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Criteria for Accreditation of Microbiological Laboratories

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1 Background Information

1.1 Purpose

This document can be considered as the “Application Document” for microbiological testing. This document has been produced the Technical Committee on Testing and Calibration KENAS as a means of promoting a consistent approach to laboratory accreditation amongst microbiological testing laboratories seeking accreditation with KENAS. This document can be considered as the “Application Document” for microbiological testing. This document has been produced KENAS as a means of promoting a consistent approach to laboratory accreditation amongst microbiological testing laboratories seeking accreditation with KENAS.

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.

1.2 Scope

This guideline 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.

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.

1.3 Role(s) and Responsibility

Role	Responsibility
All KENAS Technical Staff	Process owner
TESO	Reviewer
CMLA	Recommender
CEO	Approval
All KENAS Technical Staff	Compliance

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2 Terms and Definitions

For the purpose of this guideline, the following terms and definitions shall apply in addition to those given in ISO/IEC 17000 and the Kenya Accreditation Service Act 2019.

2.1 Acronyms and Abbreviations

KENAS	Kenya Accreditation Service
POCT	Point of Care Testing
CMLA	Chief Manager, Laboratories
MEDO	Medical Officer
KEBS	Kenya Bureau of Standards
CEO	Chief Executive Officer
GLP	Good Laboratory Practise
NSB	National Standards Body
NMI	National Metrology Institute
MR	Management Representative
TC	Technical Committee

2.2 Definition of Terms

2.2.1 Accreditation

A third-party attestation related to a conformity assessment body conveying formal demonstration of its competence to carry out specific conformity assessment tasks.

2.2.2 Committee

A group of people appointed for a specific function by a larger group or organization.

2.2.3 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.

2.2.4 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

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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. [ISO Guide 30:1992].

2.2.5 Limit of determination

Applied to quantitative microbiological tests - The lowest number of microorganisms within a defined variability that may be determined under the experimental conditions of the method under evaluation.

2.2.6 Limit of detection

Applied to qualitative microbiological tests- The lowest number of microorganisms that can be detected, but in numbers that cannot be estimated accurately.

2.2.7 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.

2.2.8 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.

2.2.9 Reference cultures

Collective term for reference strain, reference stocks and working cultures.

2.2.10 Reference strains

Microorganisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin

2.2.11 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]

2.2.12 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 characterization of a reference material. Normally a national or international standard method.

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2.2.13 Reference stocks

A set of separate identical cultures obtained by a single sub-culture from the reference strain. [ISO 11133-1:2000]

2.2.14 Relative trueness

The degree of correspondence of the results of the method under evaluation to those obtained using a recognized reference method.

2.2.15 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]

2.2.16 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]

2.2.17 Sensitivity

The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection. [ISO 13843:2000]

2.2.18 Specificity

The fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection. [ISO 13843:2000]

2.2.19 Working culture

A primary sub-culture from a reference stock. [ISO 11133-1:2000]

2.2.20 Validation

Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. [ISO 9000: 2000]

2.2.21 Verification

Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled. [ISO 9000:2000]

2.2.22 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].

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3 Criteria

3.1 Organization, Management and Personnel

3.1.1 Organization

3.1.1.1 Microbiological laboratory will include a laboratory with or part of the legally identifiable organization having a quality system which meets the relevant microbiological technology standard, providing services in permanent facilities which are part of a central laboratory network, having in place an organizational chart, internal management system , and relationship with other organizations (if applicable).

3.1.2 Personnel

3.1.1.2 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 has 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.

3.1.1.3 If the laboratory includes opinions and interpretations of test results in reports, this shall be done by authorized personnel with suitable experience and relevant knowledge of the specific application, including, for example, legislative and technological requirements and acceptability criteria.

3.1.3 Management

3.1.1.4 Laboratory management shall have responsibility for the design, implementation, maintenance, review and improvement of the quality management system (ISO 17025:2005 4.15).

3.1.1.5 The laboratory management shall ensure that all personnel have received adequate training for the competent performance of tests and operation of equipment.

