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International Accreditation Service
GUIDELINES FOR FOOD TESTING LABORATORIES
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1. INTRODUCTION

The general criteria for accreditation of laboratories are found in ISO/IEC 17025-2005, General requirements for the competence of testing and calibration laboratories.

This guideline document describes additional, specific accreditation requirements for laboratories performing analyses in the examination of food products, ingredients in the production of food, in-process food samples, environmental samples pertinent to foods (swabs, debris, scrapings, air, condensate, etc.) and final products. These specific criteria were developed to meet the needs of those testing laboratories seeking to meet national and international requirements. The information in this document may also have an impact on the laboratory's selection and use of appropriate reference materials used for quality assurance, calibrating equipment, establishing traceability and identifying parameters for validation of test methods. Further, this document can support the proper selection and participation in appropriate proficiency testing schemes.

The purposes of this document, is to specify the criteria used by IAS in the assessment of pesticides involving published methods and laboratory developed methods.

2. SCOPE

This document applies to food related testing using methods that are nationally and internationally recognized – such as AOAC International, American Public Health Association (APHA), U.S. Food and Drug Administration (FDA), U.N. FAO CODEX Alimentarius, U.S. FDA Bacteriological Analytical Manual (BAM), Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed (SANCO/12571/2013), And OECD Guidance Document on Pesticide Residue Analytical Methods. This document also applies to newly developed methods and laboratory developed methods. It is applicable to all types of laboratories, whether they are in the private sector, independent, in-house or in the government sector. In addition, this document specifically addresses testing proficiency and is not generally intended for research and/or product development laboratories, unless specified by a client and/or by regulation.

3. REFERENCES

ISO/IEC Guide 2:2004, Standardization and related activities – General vocabulary.

International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM); 2012, issued by BIPM, IEC, ISO and OIML.

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

SANCO/12571/2013 Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.

IUPAC Residue Analytical Methods

4. DEFINITIONS

Accuracy: A measure of the degree of conformity of a value generated by a specific procedure to the assumed or accepted true value and includes precision and bias.

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.

Bias: The difference between the expectation of the test results and an accepted reference value.

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

Calibration: Comparison and adjustment to a standard of known accuracy. The set of operations which establish, under specific conditions, the relationship between values of quantities by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards.

Certified Reference Material (CRM): Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure that establishes metrological traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Check Samples: Sets of samples tested by laboratories to determine if their processes are in control. A test sample with known properties of microorganisms examined on a routine basis to evaluate laboratory performance.

Client: An entity (e.g., customer, agency, company, person, etc.) that receives a test result conducted according to specified requirements.

Conformance: Compliance with specified requirements.

Control: To exercise authority over and regulate.

Controlled Document: A policy or procedure related to the documented management system that is subjected to controls to ensure that the same version of the document and any revisions are held by or available to all personnel to whom the document is applicable.

Corrective Action: Measures taken to rectify conditions adverse to quality and to eliminate recurrence.

Culture: An isolated microorganism grown on laboratory medium.

Documentation: Recorded information.

Food matrix: Components that comprise the food sample.

Food product: Any substance usually composed primarily of carbohydrates, fats, water and/or proteins that can be consumed by an animal or human for nutrition or pleasure. See Appendix 2 for examples of representative food products.

Food type: An item that is processed, partially processed or unprocessed for consumption. Appendix 2 lists various types such as raw, heat processed, frozen, fermented, cured, smoked, dry, low moisture, etc.

Food Testing Laboratory: Laboratory that performs tests on food product, ingredients, in-process samples and associated environmental samples for chemical and microbiological parameters.

Incurred Samples: Naturally-contaminated test samples.

In-process Samples: Samples in the laboratory that are in the process of being tested (not to be confused with in-process product samples from a manufacturing standpoint).

Inspection: Activities such as measuring, testing and examining one or more characteristics of a product or service and comparing these with specified requirements to determine conformity.

Internal Audit: A formal review of the performance of a management system conducted by laboratory personnel from outside of the laboratory or department under review.

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.

Method: A document that provides detailed "how to" instructions to accomplish a task.

Monitor: A substance, device or system for observing, recording or detecting the operation, condition or performance of a test procedure.

Nonconformity: The non-fulfillment or deviation of a specified requirement.

Proficiency Testing: Test materials (split samples) that are tested periodically by a number of laboratory locations to determine the proficiency of recovery, using statistical analysis where appropriate.

Quality Assurance: All those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.

Quality Control: The operational techniques and activities that are used to fulfill requirements for quality.

Quality System: The organizational structure, responsibilities, procedures, processes and resources for implementing quality management.

Qualitative method: A method that identifies analyte(s) based on chemical, biological, or physical properties; 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. Most qualitative methods are or can be made at least "semi-quantitative" to provide rough estimates of the amount of analyte present.

Quantifiable 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.

Raw Material: A material used in food processing whose properties may impact the quality of the final result.

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 such as the National Institute of Standards and Technology (NIST).*

Replicate tests: Samples of RMs or CRMs which are tested by the same analyst in duplicate or by two different analysts. In each case, the results are compared for precision.

