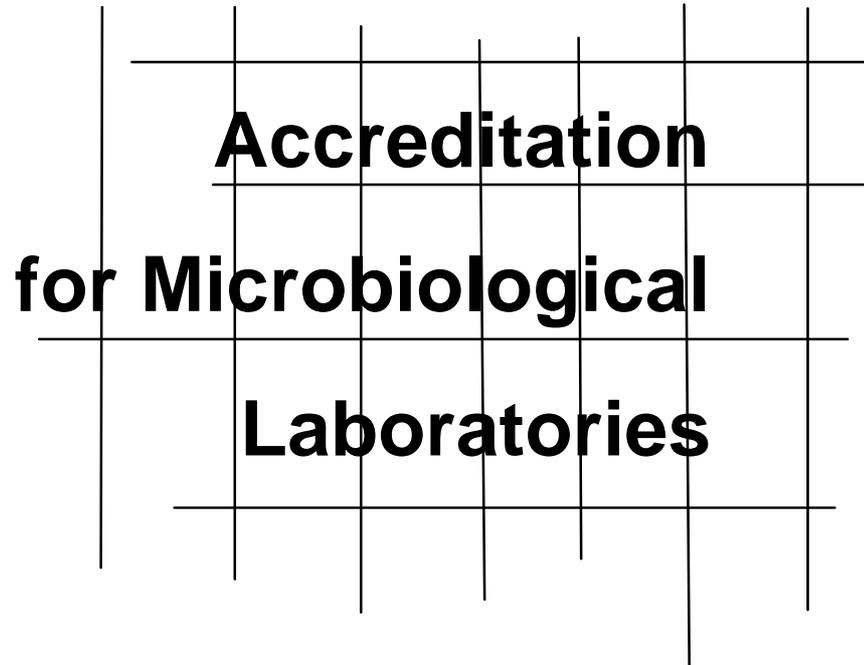# EURACHEM / EA Guide 04/10

Accreditation

for Microbiological

Laboratories

PURPOSE

This document has been produced by a joint EA/EURACHEM Working Group. It supplements ISO/IEC 17025 and provides specific guidance on the accreditation of laboratories performing microbiological testing, for both assessors and laboratories preparing for accreditation. ISO/IEC 17025 remains the authoritative documents and, in case of dispute, the individual accreditation bodies will adjudicate on unresolved matters. The guidance given in this document may be also of use to those working towards certification to the ISO 9000 series of standards.

Authorship

The publication has been prepared by the working group food of the EA Laboratory Committee in collaboration with Eurachem.

Official language

The text may be translated into other languages as required. The English language version remains the definitive version.

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Please check our website for up-to-date information http://www.european-accreditation.org/

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CONTENTS

1   Introduction and scope of document

1.1 The general requirements for accreditation are laid down in the International Standard *General requirements for the competence of testing and calibration laboratories* (ISO/IEC 17025 1st Ed., 1999), hereafter referred to as ISO 17025. All of these requirements must be met by laboratories seeking accreditation.

1.2 This document supplements ISO 17025 by providing specific guidance for both assessors and for laboratories carrying out microbiological testing. It gives detailed guidance on the interpretation of ISO 17025 for those undertaking the examination of materials, products and substances. The guidance is applicable to the performance of all objective measurements, whether routine, non-routine, or as part of research and development. Although it is written primarily for food and environmental microbiological testing, the general principles may be applied to other areas. ISO 17025 remains the authoritative document and, in cases of dispute, accreditation bodies will adjudicate on unresolved matters. The guidance given in this document may also be of use to those working towards registration under other quality standards such as GLP, GMP, GCP.

1.3 This document can be considered as the "Application Document" for microbiological testing as set out in Annex B of ISO 17025. This document has been produced jointly by EURACHEM and EA as a means of promoting a consistent approach to laboratory accreditation amongst EA member bodies, particularly those participating in the EA Multilateral Agreement.

1.4 Microbiological testing is taken to include sterility testing, detection, isolation, enumeration and identification of micro-organisms (viruses, bacteria, fungi and protozoa) and their metabolites in different materials and products, or any kind of assay using micro-organisms as part of a detection system as well as the use of micro-organisms for ecological testing. It follows that some of the guidance in this document, e.g. on laboratory environment, will need to be interpreted accordingly. This document can also provide guidance to laboratories using techniques in areas related to microbiology, such as biochemistry, molecular biology and cell culture, although there may be additional requirements for such laboratories.

