GENERAL GUIDELINES FOR THE ACCREDITATION OF FOOD AND ENVIRONMENTAL MICROBIOLOGICAL LABORATORIES

ESYD G-MICROBIOL

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1 Introduction and scope of the document

1.1 Purpose the purpose of this document is to contribute to the optimization and standardization of procedures in the assessment of microbiological tests, based on the experience obtained from the application of ISO 17025, in laboratories for microbiological testing of food and water. This document in no way replaces the standard ISO 17025 and the instructions that have occasionally published by international organizations (e.g. Eurachem), but provide clarifications on specific technical topics.

Eurachem Guide "Accreditation for Microbiological Laboratories" Edition 2013 was the basis for this guidance, along with points highlighted in the previous edition of this ESYD guide.

2 Personnel

2.1 All laboratory staff (analysts, analyst assistants) should have received adequate training to perform microbiological testing and handling of equipment and should be evaluated for performance, based on objective criteria (e.g., duplicate testing, using reference materials, etc.). The training of the staff and the evaluation should be documented.-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. This should include training in basic techniques, e.g. plate pouring, counting of colonies, aseptic technique, etc., with acceptability determined using objective criteria. The critical interval between performances of tests should be established and documented. 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.

2.2 The competence of personnel to perform tests shall be documented in relation to the results of internal and external quality control. The effectiveness of the training program, as well as the identification of further training needs, should also be evaluated based on these results.

3 Environment
See also ISO 7218 [8], paragraph 3 and Eurachem AML section 4

The laboratory should have enough space and suitable design in order to be neat and tidy, to prevent contamination of the sample and enables the staff to work comfortably.

The environmental conditions of the laboratory that may affect the quality of testing should be specified, monitored and recorded. For example, it is useful to monitoring the ambient temperature of the laboratory when is used incubators that operate at temperature 5^{o}C above the ambient temperature, for the incubation of samples in temperatures of 30^{o}C.

4 Validation and Verification of test methods

See also ISO/TS 12869 [12], ISO 7218 [8], ISO 17994 [13], ISO/TR 13843 [14], ISO 16140 [15] and EURACHEM AML section 5

4.1 Selection of method

The laboratory may choose any method that gives proven reliable results, to identify a particular microorganism. Therefore it can use: reference methods, modified methods or in house methods. It is recommended to use reference methods.

As **reference methods** are considered ISO standard methods and methods proposed by international organizations (AFNOR, AOAC, NMKL, Nordic Food Analysis of Committed, Esbo, Finland, EPA, International Dairy Federation, EU Legislation etc.).

When the lab uses a standard method should provide verification data (repeatability, reproducibility, detection limit calculation).

These methods can be used as is, without being rewritten as working document, as long as all staff that uses it has the capability of complete understanding. In case is been rewritten as working document, it should not have any deviation from the standard method.

The labs must have and use the latest versions of standard methods.

It is recommended to use standard methods to avoid the laborious process of validation of a method.

As **modified methods** are considered standard methods that have undergone slight modifications (e.g. use of substrate with a different composition, incubation temperature, using a different use for different test sample matrix). Also the combination of standard methods, leading to a modified method.

As **in house methods** are considered to be those that are developed entirely by the laboratory.

The use of modified or in house methods requires a validation and not only verification.

4.2 Verification

Data of the verification are the repeatability, reproducibility and the detection limit.

Repeatability is the closeness of the results between independent trials, obtained with the same method, using identical sample, by the same operator using the same equipment within short intervals.

Reproducibility of a method can be tested by examining, by the same analyst, three duplicate samples each day and for three consecutive days. These samples are preferable to be selected natural samples, if this is not possible they can be used for samples prepared in the laboratory.

If the method is qualitative (presence/absence) the sample should contain a small number of cells of the organism under consideration.

Acceptance criterion: RSD<0,1 ή RSD (%)<10

Reproducibility is the proximity between the results obtained with the same method, using identical sample, with different analysts using the same or different equipment.

The reproducibility of a method can be tested by examining six samples from two analysts, in duplicate. These samples, as before, are selected natural or prepared in the laboratory.

