concept of metrological traceability ensures comparability of measurement results, in different laboratories or at different times, both nationally and internationally, and provides confidence in results. Accredited laboratories must establish and maintain metrological traceability of its measurement results by means of a documented unbroken chain of calibrations, each contributing to the measurement uncertainty, linking them to an appropriate reference, usually a national or international standard. The ISO/IEC Guide 99[2] defines metrological traceability as the "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 measurement uncertainty".

Metrological traceability applies to both physical and chemical measurements. The measurement results can be from a material measure, measuring instrument, reference material, or a measuring system that defines, realises, conserves or reproduces a unit, of one or more values of a quantity, to serve as a reference. It is to be noted, that the instrument itself is not traceable, but the results produced by the instrument are. Practical guidance on the traceability of chemical measurements is provided by Eurachem/CITAC[97] and IUPAC[98].

A complete traceability chain is achieved through a calibration hierarchy consisting of primary measurement standards (or other high-level measurement standards), which are used to establish secondary measurement standards that can be used to calibrate working level standards and related measuring systems. The chain of comparison must end, where possible, at the primary standard for the realisation of the SI units and whenever possible, traceability to SI units through suitable measurement standards should be documented. This is to support the comparability of measurement results across space and time.

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[96] *ISO 17034:2016: General requirements for the competence of reference material producers*

[97] *S L R Ellison and A Williams (Eds) Eurachem/CITAC Guide: Metrological Traceability in Analytical measurement (2nd ed. 2019). ISBN: 978-0-948926-34-1. Available from www.eurachem.org*

[98] *IUPAC Technical Report "Metrological traceability of measurement results in chemistry: Concepts and implementation"; Accreditation and Quality Assurance volume 16, Article number: 473 (2011)*

[99] *BIPM: SI Brochure: The International System of Units (SI); www.bipm.org*

[100] *Thompson, S. Ellison, A. Fajgelj, P. Willetts, R. Wood, Harmonised guidelines for the use of recovery information in analytical measurements, Pure Appl. Chem., 71(2) 337-348 (1999)*

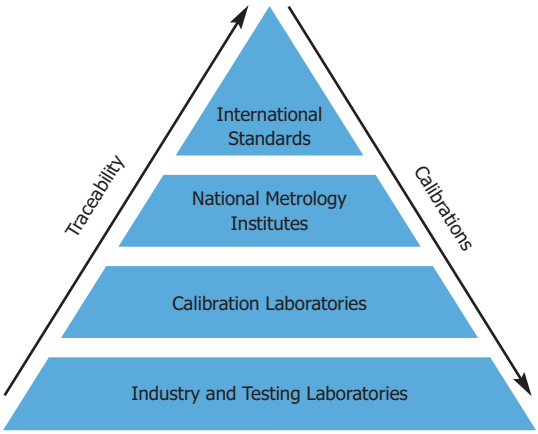


Figure 2: The traceability pyramid

The SI units are maintained by a network of National Metrology Institutes, e.g., by the International Bureau of Weights and Measures (BIPM) in Paris.

For information purposes, measurement standards are realizations of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference (VIM 3[2]). They are categorized along the calibration chain as:

1. National standards maintained by national metrological institutes and primary standards: They cannot be calibrated by another measurement standard but are compared with other primary standards
2. Secondary standards: traceable to primary standards.

Company standards

3. Reference standards: most accurate standard used in the laboratory and protected carefully
4. Transfer standards: used as an intermediate to compare standards
5. Working standard: used frequently (e.g., for daily calibration of equipment).

In general, laboratories purchase their measurement standards from commercial producers. They are supplied with certificates demonstrating their traceability to higher-level measurement standards.

Laboratories, when establishing metrological traceability in the laboratory must answer the following questions:

- What is the quantity to be measured
- Are all calibrations going back to appropriate references in an unbroken chain
- Is the measurement uncertainty evaluated for each step in the traceability chain
- Is each step of the chain performed according to appropriate methods, with recorded measurement results and the associated measurement uncertainties
- Is each step of the chain performed with the appropriate technical competence
- Are systematic measurement errors (bias) taken into account?

Laboratories ensure that measurement results are traceable to the International system of Units (SI) through:

- Calibration provided by a competent laboratory (fulfilling ISO 17025[1] requirements)

- Certified values of certified reference materials provided by a competent producer with stated metrological traceability to the SI[99] or by
- Direct realisation of the SI units ensured by comparison, directly or indirectly with national or international standards.

Details of practical realization of the definitions of some important units are given in the SI brochure[99].

Whenever possible and cost-efficient, laboratory uses accredited calibration laboratories or national metrology institutes for their equipment calibration in the knowledge that the calibration is internationally traceable. Accredited calibration services are generally considered competent. If the calibration laboratory is not accredited, the laboratory must ensure that the calibrations are adequate and that the traceability is intact. They can assess the competence of this calibration laboratory based on ISO/IEC 17025 as follows:

- Traceability of used standards, references, which are properly calibrated and provide international traceability
- Use of calibration procedures, that are scientifically sound, of known performance characteristics
- Uncertainty evaluation procedure
- Personnel carrying out the procedures, are properly trained and competent in the calibrations performed.

In case a national metrology system does not exist, calibrations with the necessary traceability can be performed by e.g., a national metrology laboratory from a nearby country.

If it can achieve traceability, the laboratory can be self-sufficient in calibration and not use any external calibration services for its equipment. Internal calibration would also have to be subject to an evaluation of its uncertainty by the laboratory just as though it were carried out by an external and accredited calibration service.

Laboratories seeking accreditation might contact their Accreditation Body for retrieving information, if a proposed calibration service is accredited to ISO/IEC 17025 and covered by a mutual recognition agreement for calibration with them. If not, the Accreditation Body might have a policy on the acceptance of calibrations from this calibration service. If this is not the case, it is to be evaluated what information the Accreditation Body would require to make a decision on the acceptability of calibrations from the proposed calibration service. This normally concerns examples of calibration certificates, information on how the calibration service establishes its traceability, what arrangements the calibration service has for measurement audit or comparisons with other calibration bodies and whether it has a management system. Essentially, the rule is to establish, as early as possible, that the proposed Accreditation Body will be likely to accept the calibrations the laboratory is proposing to rely upon.

ILAC P10:07[31] describes the ILAC policy with regard to the metrological traceability requirements of ISO/IEC 17025, and provides laboratories with guidance on how to address the traceability issue.

When metrological traceability to the SI units is not technically possible, the laboratory should demonstrate metrological traceability to an appropriate reference, for instance:

- Certified values of a certified reference material provided by a competent producer
- Results of reference measurement procedures, specified methods or consensus standards that are clearly described and accepted as providing measurement results fit for their intended use and ensured by suitable comparison methods or consensus standards specified to demonstrate metrological traceability.

However, the use of appropriate references to show that measurements are acceptably accurate is not a substitute for traceable calibration of instrumentation, since this only

tests the system at a single point.

Since some measurement results (e.g., pH, concentrations of some biological substances, hardness) have no SI units, such measurement results should be traceable to internationally agreed references (e.g., pH scale, or WHO reference materials). In this case, measurements are traced back to the relevant reference rather than to a SI unit, but provide acceptable metrological traceability in that they establish comparability between different laboratories.

In general, calibrations must be repeated at appropriate intervals (Chapter 5.5).

10.2. Metrological traceability in chemical analysis

Although traceability to SI is the ideal, it is not the only option for the start of a metrological traceability chain. Establishing chemical measurements traceability of involved values of physical quantities, such as mass, volume, and the concentration of measurement standards for instance can be readily achieved by calibration (at the level of uncertainty needed for analytical measurements), according to established procedures of the relevant equipment using measurement standards.

The problematic areas in metrological traceability are usually calibration and validation of methods in chemical analysis. This is due to the use of RMs in instrument calibration and its uncertainties (related to purity of the RM used, the preparation of a set of standards) that will be part of the uncertainty budget for the measurement results, together with the uncertainty of the calibration itself. However, the purity of the suitable calibrants (e.g., pure substances or solutions of pure substances) will only create a significant problem in case of some organic materials, where purity and stability problems can be severe, or where low uncertainty is required. For more information related to metrological traceability in chemical measurement achieving comparable results reference is provided in a guideline published by Eurachem/CITAC[97].

A major issue in chemical analysis is the 'matrix effect'[101]. Thus, besides calibration of measuring equipment, the traceability of measurement results in analytical sciences relies on validation too, to establish that the method actually measures what it is intended to measure (e.g., the mass fraction of methyl mercury in fish) and the confirmation that the measurement equation for calculating the results, including appropriate 'recovery' factors, if necessary, is valid.

Sample preparation (involving extraction, digestion, derivatisation and/or saponification; Chapter 8.3) in food analysis is challenging, since in steps of sample preparation bias (see Annex 3) can arise due to incomplete recovery of the analyte from the sample matrix, of processing losses, contamination or interferences. IUPAC describes the issue in detail and strategies to address method bias include:

- Use of primary or reference methods of known and small bias
- Comparisons with closely matched matrix CRMs
- Measurement of spiked samples and blanks
- Study of losses, contamination, interferences and matrix effects
- Collaborative studies according to ISO 5725-3[70].

[101] *Different analytical behaviour of atoms and molecules depending on their surrounding environment*

For establishing metrological traceability in the laboratory, it is relevant for laboratories to analyse: the quantity to be measured; if all calibrations going back to appropriate references in an unbroken chain; if MU is evaluated for each step in the traceability chain and if each step of the chain is performed in line with the appropriate methods and recorded with associated MUs and performed with the appropriate technical competence; and if systematic measurement errors are taken into account.

Notably, the use of spiked samples in measurement of the recovery does not necessarily completely simulate the extraction of the native analyte from the samples. For instance, when extracting solids and in case of liquid or digested biological samples, the association with carrier biomolecules can lead to a reduction in the extracted amount compared to the extraction of the same analyte spiked into a sample.

In other cases, the limitation in achieving traceability to SI derives from difficulty in evaluating bias and its uncertainty, such as the recovery of the analytes from complex matrices. One option is to define the measurand by the method and to establish traceability as described for empirical methods, see below. Such measurements have a lower level of traceability, but a smaller measurement uncertainty relative to the stated references. Alternatively, the bias is estimated and corrected with the uncertainty of the overall uncertainty evaluation. In many cases, the bias is left uncorrected, but is taken into account in the estimate of the measurement uncertainty.

Most measurement results from chemical analysis can be traceable to the mole. If the measurand is defined in operational terms, e.g., for nitrogen determination based on extractable protein, the measurement results are not traceable to the mole. In this case, the measurand is defined by the method and by variations in the protocol (e.g., a different solvent or a different conversion factor) and the traceability is to the agreed method (e.g., standard method). That is why the method must be followed exactly and to the corresponding SI units, e.g., mass and volume (the quantities used to calculate the result), to the values produced by the method and/or the values carried by a reference material. Such methods are called empirical methods.

11. REFERENCE MATERIALS AND CHEMICAL STANDARDS

ISO/IEC 17025[1], sections 6.4; 6.5; 6.6; 7.2; 7.7; ILAC G9; ISO/IEC 17043[102]; ISO Guide 35[39]; ISO/IEC 17034[96]; ISO Guide 30[103]; ISO Guide 31[104]; ISO Guide 33[105]; ISO Guide 34[106]; ISO 11133[46].

11.1. Reference materials (RM) – General

Reference materials (RMs) provide essential traceability in measurements. The use of RMs is strongly encouraged, wherever appropriate. Standards for the accreditation of testing laboratories demand the use of reference materials as important procedure to provide essential traceability (Chapter 10). RMs are also called "reference standards", "calibration standards", "standard reference materials", and "quality control materials". They are reliable quality assurance tools that improve confidence in test results.

The use of appropriate RMs enables analysts to demonstrate the traceability of results by calibrating equipment, to validate methods and to monitor the method's performance, to demonstrate quality of culture media and consistent performance of test kits. RMs are used for calibration, method validation, and measurement verification. In addition, they might be used as transfer (measurement) standards for comparison of methods, for evaluating measurement uncertainty, quality control and for training purposes.

RMs are particularly important for analytical chemistry and play a key role in the calibration of laboratory instruments by providing precise reference values and data. Since most analytical instrumentation is comparative, it requires a sample of known composition (reference material) for accurate calibration. However, a specific RM can only be used for one purpose in measurement, e.g., for Quality Assurance purposes or for calibration.

Reference materials are produced under stringent manufacturing procedures and differ from laboratory reagents in their certification and the traceability of the data provided. ISO recognizes two (2) classes of reference materials: Reference materials (RM) and certified reference materials (CRM).

A reference material (RM) is any 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 of checking methods or apparatus. For the definition of CRMs see Chapter 11.2. and Annex 1.

Since RMs are important tools for the transfer of measurement accuracy between laboratories, their property values should, where feasible, be traceable to SI units. A hierarchy of methods is used for assigning property values to materials, and their traceability can be described as follows:

[102] *ISO/IEC 17043:2010, Conformity assessment — General requirements for proficiency testing*
[103] *ISO GUIDE 30:2015, Reference materials — Selected terms and definitions*
[104] *ISO GUIDE 31:2015, Reference materials — Contents of certificates, labels and accompanying documentation*
[105] *ISO GUIDE 33:2015, Reference materials — Good practice in using reference material*
[106] *ISO GUIDE 34:2009, General requirements for the competence of reference material producers*

Table 7: Traceability of measurement methods

Measurement method	Traceability
Primary method	SI
Method of known bias	SI/International standard
Independent method(s)	Result of specified methods
Interlaboratory comparison	Result of specified method

The measurement uncertainty of the property value of a RM employed in a measurement process will contribute to the uncertainty of the final measurement. Ideally, the uncertainty associated with the property value of a RM, used for calibration purposes, should not contribute more than one third of the overall measurement uncertainty[107].

Two key types of RMs exits:

a) Single compound and multi compounds or items of established purity or properties and
b) Matrix references, which are specific types of sample where accepted values of one or more determinants have been established.

Classes of reference materials can be distinguished as primary RM, secondary RM and in-house or working RM with increasing uncertainty in this order.

Reference materials might take a variety of forms. ILAC describes the following five (5) types of reference materials:

1. Pure substances; essentially pure chemical characterised for chemical purity and/or trace impurities, e.g., 95% pure sodium chloride
2. Standard solutions and gas mixtures, often prepared gravimetrically from pure substances
3. Matrix reference materials characterised for the composition of specified major, minor or trace chemical constituents. Such materials might be prepared from matrices containing the components of interest, or by preparing synthetic mixtures
4. Physical-chemical reference materials characterised for properties such as melting point, viscosity, or optical density
5. Reference objects or artefacts, characterised for functional properties, for sensory testing such as taste, odour, flash point and hardness. This type includes microscopy specimens characterised for properties ranging from fibre type to microbiological specimens.

Whenever possible, RMs should be used in appropriate matrices.

Information about RMs is available from a number of sources. The COMAR Database contains information on more than 10,000 RMs (general term), for CRMs, RM produced under an ISO 17034 accreditation and, by a National Metrology Institute or a designated Institute. CITAC[108] is an initiative aiming to foster collaboration between existing organisations to improve the international comparability of chemical measurements. ISO REMCO, ISO's committee on RMs (www.iso.org/remco), aims for the international harmonization of RMs and

[107] *For most chemical Reference Materials produced before the late 1990s, the MU values given by the producers were most likely not estimated by the now recommended ISO (GUM) procedure. The actual uncertainty is expected to be larger than stated by a factor of 2-3, (due to use of within laboratory precision measurements only for instance).*

[108] *CITAC (Cooperation on International Traceability in Analytical Chemistry) is an international organization developing new guidelines in the area of metrology in chemistry in cooperation with international organizations such as Eurachem and IUPAC. The organisation also organizes conferences and workshops in this field.*

establishes definitions, categories, and performance characteristics of RMs for use by ISO.