3.1.1.6 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 recognized as competent to do so, or if they do so under adequate supervision.

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3.1.1.7 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 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.

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

3.2 Environment –

ISO 17025, paragraph 5.2

3.3 Premises

3.3.1 The laboratory floor plan should be configured to promote high quality work, personnel safety and efficient operations ISO15189 5.2.2

3.3.2 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.

3.3.3 Depending on the type of testing being carried out, access to the microbiological laboratory should be restricted to authorized personnel.

3.3.4 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.

3.3.5 The laboratory should be arranged so as to minimize risks of cross contamination, where these are significant to the type of test being performed. The ways to achieve these objectives 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.

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

a) Sample receipt and storage areas;
b) Sample preparation (e.g. a segregated location should be used for the preparation of powdery products likely to be highly contaminated);
c) Examination of samples, including incubation;
d) Maintenance of reference organisms;
e) Media and equipment preparation, including sterilization;
f) Sterility assessment;
g) Decontamination; and
h) Disposal.

3.3.7 The area for washing (after decontamination) may be shared with other parts of the laboratoryproviding 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 (e.g. number and type of tests carried out).

3.3.8 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

3.3.9 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 organization of the laboratory. The space should be as required according to the national regulations when available.

3.3.10 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.

3.3.11 Reduction of contamination may be achieved by having a surface that is easy to clean. Tiles are not recommended as bench coveringmaterial; instead use of continuous surface e.g. granite

a) Concave joints between the floor, walls and ceiling;
b) Minimal opening of windows and doors while tests are being carried out;
c) Sun Shases placed on the outside;
d) Easy access for cleaning of internal sun shades if it is impossible to fit them outside;
e) Fluid conveying pipes not passing above work surfaces unless placed in hermetically sealed activities casings;
f) A dust - filtered air inlet for the ventilation system;

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g) Separate hand washing arrangements, preferably non-manually controlled;
h) Cupboards up to the ceiling;
i) No rough and bare wood;
j) Wooden surfaces of fixtures and fittings adequately sealed;
k) Stored items and equipement arranged to facilitate easy cleaning
l) 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.

3.3.12 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.

3.3.13 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.

3.4 Environmental monitoring

3.4.1 The laboratory shall monitor, control and record environmental conditions as required by the relevant specifications, methods and procedures or where they influence the quality of results. Due attention shall be paid to biological sterility, dust, electromagnetic disturbances, radiation, humidity, electrical supply, temperature and sound and vibration levels as appropriate to the technical activities concerned.

3.4.2 Tests and calibrations shall be stopped when the environmental conditions jeopardize the results of the tests.

3.5 Hygiene

3.5.1 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.

3.5.2 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.

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3.5.3 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.

3.5.4 Adequate hand washing facilities should be available.

3.6 Validation of test methods

3.6.1 The validation of microbiological test methods should reflect actual test conditions.

3.6.2 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.

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

3.6.4 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).

3.6.5 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.

3.6.6 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.

3.6.7 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.

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3.6.8 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.

3.7 Uncertainty of measurement

3.7.1 The international definition for uncertainty of measurement is given in ISO International vocabulary of basic and general terms in metrology: 1993 (see Appendix B).

3.7.2 The general approach to evaluating and expressing uncertainty in testing expected by 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.

3.7.3 Microbiological tests generally come into the category of those that preclude the rigorous, metrological 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).

3.7.4 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 ina statistical manner but their importance to the variability of results should be considered also.

3.7.5 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.

3.7.6 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.

3.7.7 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.

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3.7.8 Nevertheless, individual sources of variability, e.g. consistency of reagent performance and analyst interpretation, should be identified and demonstrated to be under control.

3.7.9 Additionally, for tests where the limit of detection is an important indication of suitability, the uncertainty associated with the inoculate 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.

3.8 Equipment - maintenance, calibration and performance verification

3.8.1 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.

3.9 Maintenance

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

3.9.1 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.

3.9.2 Examples of maintenance of equipment and intervals are given in Appendix F

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

a) Disposable equipment should be clean and sterile when appropriate
b) Re-used glassware should be properly cleaned and sterilized when appropriate
c) Ideally, laboratories should have a separate autoclave for decontamination.