If the method is qualitative (presence/absence) the sample should contain a small number of cells of organism under investigation (near the detection limit).

Acceptance criterion may be the difference of the mean value of two measurements of two analysts, as log10, and should be <0,5log10.

Detection limit refers to the qualitative methods (presence/absence) and constitutes the lowest number of microorganisms, which can be detected with this method.

The detection limit, can be determined by examining the samples produced in the laboratory, with natural sample (free of the target microorganism) spiked with a known population (from a reference material) of the target microorganism (e.g., 20, 10, 5 c.f.u/gr).

If the method is specific regarding the sample matrix (e.g. milk) the verification of the method should be done in this type of matrix. If the method is horizontal, as happens with many ISO methods, the laboratory should verify the method in the al 4 categories. In water, the differentiation made between drinking and marine. Another category is sewage samples.

. In horizontal methods, the verification should include all type categories of foods. The categories are specified in Appendix A.

4.3 Validation of methods

For the validation of laboratory methods have to take account of the standards:

- ✓ ISO 16140:2003 Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods
- ✓ ISO/TR 13843:2000 Water quality – Guidance on validation of microbiological methods

On qualitative methods (presence/absence, including confirmation and identification procedures) validation should include as a minimum estimate of specificity of method, of conformity (trueness), positive and negative deviation, detection limit, the effect of the sample matrix, repeatability and reproducibility.

On quantitative methods (estimate of the population of a microorganism) validation should include as a minimum estimate of the sensitivity and specificity of the method, of trueness, positive and negative deviation, detection limit, the effect of the sample matrix, repeatability, reproducibility, linearity and robustness.

4.4. Evaluation of uncertainty of measurement

In microbiological tests are generally accepted the assessment of uncertainty based on reproducibility data. Also it can be used data from the interlaboratory in order to estimate the bias of the method.

The laboratory should have identified the sources of uncertainty of measurement and to prove that they are under control, as well as have calculated their contribution to the variation of results. Some of these sources such as pipettes, weighing and dilutions allow measurement and estimate their contribution to the overall uncertainty. Other sources, such as the stability of the sample and preparation of, cannot be measured directly and therefore cannot assess the contribution to the uncertainty of the measurements.

Microbiological laboratories should have understood the dispersion of micro-organisms in samples and if possible take more representative specimen for examination. This parameter (dispersion of microorganisms in a sample) contributes to the uncertainty of measurement, but cannot be evaluated because it is different in each sample.

The concept of uncertainty cannot be applied to qualitative methods (presence/absence). However, parameters that can affect the result as is the proper functioning of the culture media and the analyst's ability should have been identified and are under control.

In the calculation of uncertainty is preferable to used natural specimens. If this is not possible can be used artificial contaminated natural samples.

Also, the samples to be used should be of all matrixes of samples testing by the laboratory.

The uncertainty should be reassessed at regular intervals to reflect the actual conditions of the laboratory. The reassessment of the uncertainty could be done with adding new measurements and replacing older.

In the event of significant changes in the laboratory (e.g. change analyst, new version of method with different substrate), the laboratory should re-estimate the uncertainty.

All the raw data used in the calculation of uncertainty (exam date, sample type, analyst, etc.) will be recorded and archived.

In the case of microbiological laboratories performing molecular testing for the detection and quantification of genetically modified organisms (GMOs), measurement uncertainty is estimated according to JRC/IRMM Guidance EUR 22756 EN [19].

5 Equipment – maintenance, calibration and performance verification
See also Eurachem AML (section 7 and appendix A), ISO 7218 [8], paragraph 5 and ILAC P10 [20]

6 Reagents and culture media
See also ISO/TS 11133

Laboratories should ensure that the quality of reagents used is appropriate for the test concerned. They should verify the suitability of each batch of reagents critical for the test, initially and during its shelf-life, using positive and negative control organisms which are traceable to recognized national or international culture collections. Laboratories have also to include in their internal quality control scheme measures to ensure the quality of reagents and the need is to have an IQC appropriate for their work.