1.5 This document is concerned with the quality of test results and is not specifically concerned with health and safety matters. However, laboratory practices should conform to national health and safety regulations. It is important to note that in some cases health and safety issues may have an effect on quality of testing and the laboratory will be required to take this into account.

1.6 Definitions of the terms used are given in Appendix A.

2   Personnel

ISO 17025, paragraph 5.2

2.1 Microbiological testing should be either performed or supervised by an experienced person, qualified to degree level in microbiology or equivalent. Alternative qualifications may meet requirements where staff have extensive relevant experience relating to the laboratory's scope of accreditation. 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. Specific national regulations may override the guidance given in this document.

2.2 If the laboratory includes opinions and interpretations of test results in reports, this shall be done by authorised personnel with suitable experience and relevant

knowledge of the specific application, including, for example, legislative and technological requirements and acceptability criteria.

2.3 The laboratory management shall ensure that all personnel have received adequate training for the competent performance of tests and operation of equipment. This should include training in basic techniques, e.g. plate pouring, counting of colonies, aseptic technique, etc., with acceptability determined using objective criteria. Personnel may only perform tests on samples if they are either recognised as competent to do so, or if they do so under adequate supervision. On-going competence should be monitored objectively with provision for re-training where necessary. Where a method or technique is not in regular use, verification of personnel performance before testing is undertaken may be necessary. The critical interval between performance 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.

2.4 In some cases, it may be more appropriate to relate competence to a particular technique or instrument rather than to methods.

3 Environment

ISO 17025, paragraph 5.2

3.1 Premises

3.1.1   The typical laboratory is comprised of the testing facilities (where specific microbiological testing and associated activities are carried out) and ancillary facilities (entrances, corridors, administration blocks, cloak rooms and toilets, storage rooms, archives, etc). In general there are specific environmental requirements for the testing facilities.

Depending on the type of testing being carried out, access to the microbiological laboratory should be restricted to authorised personnel. Where such restrictions are in force, personnel should be made aware of:

(a) the intended use of a particular area;

(b) the restrictions imposed on working within such areas;

(c) the reasons for imposing such restrictions;

(d) the appropriate containment levels.

3.1.2   The laboratory should be arranged so as to minimise risks of cross-contamination, where these are significant to the type of test being performed. The ways to achieve these objective are, for example:

(a) to construct the laboratory to the 'no way back' layout principle;

(b) to carry out procedures in a sequential manner using appropriate precautions to ensure test and sample integrity (e.g. use of sealed containers);

(c) to segregate activities by time or space.

3.1.3 It is generally considered as good practice to have separate locations, or clearly designated areas, for the following:

- sample receipt and storage areas;

- sample preparation (e.g. a segregated location should be used for the preparation of powdery products likely to be highly contaminated);

- examination of samples, including incubation;

- maintenance of reference organisms;

- media and equipment preparation, including sterilisation;

- sterility assessment;

- decontamination.

The area for washing (after decontamination) may be shared with other parts of the laboratory providing that the necessary precautions are taken to prevent transfer of traces of substances which could adversely affect microbial growth. The need for physical separation should be judged on the basis of the activities specific to the laboratory (eg number and type of tests carried out).

Laboratory equipment should not routinely be moved between areas to avoid accidental cross-contamination. In the molecular biology laboratory, dedicated pipettes, tips, centrifuges, tubes, etc. should be located in each work area (low-medium-high DNA working environments).

3.1.4 Space should be sufficient to allow work areas to be kept clean and tidy. The space required should be commensurate with the volume of analyses handled and the overall internal organisation of the laboratory. The space should be as required according to the national regulations when available.

3.1.5 Workrooms should be appropriately ventilated and at a suitable temperature. 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.