A number of commercial suppliers provide a comprehensive range of materials, including RMs produced by other organisations, presenting them as a one-stop-shop for users. The International Atomic Energy Agency (IAEA) is the world's largest supplier of matrix reference materials characterised for radionuclides used in quality assurance of results obtained by nuclear analytical techniques. They currently are able to distribute more than 90 different RMs from the following groups: Radionuclides; Trace Elements and Methyl Mercury; Organic Compounds, and Stable Isotopes[109].

Generally, the demand for RMs exceeds supply in terms of the range of materials and availability. The option for alternative RMs is rare and the user must choose the most suitable material available. The limitation for certain reference materials requires understanding by both the users and the accreditation bodies, although the situation is improving over time.

A series of ISO documents relating to reference materials are available (Annex 2). ISO/IEC 17034[96] specifies general requirements for the competence of reference material producers, for which these organisations can seek recognition, e.g., by accreditation. RM providers, who meet the requirements of this standard, are considered competent, when accredited. Their RMs are provided with a product information sheet or certificate that specifies e.g., characteristics, homogeneity and stability for specified properties and, for CRMs, specified properties are indicated with certified values, their associated measurement uncertainty and with metrological traceability (Chapter 11.2).

11.2. Certified reference materials

Quality management systems involving laboratory accreditation under national and international accreditation standards such as ISO/IEC 17025 require metrological traceability to Certified Reference Materials (CRMs), where possible, when using reference materials for calibration, validation etc. (11.1).

Certified Reference Materials (CRMs) are samples for which the test results are firmly established and agreed, ideally on an international basis with evidence of the metrological traceability and statement of measurement uncertainty provided on the certificate for these CRMs (see also Annex 1). In order to be effective, a reference material must be typical of the samples that the laboratory tests on a routine basis. Other terminology, such as NIST (National Institute of Standards and Metrology) Standard Reference Materials or Standard Reference Material (SRMs) are regarded equivalent to CRM. In the US a classification (class O – V) based on the degree of traceability to SI has been proposed.

The key to the RM is in the acceptance. The highest level of acceptance is a certified reference material (CRM, complying with the definition of a CRM in ISO Guide 30) that is produced according to ISO Guide 35 by an organisation complying with ISO 17034[96] and where the certificate complies with ISO Guide 31[104], a guide that gives guidance on the contents of RM certificates. Metrological valid procedures for the production and certification of RMs are provided by ISO/IEC 17034 covering the production of all RMs, including CRMs (Chapter 11.1). In the ISO Guide 35, the

[109] *https://nucleus.iaea.org/sites/ReferenceMaterials/SitePages/Home.aspx*

preparation of CRM is described in more detail. The guide's information can be equally applied in the production of in-house reference materials (see Chapter 11.1). All these guides can be used for the assessment of reference material.

CRMs are sold by some national standards bureaus and similar organisations and usually are verified by highly respected reference laboratories or by interlaboratory calibration. The European Commission's Joint Research Centre (JRC) is one of the major developers and producers of reference materials in the world. It currently provides nearly 800 different CRMs under the BCR, IRMM and ERM brands[110] in the area of food and feed analysis, and environmental analysis (for more, see the Certified Reference Materials Catalogue at https://crm.jrc.ec.europa.eu/).

It is important that any CRM is produced and analysed in a technically valid manner. General steps that are required in the production of a CRM typically include:

- Collection or synthesis of material
- Sample preparation (including homogenization, stabilization, bottling etc.)
- Homogeneity testing
- Stability assessment
- Value assignment ("characterization" in ISO REMCO terms, see before).

However, users of CRMs should be aware that not all materials are produced with the same degree of rigor. Thus, details of homogeneity and stability studies, the methods used in certification, and the uncertainties and variations in the stated analyte values, usually available from the producer, should be used to judge the CRM producers reliability. The CRM must be accompanied by a certificate, which includes an estimate of the uncertainty associated with the certified value to be evaluated.

Whilst CRMs are preferred where available, their availability is limited. RMs that do not meet the criteria for CRMs are more widely available but miss the additional evidence of metrological traceability and statement of measurement uncertainty provided on the certificate. Where CRMs are not available, there are several alternative strategies. However, the main approach is participation in interlaboratory comparison (Chapter 12.3) by which laboratories are given an option to compare their data relative to other similar laboratories and, if organised properly, provide a very effective addition to the use of certified references. Accreditation bodies will always require participation in appropriate proficiency schemes (Chapter 12), and where certified references are available, their use will be expected as well.

11.3. Assessment of the suitability of reference materials

Laboratories require judging when to use a RM. They must be able to explain and justify the basis of selection of RMs and any decision not to use a RM along with demonstrating the ways for inspecting the stability of RMs until their expiry date.

For many basic test methods, especially in analytical chemistry, using a CRM is not required, since these test methods are intrinsically traceable. For instance for most titrations, traceability is provided by calibrations of the balance and the volumetric apparatus. However, methods that entail preparation steps

[110] *The ERM®, BCR®, and IRMM® Reference Materials include various segments, such as: Environment– polycyclic aromatic compounds, nitro polycyclic and oxygenated aromatic hydrocarbons, polychlorinated, etc., Water and food microbiology– milk powder; Food and agriculture– dairy products, alcohol, GMO standards, etc.*

prior to the measurement, e.g., digestion and/or distillation conducted in sample preparation must be validated by using a suitable certified reference material of the foodstuff in question, where available (Chapter 8.3).

An important factor in selecting RMs is their commutability that is the demonstrated property of an RM to behave similarly to test samples under the same measuring conditions. The concept is defined by VIM[2], further discussed in the Eurachem Guide related to quality in analytical chemistry[33]. In general, the composition of the RM should be as close as possible to that of the samples, where potential matrix interferences exist. Ideally, a method should be validated using a matched matrix RM certified in a reliable manner or otherwise, when such material does not exist, the use of a sample spiked with a RM might be acceptable (Chapters 10.2; 11.5).

CRMs must be stable and highly homogeneous and of established composition or properties. Laboratories will need CRMs, which are typical of the food they normally test. Examples are e.g., well-characterized RMs with assigned concentrations of nutrients, such as authentic food-matrix Standard Reference Materials (SRMs) from which numerous are available. They are intended for use in the validation of analytical methods for determination of nutrients in food products and for compliance with nutritional labelling laws related to proximates (e.g., energy, carbohydrates, protein, fat), fatty acids, vitamins, and elements.

Both RMs and CRMs must be clearly labelled so that they are unambiguously identified and referenced against accompanying certificates or other documentation. Information should be available indicating their shelf life, storage conditions, applicability, and restrictions of use. RMs prepared within the laboratory, e.g., as solutions, should be treated as reagents for the purposes of labelling. Laboratories should follow the manufacturer's recommendations about storage and shelf life for RMs. In addition, caution is needed as suppliers do not always provide information about all impurities and thus controlling of impurities is important, especially for trace analysis, where they might cause interferences. If laboratories use a quality control chart (Chapter 12.4) to monitor reference materials changes are shown on the charts and there is no need to carry out additional tests.

The handling of measurement standards should safeguard them against becoming contaminated or degraded. Trainings of personnel should reflect these requirements (Chapter 4).

In microbiology, sample stability is virtually impossible. However, there are certified reference cultures (Chapter 11.4), which provide a definition of particular organisms so that laboratories can verify that their test systems are adequately selective. Relatively recently, quantitative microbiology references have become readily available based on the impregnation of cultures onto plastic supports of controlled surface porosity, for more see Chapter 11.4.

For many types of analysis, calibration might be carried out using RMs prepared within the laboratory from chemicals of known purity and composition and certified e.g., but it is the users' responsibility to establish that the quality of such materials is satisfactory. Ideally, all chemicals used for RM purposes are purchased from producers with demonstrated QA systems, while laboratories confirm the quality of critical materials.

The ISO Guide 33 provides guidance and informs on good practice in the use of RMs and CRMs in analytical chemistry, especially in measurement processes. RMs/CRMs are used for assessment of precision and trueness of measurement methods, quality control, calibration, assigning values to materials, and

the establishment of conventional scales[105]. It should also be noted, that RMs, as interpreted by most ISO 17025 accreditation bodies, include materials such as standard solutions and buffers, which are frequently, purchased by laboratories from laboratory chemicals suppliers. Increasingly, accreditation bodies are insisting that laboratories should use only those from ISO Guide 34[106] accredited sources. Since competence of RM producers are increasingly widespread nowadays, many accreditation bodies start insisting that only results from RMs from accredited producers are acceptable for demonstrating traceability of measurement. In practice, RM producers will also form part of the certification chain and will need to have laboratories to monitor preparation of materials and to participate in the setting of certified values. This means that not only must the materials producer show compliance with ISO Guide 34 as regards certification but they must also have an ISO 17025 accredited laboratory. Thus, it is now very important to check with the Accreditation Body on its attitude to acceptance of materials as references before committing to buy any particular item. ISO/IEC 17043[102] contains additional information on PTs and PT providers.

11.4. Reference strains (cultures)

A reference culture is a microorganism preparation acquired from a culture type collection, also called control strains, standard cultures, reference strains, test strains, type culture or quality control strains. Reference strains are defined to at least the genus and species level, catalogued, and described according to their characteristics. They are preferably originated from food, animal feed, and the food or feed production.

Culture Collections are e.g., national organisations that preserve and provide authentic reference strains including environmental and industrially useful bacteria, plasmids and bacteriophages. A reference culture collection is a culture collection, which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections' Organization (ECCO).

Microbiological laboratories require traceable reference cultures for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. To demonstrate traceability, laboratories should use reference strains of microorganisms obtained directly from a recognised national or international collection, where these exist, for instance from the American Type Culture Collection (ATCC) Bacteriology Collection, a non-profit organization which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development (www.lgcstandards-atcc.org/). Cultures from the ATCC Bacteriology Collection are useful in a variety of applications comprising quality control organisms for commercial identification systems e.g., genomic DNA from well-characterized microbial strains suitable for amplification by Polymerase Chain Reaction (PCR). They also offer CRM and Culture Guides (www.lgcstandards-atcc.org/).

When traceable reference cultures are not readily available, commercial derivatives traceable to them could be used, if the relevant properties for its intended use have been shown by the laboratory to be equivalent at the point of use.

The typical stock culture collection might contain isolates that fall into one or more of the following categories:

- Reference strains for quality control of culture media and methods
- Isolates used in the preparation of inoculated samples and specimens for quality control (QC) and training purposes
- Reference strains for the development and validation of new methods
- Pathogens and spoilage organisms for routine testing or in the investigation of contamination problems
- Cultures used in microbiological assays
- Isolates required for research purposes.

Effective maintenance of stock cultures is essential for QC, method validation and research purposes. Repeated sub-culturing might eventually lead to contamination, loss of viability, genotypic/phenotypic changes or variation mutants. For maintaining any collection of reference cultures relevant is, that the genetic stability of the strains within is assured. Appropriate techniques (PCR, genotyping, serotyping) are used to preserve the reference microorganisms so that the desired characteristics of the strains are maintained. Reference cultures are sub-cultured preferably once but not more than five (5) times from the original culture to provide reference stocks. Records of sub-culturing must be kept with purity and biochemical checks conducted in parallel, as appropriate.

Reference stocks are used to prepare working stocks for routine work. They must not be re-frozen and re-used once thawed. Preferably, reference stocks are stored in aliquots either deep-frozen or lyophilised (freeze-dried). Freeze-drying and cryogenic storage are preferred, but might not be practical for smaller laboratories. Cryoprotectant beads allow routine laboratories to maintain a stock culture collection simply and at low cost.

Reference cultures should not be sub-cultured to replace reference stocks, unless it is required and defined by a standard method or the laboratories can provide documentary evidence that there has been no change in any relevant property. Commercial derivatives of reference strains might only be used as working cultures.

The laboratory should assign suitable staff for maintenance of reference microorganisms. Written protocols for culture maintenance should be available in the laboratory where they are used for QC and validation purposes. It is important to log any sub culturing of all stock cultures so that the collection could be maintained in good condition and replaced when necessary. ISO 11133[46] contains detailed instructions for the maintenance of microbial strains and the preparation and standardization of working cultures and inoculation suspensions (7.2). The standard specifies the optimal number of CFU per plate or membrane filter and describes how productivity ratios and limits are to be determined.

Laboratories should check the reference microorganisms they will use for validation, e.g., for purity by surface plating on appropriate non-selective and selective media and by microscopic examination of the stained smears.

Precautions are required for reference cultures of organisms held for control purposes (9.2), bearing in mind the danger of contamination of the laboratory by organisms, which are the subject of tests on samples, such as:

- Segregate storage of reference cultures in their own dedicated refrigerators and freezers
- Have a segregated area for handling the references, ideally with a laminar flow cabinet
- Use of dedicated laboratory coats and overshoes/shoes for work in the segregated area.

11.5. Use of spikes

Spikes are widely used for method validation and calibration in chemistry and microbiology and they provide a reasonable alternative to certified references, if the spiking material is adequately authenticated, ideally by certification of its purity. The spiking method is used when appropriate matrix reference material is not available, and the laboratory is able to prove the stability of the spiked material. In chemistry, the spike method (also called the addition method) is used to find the concentration of an analyte in complex matrices with possible influence that might affect the results. The matrix effect is common in food analysis (Chapter 10.2). In microbiological testing components of the sample might well affect the viability of organisms.

A spiked sample is a sample to which a known amount of the analyte has been deliberately added. The spike has the advantage that the laboratory can spike into a matrix, which is typical of its normal sample stream and ideally contains little or none of the target before spiking. If this is not possible, the spike level should be large compared to the present natural level that must be known. The spike must be thoroughly mixed and distributed homogeneously throughout the matrix.

In complex matrices, the spike might be the only alternative, however imperfect. Laboratories that are able to demonstrate good recoveries of spikes have presumably a good accuracy. The spike, at the very least, demonstrates that the laboratory would detect the material or organism being sought if it were present. As more RMs become available, accreditation bodies are, however, increasingly expecting method validation by use of RMs and are less inclined to accept validations based on spiking.

12. ENSURING THE VALIDITY OF RESULTS - QUALITY CONTROL OF PERFORMANCE

ISO/IEC 17025[1], sections 3.4; 7.7; Eurachem Proficiency Testing Scheme Guide[111]; EUROLAB Cook Book Doc. No 22[112]; ILAC P9:06[113]; EA 4/18[114]; ISO 7870 standards[115]; EA-4/21[116]; ISO 13528[117]; ISO/IEC 17043[102]; Nordtest Internal Quality Control – Handbook for Chemical Laboratories[118]; ISO Guide 80[119]. IUPAC/CITAC Guide: Selection and use of proficiency testing schemes for a limited number of participants—chemical analytical laboratories[120] .

12.1. General

The meaning of the terms "quality control" (QC) and "quality assurance" (QA) often vary according to the context. For their exact definitions, see the glossary in Annex 1. While QA is more the general concept, addressing laboratory activities to provide confidence that quality requirements will be fulfilled, QC describes the individual measures, which are used to actually fulfil the requirements.

A minimum set of analytical QC procedures should be planned, documented and conducted for all chemical testing.

Analytical QC requirements generally consist of analysis of laboratory control samples to document whether the analytical system is in control. In general, for measurement of chemical analytes, appropriate QC includes an initial demonstration of measurement system capability as well as ongoing analysis of standards and other samples to ensure the continued reliability of the analytical results. QC can take a variety of forms, both inside the laboratory (internal) and between the laboratory and other laboratories (external).