Report: Final presentation of results sent to a customer.

Ruggedness or robustness: The ability of a method to resist changes in test results when subjected to minor deviations in experimental conditions of the procedure. Ruggedness testing examines the behavior of an analytical process when subtle small changes in the environment and/or operating conditions are made, similar to those likely to arise in different test environments.

Sample: Any material brought into the laboratory for testing.

Self-Audit: A review of the performance of the management system within a limited area conducted by the personnel with responsibility for the area.

Split Samples: Unknown test samples of adequate homogeneity sub-sampled and sent to laboratories for proficiency testing.

Standard Operating Procedure: A document that specifies or describes how an activity is to be performed. It may include methods to be used and a sequence of operations.

Traceability: The property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons (e.g., all media, reagents and kits must be traceable to a result and to the appropriate Certified Reference Material, Certified Reference Culture, Reference Culture or Reference Material).

Validation: Verification, where the specified requirements are adequate for an intended use (BIPM JCGM-2012 VIM 2.45, 3rd edition 2012)

Validated method: A method whose performance characteristics (selectivity and specificity, range, linearity, sensitivity, ruggedness, accuracy and precision and quantitation and detection limits) meet the specifications related to its intended use.

Verification: Provision of objective evidence that a given item fulfils specified requirements (BIPM JCGM-2012 VIM 2.44, 3rd edition 2012) requirements have been met.

5. INTERPRETATION OF ISO/IEC 17025 REQUIREMENTS

4 Management Requirements

4.1 **Organization** (No Additions)

4.2 **Management system** (No additions)

4.3 **Document control** (No Additions)

4.4 **Review of requests, tenders and contracts** (No Additions)

4.5 **Subcontracting of tests and calibrations** (No Additions)

4.6 **Purchasing services and supplies** (No Additions)

4.7 **Service to the customer** (No Additions)

4.8 **Complaints** (No Additions)

4.9 **Control of nonconforming testing and/or calibration work** (No Additions)

4.10 **Improvement** (No Additions)

4.11 **Corrective action** (No Additions)

4.12 **Preventive action** (No Additions)

4.13 **Control of records** (No Additions)

4.14 **Internal audits**

4.14.1 The laboratory shall conduct an internal audit on an annual basis. All audit records shall be kept in the laboratory.

4.15 **Management reviews**

4.15.1 The laboratory shall conduct annual management reviews. The management review shall be conducted such that it can address the outcome of the recent internal audit.

5 Technical Requirements

5.1 **General** (No Additions)

5.2 **Personnel**

5.2.1 The laboratory shall have a selection procedure and training system to ensure technical competence of all staff members. Food testing shall be done by or supervised by competent personnel. Staff should have relevant practical work experience before being allowed to perform work covered by the scope of accreditation without supervision or before being considered as experienced for supervision of accredited work.

5.2.2 Training shall include all methods or portions of methods and techniques that each person is responsible for performing. At a minimum, each analyst shall demonstrate competency through observation by management and verification using replicate and/or check samples. For technicians performing only portions of a specific method, competency may be confirmed/verified by direct observation only. Especially for Food Microbiology labs, the interpretation of test results for identification and verification of micro-organisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

5.2.3 The continued competence of staff must be monitored/appraised using appropriate means (e.g., by objective measurements such as PT performance, blind sample performance, etc., or visual observation) as appropriate.

5.2.4 Training records shall include documentation of all relevant internal and external training and method performance verifications.

5.3 **Accommodation and environmental conditions**

5.3.1 The laboratory activities shall be arranged in such a way to minimize cross contamination and shall be segregated from other activities in the laboratory with limited access, where necessary. Within the laboratory, activities such as sample preparation, extraction and analysis need to be adequately separated from each other to avoid contamination or cross contamination:

(a) Microbiological testing areas shall be adequately separated and appropriate procedures applied to maintain sterility. Activities can be segregated by time or space. In PCR testing, PCR equipment, Master Mix preparation and DNA-RNA extraction shall be isolated from other microbiological activities.

(b) Appropriate procedures shall be applied during sample preparation for trace and ultra-trace chemical contamination testing including, but not limited to, pesticide residue analysis.

5.3.2 The laboratory shall be ventilated to reduce the levels of contamination. The laboratory test area should be air-conditioned to control humidity and temperature.

5.3.2.1 Work space temperature and test area humidity shall be monitored. The recommended relative humidity in the test area is 45-50% RH and the temperature in

the test area is 20-25°C. This may be done by natural or forced ventilation or by the use of an air conditioner. Where air conditioners are used, filters should be appropriate, inspected, maintained and replaced according to the type of work being carried out.

5.3.4 Bench tops (work surfaces) and floors shall be made of impervious, smooth, easily cleaned materials. There shall be at least six linear feet of bench or surface workspace for each analyst while working. Walls and ceilings should be made of materials that are smooth and easily cleaned.

5.3.5 There shall be at least 50 (preferably 100) foot-candles intensity at working surfaces.

5.4 **Test and calibration methods and method validation**

5.4.1 **General**

The laboratory shall have documented QA/QC procedures, including, but not limited to, sample preparation, extraction, equipment calibration and maintenance process control QC and standards for approving/rejecting results.