3.1.6 Reduction of contamination may be achieved by having:

- smooth surfaces on walls, ceilings, floors and benches (the smoothness of a surface is judged on how easily it may be cleaned). Tiles are not recommended as bench covering material;

- concave joints between the floor, walls and ceiling;

- minimal opening of windows and doors while tests are being carried out;

- sun shades placed on the outside;

- easy access for cleaning of internal sun shades if it is impossible to fit them outside;

- fluid conveying pipes not passing 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;

- cupboards up to the ceiling;

- no rough and bare wood;

- wooden surfaces of fixtures and fittings adequately sealed;

- stored items and equipment arranged to facilitate easy cleaning;

- no furniture, documents or other items other than those strictly necessary for testing activities.

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

Ceilings, ideally, should have a smooth surface with flush lighting. When this is not possible (as with suspended ceilings and hanging lights), the laboratory should have documented evidence that they control any resulting risks to hygiene and have effective means of overcoming them, e.g. a surface-cleaning and inspection programme.

3.1.7 Where laboratories are on manufacturing premises, personnel must be aware of the potential for contamination of production areas, and should demonstrate that they have taken appropriate measures to avoid any such occurrence.

3.2 Environmental monitoring

3.2.1 An appropriate environmental monitoring programme should be devised, including, for example, use of air settlement plates and surface swabbing. Acceptable background counts should be assigned and there should be a documented procedure for dealing with situations in which these limits are exceeded. Analysis of data should enable trends in levels of contamination to be determined.

3.3 Hygiene

3.3.1 There should be a documented cleaning programme for laboratory fixtures, equipment and surfaces. It should take into account the results of environmental monitoring and the possibility of cross-contamination. There should be a procedure for dealing with spillages.

3.3.2 Measures should be taken to avoid accumulation of dust, by the provision of sufficient storage space, by having minimal paperwork in the laboratory and by prohibiting plants and personal possessions from the laboratory work area.

3.3.3 Clothing appropriate to the type of testing being performed (including, if necessary, protection for hair, beard, hands, shoes, etc) should be worn in the microbiological laboratory and removed before leaving the area. This is particularly important in the molecular biology laboratory, where for example, movement from an area of high DNA load to one of low DNA load may unwittingly introduce cross-contamination. In many laboratories a laboratory coat may suffice.

3.3.4   Adequate hand washing facilities should be available.

4   Validation of test methods

4.1 The validation of microbiological test methods should reflect actual test conditions. This may be achieved by using naturally contaminated products or products spiked with a predetermined level of contaminating organisms. The analyst should be aware that the addition of contaminating organisms to a matrix only mimics in a superficial way the presence of the naturally occurring contaminants. However, it is often the best and only solution available. The extent of validation necessary will depend on the method and the application.

The laboratory shall validate standard methods applied to matrices not specified in the standard procedure.

4.2 Qualitative microbiological test methods, such as where the result is expressed in terms of detected / not detected and 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 Appendix A for definitions).

4.3 For quantitative microbiological test methods, the specificity, sensitivity, relative trueness, positive deviation, negative deviation, repeatability, reproducibility and the limit of determination within a defined variability should be considered and, if necessary, quantitatively determined in assays. 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.

4.4 Laboratories shall retain validation data on commercial test systems (kits) used in the laboratory. These validation data may be obtained through collaborative testing and from validation data submitted by the manufacturers and subjected to third party evaluation (e.g. AOAC). If the validation data are not available or not wholly applicable, the laboratory shall be responsible for completing the validation of the method.

4.5 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.

4.6 Even when validation is complete, the user will still need to verify on a regular basis that the documented performance can be met, e.g. by the use of spiked samples or reference materials incorporating relevant matrices.

5   Uncertainty of measurement

5.1 The international definition for uncertainty of measurement is given in ISO International vocabulary of basic and general terms in metrology: 1993 (see Appendix B). The general approach to evaluating and expressing uncertainty in testing expected by European accreditation bodies is one based on the recommendations produced by the International Committee for Weights and Measures (CIPM), as described in the *Guide to the Expression of uncertainty in Measurement*, 1995, ISO Geneva.

5.2 Microbiological tests generally come into the category of those that preclude the rigorous, metrologically and statistically valid calculation of uncertainty of measurement. It is generally appropriate to base the estimate of uncertainty on

repeatability and reproducibility data alone, but ideally including bias (e.g. from proficiency testing scheme results). The individual components of uncertainty should be identified and demonstrated to be under control and their contribution to the variability of results evaluated. Some components (e.g. pipetting, weighing and dilution effects) may be readily measured and easily evaluated to demonstrate a negligible contribution to overall uncertainty. Other components (e.g. sample stability and sample preparation) cannot be measured directly and their contribution cannot be evaluated in a statistical manner but their importance to the variability of results should be considered also.