Examples of appropriate QC include:

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks as a measure of freedom from contamination

[111] *B. Brookman and I. Mann (eds.) Eurachem Guide: Selection, Use and Interpretation of Proficiency Testing (PT) Schemes (3rd ed. 2021). Available from www.eurachem.org*

[112] *Cook Book 20, Planning of Activities to Ensure the Validity of Test Results 01/10/2018; Process Requirements. Available at https://www.eurolab.org/CookBooks/20.*

[113] *ILAC-P9:06/2014: ILAC Policy for Participation in Proficiency Testing Activities*

[114] *EA-4/18 :2021 Guidance on the level and frequency of proficiency testing participation*

[115] *ISO 7870-1:2019: Control charts, Part 1: General guidelines etc.; ISO 7870-2:2013. Control charts - Part 1: Shewhart control chart; see Annex 2 for more.*

[116] *EA-4/21 INF: Guidelines for the assessment of the appropriateness of small interlaboratory comparison within the process of laboratory accreditation, March 2018*

[117] *ISO 13528:2015: Statistical methods for use in proficiency testing by interlaboratory comparison*

[118] *Internal Quality Control – Handbook for Chemical laboratories (Trollboken – Troll book) (NT TR 569 – English – Edition 5.1); http://www.nordtest.info/wp/*

[119] *ISO Guide 80:2014: Guidance for the in-house preparation of quality control materials (QCMs)*

[120] *IUPAC/CITAC Guide: Selection and use of proficiency testing schemes for a limited number of participants—chemical analytical laboratories[120] (IUPAC Technical Report), Pure Appl. Chem., Vol. 82, No. 5, pp. 1099–1135, 2010. doi:10.1351/PAC-REP-09-08-15, 2010*

- Demonstration of analytical method suitability for intended use
  - Detection and quantitation limits
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte/matrix/level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)
- Demonstration of continued analytical method reliability
  - Matrix spike/matrix spike duplicate - recovery and precision data
  - QC samples (system accuracy and sensitivity at levels of concern)
  - Surrogate spikes (where appropriate)
  - Continuing calibration verification
  - Method blanks.

Quality control is usually applied after validation of a method and as part of a laboratories management system to verify that the method remains in control during routine use, and that its performance continues to be fit-for-purpose, the more since in routine analysis samples are of unknown content. The applied QC serves to detect deviations from the ideal performance. For instance, in case a laboratory might have a situation where all quality control samples are producing data within the acceptance limits, but always on one side relative to the expected value, the laboratory should imitate an investigation, since there should be a random scatter about the expected value. The finding gives an early warning of a problem with the test system that is detected before data is compromised.

QC within single chemical measurements can include using internal control verification, continuous calibration verification, internal calibration verification, and a laboratory control sample.

QC tests should run as frequently as necessary to ensure the reliability of analytical results. Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine if any additional QC might be needed. Method-specific QC requirements are described in many of the individual methods and will be referenced in any analytical protocols developed to address specific analytes and sample types of concern.

It is the responsibility of the laboratory management to establish and justify an appropriate level of QC, based on risk assessment (Chapter 2.4), taking into account the reliability of the method, the criticality of the work, and the feasibility of repeating the analysis, if the QC result is unacceptable. The level and type of QC will generally depend on the nature, criticality and frequency of the analysis, batch size, degree of automation and test difficulty, and on the lessons learnt during development and validation processes. The type and frequency of QC tests can be refined over time.

Suitable QC should be planned, implemented and reviewed to allow ongoing monitoring of day-to-day and batch-to-batch analytical performance. Laboratories usually have documented QA/QC procedures in place, including sample preparation, extraction etc. Accredited laboratories must have a procedure in place for monitoring the validity of results and the resulting data must be recorded in a way that trends are detectable. Where practicable, statistical techniques should be used to review the results for all tests included in the laboratory's scope of accreditation. The laboratories management system should include procedure(s) for identifying nonconforming work in relation to QC. Data from monitoring activities must be analysed, used to control and, if applicable, improve the laboratory's activities. If the results of the analysis of data from monitoring activities are found to be outside pre-defined criteria, appropriate action should be taken to prevent incorrect results from being reported. Therefore, the data obtained from QC activities and participation in PT should be immediately checked and interpreted. It is recommended to plot results and review trends in the data obtained from QC/PT (Chapter 12.4).

It is widely accepted, that for routine analysis, a level of internal QC of 5% is sufficient, i.e. one (1) in every 20 samples analysed should be a QC sample (Chapter 12.2). However, for robust routine methods with high sample throughput, a lower level of QC might be reasonable. For complex procedures, a level of 20% is not unusual and on occasions, even 50% might be required. In some sectors, for example water analysis, guidance on the level of required QC is available[121].

For further reading, reference is provided to the EUROLAB Cook Book Doc No. 20 on "Planning of activities to ensure the validity of test results".

12.2. Internal quality control (IQC)

The monitoring of analytical performance is an important element of quality management in the laboratory and should be planned and reviewed. Its main objective is to ensure the consistency of day-to-day results. The conformity with defined criteria is initially documented as part of the method development and validation to ensure that the analytical methods applied in routine analysis are fit for purpose each time. The IQC is then applied to ideally cover identification of internal failure, such as e.g., systematic error (Chapter 9.4).

Internal QC consists of all the procedures undertaken by laboratory personnel for the continuous monitoring of operations and measurement results in order to decide whether results are reliable enough for release. Different types of QC might be used to monitor different types of variation within the process and monitoring activities related to the validity of results include where appropriate, but are not be limited to (by ISO 17025):

- Use of reference materials or quality control materials
- Use of alternative instrumentation that has been calibrated to provide traceable results
- Functional check(s) of measuring and testing equipment
- Use of check or working standards with control charts, where applicable
- Intermediate checks on measuring equipment
- Replicate tests or calibrations using the same or different methods
- Retesting or recalibration of retained items
- Correlation of results for different characteristics of an item
- Review of reported results
- Intralaboratory comparisons (see Annex 1 for definition)
- Testing of blind sample(s).

The level of QC adopted must be demonstrably sufficient to ensure the validity of the results. A quality control plan should differentiate between activities on an on-going basis and QC checks with particular frequencies and conditions. The IQC programme must be adapted to the actual frequency of tests performed by the laboratory.

An IQC programme usually uses replicate analysis of stable test samples; blanks; standard solutions or materials similar to those used for the calibration; spiked samples (Chapter 11.5); blind samples and QC samples. QC samples are typical samples, which are sufficiently stable and homogeneous, and available in sufficient quantity, to allow repeat analysis over time. Spikes and QC samples, that are not calibrated against CRMs do not provide traceability in themselves, but demonstrate consistency of performance of the laboratory. When combined

[121] *ISO/TS 13530:2009: Water quality – Guidance on analytical quality control for chemical and physicochemical water analysis*

with satisfactory results from interlaboratory exercises, it shows that the laboratory normally agrees with its peers and it comes a very close second in establishing true traceability and is, in many situations, the only possible option.

The following definitions support a better understanding of the various QC (QA) samples and what they can mean for the analytical data[122].

Matrix Spike: Samples to which known spiked concentrations of target analytes have been added prior to sample preparation and analytical testing. The matrix spike is analyzed as a method performance assessment by measuring the effects of interferences caused by the specific sample matrix. Poor spike recoveries for Matrix Spike and Matrix Spike Duplicate samples could mean that the sample matrix is causing matrix interference issues.

Matrix Spike Duplicate: A matrix spike duplicate is an additional replicate of the matrix spike sample following the same sample preparation and analytical testing as the original sample. They are used to document the precision and bias of a method for a specific sample matrix. Following Matrix Spike and Matrix Spike Duplicate recovery results, the Relative Percent Difference (RPD) is reported for each analyte as a means of measuring reproducibility. In addition, control limits for matrix spikes and matrix spike duplicates recovery ranges could be provided for each analyte to evaluate performance.

Laboratory Control Samples: These are samples analysed to assess the laboratory performance to successfully recover target analytes from a control matrix on a purified sample material, e.g. homogenous sand or deionized water. It assesses whether the analytical procedure is in control and evaluates the laboratory capability to report unbiased measurements.

Laboratory Control Sample Duplicate: It is an additional replicate of the laboratory control sample. The results are generated to monitor the accuracy and precision of the analytical process on a purified material. Following Laboratory Control Samples and Laboratory Control Sample Duplicates recovery results, the Relative Percent Difference could be reported for each analyte (measurement of reproducibility), control limits for the laboratory control sample, and its duplicates recovery ranges for each analyte to evaluate the performance.

Surrogate Spike: They are typically used to measure the performance of organic testing by GC, GC-MS, and HPLC. Surrogate spikes of known concentrations are added to primary samples, which are then analysed and reported. The surrogate recoveries assess sample matrix interference effects and laboratory performance. The analytes selected as surrogates mimic the behaviour of the target analytes throughout sample preparation and analysis, but are not normally found in the environment.

Method Blanks: A method blank is a QC sample that is deionized water or contaminant-free homogenous sand. The method blank is prepared and analysed following the sample.

The following is indicated by QC samples, blanks and duplicates:

- QC samples, analysed at intervals in the sample batch, will indicate drift in the system
- QC samples with established values and acceptance limits can be tested along with unknown samples as a performance check. As long as the QC sample result is acceptable, it is likely that results from samples in the same batch, as the QC sample, can be taken as reliable.
- Use of various types of blank will indicate any contribution to the instrument signal from sources other than the analyte
- Duplicate analyses of routine test samples will give a check of repeatability.

[122] *https://www.meritlabs.com/blog/2018/2/21/helping-you-understand-quality-assurance-quality-control-samples*

A programme of periodic checks is necessary to demonstrate that variability (e.g., between analysts and between equipment, materials etc.) is under control.

For analyses performed infrequently, a full system validation should be performed on each occasion. This might typically involve the use of a RM containing a certified or known concentration of analyte, followed by replicate analyses of the sample and a spiked sample (a sample to which a known amount of the analyte has been deliberately added; Chapter 11.5).

Analyses undertaken more frequently should be subject to systematic QC procedures incorporating the use of control charts. The use of control charts is particularly recommended for monitoring the results obtained from the analysis of QC samples (Chapter 12.4). Where possible, tests should incorporate controls to monitor performance, using data from RMs and spiked samples to be plotted to assist in the evaluation of trends visually.

In the case of methods where neither CRMs nor effective spikes are available, consensus standards, recognised by all parties or industries concerned could be used. They are not traceable in a strict sense, but are used to ensure consistency of data within the industry sector and hence form a basis for agreement when testing against product quality standards.

Replicating determinations (e.g., with retained items) by the same method only provides confidence in the results when the risk of repeating errors is carefully considered and systematic errors are excluded.

Interlaboratory comparison could be applied to organising, performing and evaluating measurements or tests on the same or similar items within the same laboratory in accordance with predetermined conditions (ISO 17025; Annex 1).

Determinations by different methods that lead to comparable answers are another approach to testing that could provide confidence in results. If for some items, different methods might give different results, the "correct" result is defined in terms of a reference method, which is tightly specified.

For microbiological laboratories internal QC programme routinely involves e.g.,

- The use of spiked samples with variable contamination levels, including target and background flora
- The use of spikes/naturally contaminated samples from a range of matrices
- The use of reference materials (including proficiency testing scheme test materials)
- Replicate testing
- Replicate evaluation of test results, i.e. counting of colonies in petri dishes by two analysts.

If a laboratory is accredited for a test that it is rarely requested, an on-going internal quality control programme might be inappropriate. More suitable is a scheme for demonstrating satisfactory performance carried out in parallel with the testing. However, this does not eliminate the need by the laboratory to participate in PT schemes at acceptable frequency (Chapter 12.3). In any case, the laboratory should be aware of the inherent risk associated with such an approach and take all appropriate measures.

For further reading in the area, reference is provided to the ISO Guide 80[119] for the in-house preparation of quality control materials (QCM) and to the Nordtest Internal Quality Control – Handbook for Chemical Laboratories. The ISO Guide 80 outlines the essential characteristics of reference materials for quality control purposes, and describes the processes by which they can be prepared by competent personnel within the facility in which they will be used (e.g., where instability due to transportation conditions is avoided). The content of this guide also applies to inherently stable material and transport.

12.3. External quality assessment (Proficiency Testing)

Laboratories must monitor their performance by comparison with results of other laboratories, where available and appropriate according to ISO 17025 standard requirement. Monitoring activities involving other laboratories refer to participation in proficiency testing (PT) and other types of interlaboratory comparisons (for definitions see Annex 1). This monitoring should be planned and reviewed as part of the laboratories' management system. It is recognized as an important means of observing the degree of equivalence of measurement results at national and international levels.

The most commonly employed type of external quality assessment (EQA) is Proficiency Testing (PT). PTs are regularly organised interlaboratory comparisons to assess the performance of analytical laboratories. PT schemes are available for most of the typically performed laboratory tests in food chemistry and microbiology nowadays. The regular participation in PTs by laboratories is a recognised way to a) monitor performance against both the laboratories own requirements, the validity of the whole quality system, including the competence of the analytical personnel and b) the standard of peer laboratories. It supports to highlight variation between laboratories (reproducibility) and, in some circumstances to detect systematic errors (laboratory bias).

As an obligatory requirement by Accreditation Bodies, accredited laboratories must regularly participate in PT schemes relevant to their scope of accreditation and with appropriate matrices. National Accreditation Bodies have issued specified policies and guidelines related to laboratories PT participation as part of the accreditation process (for more see ILAC P9:06123). National Accreditation Bodies might require a minimum participation in PT schemes by laboratories, but it is the responsibility of the laboratory to demonstrate that the frequency and extent of their participation is appropriate for their scope of accreditation. In addition, Accreditation Bodies can specify participation in a particular PT scheme as a requirement for accreditation.

Otherwise, the laboratory should participate in interlaboratory comparisons (other than PTs) organised by a sufficient number of other laboratories based on a well-documented protocol (see below for more).

It is the laboratory's responsibility to select the most appropriate scheme and to check and evaluate the quality of the PT provider. In the PT process, laboratories receive samples from a PT provider, an organization that can be non-profit or for-profit and formed specifically to provide PTs to customers. Other providers of PTs include central reference laboratories, such as e.g., the EU Reference Laboratories[123], government health agencies, and manufacturers of test kits or instruments.

In a typical PT programme, challenge samples are sent at regular intervals to members of a group of laboratories for analysis and/or identification, whereby each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratories and, if agreed, to others. The participating laboratories use the information regarding their performance to make appropriate changes and improvements, related to the test or laboratory work, if necessary.

[123] *E.g. European Union Reference Laboratories Food Safety overview at https://ec.europa.eu/food/horizontal-topics/european-union-reference-laboratories_en*

Since PT is a tool to measure laboratory performance, there must be no difference in the treatment of PT samples. PT providers make every effort to produce samples that exactly mimic, or closely resemble usual samples received.

The key principles ensuring the appropriateness of participation in PT schemes are:

- The PT scheme in which a laboratory participates should resemble as closely as possible the laboratory's routine work, for example, in terms of sample matrix, characteristics and levels; any differences should be noted and accounted for
- Laboratories should treat PT items as routine samples
- PT samples must be processed by normal testing method(s) and involve personnel who routinely perform the testing
- To be successful, PT instructions must be followed carefully by the laboratory, including those for transport and storage, and all paper work must be completed accurately and results submission deadlines met
- The evaluation and interpretation of the performance in a PT scheme should take into account the risk associated with the measurement
- All unsatisfactory or repeated questionable results must be thoroughly investigated so that the laboratory can understand the reasons for poor performance and correct as necessary
- The performance of a laboratory over several rounds of a PT scheme and analysis of trends is paramount to determining the successfulness of participation
- The PT scheme documentation, such as scheme protocols, must provide clear information for all parties to understand how the PT scheme operates
- The PT provider should be open to discussion amongst interested parties
- Laboratories should view PT participation as an educational tool, using the PT scheme results in the improvement process and providing feedback to staff
- All PT results, as well as corrective actions, must be recorded by the laboratory and records are to be maintained for an appropriate period.

PT participation is valuable only if the information received is directed towards improvement in the laboratory, used to assess laboratory bias and to check the validity of the whole quality system. Irrespective of an eventual classification of the PT results as satisfactory or unsatisfactory by the organiser of an Interlaboratory Comparison (ILC), the participating laboratory should carefully analyse its results on the basis of its criteria. If the result then turns out to be unsatisfactory, the laboratory should take appropriate corrective actions and should satisfy itself that these actions have been effective. Additionally, the results of an ILC should be used to verify or improve the estimates of measurement uncertainty of the used test procedures.