7 Reference materials and reference cultures
See also ISO/TS 11333 [21] and EURACHEM AML section 9

7.1 Reference materials
Reference materials and certified reference materials (see definition in Appendix A) provide essential traceability in measurements and are used, for example:
• to demonstrate the accuracy of results;
• to calibrate equipment;
• to monitor laboratory performance;
• to validate methods;
• to enable comparison of methods;
• to demonstrate quality of culture media;
• to demonstrate consistent performance of kits.

If possible, reference materials should be used in appropriate matrices.

7.2 Traceable reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. To demonstrate traceability, laboratories shall use reference strains of micro-organisms obtained directly from a recognized national or international collection, where these exist. Where traceable reference cultures are not readily available, commercial derivatives traceable to them could alternatively be us ed, provided that the relevant properties for its intended use have been shown by the laboratory to be equivalent at the point of use. Reference strains may be sub-cultured once to provide reference stocks.

8 Sampling
See also ISO 7218, paragraph 8 and ISO 19458

Sampling should only be performed by trained personnel. Whenever the laboratory is responsible for sampling, the personnel to be involved shall also be authorized for sampling. It should be carried out aseptically using sterile equipment. Environmental conditions, for instance air contamination and temperature, should be monitored and recorded at the sampling site. Time of sampling should be recorded.

9 Sample handling and identification

See also ISO 7218 [8], paragraph 8, ISO 6887 [23] and ISO 19458 [22]

9.1 The laboratory shall have procedures that cover the delivery of samples and sample identification. 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 and the condition of the sample should be indicated on the test report.

9.2 The laboratory shall record all relevant information, in particular the following:

(a) date and, where relevant, the time of receipt;

(b) condition of the sample on receipt and, when necessary, temperature;

(c) characteristics of the sampling operation (sampling date, sampling conditions, etc.).

9.3 General guidance for sub-sampling by the laboratory immediately prior to testing is given in ISO 6887 and ISO 7218). Sub-sampling procedures should be designed to take account of uneven distribution of micro-organisms.

9.4 Laboratory sample portions that are known to be highly contaminated should be decontaminated prior to being discarded.

10 Disposal of contaminated waste
See also ISO 7218

The correct disposal of contaminated materials may not directly affect the quality of sample analysis, although procedures should be designed to minimize the possibility of contaminating the test environment or materials. However, it is a matter of good laboratory management and should conform to national/international environmental or health and safety regulations.

11 Quality assurance of results/quality control of performance

See also EA-4/18

11.1 Internal quality control

11.1.1 Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results day-to-day and their conformity with defined criteria.

11.1.2 A program of periodic checks is necessary to demonstrate that variability (i.e. between analysts and between equipment or materials etc.) is under control. All tests included in the laboratory's scope of accreditation need to be covered. The program may involve:

- 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.

The internal quality control program must be adapted to the actual frequency of tests performed by the laboratory. It is recommended that, where possible, tests should incorporate controls to monitor performance. It is also advised that data from reference materials and spiked samples be plotted to assist in the evaluation of trends in a visual manner.

In special instances, it is recognized that for a test that it is rarely called on to do, an on-going internal quality control program may be inappropriate. In such cases, a scheme for demonstrating satisfactory performance which is carried out in parallel with the testing may be more suitable. In any case, the laboratory should be aware of the inherent risk associated with such an approach and take all appropriate measures.

11.2 External quality assessment (proficiency testing)

11.2.1 Laboratories should regularly participate in proficiency testing (PT), relevant to their scope of accreditation and according to ESYD/PDI.

12 Test reports
ISO/IEC 17025, paragraph 5.10
See also ISO 19036 [9], ISO 8199 [11], ISO 7218 [8]

Appendix A Matrix categories for food and water samples

Category **1**	Fluids and powders (e.g. milk, dried milk etc)
Category **2**	Mixed solid substances (e,g. minced meat, meat products, MDM, ice creams, whipped cream etc)
Category **3**	Small (or very small) solid substances e.g Dried parsley and mushrooms, shredded carrots & celeriac, lettuce, shrimp, cereals, feedstuffs, chopped nuts, etc
Category **4**	Other solid substances (Meat (not ground), cheese, pastry, etc.
Category **5**	Potable water, surface and swimming pool water
Category **6**	Sea water
Category **7**	Waste water

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Other references for further reading

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