5.3 It is expected that accredited microbiological testing laboratories will have an understanding of the distributions of organisms within the matrices they test and take this into account when sub-sampling. However, it is not recommended that this component of uncertainty is included in estimates unless the client's needs dictate otherwise. The principal reasons for this are that uncertainty due to distribution of organisms within the product matrix is not a function of the laboratory's performance and may be unique to individual samples tested and because test methods should specify the sample size to be used taking into account poor homogeneity.

5.4 The concept of uncertainty cannot be applied directly to qualitative test results such as those from detection tests or the determination of attributes for identification. Nevertheless, individual sources of variability, e.g. consistency of reagent performance and analyst interpretation, should be identified and demonstrated to be under control. Additionally, for tests where the limit of detection is an important indication of suitability, the uncertainty associated with the inocula used to determine the limit should be estimated and its significance evaluated. Laboratories should also be aware of the incidence of false positive and false negative results associated with the qualitative tests they use.

6 Equipment - maintenance, calibration and performance verification

ISO 17025, paragraph 5.5

As part of its quality system, a laboratory is required to operate a documented programme for the maintenance, calibration and performance verification of its equipment.

6.1 Maintenance
(Guidance on maintenance of equipment can be found in ISO 7218.)

6.1.1 Maintenance of essential equipment shall be carried out at specified intervals as determined by factors such as the rate of use. Detailed records shall be kept. Examples of maintenance of equipment and intervals are given in Appendix F.

6.1.2 Attention should be paid to the avoidance of cross-contamination arising from equipment, e.g.:

- disposable equipment should be clean and sterile when appropriate;

- re-used glassware should be properly cleaned and sterilised when appropriate;

- ideally, laboratories should have a separate autoclave for decontamination. However, one autoclave is acceptable provided that adequate precautions are taken to separate decontamination and sterilisation loads, and a documented cleaning programme is in

place to address both the internal and external environment of the autoclave.

6.1.3 Typically, the following items of equipment will be maintained by cleaning and servicing, inspecting for damage, general verification and, where relevant, sterilising:

- 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, homogenisers, fridges, freezers;

- volumetric equipment - pipettes, automatic dispensers, spiral platers;

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

6.2 Calibration and performance verification

6.2.1 The laboratory must establish a programme for the calibration and performance verification of equipment which has a direct influence on the test results. The frequency of such calibration and performance verification will be determined by documented experience and will be based on need, type and previous performance of the equipment. Intervals between calibration and verification shall be shorter than the time the equipment has been found to take to drift outside acceptable limits. Examples of calibration intervals and typical performance checks for various laboratory instruments are given in Appendix D and Appendix E.

6.2.2 Temperature measurement devices

(a) Where temperature has a direct effect on the result of an analysis or is critical for the correct performance of equipment, temperature measuring devices, e.g. liquid-in-glass thermometers, thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves, shall be of an appropriate quality to achieve the accuracy required.

(b) Calibration of devices shall be traceable to national or international standards for temperature. Where the accuracy permits, devices that can be demonstrated to conform to an appropriate and nationally or internationally accepted manufacturing specification may be used (e.g. ISO 1770 for liquid-in-glass thermometers). Such devices may, for example, be used for monitoring storage fridges and freezers and also incubators and water baths where acceptable tolerance around the target temperature permits. Verification of the performance of such devices is necessary.

6.2.3 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 shall be established initially and documented, in particular with respect to typical uses (for example position, space between, and height of, stacks of Petri dishes). The constancy of the characteristics recorded during initial validation of the equipment shall be checked and recorded after each significant repair or modification.

Laboratories shall monitor the operating temperature of this type of equipment and retain records.

6.2.4 Autoclaves, including media preparators

The following outlines the generally expected approach to calibration and the establishment and monitoring of performance. However, it is recognised that quantitative testing of materials and items processed by autoclaving, able to comment suitably on variation within and between batches may also provide equivalent assurance of quality.