5.4.2 **Selection of methods**

The laboratory shall use test methods that meet the needs of the client. Where possible, these methods shall comply with the essential/critical elements of regulatory requirements, international, national and/or regional standards. Where no method is specified, the laboratory shall use an appropriate method that is traceable to a recognized, validated method.

For multi-residue testing of pesticides, several official methods exist and their use is highly recommended. These methods have an appendix with the active ingredients that may be determined. The laboratory shall be able to cover at least 75% of these substances and be able to prove that they may be detected with the necessary sensitivity in the accredited food commodity groups (see Table X).

Official multi-methods for pesticide residue testing:

DIN EN 15662	Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method
DIN EN 12393-1,2,3	Multiresidue methods for the gas chromatographic determination of pesticides residue

DIN EN 1528-1,2,3,4	Determination of pesticides and polychlorinated biphenyls (PCBs) in fatty food
AOAC official method 970.52	Determination of Organochlorine and Organophosphorus Pesticide Residues (non-fatty-foods – Multiresidue Method for the Gas Chromatography)

5.4.2.1 All methods not taken from authoritative, validated sources shall be agreed upon by the laboratory and client with clearly defined expectations and requirements. Validation of the appropriateness of these methods (including nonstandard, commercialized tests systems (kits) and new test methods) shall be performed and documented and shall be subject to review and agreement with the client to ensure that the range and accuracy of values obtainable from the method (e.g., detection limit, selectivity, matrix effects, repeatability/reproducibility, ease of use, etc.) are relevant to the client's needs. Where methods exist that are superior to officially recognized methods, these methods may be used if agreed upon with the client and validated as meeting their intended purpose.

Note: *If a modified version of a method is required to meet the same specification as the original method, then comparisons should be carried out using replicates to ensure that this is the case. Experimental design and analysis of results must be statistically valid.*

5.4.2.2 The laboratory shall validate standard methods applied to food matrices not specified in the standard procedures.

Note: *Qualitative test methods, such as where the result is expressed in terms of detected/not detected including confirmation and identification procedures, should be validated by determining, if appropriate, the specificity, relative trueness, positive deviation, negative deviation, limit of detection, matrix effect, repeatability and reproducibility.*

See Annex1.

5.4.2.3 The laboratory shall validate internally developed software used for calculations.

5.5 **Equipment**

5.5.1 The laboratory shall be furnished with all items of sampling, measurement and test equipment required for the correct performance of the tests, including sampling, preparation of test items, processing and analysis of test data.

5.5.2 All equipment (especially those items having an impact on the uncertainty of the results) listed in the methods shall meet the specifications relevant to the method and shall be calibrated and/or verified against those specifications. The frequency of calibration of equipment shall be appropriate to the operation of the equipment to achieve the accuracy relevant to the method.

5.5.3 The laboratory shall have documented procedures for the handling, transport, storage and use of measuring equipment to prevent contamination or deterioration.

5.5.4 The laboratory shall document maintenance schedules and procedures. Maintenance records shall be maintained. All critical equipment, where appropriate, shall undergo regular maintenance and service. The following equipment, but not limited to, are examples of critical equipment requiring regular maintenance and servicing as specified: refrigerators, freezers where samples and certified reference samples are stored, ovens, incubators, water baths, centrifuges, pH meters, balances, analytical instruments such as chromatographs and spectrometers. The laboratory's water source shall be tested to ensure that it meets requirements for chemical and microbiological testing (e.g., MilliQ water for chemical and sterile water for microbiology). Typically, the following items of equipment will be maintained by cleaning and servicing, inspecting for damage, general verification and, where relevant, sterilizing:

- general service equipment – filtration apparatus, glass or plastic containers (bottles, test tubes), glass or plastic Petri dishes, sampling instruments, wires or loops of platinum, nickel/chromium or disposable plastic;

- water baths, incubators, microbiological cabinets, autoclaves, homogenizers, fridges, freezers;

- volumetric equipment – pipettes, automatic dispensers, spiral platens;

- measuring instruments – thermometers, timers, balances, pH meters, colony counters.

5.6 Measurement Traceability

5.6.1 **General**

The laboratory shall have a program for calibrating/verifying the performance of all critical equipment and certified reference standards, traceable to national standards.

5.6.2 **Specific requirements**

Reference standards (e.g., reference thermometers, weights, etc.) shall be used for calibration or verification purposes only.

5.6.3 **Reference standards and reference materials**

Certified reference standards shall be traceable to a nationally or internationally recognized body or provider accredited to ISO Guide 34.

Each “pure” standard material used in the laboratory must be uniquely identified, its expiry date recorded and stored appropriately, preferably in a freezer, with light and moisture excluded, i.e., under conditions that minimize the rate of degradation.

Stock standards should be prepared with not less than 10 mg of the “pure” standard using a 5 decimal place balance.

- Where appropriate and practicable, equipment performance parameters (e.g., temperature of ovens, signal responses of gas chromatographs) shall be verified on a set frequency (e.g., daily) and recorded.