3.9.4 However, one autoclave is acceptable provided that adequate precautions are taken to separate decontamination and sterilization loads, and a documented cleaning programme is in place to address both the internal and external environment of the autoclave.

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

a) 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;
b) Water baths, incubators, microbiological cabinets, autoclaves, homogenizers, fridges, freezers;

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c) Volumetric equipment - pipettes, automatic dispensers, spiral platers;
d) Measuring instruments - thermometers, timers, balances, pH meters, colony counters.

3.10 Calibration and performance verification

3.10.1 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.

3.10.2 Temperature measurement devices- 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.

3.10.3 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 ofsuch devices is necessary.

3.10.4 Incubators, water baths, ovens - The stability of temperature, uniformity of temperature distribution and time required to achieveequilibrium conditions in incubators, water baths, ovens and temperature controlled roomsshall 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.

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

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3.10.6 Autoclaves, including media preparators - The following outlines the generally expected approach to calibration and the establishment and monitoring of performance.

a) Autoclaves should be capable of meeting specified time and temperature tolerances. Pressure cookers fitted only with a pressure gauge are not acceptable.
b) Sensors used for controlling or monitoring operating cycles require calibration and theperformance of timers verified.
c) 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) orwhere indicated by the results of quality control checks on media.
d) 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 useof two sensors, one adjacent to the control probe and one remote from it would generally be considered appropriate.
e) Validation and re-validation should consider the suitability of come-up and comedowntimes as well as time at sterilization temperature.
f) Clear operating instructions should be provided based on the heating profiles determined for typical uses during validation/re-validation. Acceptance/rejection criteriashould be established and records of autoclave operations, including temperature and time, maintained for every cycle.
g) Monitoring may be achieved by one of the following:
    i. using a thermocouple and recorder to produce a chart or printout; thattemperature.
    ii. 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 sterilization /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.

3.10.7 Weights and balances

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

3.10.8 Volumetric equipment

(a) Volumetric equipment such as automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable

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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 recognized and relevant quality system. After initial validation ofthe suitability of the equipment, it is recommended that random checks on accuracyare carried out. If the supplier has not a recognized quality system, laboratories should check each batch of equipment for suitability.

3.10.9 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.

3.11 Reagents and culture media

3.11.1 ISO 17025, paragraph 4.6 and 5.5 Reagents

Laboratories should ensure that the quality of reagents used is appropriate for the testconcerned. 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

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or international culture collections.

3.11.2 In – house prepared media - The suitable performance of culture media, diluents and other suspension fluidsprepared in-house should be checked, where relevant, with regard to:

a) Recovery or survival maintenance of target organisms,
b) Inhibition or suppression of non-target organisms,
c) Biochemical (differential and diagnostic) properties,
d) Physical properties (e.g. pH, volume and sterility).

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

3.11.3 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 deionized, or reverse osmosis produced water, free from bactericidal, inhibitory or interfering substances, should be used for preparation unless the test method specifies otherwise.

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

3.11.5 Ready – to – use – media - 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.

3.11.6 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:

a) Name of the media and list of components, including any supplements
b) Shelf life and the acceptability criteria applied
c) Storage conditions
d) Sample regime / rate
e) Sterility check
f) Check of growth of target and non-target control organisms used (with their culture collection references) and acceptability criteria
g) Physical checks and the acceptability criteria applied critical for the test, initially and during its shelf life, using positive and negative control

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organisms which are traceable to recognized national or international culture collections.

h) Date of issue of specification

3.11.7 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.

3.11.8 Where the manufacturer of media procured ready to use or partially complete is covered by a recognized 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.

3.12 Labelling

3.12.1 Laboratories shall ensure that all reagents (including stock solutions), media, diluents, and other suspending fluids are adequately labeled 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.

3.13 Reference materials and reference cultures

ISO 17025, paragraph 5.6.3

3.13.1 Reference materials - Reference materials and certified reference materials (see definition in Appendix A) provide essential traceability in measurements and are used, for example;

a) To demonstrate the accuracy of results,

b) To calibrate equipment,

c) To monitor laboratory performance,

d) To validate methods, and

e) To enable comparison of methods.