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

(b) Initial validation should include performance studies (spatial temperature distribution surveys) for each operating cycle and each load configuration used in practice. This process must be repeated after significant repair or modification (e.g. replacement of thermo-regulator probe or programmer, loading arrangements, operating cycle) 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 location differences to be demonstrated. In the case of media preparators, where uniform heating cannot be demonstrated by other means, the use of two sensors, one adjacent to the control probe and one remote from it, would generally be considered appropriate. Validation and re-validation should consider the suitability of come-up and come-down times as well as time at sterilisation temperature.

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

(d) Monitoring may be achieved by one of the following:

(i) using a thermocouple and recorder to produce a chart or printout;

(ii) 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 may be checked by the use of chemical or biological indicators for sterilisation/decontamination purposes. Autoclave tape or indicator strips should be used only to show that a load has been processed, not to demonstrate completion of an acceptable cycle.

6.2.5 Weights and balances

Weights and balances shall be calibrated traceably at regular intervals (according to their intended use).

6.2.6 Volumetric equipment

(a) Volumetric equipment such as automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable pipettes may all be used in the

microbiology laboratory. Laboratories should carry out initial verification of volumetric equipment and then make regular checks to ensure that the equipment is performing within the required specification. Verification should not be 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.

(b) 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 that random checks on accuracy are carried out. If the supplier has not a recognised quality system, laboratories should check each batch of equipment for suitability.

6.2.7 Other equipment

Conductivity meters, oxygen meters, pH meters and other similar instruments should be verified regularly or before each use. The buffers used for verifications purposes should be stored in appropriate conditions and should be marked with an expiry date.

Where humidity is important to the outcome of the test, hygrometers should be calibrated, the calibration being traceable to national or international standards.

Timers, including the autoclave timer, should be verified using a calibrated timer or national time signal.

Where centrifuges are 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.

7 Reagents and culture media

ISO 17025, paragraph 4.6 and 5.5

7.1 Reagents

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 recognised national or international culture collections.

7.2 In – house prepared media

7.2.1 The suitable performance of culture media, diluents and other suspension fluids prepared in-house should 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).

Quantitative procedures for evaluation of recovery or survival are to be preferred (see also ISO 11133 Part 1 and 2).

7.2.2 Raw materials (both commercial dehydrated formulations and individual constituents) should be stored under appropriate conditions, e.g. cool, dry and dark. All containers, especially those for dehydrated media, should be sealed tightly. Dehydrated media that are caked or cracked or show a colour change should not be used. Distilled deionised, or reverse osmosis produced water, free from bactericidal, inhibitory or interfering substances, should be used for preparation unless the test method specifies otherwise.

7.2.3 Shelf life of prepared media under defined storage conditions shall be determined and verified.

7.3 Ready – to – use – media

7.3.1 All media (and diluents and other suspension fluids) procured ready to use or partially complete require validating before use. Evaluation of performance in recovery or survival of target organisms and the inhibition or suppression of non-target organisms needs to be fully quantitative; attributes (e.g. physical and biochemical properties) should be evaluated using objective criteria.

7.3.2 As part of the validation, the user laboratory needs to have adequate knowledge of the manufacturer's quality 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

- Storage conditions

- Sample regime / rate

- Sterility check

- Check of growth of target and non-target control organisms used (with their culture collection references) and acceptability criteria

- Physical checks and the acceptability criteria applied

- Date of issue of specification

7.3.3 Batches of media should be identifiable. Each one received should be accompanied by evidence that it meets the quality specification. The user laboratory should ensure that it will be notified by the manufacturer of any changes to the quality specification.

7.3.4 Where the manufacturer of media procured ready to use or partially complete is covered by a recognised quality system (e.g. ISO 9000-series registered), checks by the user laboratory of conformance of supplies with the specification defined through initial validation may be applied in accordance with the expectation of consistency. In other circumstances, adequate checks would be necessary on every batch received.

7.4 Labelling

Laboratories shall ensure that all reagents (including stock solutions), media, diluents, and other suspending fluids are adequately labelled to indicate, as appropriate, identity, concentration, storage conditions, preparation date, validated expiry date and /or recommended storage periods. The person responsible for preparation should be identifiable from records.

8   Reference materials and reference cultures

ISO 17025, paragraph 5.6.3

8.1 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, and
- to enable comparison of methods.