- Analytical equipment such as Chromatographs, Spectrometers etc. shall be calibrated and the frequency of calibration shall be appropriate to the operation of the equipment to achieve the accuracy relevant to the method.

5.7 Sampling (No Additions)

5.8 Handling of test and calibration items

5.8.2 The laboratory’s system for the identification of samples shall include the following information, records of which must be retained throughout the testing life of the sample:

5.8.2.1 Unique and unambiguous sample identification (usually a number or alpha numeric identification or a combination of both);

5.8.2.2 Name of the client to whom the final report will be sent;

5.8.2.3 Inspector’s name or sample source and date of sampling;

5.8.2.4 Identification number or description from field inspector;

5.8.2.5 Product description;

5.8.2.6 Tests desired and/or methods requested;

5.8.2.7 Date of receipt;

5.8.2.8 Delivery carrier;

5.8.2.9 Sample condition and physical appearance (including temperature);

5.8.2.10 Laboratory sample identification number.

5.8.4 It is advisable to keep a portion of non-homogenized sample in reserve in case of necessary confirmations of measurements until the final test result is finished.

5.8.4.1 The laboratory shall have documented procedures for the handling, sampling, transport, storage, preparation, retention and disposal of test items.

5.8.4.2 Sample handling and storage procedures shall include precautions for preventing cross contamination and deterioration.

5.8.4.3 Sample preparation and homogenization should not have a significant effect on the degradation of certain pesticides. Sample preparation shall ensure that the sample is homogeneous enough so that sub sampling is acceptable.

5.8.4.4 Where there is evidence that comminution (cutting and homogenization) at ambient temperature has a significant influence on the degradation of certain pesticide residues, it is recommended that samples are homogenized at low temperature (e.g., frozen and/or in the presence of “dry ice”).

Note: *Where comminution is known to affect residues (e.g., dithiocarbamates or fumigants) and practical alternative procedures are not available, the test portion should consist of whole units of the commodity, or segments removed from large units. For all other analyses, the whole laboratory sample (food in most cases 1-2 kg; feed 0.5-2 kg) needs to be comminuted.*

To improve the extraction efficiency of low moisture containing commodities (e.g., cereals, spices, dried herbs), it is recommended to achieve small particle sizes (e.g., using ≤1 mm sieves in rotor mills). Milling should be performed in a way avoiding extensive heating of the samples as heating can cause losses of certain pesticides.

5.8.4.5 The extraction process and extraction efficiency as well as steps such as clean-up, concentration, dilution of extract and storage of extract needs to be documented.

5.8.4.6 If long term storage is required, it is advisable that in order to maintain integrity samples should be kept in a freezer (approx. -18ºC).

5.8.4.7 Samples must be separated from each other, and from other sources of potential contamination, during transit to and storage at the laboratory.

5.8.4.8 A reagent blank should be analyzed in conjunction with every batch of samples analyzed.

5.8.4.9 Volumetric equipment, such as flasks, pipettes and syringes must be cleaned scrupulously, especially for re-use. As far as practicable, separate glassware, etc., should be allocated to standards and sample extracts, in order to avoid cross-contamination. Avoid using excessively scratched or etched glassware. Solvents used

for fumigant residues analysis should be checked to ensure that they do not contain the analyte.

5.8.4.10 Where the analyte occurs naturally, or as a contaminant, or is produced during the analysis (e.g., biphenyl in herbs, inorganic bromide in all commodities, sulphur in soil, or carbon disulfide produced from the Brassicaceae), low-level residues from pesticide use cannot be distinguished from background levels. Natural occurrence of these analytes must be considered in the interpretation of results. Dithiocarbamates, ethylenethiourea or diphenylamine can occur in certain types of rubber articles and this source of contamination must be avoided.

5.8.4.11 The Lowest Calibration Level (LCL) must be equal to or lower than the Reporting Limit (RL).

5.8.4.12 Bracketing calibration must be used unless the determination system has been shown to be free from significant drift proven by relative (internal standardization) response.

5.8.4.13 Multi-level calibration is preferred. However, single-level calibration may provide more accurate results if the detector response of the analyte in the sample extract is close to the response of the single-level calibration standard (within ± 30%).

5.8.4.14 All targeted analytes should be injected in every batch of samples, at least the level corresponding to the RL. Sufficient response at this level is required and should be checked to avoid false negatives.

Note: *If this requires a disproportionate effort, the determination system must be calibrated with a minimum number of representative analytes taking into account that reliance only on representative analytes is associated with an increased risk of incorrect results, especially false negatives. Therefore, representative analytes must be chosen very carefully, to provide enough evidence that acceptable performance is achieved for all other analytes.*

The choice of the representative analytes should be made according to the probability of finding residues in the sample and the physico-chemical characteristics of the analytes, i.e., analytes likely to give the poorest and most variable response.

The representative analytes to be calibrated in each batch must be at least 15 analytes plus 25% of the total number of analytes included in the analytical scope of each determination system. For example, if the analytical scope of an instrument method covers 40 analytes, the determination system must be calibrated with at least 25 representative analytes. If the scope of analysis in the determination system is 20 or less, then all analytes should be calibrated).