PT schemes have some limitations and it is not appropriate to use PTs as the only means for evaluating the quality of a laboratory (Chapter 12.2). PT will not detect all problems in the laboratory, particularly not those that address the pre-examination and post examination procedures. PT results are affected by variables not related to samples, including preparation of the sample, matrix effects, clerical functions, selection of statistical methods of evaluation, and peer group definition. Quite often, unsatisfactory results in PT participation relate to clerical errors, including transcription errors, mislabelling, decimal error and/or results reported in the wrong units that can be easily eliminated, if analysed as such. In addition, a single unacceptable result does not necessarily indicate that a problem exists in the laboratory.

The value of a PT is only as good as the schemes themselves. Laboratories are encouraged to subscribe to ISO/IEC 17043[102] accredited PT schemes. Accreditation bodies are increasingly insisting that, where an appropriate accredited PT scheme exists, it should be used. Otherwise, PT providers should only be used where the laboratory has assessed their competency. Annex 3 to ISO 17043 is a useful reference for laboratories, as it gives guidance on the issues to be considered when choosing a PT provider. The statistical aspects of PT schemes are described in ISO 13528[117].

Information about a large number of schemes and PT providers can be found in the EPTIS database operated by BAM[124], a German material research institute. PT providers that meet the requirements of ISO/IEC 17043 are considered to be competent. However, for emerging fields of analysis or rare applications in particular, a fully appropriate PT scheme might not be available. These, and other limitations, are considered in an EA guidance document (EA-4/18) on the level and frequency of participation in PT[114]. The guidance document is providing useful information about the use of sub-disciplines to facilitate the optimization of the extent of participation in PT, e.g., an area of technical competence defined by a minimum of one measurement technique, property and product.

There is also guidance from IUPAC/CITAC on the selection and use of proficiency testing schemes for a limited number of participants[120] and an EA Guidelines for small interlaboratory comparison within the process of laboratory accreditation[116] that can be used for setting up interlaboratory comparison schemes.

Even when effectively monitoring the consistency of the laboratory's own performance, it is in the interest of any laboratory to test this assumption from time to time by exchanging samples with other laboratories and comparing results (Chapter 12.3). Interlaboratory comparisons may be informal, in that a group of laboratories will exchange samples on an ad hoc basis, or can be a formal exercises organised by the participating laboratories or a third party PT provider circulating a sample for comparison. Recognition of the laboratory's competence will normally not condition any particular level of performance in interlaboratory comparison, but will require the laboratory to have a procedure for evaluating the results from its participation and for responding to any problems revealed. There must also be records showing that the results were evaluated and what action was taken to remedy problems.

For more information related to the interpretation of the results from PT participation, reference is provided to the EUROLAB Cook Book Doc No. 4 on the "Use of interlaboratory comparison data by laboratories: Part A" and to the PT Eurachem guide on selection, use and interpretation of PT schemes[111]. The latter offers guidance and practical information on how to select, use and interpret PT schemes. The main topics covered by the Guide are: the aims and benefits of participation in PT schemes; selecting the most appropriate PT scheme; understanding the basic statistics and performance scoring used by the PT providers; and using and interpreting the PT results in order to improve the overall performance of the laboratory.

[124] *(https://www.eptis.bam.de/en/index.htm (www.eptis.bam.de)*

12.4. Quality control charts

The data obtained regularly from quality control (QC) materials are evaluated by control charts. Control charts are graphical and analytic tools for monitoring process variation. Control charts illustrate change over time and are extremely valuable in monitoring the total of the performance of the analyst, the instruments and the test procedure. They quickly assess if the result from a QC sample is acceptable. They can be utilized by any laboratory by plotting results on a chart, e.g. results from HPLC measurements.

Frequently used control charts are X-charts and R-charts. They are a pair of control charts where continuous or variable data is collected in rational subgroups and used to monitor the mean and variation of a process based on samples taken in a given time. The X-bar chart measures between-sample variation (signal), while the R chart measures within-sample variation (noise).

X-charts are used when the same or similar items are analyzed for quality control.

R-charts are used to control ranges of replicate measurement results. They indicate how the range of the subgroups changes over time. This is utilized to monitor process variability.

X-charts known as Shewhart charts consist of a central line representing the mean value for the QC sample (also called mean value control chart). There are two other lines, warning limits and action limits set at ±2s and ±3s (standard deviation) about the mean value respectively (where s is an experimentally obtained estimate of the standard deviation or a target standard deviation based on a requirement).

Detailed criteria for assessing QC results against the limits are required to enable the laboratory to make best use of the QC results and to take appropriate action, when necessary. In order to set realistic limits on the control chart, the initial measurements made on the QC sample to estimate the standard deviation must reflect the way the method is actually intended to be used on a day to-day basis. At least 20 data points must be collected over a 20 to 30 day period to start a control chart. Otherwise, the experimentally obtained standard deviation will be unrealistically small, resulting in limits being set on the chart that cannot be complied with in normal use. Since the initial estimate of "s" is often based on a relatively small dataset, it is generally advisable to reassess the limits after one year or when sufficient results are collected.

The objective of reviewing control charts is to catch problems, to make corrections before the situation has become "out of control" and to investigate systematic trends. An assigned responsible person should review all charts on a routine basis.

For more information related to quality control charts reference is provided to the EURACHEM/CITAC Guide CG4 on Quantifying Uncertainty in Analytical Measurement[72], and to the ISO 7870 standards[115] (see Annex 2). ISO 7870-2:2013 is a guide for the use and understanding of the Shewhart control chart approach to the methods for statistical control of a process. The use of warning limits, analysis of trend patterns and process capability is briefly introduced that is consistent with the Shewhart approach. Other types of control charts, an overview of the basic principles and concepts, can be found in ISO 7870-1[115], along with key elements and the philosophy of the control chart approach.

13. REPORTING OF RESULTS

ISO/IEC 17025[1], section 7.8; ISO 19036[75]; ISO 8199[125]; ISO 7218[17]; ISO/IEC GUIDE 98-4[126]; EUROLAB Technical Report No.1/2017[127]; ILAC-G8:09[128]; EUROLAB Cook Book No 8[129]; EURACHEM Guide: Use of uncertainty information in compliance assessment[93].

Proper reporting of results is as important as the performed test itself. Accredited laboratories must meet certain requirements related to reporting of their results. In general, results must be reported accurately, clearly, unambiguously, and objectively for each test or series of tests carried out.

Results are usually reported in a test report called as such or "test results", or "a report of test results" and can be issued as hard copies or by electronic means. The test report should include all the information requested by the customer for the interpretation of the test results and equally all information required by the method used.

Each test report should include at least the following information, unless the laboratory has valid reasons for not doing so:

- A title; name and address of the laboratory
- The location of performance of the laboratory activities
- Unique identification of the test report that all its components are recognized as a portion of a complete report and a clear identification of the end of the report
- The name and contact information of the customer
- Identification of the method used
- A description, unambiguous identification of the test item
- A date of the receipt of the test item and the date of sampling where this is critical to the validity and application of the results
- The date of performance of the laboratory activity
- The date of issue of the report
- Reference to sampling plan and sampling method used by the laboratory or other bodies where these are relevant to the validity and application of the result
- A statement that results relate only to the items tested
- The results with units of measurement
- Additions to, deviations, or exclusions from the method
- Identification of the person(s) authorizing the report
- Clear identification when the results are from external providers.

It is possible to provide the test report in a more simplified way, but only if this is agreed with the customer. Results that are not reported should be readily available.

The laboratory is responsible for the test report, except for the information provided by the customer. Data provided by the customer are clearly identified, e.g., the report contains a disclaimer that the information is supplied by the customer and can affect the validity of the results. Where the laboratory has not been responsible for the sampling stage, the report

[125] *ISO 8199:2018, Water quality — General requirements and guidance for microbiological examinations by culture*
[126] *ISO/IEC GUIDE 98-4:2012, Uncertainty of measurement — Part 4: Role of measurement uncertainty in conformity assessment*
[127] *EUROLAB Technical Report No.1/2017 - Decision rules applied to conformity assessment*
[128] *ILAC-G8:09/2019 -Guidelines on Decision Rules and Statements of Conformity*
[129] *EUROLAB Cook Book No8 - Determination of Conformance with Specifications or Limit Values, 01/09/2018; https://www.eurolab.org/CookBooks/8*

states that the results apply to the sample as received. In addition, test reports for the interpretation of the results, where necessary, should include the following:

- Information on specific test conditions, such as environmental conditions
- Where relevant, a statement of conformity with requirements/specifications
- Where applicable, the measurement uncertainty, is presented in the same unit as that of the measured or in a term relative to the measurand (e.g., percent), when:
  - It is relevant to the validity of application of test results
  - A customer's instruction so requires, or
  - The measurement uncertainty affects conformity to a specification limit
- Opinions/interpretations, where appropriate and needed
- Additional information by specific methods, customers or groups of customers that might be required.

In addition to that, where the laboratory is responsible for the sampling activity, the report should include the following, where necessary for the interpretation of test results:

- The date of sampling
- Unique identification of the item or material sampled (including the name of the manufacturer, the model or type of designation and serial numbers/Item type identification code as appropriate)
- The location of sampling, including any diagrams, sketches or photographs
- A reference to the sampling plan and sampling methods
- Details of any environmental conditions during sampling that affect the interpretation of the results
- Information required evaluating measurement uncertainty for subsequent testing.

ISO 7218[17] specifies information related to expressing test result in microbiological testing. For quantitative methods, results are expressed as number of colony forming units (CFU) per volume or grams of sample analysed. Below 10 CFU per plate, precision decreases significantly and laboratories are advised to reflect this on their test reports. If the result of the enumeration is negative, it should be reported as "not detected for a defined unit" or "less than the detection limit for a defined unit". If preferred, and in order to comply with national technical and health regulations, the result might also be reported as "zero for a defined unit". Qualitative test results in microbiology are reported as "detected/not detected in a defined quantity or volume". They might also be expressed as "less than a specified number of organisms for a defined unit" where the specified number of organisms exceeds the detection limit of the method and this has been agreed with the customer. At decimal dilutions the lowest reportable result (for 1 colony on the plate) is 10, negative result would therefore be reported at <10 CFU/g (mL).

Where an estimate of the measurement uncertainty of the test result is expressed on the test report, any limitations (particularly if the estimate does not include the component contributing to the distribution of microorganisms within the sample) have to be made clear to the customer.

Laboratories should check if standards they use have their own specific requirements regarding the expression of test results.

In case of providing opinions and interpretations, the laboratory must ensure that only authorized personnel release the respective statement and that the basis upon which these opinions and interpretation were made is documented. Laboratories at best have already personnel with required qualifications and experience as well as guidelines for any routine interpretations and judgements. For opinions and interpretations that are directly communicated by dialogue with the customer, a record of the dialogue is retained. The expression in reports should be based on the results obtained from the tested item and clearly identified as such. It is important to distinguish opinions and interpretations from statements of inspections and product

certifications as intended in ISO/IEC 17020[130] and ISO/IEC 17065[131], and from statements of conformity as referred to in Chapter 13.1.

Before stating measurement uncertainties (where it is relevant to the validity or application of the test results or a customer's instruction requires it for a statement of conformity), the laboratory should have a procedure on estimation of uncertainty of measurement in place and personnel should be trained to carry out these estimations (see Chapter 9.5).

Test results must be checked, reviewed and authorized prior to release. In case the issued test report needs to be changed, amended or re-issued specific rules apply. For instance an amendments to a report will be a further document which includes the statement "Amendment to Report N"....or an equivalent form of wording. If it is necessary to issue a completely new report, it must be uniquely identified, and containing a reference to the original version and the previous /original version should be archived together with the amended version.

13.1. Decision rule - Reporting statements of conformity

When testing is performed as conformity assessment and test results contain a decisive statement of conformity (such as "target value achieved" or "test failed"), a decision with regard to fulfilment of the specifications is required. The key to the assessment of compliance is the concept of "Decision rules". Laboratories must have a documented decision rule, to be employed when a statement of conformity to a specification or standard requires it, taking into account the level of risk (such as false accept and false reject and statistical assumptions) associated with the employed decision rule. For addressing statements of conformity, an associated decision rule is already required in the review phase and before the laboratory takes up activities.

The concept of "Decision rules" gives a prescription for the compliance or noncompliance with a specification limit, taking into account the acceptable level of the probability of making a wrong decision. It describes how measurement uncertainty is allocated with regard to the acceptance or rejection of a product according to its specification and the result of a measurement before providing the statement on conformity.

Decision rules are developed, verified and validated in a way that the decision is ideally based on objective evidence and less on individual knowledge or experience of personnel. Decision rules can require complex calculations performed by software. They have to be appropriate and applicable either to the accuracy of the laboratory's methods and outcomes as well as to the customer's requirements for conformity.

When agreeing on the decision rule, the associated risk for false accept or false reject has to be taken into account (see Chapter 2.4). Where the decision rule is prescribed by the customer or by regulations or normative documents, a further consideration of the level of risk is not necessary.

The laboratory must report on the statement of conformity, such that the statement clearly identifies:

[130] *ISO/IEC 17020:2012- Conformity assessment — Requirements for the operation of various types of bodies performing inspection*

[131] *ISO/IEC 17065:2012, Conformity assessment — Requirements for bodies certifying products, processes and services*

- To which results the statement of conformity applies
- Which specifications, standards or parts thereof are met or not met
- The decision rule applied (unless it is inherent in the requested specification or standard).

Further guidance on decision rules, statements of conformity and requirements are provided by ISO/IEC Guide 98-4[126], the EUROLAB Technical Report 1/2017[127] and ILAC G8:09[128] for instance. These provide guidance on setting appropriate criteria for unambiguous decisions on compliance given results with associated uncertainty information. Cases involving decisions based on multiple measurands are not considered. The ISO/IEC Guide 98-4137 details on assessing the conformity of an item (entity, object or system) with specified requirements, while ILAC G8:09 assumes that where the measurand implies a sampling requirement, the uncertainty includes components arising from sampling.

The EUROLAB Cook Book No8[129] informs on the following process requirements and relevance: 1. To identify what should be proved with the conformity assessment and by the decision rule: compliance or non compliance with a specification or with a limit value. Based on the answer, either the supplier's risk (α) or the consumer's risk (ß) has to be specified. 2. Deviations requested by the customer should not affect the integrity of the laboratory or the validity of the results. If the laboratory perceives a decision rule prescribed by the customer to be inappropriate, this should be discussed during contract review.

The EURACHEM Guide "Use of uncertainty information in compliance assessment"[93] takes into account the developments in other international guides and standards, including ILAC G8:09, and JCGM 106[132]. The guide is applicable to decisions on compliance with regulatory or manufacturing limits where a decision is made based on a decision rule together with a measurement value and the associated measurement uncertainty. It includes a discussion and general recommendations, including the use of "guard bands" to improve the probability of correct acceptance or correct rejection. This is followed by more detailed guidance on establishing rules for interpretation and by several examples.

Based on the decision rules, an "Acceptance zone" and a "Rejection zone" can be determined, such that if the measurement result lies in the acceptance zone the item is declared compliant and if in the rejection zone it is declared noncompliant. The limits of the acceptance zone are called "acceptance" limits'. A decision rule should have a well-documented method of determining the location of acceptance and rejection zones, ideally including acceptable levels of probability (P) that the value of the measurand 1) lies within the specification limit or 2) lies outside the specification limit.

Decision rules could come from legislation. In case there is no legislative bases for a decision, the conformity assessment body needs to decelerate and agree with the customer on the decision rule whether to take classical or guard band approach.

For additional information related to decision rules reference is provided to:

- ASME B89.7.3.1: Guidelines for Decision Rules: Considering Measurement Uncertainty, Determining Conformance to Specifications (2001)
- Technical Document on Decision Limits (DL) for the Confirmatory Quantification of Threshold Substances by the WADA's Laboratory Expert Group (LabEG) WADA TD2019DL.

[132] *BMPI JCGM 106:2012, Evaluation of measurement data – The role of measurement uncertainty in conformity assessment, Joint Committee for Guides in Metrology, 2012*

14. LABORATORY ACCREDITATION

ISO/IEC 17025[1]; ISO/IEC 17011[133].

Accreditation is the last level of public control in the European conformity assessment system. It is designed to ensure that conformity assessment bodies (e.g., laboratories) have the technical capacity to perform their duties. Used in regulated sectors and voluntary areas, accreditation increases trust in conformity assessment. It reinforces the mutual recognition of products, services, systems, and bodies across the EU.