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

8.2 Reference cultures

8.2.1   Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. Traceability is necessary, for example, when establishing media performance for test kit and method validations. To demonstrate traceability, laboratories must use reference strains of micro-organisms obtained directly from a recognised national or international collection, where these exist. Alternatively, commercial derivatives for which all relevant properties have been shown by the laboratory to be equivalent at the point of use may be used

8.2.2   Following the guidance in ISO 11133-1, reference strains may be sub-cultured once to provide reference stocks. Purity and biochemical checks should be made in parallel as appropriate. It is recommended to store reference stocks in aliquots either deep-frozen or lyophilised. Working cultures for routine use should be primary subcultures from the reference stock (see Appendix C on preparation of working stocks). If reference stocks have been thawed, they must not be re-frozen and re-used.

8.2.3   Working stocks should not be sub-cultured unless it is required and defined by a standard method or laboratories can provide documentary evidence that there has been no change in any relevant property.

Working stocks shall not be sub-cultured to replace reference stocks. Commercial derivatives of reference strains may only be used as working cultures.

9   Sampling

ISO 17025, paragraph 5.7

9.1   In many cases, testing laboratories are not responsible for primary sampling to obtain test items. Where they are responsible, it is strongly recommended that this sampling be covered by quality assurance and ideally by accreditation.

9.2 Transport and storage should be under conditions that maintain the integrity of the sample (e.g. chilled or frozen where appropriate). The conditions should be monitored and records kept. Where appropriate, responsibility for transport, storage between sampling and arrival at the testing laboratory shall be clearly documented. Testing of the samples should be performed as soon as possible after sampling and should conform to relevant standards and/or national/international regulations.

9.3 Sampling should only be performed by trained personnel. 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.

10 Sample handling and identification

ISO 17025, paragraphs 5.7 and 5.8

10.1 Microbial flora may be sensitive to factors such as temperature or duration of storage and transport, so it is important to check and record the condition of the sample on receipt by the laboratory.

10.2 The laboratory should 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, torn packaging or deficient labelling, the laboratory should consult with the client before deciding whether to test or refuse the sample. In any case, the condition of the sample should be indicated on the test report.

10.3 The laboratory should record all relevant information and particularly the following information:
   (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).

10.4 Samples awaiting test shall be stored under suitable conditions to minimise changes to any microbial population present. Storage conditions should be defined and recorded.

10.5 The packaging and labels from samples may be highly contaminated and should be handled and stored with care so as to avoid any spread of contamination.

10.6 Sub-sampling by the laboratory immediately prior to testing is considered as part of the test method. It should 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 uneven distribution of micro-organisms (general guidance given in ISO 6887 and ISO 7218).

10.7 A procedure for the retention and disposal of samples shall be written. Samples should be stored until the test results are obtained, or longer if required. Laboratory sample portions that are known to be highly contaminated should be decontaminated prior to being discarded (see 11.1).

11 Disposal of contaminated waste

11.1   The correct disposal of contaminated materials may not directly affect the quality of sample analysis, although procedures should be designed to minimise 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 (see also ISO 7218).

12 Quality assurance of results/quality control of performance

ISO 17025, paragraph 5.9

12.1   Internal quality control

12.1.1 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.

12.1.2 A programme 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 programme may involve:

- the use of spiked samples

- the use of reference materials (including proficiency testing scheme materials)

- replicate testing

- replicate evaluation of test results

The interval between these checks will be influenced by the construction of the programme and by the number of actual tests. It is recommended that, where possible, tests should incorporate controls to monitor performance.

12.1.3 In special instances, a laboratory may be accredited for a test that it is rarely called on to do. It is recognised that in such cases an ongoing internal quality control programme may be inappropriate and that a scheme for demonstrating satisfactory performance which is carried out in parallel with the testing, may be more suitable.

12.2   External quality assessment (proficiency testing)

12.2.1 Laboratories should regularly participate in proficiency testing which are relevant to their scope of accreditation, preference should be given to proficiency testing schemes which use appropriate matrices. In specific instances, participation may be mandatory.

12.2.2 Laboratories should use external quality assessment not only to assess laboratory bias but also to check the validity of the whole quality system.