5.8.4.15 There are several different approaches for quantification.

- Calibration using solvent standards
- Calibration using matrix matched standards
- Calibration using internal standards
- Standard addition

The choice of calibration technique employed is dependent on several factors and can differ for different analytical methods.

5.9 **Assuring the quality of test and calibration results**

5.9.1 The laboratory quality control procedure for monitoring the validity of the test shall be appropriate to the relevancy of the method. The quality control procedure shall be planned, documented and include, but not limited to, the following:

- Use of certified reference materials traceable to nationally or internationally recognized body or provider accredited to ISO Guide 34.
- Where practicable, participate in proficiency testing programs or inter-laboratory comparison program.
- All targeted analytes should be injected in every batch of samples, in one or more QC samples

Note: *(If this requires a disproportionate effort, the determination system must be checked with a minimum number of representative analytes taking into account that a reliance only on representative analytes is associated with an increased risk of incorrect results, especially false negatives. Therefore representative analytes must be chosen very carefully, to provide enough evidence that acceptable performance is achieved for all other analytes.*

The choice of the representative analytes should be made according to the probability of finding residues in the sample and the physico-chemical characteristics of the analytes, i.e., analytes likely to give the poorest and most variable response.

The representative analytes to be calibrated in each batch must be at least 15 analytes plus 25% of the total number of analytes included in the analytical scope of each determination system. For example, if the analytical scope of an instrument method covers 40 analytes, the determination system must be calibrated with at least 25 representative analytes. If the scope of analysis in the determination system is 20 or less, then all analytes should be calibrated).

- Quality control data are recorded in such a way to detect trends and where applicable, statistical techniques are applied to review the results.

- Trend monitoring shall be planned, documented and where practicable control charts are applied to detect trend and manage quality control data within the pre-defined criteria

- Participation in Inter-laboratory/Proficiency testing programs (see related IAS Policy).

5.9.2 Proficiency testing for pesticide single- and multi-residue testing

Before first accreditation, the laboratory must have participated successfully in at least one proficiency test for every single residue method and at least one proficiency test for the multi-residue methods in each group of food commodities (see Table X). If no proficiency scheme is available on the marked, comparative testing with other accredited laboratories must be performed.

The laboratory shall participate with success at least once a year in a proficiency test for every single-residue method and in each group of food commodities for the multi-residue methods.

Successful participation is defined as following:

- No false positive or false negative result must be reported

- Single residue method:

    - Incurred parameters: The reported result must have a z-score between -2 and +2.

    - Spiked parameters: The reported result must have a z-score between -2 and +2. If a reported result is <-2 or >+2, the reported result must be between 70% and 120% of the spiked value.

- Multi-methods: At least n-1 parameters must meet the following criteria (n= number of spiked substances):

    - Incurred parameters: The reported result must have a z-score between -2 and +2.

    - Spiked parameters: The reported result must have a z-score between -2 and +2. If a reported result is <-2 or >+2, the reported result must be between 70% and 120% of the spiked value.

Table X: Food Commodity Groups in Pesticide Residue Testing

No.	Group of food commodity	Characteristics	Example matrices
1	**Fruits and vegetables**		
1a	Fruits and vegetables	High water content	Apples, banana, cherries, onions, cucumber, lettuce, mushrooms, cabbage
1b	Acid fruits	High acid and water content	Lemons, oranges, strawberries, kiwi, pineapple
1c	Dried fruits, honey	High sugar content and low water content	Honey, raisins, dried apricots, fruit-marmalade
2	**Cereals and cereal products, dried legumes**	Low water and fat content, high starch and/or protein content	Rice, maize, wheat, cakes, muesli, cornflakes, dried legumes, cereals, bread
3	**Oils, Oilseeds and fatty food**	High fat content	Oils, fats, olives, avocado, nuts
4	**Special matrices**		Cacao, hops, coffee, tea, spices
5	**Food of animal origin**		
5a	Meat, Fish, Shellfish		
5b	Milk, milk-products		Milk, cheese, yogurt, cream, butter
5c	Eggs		

5d	Fat from food or fat of animal origin		Lard, butter, fish oil, cod liver oil, suet
6	**Baby food**	Lower LOD necessary (generally 0,001 mg/kg)	
7	**Feed**	Low water content, high starch or protein content	

5.10 Reporting the Results (No Additions)

APPENDIX 1
METHOD VALIDATION & VERIFICATION GUIDELINES
FOOD TESTING LABORATORIES

A1. INTRODUCTION

Method validation is a confirmation through objective evidence that a method is fit for the intended purpose. Hence method validation is an essential requirement for the accreditation of food testing laboratories to ISO/IEC 17025.

Validated analytical methods for the quantitative evaluation of analytes (chemical or microbiological) are a requirement for the accreditation of food test methods. Accordingly food testing laboratories accredited to ISO/IEC 17025 must demonstrate validity of all accredited methods. Validation of these methods includes performing all procedures that demonstrate that a particular method used for quantitative measurement of specific analytes in a given matrix is reliable and reproducible for the intended use.