Many countries, including the EU require that laboratories providing testing services to competent authorities for the control and testing of foodstuff must be accredited to the ISO 17025 standard for the test methods involved.

14.1. Accreditation

Accreditation is the independent evaluation of conformity assessment bodies against recognised standards to carry out specific activities to ensure their impartiality and competence. Through the application of national and international standards, government, producers and consumers can have confidence in the calibration and test results, inspection reports and certifications provided.

An Accreditation Body grants accreditation to a laboratory for a specified set of activities (i.e. tests) based on an assessment of that laboratory by them against the ISO 17025 requirements. Such assessments will typically involve an examination of the methods in use, the facilities/environment, equipment and personnel involved, and the means of controlling the procedures being performed. Furthermore, the management system and the related documentation of the laboratory will be examined.

The methods will be examined to ensure they are technically appropriate for the intended purpose, that they have been validated and documented clearly and unambiguously, and that their performance is under control (e.g., through the use of QC charts). The performance of tests might be witnessed to ensure documented procedures are being followed and interpreted in a consistent way. The laboratory's performance in PT schemes or other interlaboratory comparisons will also be on focus. Assessment might additionally include a 'performance audit' or 'measurement audit', where the laboratory is required to analyse specific samples and achieve acceptable levels of accuracy.

Accreditation is viewed by some laboratories as uneconomical in terms of time and resources. However, on the other hand, it allows for increased confidence in the test results and professional recognition of the quality of the services provided. The documentation prepared in support of accreditation makes very useful training aids for new staff or those being developed to take on broader duties. In addition, the assessment by a third-party Accreditation Body verifies that tests are done correctly by trained laboratory professionals. Anyhow, obtaining this standard requires total management support and investment, appropriate accommodation, equipment and supporting facilities and welltrained/well-supervised staff.

[133] *ISO/IEC 17011:2017, Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies.*

ISO 17025 demonstrates that a laboratory is capable of providing consistently valid results, that the individuals performing the work are competent, and that all accredited measurement results can be traced back to the International System of Units (SI) or appropriate references. This is the primary objective for the customers, so that results are accepted between countries. Accredited status allows for the exchange of data and its acceptance that it is from a source that has attained, and maintains through appropriate procedures, a recognized standard of delivery. Further to that, it is creating a proactive risk-based business and quality culture. Defined activities, policies, and quality objectives are the foundation for the strategic direction of the organization. A culture of risk-based thinking drives cost-effective operations and evidence-based decision-making. The laboratory must plan actions to address risks and drive improvements and ensure that major quality risks related to tests are known and controlled (carried out the same way every time).

The requirements for ISO 17025 implementation are detailed in Annex 4; a simple checklist can easily be prepared from the ISO 17025 standard requirements to enable auditing or monitoring of the degree of preparedness or compliance of an organization for accreditation. The Eurachem/CITAC Guide: Guide to Quality in Analytical Chemistry: An Aid to Accreditation[35] provides an Appendix A: "Quality audit: Areas of particular importance to a chemistry laboratory and its assessment process" that can be used to examine whether the procedures in the laboratory are fit-for purpose.

14.2. Accreditation Body

A conformity assessment (demonstration that specified requirements relating to a product, process, service, person, system or body are fulfilled) should be carried out by the third-party organizations. Such organizations are usually national accreditation bodies.

Accreditation bodies are established in many economies with the primary purpose of ensuring that conformity assessment bodies are subject to oversight by an authoritative body. They are authorized to assess and confer accredited status to laboratories for instance.

Accreditation of conformity assessment bodies is based on harmonised standards defining competence criteria for:

- The national accreditation body and each category of conformity assessment body (such as laboratories or certification bodies)
- Sector-specific requirements
- Guidance drawn up by regional and international organisations of accreditation bodies.

Each Accreditation Body has established procedures against which it operates and assesses laboratories and grants accreditation. To ensure that these Accreditation Bodies operate to a high level of competence and that they apply the standards in a consistent and equivalent manner, they are required to meet the requirements of ISO/IEC 17011, be a member of the International Accreditation Forum (IAF) and a signatory to a Multilateral Recognition Arrangement (MRA). The selection criteria for assessors appointed by accreditation bodies are specified in ISO/IEC 17011. These include the requirement for technical expertise in the specific areas of operation being assessed.

Accreditation bodies that have been peer evaluated as competent sign regional and international arrangements to demonstrate their competence. These accreditation bodies then assess and accredit conformity assessment bodies to the relevant standards. The arrangements support the provision of local or national services, such as providing safe food and clean drinking water, energy, delivering

health and social care or maintaining an unpolluted environment. In addition, the arrangements enhance the acceptance of products and services across national borders, thereby creating a framework to support international trade through the removal of technical barriers.

The benefit of accreditation is that it enables potential customers to have confidence in the quality of the work performed by the laboratory. Since the introduction of formal requirements for the competence of laboratories, the endorsement conferred by accreditation and other assessments has gained worldwide recognition and plays an important role in trade.

In order to be internationally recognized, accreditation bodies must be party to the International Laboratory Accreditation Cooperation (ILAC) mutual recognition agreement (MRA) and/or one of the regional mutual recognition organizations, such as EA (European co-operation for Accreditation), APAC (North and South America, Asia Pacific Accreditation Cooperation), AFRA (African Accreditation Cooperation), etc. The accreditation bodies that are signatories to the ILAC MRA have been peer evaluated in accordance with the requirements of ISO/IEC 17011 to demonstrate their competence. The ILAC MRA Signatory Search[134] provides a current list of all accreditation bodies that are signatories to the ILAC MRA, including their contact details, the scope of their recognition and the initial date of signing the ILAC MRA.

Many laboratory accreditation bodies have signed a Multilateral Agreement (MLA) with European Accreditation (EA) members, and/or a MRA under the International Laboratory Accreditation Cooperation (ILAC). ILAC is the international organisation for accreditation bodies operating in accordance with ISO/IEC 17011 and involved in the accreditation of conformity assessment bodies including testing laboratories (using ISO/IEC 17025), proficiency testing providers (using ISO/IEC 17043[102]) and reference material producers (using ISO 17034[96]). The international arrangements are managed by ILAC in the fields of calibration, testing, medical testing, inspection, proficiency testing providers and reference material producers accreditation and by the International Accreditation Forum (IAF). The latter is a worldwide association of accreditation bodies and other bodies interested in conformity assessment in the fields of management systems, products, processes, services, personnel, validation and verification and other similar programs of conformity assessment. Both organisations, ILAC and IAF, work together and coordinate their efforts to enhance the accreditation and the conformity assessment worldwide. ILAC works closely with the regional co-operation bodies involved in accreditation, notably EA in Europe, APAC in the Asia-Pacific, Inter American Accreditation Cooperation) in the Americas, AFRAC in Africa, SADCA (Southern African Development Community Accreditation Services) in Southern Africa, and ARAC in the Arab region.

In the European Union, the organisation of accreditation and requirements for accreditation are established by Regulation 765/2008[135] promoting a uniformly rigorous approach to accreditation across EU countries. This consensus is normally reflected in a CERTIF[136] document on a specific topic. As a result, an accreditation certificate is enough to demonstrate the technical capacity of a conformity assessment body.

The main principles of accreditation are:

[134] *https://ilac.org/signatory-search/*

[135] *Regulation (EC) No 765/2008 of the European Parliament and of the Council of 9 July 2008 setting out the requirements for accreditation and market surveillance relating to the marketing of products and repealing Regulation (EEC) No 339/93*

[136] *A certified document is a document that's been signed by a professional or someone of 'good standing' to confirm that it's a 'true copy' of an original document*

- One Accreditation Body per EU country (it is possible, however, to use another country's national Accreditation Body)
- Accreditation is a public sector activity and a not-for-profit activity
- There is no competition between national accreditation bodies
- Stakeholders are represented
- Accreditation is the preferred means of demonstrating technical capacity of notified bodies in the regulated area.

Within the European accreditation infrastructure, national accreditation bodies are members of the European co-operation for Accreditation (EA) that cooperates with the European Commission. EA's tasks include:

- Setting up and managing a peer evaluation system of national accreditation bodies
- Providing technical assistance to the Commission in accreditation.

To provide a framework for cooperation, the European Commission, the European Free Trade Association (EFTA), EU countries and EA signed cooperation guidelines for joint work and a framework partnership agreement 2018-2022 to implement objectives related to accreditation and market surveillance. An assessment (between 2013 and 2017) confirmed that the created accreditation infrastructure provided added value for the Single Market and international trade and has established a trustworthy and stable accreditation system, supported by industry and the conformity assessment community. It ensures that products meet the applicable requirements, removes barriers for conformity assessment bodies and helps entrepreneurial activities to flourish in Europe.

Usually there is one organization responsible for delivery of accreditation service in the country (see above). Exceptions are Canada with two accreditation bodies and USA with several multidisciplinary accreditation bodies. In the South Caucasus Region the following accreditation bodies exist:

- ARMNAB, the "National Accreditation Body", Ministry of Economy of the Republic of Armenia, is a state non-commercial organization that provides accreditation service to conformity assessment bodies within the Republic of Armenia. ARMNAB is a Member of EA (B category). Web page: http://www.armnab.am
- AZAK is the national Accreditation Body of the Republic Azerbaijan recognized by the State to perform accreditation activity in Azerbaijan. The Azerbaijan Accreditation Centre is a Member of EA (B category) and an Associate Member of ILAC. Web page: http://www.accreditation.gov.az
- The LEPL "Unified National Accreditation Body - Accreditation Center" (GAC) is a national public authority operating under the Ministry of Economy and Sustainable Development of Georgia. GAC is a Member of EA (B category) and a full member of ILAC and IAF. Web page: http://www.gac.gov.ge

For more information related to activities of these accreditation bodies above reference is provided to their respective websites.

14.3. ISO/IEC 17025 requirements

Understanding "Standard" as "a level of quality", these are written instructions providing requirements on specifications of various products, processes, systems, services, and persons. ISO (International Organization for Standardization), one of the biggest suppliers of International Standards, is an independent, non-governmental organization, founded in 1947, composed of the representatives from standards organizations of 165 member countries. ISO in cooperation with different organizations and committees elaborates and publishes standards for every aspect of human life, such as Quality Management Standards (ISO 9000 series), Environmental Management Standards (ISO 14000 series), Food Safety Management Standards (ISO 22000 series) and others among them Conformity Assessment Standards (ISO 17000 series). International standards promote the development of industry, facilitate the global trade and are helpful to human health and environment. Government, business and consumers, all of them, will benefit from the implementation of International standards. ISO international standards are voluntary and they do not replace national laws.

Working in compliance with International standards is essential to ensure validity and comparability of results globally. ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories" is the main standard for testing (and calibration) laboratories. It specifies the general requirements for the competence, impartiality and consistent operation of laboratories (ISO 17025, 1. scope) and contains requirements for laboratories to enable them to demonstrate that they operate competently, and are able to generate valid results.

The standard is developed by ISO and IEC (International Electrotechnical Commission) under the management of the ISO committee on conformity assessment. It is applicable to all organizations performing laboratory activities, regardless of the number of personnel.

The ISO/IEC 17025 in its latest version consists of eight (8) main sections and of two (2) annexes (see Annex 4). The sections 1 – 3 cover: Scope, normative references and terms and definitions and are introductory. The following five main sections contain the requirements for laboratory accreditation.

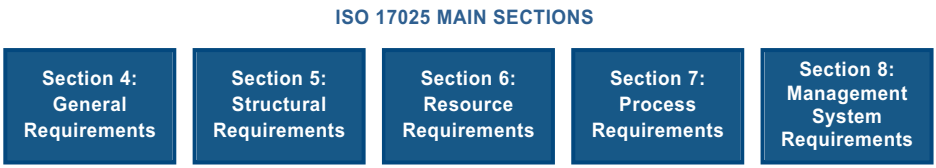


Figure 3: ISO 17025 main sections

Section 4: General requirements

This section covers impartiality and confidentiality, two requirements that are vital for maintaining the trust and confidence that the users of tests place in the laboratories they use. Impartiality implies that the laboratory will not allow commercial, financial, or other pressures to compromise the quality of results. Internal issues, personal relationships, or other conflicts of interest are addressed and resolved. Confidentially requires the laboratory to keep all results and information private.

Section 5: Structural Requirements.

This section defines the basic organizational components of a laboratory, its range of

activities, and its commitment to an effective management system. It states that an accredited laboratory must be a legal entity or part of a legal entity, which is responsible for its testing activities. Section 5 sets management's responsibilities in an accredited laboratory and their responsibilities to customers, regulatory authorities, and organizations that provide recognition. It also defines the basic requirements for personnel, the authority given to them, and the resources needed to carry out their duties.

Section 6: Resource Requirements

The section is addressing the requirements for the laboratory to have available (reference to sub-chapters of the ISO 17025 standard are indicated in the brackets):

- Personnel (6.2)
- Facilities and environmental conditions (6.3)
- Equipment (6.4)
- Metrological traceability (6.5), and
- Externally provided products and services (6.6) necessary to perform its laboratory activities.

Section 7: Process Requirements

This section covers 11 core processes to improve efficiency. The section begins with the Review of Requests, Tenders and Contracts. The Selection, Verification and Validation of Methods is one of the most technical and most important parts of the standard. Sampling, the handling of test items, and technical record keeping are covered here as well as measurement uncertainty. Ensuring the validity of results is the quality monitoring and control function in the laboratory. Several tools for monitoring are listed, and the requirements for proficiency testing are explained. The standard goes into much detail regarding the reporting of results. Requirements are laid out for dealing with complaints and nonconforming work. A strong aspect is on Control of Data and Information Management.

Section 8: Management System Requirements

Options A & B come in. Option B applies if the laboratory is part of a larger organization, or if it has its own effective management system in accordance with ISO 9001:2015. Here, the management system requirements specified in sections 8.2 to 8.9 are covered by the existing management system, as long as laboratory activities are included and the laboratory is capable of demonstrating its fulfilment of ISO 17025 sections 4 to 7. If the laboratory's management system is independent of any other management system, Option A applies and the laboratory must comply with Section 8 requirements related to the management system requirements. Option A is aligned with the new version of ISO 9001:2015, especially regarding risk-based thinking and addresses as a minimum the requirements of sections 8.2 to 8.9 of the ISO 17025 standard, such as:

- Management system documentation (8.2)
- Control of management system documents (8.3), e.g., policies, objectives
- Control of records (8.4)
- Actions to address risks and opportunities (8.5 see also subchapter 3.3)
- Improvement (8.6)
- Corrective action (8.7)
- Internal audits (8.8)
- Management review (8.9).

In the Annexes A and B of ISO 17025 additional information is provided on the issues of metrological traceability and management system options.

For further reading related to the standard requirements, reference is provided to the ISO 17025 standard itself and related guidance documents, especially as to the management system aspects (see Annex 2). Since the ISO 17025 standard only provides general requirements, it is best to consult guidelines for auditing management systems to fill in the details for internal audits for instance.

14.4. The accreditation procedure

The accreditation procedure starts with the laboratory engaging with an Accreditation Body (Chapter 14.2) that will provide the necessary service.

The selected Accreditation Body will liaise with the organization applying for accreditation and a timescale for the submission of the primary documentation will be agreed. A pre-assessment (if requested) to observe the general standard of operation and to highlight significant problems will generally follow, after which the main accreditation audit takes place during which the laboratory operation will be observed and the generated data scrutinized. At the end of the audit, the organization will be told whether it has succeeded or failed; a written report from the Accreditation Body will follow. This report will detail any non-compliances observed (generally where the procedure is inadequate or ineffective or where its implementation could be improved), together with a timescale for the resolution of these nonconformities. Following confirmation and evidence of the closure of these non-compliances, a date will be given for the formal award of the accreditation.

The accreditation certificate that is issued to the laboratory confirms that the laboratory meets the requirements of the international standard ISO/IEC 17025:2017, while demonstrating technical competence in the field of "Testing", further referring to the accompanying scope of accreditation and types of activities to which this accreditation applies. The scope of an accreditation is granted for specific and detailed conformity assessment activities.