13 Test reports

ISO 17025, paragraph 5.10

13.1   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". The result should not be given as "zero for a defined unit" unless it is a regulatory requirement. Qualitative test results should be reported as "detected/not detected in a defined quantity or volume". They may 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 client.

13.2   Where an estimate of the uncertainty of the test result is expressed on the test report, any limitations (particularly if the estimate does not include the component contributed by the distribution of micro-organisms within the sample) have to be made clear to the client.

Appendix A   Glossary of Terms

Calibration	Set of operations that establish, under specified conditions, the relationship between values of quantities indicated 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 NOTES 1 The result of a calibration permits either the assignment of values of measurands to the indications or the determination of corrections with respect to indications. 2 A calibration may also determine other metrological properties such as the effect of influence quantities. 3 The result of a calibration may be recorded in a document, sometimes called a calibration certificate or a calibration report. [VIM: 1993 ISO International vocabulary of basic and general terms in metrology]
Certified reference material	Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes traceability to an accurate realisation 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. [ISO Guide 30:1992]
Limit of determination	Applied to quantitative microbiological tests - The lowest number of micro-organisms within a defined variability that may be determined under the experimental conditions of the method under evaluation.
Limit of detection	Applied to qualitative microbiological tests- The lowest number of micro-organisms that can be detected, but in numbers that cannot be estimated accurately.
Negative deviation	Occurs when the alternative method gives a negative result without confirmation when the reference method gives a positive result. This deviation becomes a false negative result when the true result can be proved as being positive.
Positive deviation	Occurs when the alternative method gives a positive result without confirmation when the reference method gives a negative result. This deviation becomes a false positive result when the true result can be proved as being negative.
Reference cultures	Collective term for reference strain, reference stocks and working cultures.
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 11133-1:2000] Normally obtained from a recognised national or international collection.

Reference material	Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. [ISO Guide 30:1992]
Reference method	Thoroughly investigated method, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have accuracy and precision commensurate with its intended use and that can therefore be used to assess the accuracy of other methods for the same measurement, particularly in permitting the characterisation of a reference material. Normally a national or international standard method.
Reference stocks	A set of separate identical cultures obtained by a single sub-culture from the reference strain. [ISO 11133-1:2000]
Relative trueness	The degree of correspondence of the results of the method under evaluation to those obtained using a recognised reference method.
Repeatability	Closeness of the agreement between the results of successive measurements of the same measurand under the same conditions of measurement. [VIM: 1993 ISO International vocabulary of basic and general terms in metrology]
Reproducibility	Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement. [VIM: 1993 ISO International vocabulary of basic and general terms in metrology]
Sensitivity	The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection. [ISO 13843:2000]
Specificity	The fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection. [ISO 13843:2000]
Working culture	A primary sub-culture from a reference stock. [ISO 11133-1:2000]
Validation	Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. [ISO 9000: 2000]
Verification	Confirmation , through the provision of objective evidence, that specified requirements have been fulfilled. [ISO 9000:2000]

Appendix B **References**

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

2. ISO 7218, Microbiology of food and animal feeding stuffs - General rules for microbiological examinations.

3. ISO 6887-1, Preparation of dilutions.

4. ISO Guide 30, Terms and definitions used in connection with reference materials.

5. ISO 9000, Quality management systems - fundamentals and vocabulary.

6. VIM: 1993, ISO international vocabulary of basic and general terms in metrology.

7. ISO (CIPM):1995, Guide to the expression of uncertainty in measurements .

8. Draft ISO/DIS 16140, Food microbiology. Protocol for the validation of alternative methods.

9. ISO 13843, Water quality – Guidance on validation of microbiological methods.

10. ISO 11133-1, Microbiology of food and animal feeding stuffs. Guidelines on preparation and production of culture media. Part 1- General guidelines on quality assurance for the preparation of media in the laboratory.

11. Draft ISO/FDIS 11133-2, Microbiology of food and animal feeding stuffs. Guidelines on preparation and production of culture media. Part 2- Practical guidelines on performance testing on culture media.

12. EN 12741, Biotechnology- Laboratories for research, development and analysis – Guidance for biotechnology laboratory operations.