Fundamental parameters for method validation include but not limited to, the following:

- Accuracy
- Precision (as repeatability and reproducibility)
- Selectivity/Specificity
- Linearity (Calibration)
- Limit of Detection (LOD)
- Limit of Quantitation
- Robustness

The standard recognizes that validation is a balance between costs, risks and technical possibilities (Clause 5.4.5.3 Note 3). The extent of validation required will depend on method under consideration and the intended application. A newly developed in-house method will require a comprehensive validation whereas minor modification to an existing validated method may only require a confirmation of accuracy and precision.

Method validation is a requirement for accrediting:

- non-standard methods,
- laboratory designed/developed methods,

- standard methods modified or amplified by the laboratory and
- addition of new analyte(s) or matrix outside the scope of the existing validated method.

A food testing laboratory that wishes to use a reference method that has been extensively validated (e.g., AOAC, Codex Alimentarius, etc.) is required to confirm that it can properly operate standard methods before conducting the reference method. The laboratory shall demonstrate its ability to achieve the performance criteria of the standard method under their own testing conditions. This confirmation can be via "verification" of certain key performance characteristics of the standard method. If the standard method changes, the confirmation shall be repeated.

Method verification (or simply verification) is a partial validation and hence is less extensive than what is required for the method validation. The verification shall be appropriate to the intended purpose and can be demonstrated by achieving certain specific performance characteristics of the standard method under the laboratory's own testing conditions. More useful guidelines on method verification are provided in an AOAC publication (Reference 2).

The laboratory, through the use of specific laboratory investigations, should demonstrate that the performance characteristics of a method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method. When changes are made to a previously validated method, additional validation may be needed.

The laboratory shall document the procedure for validation and verification. Methods once validated or verified must be supported by performance verification during routine analysis (analytical quality control) and on-going method verification.

This document provides guidance on how method validation and method verification may be investigated and evaluated. This guidance is intended to be applied to all fields of food testing using chemical and microbiological methods of analysis.

A2. DEFINITION OF VALIDATION PARAMETERS

To be fit for the intended purpose, the method should meet standards for certain validation parameters. Typical validation characteristics for food analytical methods that should be considered are provided above. These parameters are defined below.

Accuracy (as Bias or Recovery)

Accuracy is defined as closeness of agreement between a measured quantity value and a true quantity value of a measurand. A measurement is said to be more accurate when it offers a smaller measurement error (smaller bias). This approach to accuracy determination in testing is feasible if matrix matched reference materials are available. A more common approach in food testing for accuracy determination is the recovery studies.

Recovery is the amount measured as a percentage of the amount of analyte(s) (active substance and relevant metabolites) initially added (“spike”) to a sample of the appropriate matrix, which contains either no detectable level or a known detectable level (preferably at low levels) of the specific analyte(s). Recovery experiments provide information on both precision and trueness (bias), and thereby the accuracy of the method.

Selectivity (Specificity)

Selectivity refers to the extent to which the method can be used to determine particular analyte(s) in mixtures or matrices without interferences from other components of similar behaviour. The words ‘Selectivity’ and ‘Specificity’ are interchangeable. Evidence should be provided that the substance quantified is the intended analyte.

Calibration

Calibration refers to the ability of a detection system to produce an acceptable, well defined, correlation between the instrumental response and the concentration of the analyte in the sample. The analyte concentration to be measured should be within the defined dynamic range (Linearity) of the instrument.

Linearity

Linearity is defined as the ability of the method to obtain test results proportional to the concentration. Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components.

Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method. The acceptable linear regression factor (R or r should be defined.

Range

The range of an analytical procedure is the interval from the upper to the 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. The range is normally expressed in the same units as the test results (for example percentage, parts per million) obtained by the analytical method.

Repeatability (Precision)

Repeatability refers to the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. The repeatability (within-run effect) includes contributions from any part of the procedure that varies within a run, including contributions from normal gravimetric and volumetric errors, heterogeneity of the test material, and other procedural errors during the analysis.

Reproducibility (Precision)

Reproducibility refers to the closeness of agreement between independent results obtained with the same method on identical test material obtained but under different conditions. Within-laboratory or intra-laboratory reproducibility or single-laboratory reproducibility (run effect) contributes to day-to-day variations in the analytical system due to changes of analyst, batches of reagents, recalibration of instruments and laboratory environment (e.g., temperature changes). Between-laboratory or inter-laboratory or multiple-laboratory reproducibility (laboratory effect) contributes to additional variations, such as variations in calibration standards, differences between local interpretations of a protocol, differences in equipment or reagent source, or environmental factors, such as differences in average climatic conditions.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure's reliability during normal usage. Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, such as pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

Limit of Detection (LOD)

The limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. The limit of detection of an analytical procedure is the lowest amount of an analyte in a sample that can be detected but not necessarily quantitated. At the limit of detection, a positive identification can be achieved with reasonable and/or previously determined confidence in a defined matrix using a specific analytical method. The LOD is typically not required. However, if needed for a refined assessment (or some other purpose), an explanation of how the LOD was derived should be provided which should be appropriate to the method and statistically justified.