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

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3.13.2 Reference cultures

3.13.2.1 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 microorganisms obtained directly from a recognized 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.

3.13.2.2 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 lyophilized. 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.

3.13.2.3 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.

3.13.2.4 Working stocks shall not be sub-cultured to replace reference stocks.

3.13.2.5 Commercial derivatives of reference strains may only be used as working cultures.

3.14 Sampling

ISO 17025, paragraph 5.7

3.14.1 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.

3.14.2 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.

3.14.3 Testing of the samples should be performed as soon as possible after sampling and should conform to relevant standards and/or national/international regulations.

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3.14.4 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.

3.15 Sample handling and identification

ISO 17025, paragraphs 5.7 and 5.8

3.15.1 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.

3.15.2 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

3.15.3 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.).

3.15.4 Samples awaiting test shall be stored under suitable conditions to minimize changes to any microbial population present.

3.15.5 Storage conditions should be defined and recorded.

3.15.6 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.

3.15.7 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.

3.15.8 Sub-sampling procedures should be designed to take into account uneven distribution of micro-organisms (general guidance given in ISO 6887 and ISO 7218).

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3.15.9 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).

3.16 Disposal of contaminated waste

3.16.1 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.

3.16.2 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).

3.17 Quality assurance of results/quality control of performance

ISO 17025, paragraph 5.9

3.17.1 Internal quality control

3.17.1.1 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

3.17.1.2 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:

a) The use of spiked samples
b) The use of reference materials (including proficiency testing scheme materials)
c) Replicate testing
d) Replicate evaluation of test results

3.17.1.3 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.

3.17.1.4 In special instances, a laboratory may be accredited for a test that it is rarely called on to do. It is recognized 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.

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3.18 External quality assessment (proficiency testing)

3.18.1 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.

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

3.19 Test reports

ISO 17025, paragraph 5.10

3.19.1 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”.

3.19.2 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.

3.19.3 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.

4 Associated Documents

Ref	Document Identifier	Document Title
1.	ISO 15189	Medical Laboratories – Requirements for Quality and Competence
2.	ISO/IEC 17011	Conformity Assessment-General requirements for accreditation bodies accrediting conformity assessment bodies
3.	ISO/IEC 17025	Conformity Assessment – General requirements for Testing and Calibration Laboratories
4.	KENAS-TS-F-004	Confidentiality Form
5.	ISO 7218:2007	Microbiology of food and animal feeding stuffs - General rules for microbiological examinations.
6.	ISO 6887-1:1999	Preparation of dilutions.

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Ref	Document Identifier	Document Title
7.	ISO Guide 30:2015	Terms and definitions used in connection with reference materials.
8.	ISO 9000:2015	Quality management systems - fundamentals and vocabulary.
9.	JCGM 200:2012	International Vocabulary of Metrology – Basic and General Concepts andAssociated Terms (VIM 3rd edition)
10.	ISO (CIPM):1995	Guide to the expression of uncertainty in measurements.
11.	ISO 16140-2:2016	Food microbiology. Protocol for the validation of alternative methods.
12.	ISO 13843:2000	Water quality – Guidance on validation of microbiological methods.
13.	ISO 11133-1:2014	Microbiology of food and animal feeding stuffs. Guidelines on preparationand production of culture media. Part 1- General guidelines on quality assurance for the preparation of media in the laboratory
14.	ISO 11133-2: 2014	Microbiology of food and animal feeding stuffs. Guidelines on preparation and production of culture media Part 2- Practical guidelines on performance testing onculture media
15.	BS EN 12741:1999	Biotechnology- Laboratories for research, development and analysis – Guidance for biotechnology laboratory operations
16.	ISO 15189:2012	Medical Laboratories – Particular requirements for quality and competence.

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5 Revision/ Amendment Records

Date	Ver	Revised By	Reason for Revision
10/10/2022	01	RSQ	Newly developed/formatted document