Appendix C **General use of reference cultures**

Reference strain from source recognised by accreditation body

Sub-Cultured once

Not allowed

*

Reference stocks

Freeze dried, liquid nitrogen storage, deep frozen etc.
Specified conditions and recommended storage times

*

Kept under specified conditions

Sub-cultured once

Thaw / reconstitute

*

Working culture

Specified conditions and recommended storage times

Routine use

* Parellel purity checks and biochemical tests as appropriate

All parts of the process shall be fully documented and detailed records of all stages must be maintained

Appendix D   Guidance of calibration and calibration checks

This information is provided for guidance purposes and the frequency will be based on
the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
Reference thermometers (liquid-in-glass)	Full traceable re-calibration Single point (e.g. ice-point check)	Every 5 years Annually
Reference thermocouples	Full traceable re-calibration Check against reference thermometer	Every 3 years Annually
Working thermometers & Working thermocouples	Check against reference thermometer at ice-point and/or working temperature range	Annually
Balances	Full traceable calibration	Annually
Calibration weights	Full traceable calibration	Every 5 years
Check weight(s)	Check against calibrated weight or check on balance immediately following traceable calibration	Annually
Volumetric glassware	Gravimetric calibration to required tolerance	Annually
Microscopes	Traceable calibration of stage micrometer (where appropriate)	Initially
Hygrometers	Traceable calibration	Annually
Centrifuges	Traceable calibration or check against an independent tachometer, as appropriate	Annually

Appendix E Guidance on equipment validation and verification of performance

This information is provided for guidance purposes and the frequency will be based on
the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
Temperature controlled equipment (incubators, baths, fridges, freezers)	(a) Establish stability and uniformity of temperature (b) Monitor temperature	(a) Initially, every 2 years and after repair/modification (b) Daily/each use
Sterilising ovens	(a) Establish stability and uniformity of temperature (b) Monitor temperature	(a) Initially, every 2 years and after repair/modification (b) Each use
Autoclaves	(a) Establish characteristics for loads/cycles (b) Monitor temperature/time	(a) Initially, every 2 years and after repair/modification (b) Each use

Type of equipment	Requirement	Suggested frequency
Safety cabinets	(a) Establish performance (b) Microbiological monitoring (c) Air flow monitoring	(a) Initially, every year and after repair/modification (b) Weekly (c) Each use
Laminar air flow cabinets	(a) Establish performance (b) Check with sterility plates	(a) Initially, and after repair/modification (b) Weekly
Timers	Check against national time signal	Annually
Microscopes	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 weight	Daily/each use
De-ionisers and reverse osmosis units	(a) Check conductivity (b) Check for microbial contamination	(b) Weekly (b) Monthly
Gravimetric diluters	(a) Check weight of volume dispensed (b) Check dilution ratio	(a) Daily (b) Daily
Media dispensers	Check volume dispensed	Each adjustment or replacement
Pipettors/pipettes	Check accuracy and precision of volume dispensed	Regularly (to be defined by taking account of the frequency and nature of use)
Spiral platers	(a) Establish performance against conventional method (b) Check stylus condition and the start and end points (c) Check volume dispensed	(a) Initially and annually (b) Daily/each use (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	Each use
Laboratory environment	Monitor for airborne and surface microbial contamination using, e.g. air samplers, settle plates, contact plates or swabs	Weekly

Appendix F   Guidance on maintenance of equipment

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
(a) Incubators (b) Fridges (c) Freezers, ovens	Clean and disinfect internal surfaces	(a) Monthly (b) When required (e.g. every 3 months) (c) When required (e.g. annually)
Water baths	Empty, clean, disinfect and refill	Monthly, or every 6 months if biocide used
Centrifuges	(a) Service (b) Clean and disinfect	(a) Annually (b) Each use
Autoclaves	(a) Make visual checks of gasket, clean/drain chamber (b) Full service (c) Safety check of pressure vessel	(a) Regularly, as recommended by manufacturer (b) Annually or as recommended by manufacturer (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 (b) Service	(a) Each use (b) Annually
Stills	Clean and de-scale	As required (e.g. every 3 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 (b) Decontaminate, clean and sterilise	(a) Annually (b) Each use
Laboratory	(a) Clean and disinfect working surfaces (b) Clean floors, disinfect sinks and basins (c) Clean and disinfect other surfaces	(a) Daily, and during use (b) Weekly (c) Every 3 months