The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small

differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass.

Limit of quantitation (LOQ)

Limit of quantitation (LOQ), defined from a regulatory perspective as the lowest concentration tested and quantified such that an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation (RSD) is obtained. The LOQ should be low enough to achieve the intended purpose of the method. From an analytical perspective, 6-10 times the standard deviation of the noise provides an estimate of the LOQ, which is then verified by the fortification experiments. If not stated otherwise, this document refers to the LOQ from the regulatory perspective.

For method validation, the following validation tools should be used to generate method performance characteristics:

Blanks: Use of various types of blanks enables assessment of how much of the result is attributable to the analyte in relation to other sources. Blanks are analyzed and compared to the limit of detection.

Reference materials and certified reference materials: The use of known reference materials (when available and applicable) can be incorporated to assess the accuracy or bias of the method, as well as for obtaining information on interferences.

Matrix Blank: A substance that closely matches the samples being analyzed with regard to matrix components. Matrix blanks are used to establish background level (presence or absence) of analyte(s) and to verify that sample matrix and equipment used does not interfere with or affect analytical signal.

Matrix Spikes (Laboratory Fortified Matrix): Recovery determinations can be estimated from fortification or spiking with a known amount of analyte and calculation of spike recoveries. (Note: spike recovery may not be truly representative of recovery from naturally incurred analytes). Matrix effects can also be assessed with these samples. Accuracy or bias and precision are calculated from these results. The data can also be used to evaluate robustness of the method resulting from changes in the sample matrix.

Incurred Samples: Samples that contain (not laboratory fortified) the analyte(s) of interest (if available) may also be used to evaluate precision and bias (if analyte concentration(s) are reliably known). Analyte recovery can also be evaluated through

successive extractions of the sample and/or comparison to another analytical procedure with known bias.

A3. METHOD VALIDATION PROTOCOL

All food testing methods (chemistry and microbiology) shall be fully validated or verified by the laboratory for “fitness of purpose” prior to implementation of the methods. This validation or verification shall be according to fully documented procedures.

Standard published method used verbatim does not require a full validation, however, the laboratory shall confirm that it can properly operate the standard method before introducing the tests. Confirmation can be achieved through method verification of specific performance characteristics of the standard methods. If the standard method changes, the confirmation shall be repeated.

Conditions for full validation:

a. Standard published method modified or amplified (exemption: editorial modifications) to the extent that the modification or amplification directly affect the performance of the test.

b. Standard published method or previously validated method applied to different matrices, analytes, concentration range or conditions outside the scope of the published method.

c. In-house developed methods.

d. Method published in scientific journals without appropriate performance data.

e. Vendor test kits without appropriate performance data.

Conditions for verification (confirmation):

a. Standard published method used without modification.

b. Method published in scientific journals with appropriate performance data.

c. Vendor test kits with appropriate performance data.

Extent of validation and verification:

Methods shall be validated or verified using certified reference materials (where available) or materials of known characteristics.

Validation of quantitative methods, shall include, but not limited to, the following attributes as appropriate:

• Accuracy	• Linearity
• Range	• Reproducibility (Precision)
• Repeatability (Precision)	• Robustness
• Limit of Detection	• Limit of Quantitation
• Estimation of measurement uncertainty	• Reporting limits
• Selectivity	

In addition, where applicable, the following shall be taken into consideration during validation process:

- Matrix effect
- Interference
- Sample homogeneity

Verification of quantitative methods, shall include, but not limited to, the following as appropriate:

- Accuracy
- Precision
- Limit of Detection
- Measurement uncertainty estimate

For validation or verification of qualitative methods the following attributes, should be taken into consideration where applicable:

- Specificity
- Contamination
- Detection limit
- Rate of false positive and false negative
- Repeatability
- Interferences

For pesticides in food method the validation or verification shall be extended to include all commodity categories within the same commodity group for which accreditation is sought. A guidance table is provided below.

Commodity groups	Typical commodity categories within the group	Typical representative commodities within the category
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprouts, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Forage/fodder crops	Fresh alfalfa, fodder vetch, fresh sugar beets
	Fresh legume vegetables	Fresh peas with pods, petit pois, mange tout, broad beans, runner beans, French beans
	Leaves of root and tuber vegetables	Sugar beet and fodder beet tops
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrots, potatoes, sweet potatoes

Commodity groups	Typical commodity categories within the group	Typical representative commodities within the category
2. High acid content and high water[(1)] content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, blackcurrant, red currant, white currant, grapes
	Other	Kiwifruit, pineapple, rhubarb
3. High sugar and low water content[(2)]	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts, chestnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame, etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahina, hazelnut paste
	Oils from tree nuts, oil seeds and oily fruits	Olive oil, rapeseed oil, sunflower oil, pumpkin seed oil
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grain; maize, rice, wholemeal bread, white bread, crackers, breakfast cereals, pasta