The detailed description of the scope of accreditation is set out in the Annex to the accreditation certificate and is published in the database of accredited bodies. It would list for instance: The test area (field of testing), such as microbiological or chemical testing e.g.; the materials and products; specific tests and/or properties measured, standard methods, and techniques/key equipment and technology. The Accreditation Bodies have guidelines for the accreditation certificate and corresponding scope of accreditation, its formulation and evaluation.

The requirements for accreditation are broad and necessitate diligence in ensuring compliance with the procedures developed to meet those requirements. ISO 17025 accreditation is specific to individual test methods and for a laboratory involved in testing for a wide range of analytes, each of the test methods should be accredited to satisfy customer requirements.

The main requirements for a laboratory that are assessed (by management and technical assessors) working for the Accreditation Body are:

- A (quality) management system; a suitable laboratory environment
- Educated, trained and skilled personnel
- Training procedures and records
- Specifications for reagents, calibrants and measurement standards (including RMs)
- Equipment suitably maintained and calibrated
- Procedures for sampling (where the laboratory is responsible for this activity)
- Procedures for sample handling
- Documented and validated methods
- Decision rules
- Customer relationship and use of methods
- Metrological traceability of results
- Evaluation of measurement uncertainty
- Internal quality control procedures
- Participation in proficiency testing (PT)/external quality assessment (EQA)
- Procedures for checking and reporting results
- Procedures for implementing corrective actions; risk assessment

- Internal audit and review procedures

The principle behind the assessment is to ensure that the complete operational infrastructure is adequate for the provision and maintenance of a testing service that is fully competent. The laboratory demonstrates competence through the quality assurance measures that have been put in place and through the measures that it takes to ensure effective liaison with its customers and via the confidentiality of the service to that customer.

Accreditation is an ongoing process that guarantees the competence of the laboratories. It is based on demonstrating ongoing compliance with the requirements. The accreditation cycle usually covers two to five years, depending on the decision of the Accreditation Body. During this period, the Accreditation Body performs annual surveillance assessment to ensure that the accredited organization continues working under the requirements of the standards and to allow for the accreditation of additional test procedures. Application for accreditation of additional test procedures is generally much more straightforward than the initial application as the organizational documentation and associated procedures are already in place.

Some accreditation bodies allow flexible scopes of accreditation. They provide a mechanism to allow a laboratory to undertake new or modified activities within its scope of accreditation, even though the specific conformity assessment activities may not be explicitly stated on its schedule of accreditation. The degree of flexibility awarded can vary between technical disciplines and conformity assessment activities.

ANNEX 1: GLOSSARY OF TERMS

In the context of this document, the following terms are defined:

Accreditation	Third-party attestation related to a conformity assessment body conveying formal demonstration of its competence to carry out specific conformity assessment tasks (ISO/IEC 17000), e.g., that a laboratory is competent to carry out tests.
Analyte	Component measured by the method of analysis. In the case of microbiological methods, it is the microorganism or associated by-products (e.g., enzymes or toxins).
Audit	A systematic and independent examination to determine whether quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives.
Calibration	Operation that, under specified conditions, in a first step, establishes a relation between the quantity 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 (VIM 3).
Certified Reference Material (CRM)	A CRM is a Reference Material (RM) that is characterized by a metrological valid procedure for one or more specified properties, accompanied by RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability (ISO Guide 30:2015). Note: The concept of value includes a nominal property or a qualitative attribute such as identity or sequence. Uncertainties for such attributes may be expressed as probabilities or levels of confidence. Metrologically valid procedures for the production and certification of RMs are, among others, given in ISO Guides 34 and 35.
Comminution	It is the reduction of solid materials from one average particle size to a smaller average particle size, by crushing, grinding, cutting, vibrating, or other processes.
Contaminants	Any substance not intentionally added to food that is present in food as a result of the production, manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. Food containing a contaminant in an amount which is unacceptable from the public health viewpoint and in particular at a toxicological level shall not be placed on the market (Council Regulation (EEC) No. 315/93[137]).
Conformity Assessment	Demonstration that specified requirements relating to a product, process, service, person, system or body are fulfilled (ISO). Conformity assessment activities might include: Testing, Surveillance, Inspection, Auditing, Certification, Registration, Accreditation.
Client	An entity (e.g., customer, agency, company, person, etc.) that receives a test result conducted according to specified requirements.
Culture	An isolated microorganism grown on a laboratory medium.

[137] *Council Regulation (EEC) No. 315/93 laying down Community procedures for contaminants in food*

Data Integrity	Refers to the accuracy and consistency (validity) of data over its lifecycle. It is assurance that results reported by the laboratory are accurate, complete, and true representations of the laboratory sample and analysis.
Decision Rule	Rule that describes how measurement uncertainty is accounted for when stating conformity with a specified requirement (ISO/IEC 17025). A documented rule that describes how measurement uncertainty will be allocated with regard to accepting or rejecting a product according to its specification.
Food Matrix	Components that comprise the food sample.
Food Testing Laboratory	Laboratory that performs tests on food product, ingredients, in-process samples and associated environmental samples for chemical and microbiological parameters.
Intralaboratory Comparison	Organization, performance and evaluation of measurements or tests on the same or similar items within the same laboratory in accordance with predetermined conditions (ISO 17025).
Interlaboratory Comparison	Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions (ISO/IEC 17043:2010, 3.4).
Laboratory Sample	Primary material delivered to the laboratory (sample or subsample(s)). A sub-sample might be: a portion of the sample obtained by selection or division; or an individual unit of the lot taken as part of the sample; or the final unit of multistage sampling.
Laboratory Information Management System (LIMS)	The computer and software system used to identify, schedule, prioritize, perform calculations, generate reports, store results and perform any other function necessary to control the flow of a sample through the laboratory.
Management System	Is a set of interrelated or interacting elements of an organization to establish policies and objectives, and processes to achieve these objectives (ISO 9000). In practice, the terms 'management system' and 'quality management system' are often used interchangeably.
Measurand	Quantity intended to be measured (VIM). The specification of the measurand should be sufficiently detailed to avoid any ambiguity.
Measurement Uncertainty	Non-negative parameter characterising the dispersion of the quantity values being attributed to a measurand, based on the information used (VIM). Parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand (GUM).
Measurement Standard	Realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference (VIM 3).
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 measurement uncertainty (VIM 3).
Non-standard Method	A method that is not taken from authoritative and validated sources, e.g., methods from scientific journals and unpublished laboratory-developed methods.

Official Controls Laboratory	A laboratory that conducts measurements and tests, which result in qualitative and/or quantitative analysis findings that might be used to interpret and enforce and/or be used as evidence to determine whether there has been a violation of a law or administrative rule or regulation adopted by a governmental agency pursuant to authority conferred by law.
Performance Characteristic	Functional quality that can be attributed to an analytical method. This might be specificity, accuracy, trueness, precision, repeatbility, reproducability, recovery; see Annex 3 for more.
Proficiency Testing (PT)	Evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons (ISO/IEC 17043).
Quality	Degree to which a set of inherent characteristics fulfils requirements (ISO 9000).
Quality Assurance (QA)	Part of quality management focused on providing confidence that quality requirements will be fulfilled (ISO 9000). All those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.
Quality Control (QC)	Part of quality management focused on fulfilling quality requirements (ISO 9000). QC procedures relate to ensuring the quality of results obtained for specific samples or sets of samples. The operational techniques and activities that are used to fulfil requirements for quality.
Qualitative Method	Method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a certain amount of sample.
Quality System	The organizational structure, responsibilities, procedures, processes and resources for implementing quality management.
Quantitative Method	A method that provides an estimate of the amount of analyte present in the test sample, expressed as a numerical value in appropriate units, with trueness and precision, which are fit for the purpose.
Reference Material (RM)	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. Properties can be quantitative or qualitative, e.g., identity of substances or species. Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control (ISO Guide 30:2015).
Reagent	Substance or compound that is added to a system in order to bring about a chemical reaction, or added to see if a reaction occurs (International Union of Pure and Applied Chemistry, IUPAC).
Reference Culture	A microorganism preparation that is acquired from a type culture collection.
Reference Standard	A standard, generally having the highest metrological quality available at a given location in a given organization, from which measurements are made or derived. Note: Generally, this refers to recognized national or international traceable standards provided by a standards producing body, e.g., the National Institute of Standards and Technology (NIST).

Reference Strains	Microorganisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin (ISO 11133[50]). Normally they are obtained from a recognised national or international culture collections.
Report	Final presentation of results sent to a customer.
Replicate Test	When samples are tested by the same analyst in duplicate or by two different analysts. In each case, the results are compared for precision.
Risk and Opportunities	Risk: Effect of uncertainty on objectives. Note: An effect is a deviation from the expected, positive and/or negative. Objectives can have different aspects (such as financial, health and safety, and environmental goals) and can apply at different levels (such as strategic, organization-wide, project, product and process). Risk is often expressed in terms of a combination of the consequences of an event (including changes in circumstances) and the associated likelihood of occurrence. Uncertainty is the state, even partial, of deficiency of information related to, understanding or knowledge of, an event, its consequence, or likelihood (ISO Guide 73:2009). Opportunity: An event with potential positive consequences for the organization. A risk is a potential for a loss. An opportunity is a potential for a gain. Both play a role in decision making, strategy formation and management. The goal of the strategy is not to maximize opportunity and the goal of risk management is not to minimize risk.
Sample	A portion of material selected to represent a larger body of material.
Sample Handling	Referring to the manipulation to which samples are exposed during the sampling process, from the selection from the original material through to the disposal of all samples and test portions.
Sample Preparation	Procedures followed to select the test portion from the laboratory sample. In analytical chemistry that is the processes in which a representative piece of material is extracted from a larger amount and prepared for analysis.
Sampling	The process of collecting sample(s).
SI	International System of Units (SI). International decimal system of weights and measures derived from and extending the metric system of units. Adopted by the 11th General Conference on Weights and Measures (CGPM) in 1960.
Standard Operating Procedures (SOP)	Established or prescribed methods to be followed routinely for the performance of designated operations, processes. A detailed set of instructions, which describes how to carry out a task, procedure. A document that specifies or describes how an activity is to be performed. It might include methods to be used and a sequence of operations.
Standard Method	Those published by international, regional or national standards-writing bodies; by reputable technical organizations; in legal references; and official published methods.
Subsample	A portion of the sample obtained by selection or division; an individual unit of the lot taken as part of the sample or the final unit of multistage sampling.

Test Item	An item is the basic unit of interaction on a test. An article that is subject of a study (OECD Series on Principles of Good Laboratory Practice (GLP) and Compliance Monitoring, No. 19: Management, Characterisation and Use of Test Items).
Test Portion	This refers to the actual material weighed or measured for the analysis.
Test Sample	Is prepared from the laboratory sample, from which test portions are removed for analysis.
Testing	One or more characteristics of an object of conformity assessment, according to a procedure. There is a specified way to carry out the testing procedures.
Traceability	Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties (VIM).
Validation	The confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (see ISO 17025[1]). In practice, method validation is conducted by evaluating a series of method performance characteristics, such as precision, trueness, accuracy, selectivity/specificity, linearity, operating range, recovery, LOD, limit of quantification (LOQ), sensitivity, ruggedness/robustness, and applicability.
Verification	Provision of objective evidence that a given item fulfils specified requirements (VIM; ISO 17025, BIPM JCGM-2012 VIM 2.44, 3rd edition 2012). Verification (secondary validation) takes place when a laboratory proceeds to implement a method developed elsewhere. Verification focuses on gathering evidence that the laboratory is able to meet the specifications established in primary validation (adopted from ISO 13843). As per VIM verification is understood as the "provision of objective evidence that a given item fulfils specified requirements", hence is directed at the test item itself, whether a process, measurement procedure, material, compound, or measuring system.
Working Culture	A primary sub-culture from a reference culture stock (ISO 11133).

ANNEX 2: FURTHER READING

This Manual is based on a number of different sources of information that are listed below, and might be consulted for additional information as required.

Brochures and Guidelines, Publications

1. A. Williams and B. Magnusson (Eds.) Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment (2nd ed. 2021). ISBN 978-0-948926-38-9. Available from www.eurachem.org
2. American Chemical Society: Guidelines for chemical laboratory safety in academic institution, published by American Chemical Society 1155 Sixteenth Street, NW Washington, DC 2003, Copyright 2016
3. AOAC International guidelines for laboratories performing microbiological and chemical analysis of food, dietary supplements, and pharmaceuticals – An aid to interpretation of ISO/IEC 17025:2017; August 2018
4. ASME B89.7.3.1: Guidelines for Decision Rules: Considering measurement uncertainty, determining conformance to specifications (2001)
5. B. Brookman and I. Mann (eds.) Eurachem Guide: Selection, use and interpretation of Proficiency Testing (PT) schemes (3rd ed. 2021). Available from www.eurachem.org
6. B. Magnusson and U. Ornemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – A laboratory guide to method validation and related topics (2nd ed. 2014). ISBN 978-91-87461-59-0. V. Barwick (ed.), Planning and reporting method validation studies – Supplement to Eurachem Guide on the fitness for purpose of analytical methods (1st ed. 2019)
7. H. Cantwell (ed.) Blanks in method validation – Supplement to Eurachem Guide on the fitness for purpose of analytical methods, (1st ed. 2019). Available from www.eurachem.org.
8. FDA's Bacteriological analytical manual (the BAM), Chapter 1: Food sampling/preparation of sample homogenate bacteriological analytical manual (BAM); https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparationsample-homogenate
9. Barwick (Ed), Eurachem/CITAC Guide: Guide to quality in analytical chemistry: An aid to accreditation (3rd ed. 2016). ISBN 978-0-948926-32-7. Available from www.eurachem.org.
10. BIPM JCGM 106:2012, Evaluation of measurement data — The role of measurement uncertainty in conformity assessment
11. BIPM: SI Brochure: The International System of Units (SI); www.bipm.org
12. BMPI JCGM 106:2012, Evaluation of measurement data – The role of measurement uncertainty in conformity assessment, Joint Committee for Guides in Metrology, 2012
13. Da-Wen Sun (Ed.): Modern techniques for food authentication; 2013, ISBN 78-0-12-814264-6
14. EEE/RM/062rev3: The selection and use of reference materials, a basic guide for laboratories and accreditation bodies, Eurachem
15. EURACHEM/CITAC Guide: Measurement uncertainty arising from sampling. A guide to methods and approaches. Produced jointly with EUROLAB, Nordtest and the UK RSC Analytical Methods Committee. First Edition 2007
16. EURACHEM Guide: Use of uncertainty information in compliance assessment Draft 21-02-25 Second edition (2021)

17. Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: A guide to methods and approaches Eurachem (2007). ISBN 978 0 948926 26 6. Available from the Eurachem Secretariat
18. Eurachem: Terminology in analytical measurement: Introduction to VIM 3, First Edition 2011 EUROLAB Cook Books, short documents on quality issues to help laboratories comply with ISO/IEC 17025; https://www.eurolab.org/pubs-cookbooks¸Cook Book Nº18 An introduction to risk consideration; Cook Book Nº7 Management reviews; Cook Book No 6 and No 11 on how to assess the competence of staff and how to introduce new staff members; Cook book No 08 Determination or conformance with specifications or limit values, 01/09/2018; Cook Book Doc No. 4 on the use of interlaboratory comparison data by laboratories; Cook Book" – Doc No. 20 on planning of activities to ensure the validity of test results Cook Book" – Doc No. 20 on planning of activities to ensure the validity of test results
19. EUROLAB Handbook ISO/IEC 17025:2017 https://www.aphl.org/programs/food_safety/laboratoryaccreditation/Documents/EUROLAB%20Handbook%20ISO%20IEC%2017025%202017.pdf
20. EUROLAB Technical Report No. 2/2006, Guidance for the management of computers and software in laboratories with reference to ISO/IEC 17025/2005, EUROLAB (2006). Available from www.eurolab.org
21. EUROLAB Technical Report No.1/2017 - Decision rules applied to conformity assessment. January 2017. eurolab-d.de/files/eurolab_technial_report_no.1-decision_rules_applied_to_conformity_assessment-2017_final.pdf
22. FAO and IFIF. 2010. Good practices for the feed industry – Implementing the Codex Alimentarius code of practice on good animal feeding. FAO Animal Production and Health Manual No. 9. Rom; Section 5 Methods of sampling and analysis; www.fao.org/3/i1379e/i1379e05.pdf
23. Food and Drug Administration Office of Regulatory Affairs, ORA Laboratory Manual Volume I; https://www.fda.gov/media/73912/download FOOD AND DRUG ADMINISTRATION OFFICE OF REGULATORY AFFAIRS ORA Laboratory Manual Volume II, Document Number: ORA-LAB.5.3, Revision #: 02, Revised: 05/15/2019, Title: Facilities and Environmental Conditions
24. Food/Feed Testing Laboratories best practices manual (Draft) – www.fda.gov/media/88973/download
25. Froese, C (2016): Manual on laboratory quality assurance. CRFM Special Publication. No.15. 59pp. ISSN: 1995-4875. ISBN: 978-976-8257-39-0
26. GUM, Guide to the expression of uncertainty in measurement, issued by BIPM, IEC, IFCC, ISO, IUPAC, IUPAP and OIML
27. I. Kuselman, F. Pennecchi, C. Burns, A. Fajgelj, P. de Zorzi, IUPAC/CITAC Guide: Investigating out of-specification test results of chemical composition based on metrological concepts (IUPAC Technical Report), Pure Appl. Chem., 84(9), 1939-1971 (2012)
28. ICH Q2 (R1) Validation of analytical procedures: Text and methodology – Step 5 (CPMP/ICH/381/95); https://www.ema.europa.eu/en/ich-q2-r1-validation-analytical-procedurestext-methodology
29. Internal quality control, Handbook for chemical laboratories, Edition 5.1, Nordtest Report TR569, 2018 (www.nordtest.info)
30. International acccreditation service: Guidelines for food testing laboratories, August, 2015; https://www.iasonline.org/wp-content/uploads/2017/06/Guidelines-for-Food-Testing-Laboratories-Aug-2015-1.pdf
31. International vocabulary of metrology – Basic and general concepts and associated terms (VIM 3rd edition), JCGM 200:2012, www.bipm.org

32. International vocabulary of terms in legal metrology (VIML), OIML V1:201
33. ISA-TR52.00.01 Recommended environments for standards laboratories. ISBN: 1-55617-977-4
34. ISO/IEC GUIDE 99:2007: International vocabulary of metrology - Basic and general concepts and associated terms (VIM); JCGM (Joint Committee for Guides in Metrology) 200:2012, International vocabulary of metrology – Basic and general concepts and associated terms (VIM)
35. IUPAC Technical Report "Metrological traceability of measurement results in chemistry: Concepts and implementation"; Accreditation and Quality Assurance volume 16, Article number: 473 (2011)
36. IUPAC/CITAC Guide: Selection and use of proficiency testing schemes for a limited number of participants—chemical analytical laboratories (IUPAC Technical Report), Pure Appl. Chem., Vol. 82, No. 5, pp. 1099–1135, 2010. doi:10.1351/PAC-REP-09-08-15, 2010
37. Jan W. Kretzer et al: Sample preparation – An essential prerequisite for high-quality bacteria detection; Principles of bacterial detection: Biosensors, recognition receptors and microsystems, 2008
38. Joint BIPM, OIML, ILAC and ISO declaration on metrological traceability, 2011, updated and resigned on 13 November 2018 during the 26th meeting of the CGPM, held in Versailles (www.ilac.org)
39. Joint Committee for Guides in Metrology 200:2012, International vocabulary of metrology – Basic and general concepts and associated terms (VIM); 3rd edition 2008 version with minor corrections
40. Leaflet Eurachem: The importance of method validation, https://www.eurachem.org/index.php/publications/leaflets/mnu-il-mv
41. M. Eleftheriadou and K. C. Tsimillis (Eds), Eurachem guide: Accreditation for microbiological laboratories, Second edition (2013), ISBN: 978-91-87017-92-6. Available from www.eurachem.org.
42. M. H. Ramsey and S. L. R. Ellison (eds.), Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: A guide to methods and approaches, Eurachem (2007). ISBN 978-0-948926-26-6. Available from www.eurachem.org
43. NMKL (Nordic Committee on Food Analysis) Procedure No. 12: Guide on sampling for analysis of foods. www.nmkl.org
44. Nordtest, NT Technical report, TR 606 (2007): Uncertainty from sampling – A Nordtest handbook for sampling planners on sampling quality assurance and uncertainty estimation
45. OECD Series on Principles of good laboratory practice (GLP) and compliance monitoring No. 19: Management, characterisation and use of test items
46. Official Medicine Control Laboratories (OMCL) Network of the Council of Europe. Qualification of equipment. PA/PH/OMCL (08) 73-2R Core document and Annexes; https://www.edqm.eu/en/quality-management-guidelines-86.html
47. Official methods of analysis, 21st Edition (2019), https://www.aoac.org/official-methods-ofanalysis-21st-edition-2019/
48. S L R Ellison and A Williams (Eds) Eurachem/CITAC Guide: Metrological traceability in analytical measurement (2nd ed. 2019). ISBN: 978-0-948926-34-1. Available from www.eurachem.org
49. S. L. R. Ellison, A. Williams (eds.), Eurachem/CITAC Guide CG4: Quantifying uncertainty in analytical measurement, (3rd ed. 2012). ISBN 978-0-948926-30-3. Available from www.eurachem.org

50. Simultaneous determination of 16 mycotoxins in cereals using a Triple Quadrupole LC/MS system and e-Method. Agilent Application Note Food Testing and Agriculture; Authors: Ye Jin, Wu Yu, Wang Songxue, Academy of State Administration of Grain, China Guo Qilei, Lu Meiling, Wu Cuiling,Chen Yuhong, Agilent Technologies; https://www.agilent.com/cs/library/applications/5991-7862EN.pdf
51. Somenath Mitra. P (Ed), Sample preparation techniques in analytical chemistry, Chemical analysis; v. 162. ISBN 0-471-32845-6
52. Strayer University* School Info Washington, District of Columbia: Food analysis lecture notes – Chapter 1 Analysis of food products
53. Technical Document on Decision Limits (DL) for the confirmatory quantification of threshold substances by the WADA's Laboratory Expert Group (LabEG) WADA TD2019DL
54. The EURACHEM Guide: Use of uncertainty information in compliance assessment Draft 21-02-25 Second edition (2021)
55. Thomas P J Linsinger, European Commission: Use of recovery and bias information in analytical chemistry and estimation of its uncertainty contribution; TrAC Trends in Analytical Chemistry 27(10):916-923, 2008
56. Thompson, S. Ellison, A. Fajgelj, P. Willetts, R. Wood, Harmonised guidelines for the use of recovery information in analytical measurements, Pure Appl. Chem., 71(2) 337-348 (1999)
57. United Nations Industrial (UNIDO) Guide: TESTED & ACCEPTED - Implementing ISO/IEC 17025:2017; Guide ISO 17025-2017_online.pdf
58. V J Barwick and E Prichard (Eds), Eurachem Guide: Terminology in analytical measurement – Introduction to VIM 3 (2011). ISBN 978-0-948926-29-7. Available from www.eurachem.org
59. W. Horwitz, Nomenclature for sampling in analytical chemistry (IUPAC Recommendations 1990), Pure Appl. Chem., 62(6), 1193-1208 (1990)
60. WHO: Laboratory biosafety manual; https://www.who.int/csr/resources/publications/biosafety/Labbiosafety.pdf

Laboratory Accreditation documents

1. EA-2/18: INF 2015, Guidelines for accreditation bodies on the contents of the scopes of accreditation for proficiency testing providers, 2015 (www.european-accreditation.org)
2. EA-4/18, Guidance on the level and frequency of proficiency testing participation, 2010 (www.european-accreditation.org)
3. EA-4/21 INF:2018, Guidelines for the assessment of interlaboratory comparisons with few participants organised by laboratories and measurement audits by accreditation bodies in the process of laboratory accreditation (www.european-accreditation.org)
4. EA-4/22 G:2018, EA Guidance on accreditation of pesticide residues analysis in food and feed; Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed by the EU. SANTE/11813/2017
5. EA-4/23 INF:2019: Assessment and accreditation of opinions and interpretations using ISO/IEC 17025:2017
6. ILAC G17:01/2021, ILAC Guidelines for measurement uncertainty in testing
7. ILAC P10:07/2020, ILAC Policy on metrological traceability of measurement results
8. ILAC P9:06/2014 ILAC Policy for participation in proficiency testing activities
9. ILAC-G24/OIML D 10:2007 (E), Guidelines for the determination of calibration intervals of measuring instruments, https://www.oiml.org/en/files/pdf_d/d010-e07.pdf

10. ILAC-G8:09/2019, Guidelines on Decision Rules and statements of conformity
11. ILAC-P14:09/2020: ILAC Policy for measurement uncertainty in calibration; https://ilac.org/publications-and-resources/ilac-policy-series/

International Standards

1. ISO/IEC 17000:2020 , Conformity assessment — Vocabulary and general principles
2. ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories
3. ISO 9000:2015, Quality management systems — Fundamentals and vocabulary
4. ISO 9001:2915, Quality management systems — Requirements
5. ISO/IEC GUIDE 99:2007: International vocabulary of metrology - Basic and general concepts and associated terms (VIM)
6. ISO 17678:2019 [IDF 202:2019], Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides
7. ISO 31000:2018, Risk management- Guidelines
8. ISO Guide 73:2009, Risk management – Vocabulary
9. ISO/IEC 31010:2019, Risk management – Risk assessment technique
10. ISO 7218: 2007+ Amd 1:2013, Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
11. ISO 10012:2003 Measurement management systems — Requirements for measurement processes and measuring equipment
12. ISO 8655-1:2022, Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations;
13. ISO 8655-2:2022, Part 2: Pipettes;
14. ISO 8655-3:2022, Part 3: Burettes; ISO 8655-4:2022, Part 4: Dilutors;
15. ISO 8655-5:2022, Part 5: Dispensers; ISO 8655-6:2022, Part 6: Gravimetric reference measurement procedure for the determination of volume;
16. ISO 8655-7:2022, Part 7: Alternative measurement procedures for the determination of volume;
17. ISO 8655-8:2022, Part 8: Photometric reference measurement procedure for the determination of volume
18. ISO 8655-9:2022, Part 9: Manually operated precision laboratory syringes.
19. SO 4787:2021, Laboratory glass and plastic ware — Volumetric instruments — Methods for testing of capacity and for use
20. ISO 6887-1:2017, Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions; Part 2: Specific rules for the preparation of meat and meat products
21. ISO 11133:2014, Microbiology of food, animal feed and water – preparation, production, storage and performance testing of culture media
22. ISO 19458:2006, Water quality — Sampling for microbiological analysis
23. ISO/TS 17728:2015, Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples
24. ISO 24333:2009 - Cereals and cereal products — Sampling
25. ISO 707:2008, Milk and milk products — Guidance on sampling
26. ISO 13843:2017, Water quality — Requirements for establishing performance characteristics of quantitative microbiological methods

27. ISO 16140-1:2016, Microbiology of the food chain — Method validation — Part 1: Vocabulary. Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.
28. ISO 16140-3:2020: Microbiology of the food chain — Method validation- Part 3, Protocol for the verification of reference methods and validated alternative methods in a single laboratory.
29. ISO 16140-4:2020: Microbiology of the food chain — Method validation- Part 4, Protocol for method validation in a single laboratory.
30. ISO 16140-5:2020: Microbiology of the food chain — Method validation - Part 5, Protocol for factorial interlaboratory validation for non-proprietary methods.
31. ISO 16140-6:2019: Microbiology of the food chain — Method validation- Part 6, Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.
32. ISO 19036:2019, Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations
33. ISO 17994:2014, Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods
34. ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparison
35. ISO 8199:2018, Water quality — General requirements and guidance for microbiological examinations by culture
36. ISO/IEC 17020:2012, Conformity assessment — Requirements for the operation of various types of bodies performing inspection
37. ISO/IEC 17065:2012, Conformity assessment — Requirements for bodies certifying products, processes and services
38. ISO/IEC 17011:2017, Conformity assessment — Requirements for accreditation bodies accrediting conformity assessment bodies
39. ISO 7002:1986, Agricultural food products — Layout for a standard method of sampling from a lot
40. ISO Guide 80:2014, Guidance for the in-house preparation of quality control materials (QCMs)
41. ISO ISO/IEC 17034:2016, General requirements for the competence of reference material producers
42. ISO Guide 30:2015, Reference materials — Selected terms and definitions
43. ISO Guide 31:2015, Reference materials — Contents of certificates, labels and accompanying documentation
44. ISO Guide 32:1997, Calibration in analytical chemistry and use of certified reference materials
45. ISO Guide 33: 2015, Reference materials — Good practice in using reference material
46. ISO GUIDE 34:2009, General requirements for the competence of reference material producers
47. ISO Guide 35:2017, Reference materials — Guidance for characterization and assessment of homogeneity and stability
48. ISO 7870-1:2014, Control charts — Part 1: General guidelines
49. ISO 7870-2:2013, Control charts — Part 1: Shewhart control charts
50. ISO 7870-3:2012, Control charts — Part 3: Acceptance control charts
51. ISO 7870-4:2011, Control charts — Part 4: Cumulative sum charts
52. ISO/TS 13530:2009, Water quality — Guidance on analytical quality control for chemical and physicochemical water analysis
53. ISO 5725-1:1994, Accuracy (trueness and precision) of measurement methods and results — Part 1 General principles and definitions
54. 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

55. ISO 5725-3, Accuracy (trueness and precision) of measurement methods and results — Part 3: Intermediate measures of the precision of a standard measurement method
56. ISO 5725-4, Accuracy (trueness and precision) of measurement methods and results — Part 4: Basic methods for the determination of the trueness of a standard measurement method
57. ISO 5725-6, Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values
58. ISO 19011:2018, Guidelines for auditing management systems
59. ISO/IEC 17043:2010, Conformity assessment — General requirements for proficiency testing
60. ISO 21748:2017, Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty evaluation
61. ISO 29201:2012, Water quality – The variability of test results and the uncertainty of measurement of microbiological enumeration methods
62. ISO/IEC 17011:2017, Conformity assessment - Requirements for accreditation bodies accrediting conformity assessment bodies
63. ISO/IEC GUIDE 98-1:2009 Uncertainty of measurement — Part 1: Introduction to the expression of uncertainty in measurement
64. ISO/IEC Guide 98-3:2008, Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)
65. ISO/IEC GUIDE 98-4:2012, Uncertainty of measurement — Part 4: Role of measurement uncertainty in conformity assessment
66. ISO 3951-1:2022, Sampling procedures for inspection by variables – Part 1: Specification for single sampling plans indexed by acceptance quality limit (AQL) for lot-by-lot inspection for a single quality characteristic and a single AQL
67. ISO 3951-2:2013, Sampling procedures for inspection by variables – Part 2: General specification for single sampling plans indexed by acceptance quality limit (AQL) for lot-by-lot inspection of independent quality characteristics
68. ISO 3951-3:2007, Sampling procedures for inspection by variables – Part 3: Double sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection
69. ISO 3951-4:2011, Sampling procedures for inspection by variables – Part 4: Procedures for assessment of declared quality levels
70. ISO 3951-5:2006, Sampling procedures for inspection by variables – Part 5: Sequential sampling plans indexed by acceptance quality limit (AQL) for inspection by variables (known standard deviation)
71. ISO 2859-1:1999 + Cor. 1:2001 + Amd.1:2011, Sampling procedures for inspection by attributes — Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection
72. ASTM D1193 – 06 (2018) Standard Specification for Reagent Water
73. ISO 13528:2015: Statistical methods for use in proficiency testing by interlaboratory comparison
74. CAC/GL 50-2004, General guidelines on sampling, www.fao.org