Commodity groups	Typical commodity categories within the group	Typical representative commodities within the category
6. “Difficult or unique commodities”		Hops Cocoa beans and products thereof, coffee, tea Spices
7. Meat and Seafood	Red meat White meat Offal(3) Fish Crustaceans	Beef, pork, lamb, game, horse Chicken, duck, turkey Liver, kidney Cod, haddock, salmon, trout Shrimp, scallop, crab
8. Milk and milk products	Milk Cheese Dairy products	Cow, goat and buffalo milk Cow, goat cheese Yogurt, cream
9. Eggs	Eggs	Chicken, duck, quail, goose eggs
10. Fat from food of animal origin(3)	Fat from meat Milk fat(4) Fish oil	Kidney fat, lard Butter Cod liver oil

[Footnotes to be included in a later draft]

Once the method is validated or verified, the laboratory shall record the results with a statement signed and dated by an approving authority as to whether the method is fit for the intended purpose. The laboratory may include a report summarizing the validation/verification data.

Once the method is validated or verified and implemented it shall be periodically reviewed to confirm the ongoing “fitness for purpose” of the method. The laboratory shall define and document the frequency and the review process, preferably in the method validation procedure.

A4. SUMMARY

The rigour of validation of a method should be such that the test results produced by the method are technically sound and meet the client’s needs. Hence, for validation study all parameters are not necessarily assessed for all methods. In such cases, the selection of parameters for validation should be appropriate to determine the fitness of the method for the intended use.

Method should be validated or verified using certified reference materials (CRMs) when available. Reference materials from inter-laboratory studies such as those obtained from proficiency testing programs can be used if suitable CRMs are not available.

When reference materials are not available, method bias can be assessed through recovery studies. Recovery can be assessed by spiking blank samples or duplicate test sample with a known amount of analyte or organism.

When adopting previously validated method (standard method) such as those published in AOAC, CODEX, ASTM, etc., the laboratory is not required to conduct a full validation, however, it is expected to confirm through verification that the performance characteristics of the standard method are achievable in their laboratory. If the standard method changes, the confirmation should be repeated.

For method verification, as a minimum, the laboratory should determine that the bias and precision are fit for the purpose. For trace analyses, the confirmation should also verify that the LOD and LOQ achievable are appropriate for the application.

Table 1 Approach to Validation and Verification for Food Testing Laboratories

METHOD	REQUIREMENT
1. Standard published method 2. Method published in scientific journals with validation data 3. Instrument manufacturer's published technical paper with validation data 4. Commercial test kit third party validated or approved by regulatory agencies	No validation required. Verify to confirm the performance characteristics are achievable. Validation may be required if the method changes (revised) and if the revision/s are significant.
1. Standard published method subject to in-house modification 2. Standard method applied outside the scope of the standard method (e.g., different matrices, analytes or conditions)	Validation is required. The rigor of validation will depend on the extent of the modification/s.
1. In-house developed method 2. Method published in scientific journals without validation data 3. Instrument manufacturer's published technical paper without appropriate validation data 4. Commercial test kit with no performance data or incomplete	Full validation required.

Table 2 Recommended Analytical Requirements for Method Validation

Parameter	Procedure	Determination
Accuracy (bias, recovery)	Analysis of: CRMs/RMs, Analyte Spikes, PT samples	Minimum of 7 replicate Reference materials should be matrix and concentration matched with test samples
Selectivity (Specificity)	Evaluate potential interferences. Analysis of spike samples containing potential interferences.	If method development has evaluated this then recommend one test (preferably with a duplicate)
Sensitivity (analytical instrumental)	Calibration curve using traceable calibration standards	The slope of the linear calibration curve.
Calibration	Calibration curves established using traceable calibration standards	Calibration verification using a traceable standard prepared from a source other than what is used for preparing calibration standards
Linearity	Calibration curve using traceable calibration standards	Duplicate measurement of six or more concentration ranges over the expected range. The linearity is defined by the regression factor "r" which should be greater than 0.99
Range	Calibration curve using traceable calibration standards. Also bias and LOQ should be evaluated	The concentration range between LOQ and the upper limit of the linear curve
Precision Reproducibility Repeatability	Repeated analysis of CRMs, samples preferably containing analyte concentration in the mid-range of the calibration curve and most relevant to the application of the method	Minimum of 7 replicates for each matrix
Robustness (Ruggedness)	Consider all steps of the method which if varied marginally can affect results (e.g. environmental conditions)	Repeat testing with small changes to the defined steps and determine the variation
Limit of Detection (LOD)	Analysis of samples containing low concentrations of analytes	Minimum 7 replicates of blank or sample containing analyte concentration estimated to equal or twice the LOQ
Limit of Quantitation		

Keep comprehensive records of method validation/verification, including the procedures used for validation, worksheets and records, the result obtained. Documentation for method validation shall include, but not limited to:

- Description of the analytical method used, including sample preparation, extraction and analysis,
- Description of the preparation of calibration standards, QC, blanks, etc.,
- Description of potential interferences,
- Summary of tests performed to determine accuracy, precision, recovery, selectivity, limit of quantification and calibration,
- Signed statement indicating the fitness of the method to the intended purpose.

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End of Report