Useful links

1. CITAC – *www.citac.ws*
2. EURACHEM – *www.eurachem.org*
3. ISO – *www.iso.org*
4. EPTIS – *www.eptis.org*
5. AOAC – *www.aoac.org - http://www.eoma.aoac.org/*

6. ILAC – *www.ilac.org*
7. APLAC – *www.ianz.govt.nz/aplac*
8. EA – *www.european-accreditation.org*
9. BIPM – *www.bipm.org*
10. OECD – *www.oecd.org; www.oecd.org/chemicalsafety/testing*
11. Eurachem – *www.eurachem.org*
12. EUROLAB – *www.eurolab.org*
13. WTO – *www.wto.org*
14. EU food safety – *https://ec.europa.eu/food/horizontal-topics/general-food-law_en*
15. EU Reference Laboratories – *www.ec.europa.eu/jrc/en/eurls; ec.europa.eu/food/ref-labs_en*
16. Joint Research Center EU JCR – *www.crm.jrc.ec.europa.eu/*
17. Nordtest Nordic cooperation – *www.nordtest.info*
18. Codex Alimentarius, International Food Standards – *www.fao.org/fao-who-codexalimentarius/en/*

Online training courses (free of charge)

- Web based training on measurement uncertainty and accreditation - www.mutraining.com
- Online version of the 2nd edition Eurachem guide to measurement uncertainty on behalf of the Eurachem measurement uncertainty and traceability working group - www.measurementuncertainty.org (currently the site is offline pending a re-build); see https://eurachem.org/index.php?option=com_content&view=article&id=141
- Introductory course on estimation of measurement uncertainty, specifically related to chemical analysis (analytical chemistry) - https://sisu.ut.ee/measurement/uncertainty - MOOC: Estimation of measurement uncertainty in chemical analysis (analytical chemistry) course (ut.ee)

ANNEX 3: METHOD PERFORMANCE CHARACTERISTICS [71]

Performance Characteristic	Definition
Accuracy	It describes how close a single measurement result is to the true quantity value (VIM 2.11) and includes the effect of both precision and trueness. When the term is applied to sets of measurements of the same measurand, it involves a component of random error and a component of systematic error. In this case trueness is the closeness of the mean of a set of measurement results to the actual (true) value and precision is the closeness of agreement among a set of results. ISO 5725-1 and VIM avoid the use of the term "bias". Accuracy is often evaluated by repetitively spiking the matrix or placebo with known levels of analyte standards at or near target values. The fraction or percentage of added analyte recovered from a blank matrix is often used as the index of accuracy. Added analyte, however, might not always reflect the condition of the natural analyte in the materials submitted for analysis (Official Methods of Analysis of AOAC International).
Bias	The difference between the expectation of the test results and an accepted reference value. Bias is the total systematic error in contrast to a random error with one or more systematic error components contributing to the bias. A larger systematic error difference from the accepted reference value creates a larger bias value. Bias is determined by comparing the mean of the results from the method with a suitable reference value. This can be achieved by a) analysis of reference materials; b) recovery experiments using spiked samples over a range of concentrations; c) comparison with results obtained with another method for which bias or trueness is known and from PT results after certain amount of participations with the same testing parameter. There is method bias and laboratory bias. Method bias arises from systematic errors inherent to the method, irrespective of which laboratory uses it. Laboratory bias arises from additional systematic errors specific to the laboratory and its interpretation of the method. In isolation, a laboratory can only estimate the combined (total) bias from these two sources.
Limit of Detection, LOD	According to IUPAC, it is the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank. It is often taken as the blank value (or background) plus 3 times the standard deviation of the blank measurement under repeatability conditions. When the LOD is calculated, it should be stated what definition and method are used. Definitions that are more rigorous require consideration of false positives as well as false negatives (Official Methods of Analysis of AOAC International).
Limit of Quantification, LOQ	It is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision, accuracy and an acceptable level of measurement uncertainty. The detection limit should be established using an appropriate measurement standard or sample, e.g., it is usually the lowest point on the calibration curve (excluding the blank). The LOQ can be determined based on the blank value (or background) with 5, 6 or 10 times the standard deviations of the (at least 6) blank measurement in repeatability conditions. By the Signal-to-Noise approach, the measured signals from samples with known low concentrations of analyte are compared with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

Linearity	The ability of an analytical method to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (USP NF). For qualitative methods, there is likely to be a concentration threshold below which positive identification becomes unreliable. The response range should be examined by testing a series of samples and measurement standards, consisting of sample blanks, and samples containing a range of analyte levels. At each concentration level, it will be necessary to measure approximately 10 replicates. A response curve of % positive (or negative) results versus concentration should be constructed. From this it will be possible to determine the threshold concentration at which the test becomes unreliable. For quantitative methods linearity is determined by the measurement of samples with analyte concentrations spanning the claimed range of the method. The results are used to calculate a regression line against analyte calculation using the least squares method. It is convenient if a method is linear over a particular range but it is not an absolute requirement. Where linearity is unattainable for a particular procedure, a suitable algorithm for calculations should be determined.
Measurement Uncertainty	Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used (VIM 2.26). Measurement uncertainty provides a quantitative indication of the quality of a measurement result. Synonyms are 'uncertainty' and 'uncertainty of measurement'. This definition expresses the fact that parameters used to describe the dispersion of distributions, e.g., standard deviations, are always positive or zero. The statement, 'based on the information used', explains why it is necessary to declare what is included in the estimate of measurement uncertainty. This does not mean we can choose what to include and what to leave out. There are many approaches to evaluating measurement uncertainty and these are described in the literature[78,84; 138]
Precision	Measurement precision is related to random measurement error (VIM 2.19) and is a measure of how close results are to one another. It is usually expressed by standard deviation (or relative standard deviation) calculated from results obtained by carrying out replicate measurement on a suitable material under specified conditions. Precision is generally dependent on analyte concentration and should be determined at a number of concentrations across the range of interest. VIM 3 defines three measurement conditions: repeatability condition (VIM 2.20), intermediate precision condition (VIM 2.22) and reproducibility condition (VIM 2.24).Precision expresses the closeness of agreement (degree of scatter) among a series of measurements obtained from multiple testing of a homogeneous test sample under the method's established conditions. It should be investigated with homogeneous test samples, representative of the matrixes to which the method will be applied and containing the expected range of analyte concentrations within these matrixes. If it is not possible to obtain homogeneous test samples, however, precision may be investigated using test samples artificially prepared in the laboratory to simulate the original test samples (Official Methods of Analysis of AOAC International).
Sensitivity	Is the change in instrument response which corresponds to a change in the measured quantity (e.g., analyte concentration). Sensitivity is the change in measured signal for unit change in concentration and can be obtained from the calibration curve.

[138] *Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation, Eurolab 2007/1, 2007, www.eurolab.org*

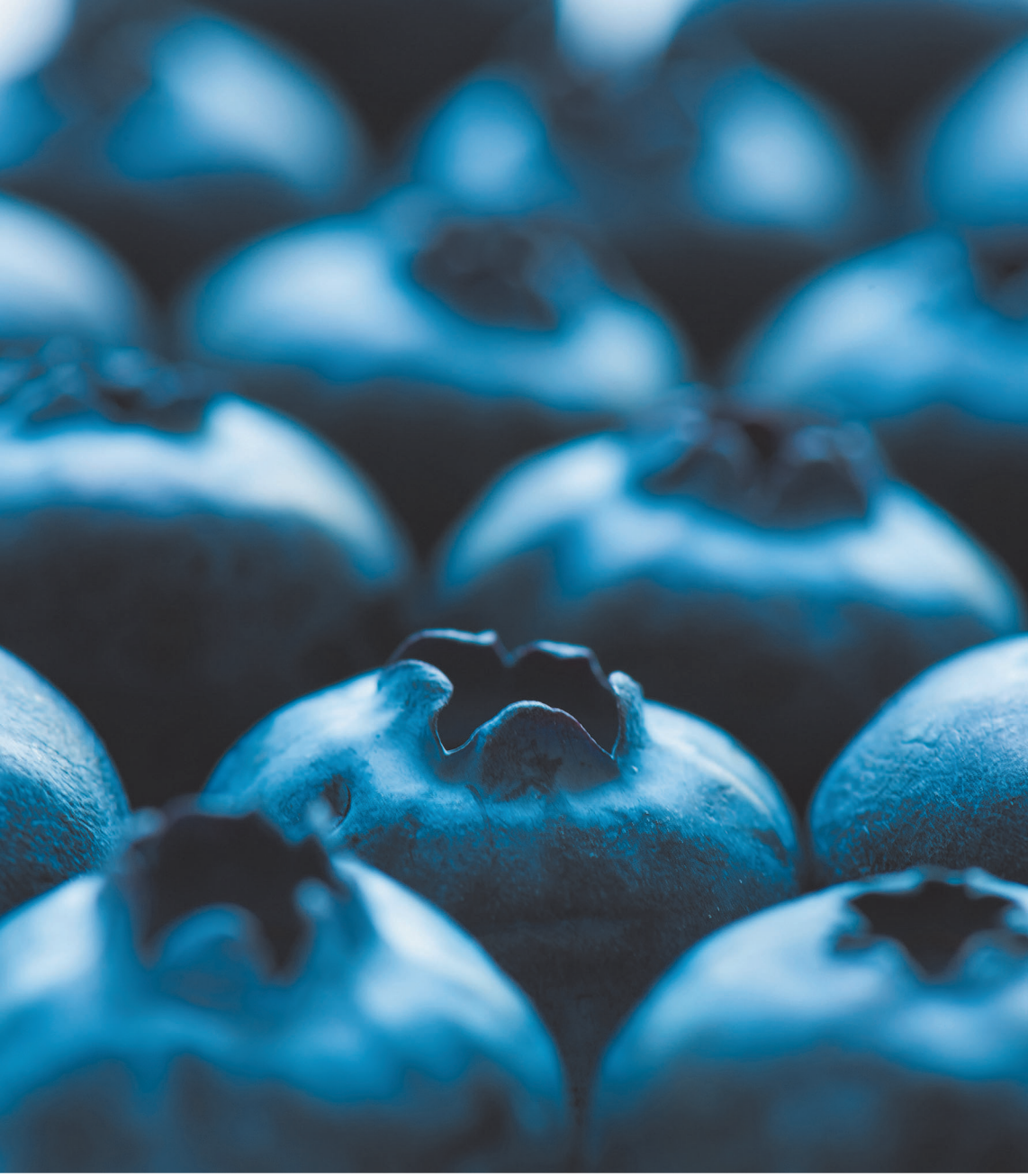
Repeatability	Is a measure of the variability in results when a single analyst performs a measurement using the same sample, equipment over a short timescale. Estimates of measurement repeatability (VIM 2.21) and intermediate measurement precision (VIM 2.23) are obtained in a single laboratory. Repeatability condition of measurement refers to measurements made on portions of the same material by a single analyst, using the same procedure, under the same operating conditions over a short time period. Measurement repeatability is often used to provide an estimate of within-batch variability in results. Since measurement repeatability only reflects the variation in results over a short time period it is likely to underestimate the variability in results obtained when the measurement procedure is used routinely. Assuming appropriate intermediate measurement conditions have been used during the validation study, the intermediate measurement precision provides a more realistic estimate of the long-term variability of measurement results in the laboratory.
Reproducibility	Is a measure of variability in results between laboratories. Reproducibility condition can be defined as condition of measurement out of a set of conditions that includes different locations, operations, measurement systems, and replicate measurements on the same and similar objects (VIM 2.24). Reproducibility might be expressed quantitatively in terms of the dispersion characteristics of the results.
Intermediate Precision	Intermediate precision can be placed between repeatability and reproducibility. It gives an estimate of the variation in results by measurements made in a single laboratory but under conditions that are more variable than repeatability conditions. Under intermediate measurement conditions, measurements are made on portions of the same material using the same procedure, but over an extended time period and possibly by different analysts who may be using different pieces of equipment. Intermediate measurement precision is often used to provide an estimate of between-batch variability. Intermediate measurement conditions are user-defined and the conditions used should always be recorded (also within-laboratory reproducibility for intermediate precision). Demonstration of intermediate precision is through QC charts.
Ruggedness (robustness)	Where different laboratories use the same method, they inevitably introduce small variations in the procedure, which may or may not have a significant influence on the performance of the method. Ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Here "a ruggedness test" can be used. The ruggedness of a method is tested by deliberately introducing small changes to the method and examining the consequences. A large number of factors may need to be considered, but because most of these will have a negligible effect, it will normally be possible to vary several at once. Ruggedness is normally evaluated by the originating laboratory, before other laboratories collaborate.
Selectivity (analytical selectivity)	Relates to "the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other compounds of similar behaviour (selectivity in analytical chemistry IUPAC recommendations 2001; Pure Appl. Chem 2001, 73(8), 1381). A method, which is selective for an analyte or group of analytes is specific. Method selectivity is usually investigated by studying its ability to measure the analyte of interest in the samples to which specific interferences have been deliberately introduced (those that are likely present in the sample) or by studying the ability of the method to measure the analyte compared to other independent methods (comparative techniques), especially if those methods are based on significantly different principles of measurement. Establishing selectivity is by comparing the response of the analyte in a test mixture with the response of a solution containing only the analyte.

Specificity	According to the official guideline to be applied for method validation ICH Q2 (R1, Annex 2): "Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present", such as impurities, degradation products and matrix components.
Trueness	Is an expression of how close the mean of an infinite number of results (produced by the method) is to a reference value. Since it is not possible to take an infinite number of measurements, trueness cannot be measured. However, it is possible to make a practical assessment of trueness in terms of "bias".
Working Range	The working range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. It is the interval over which the method provides results with an acceptable uncertainty. The lower end of the working range is LOD, the upper end is defined by concentrations at which significant anomalies in sensitivity are observed. For assessing the method working range, samples with known concentrations (which covers the range of interest) and sample blank should be analysed according to the method and results plotted against the known/reference concentrations of the sample. Method working range, upper and lower boundaries should be assessed by visual inspection of the plot supported by statistics and a residuals plot from a linear regression. For quantitative analysis, the working range for a method is determined by examining samples with different analyte concentrations and determining the concentration range for which acceptable uncertainty can be achieved. The working range is generally more extensive than the linear range, which is determined by the analysis of a number of samples of varying analyte concentrations and calculating the regression from the results, usually using the method of least squares. The relationship of analyte response to concentration does not have to be perfectly linear for a method to be effective. For methods showing good linearity it is usually sufficient to plot a calibration curve using measurement standards at 5 different concentration levels (+ blank). More measurement standards will be required where linearity is poor. In qualitative analysis, it is common place to examine replicate samples and measurement standards over a range of concentrations to establish at what concentration a reliable cut-off point can be drawn between detection and non-detection.

Annex 4: ISO 17025[1] Table of contents (main headings)

Content	Clause No
Foreword	
Introduction	
1 Scope	
2 Normative references	
3 Terms and definitions	
4 General requirements	
Impartiality	4.1
Confidentiality	4.2
5 Structural requirements	
Legal entity	5.1
Laboratory management	5.2
Range of laboratory activities	5.3
Conformity	5.4
Organisational structure	5.5
Personnel responsible for management system	5.6
Responsibility laboratory management	5.7
6 Resource requirements	
General	
Personnel	6.2
Facilities and environmental conditions	6.3
Equipment	6.4
Metrological traceability	6.5
Externally provided products and services	6.6

7 Process requirements	
Review of requests, tenders and contracts	7.1
Selection, verification and validation of methods	7.2
Sampling	7.3
Handling of test or calibration items	7.4
Technical records	7.5
Evaluation of measurement uncertainty	7.6
Ensuring the validity of results	7.7
Reporting of results	7.8
Complaints	7.9
Nonconforming work	7.10
Control of data and information management	7.11
8 Management system requirements	
Options	8.1
Management system documentation (Option A)	8.2
Control of management system documents (Option A)	8.3
Control of records (Option A)	8.4
Actions to address risks and opportunities (Option A)	8.5
Improvement (Option A)	8.6
Corrective actions (Option A)	8.7
Internal audits (Option A)	8.8
Management reviews (Option A)	8.9



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