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WHO GOOD PRACTICES FOR PHARMACEUTICAL MICROBIOLOGY LABORATORIES

DRAFT FOR COMMENT

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During the past few years we have moved more towards an electronic system for sending out our working documents for comment, for convenience and in order to speed up the process. If you do not already receive our documents electronically, please let us have your e-mail address (to bonnyw@who.int) and we will add it to our electronic mailing list.

SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/09.297/Rev.2:

WHO GOOD PRACTICES FOR PHARMACEUTICAL MICROBIOLOGY LABORATORIES

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Further follow-up as necessary	

BACKGROUND

The WHO Expert Committee on Specifications for Pharmaceutical Preparations adopted in 2009 a revised version of the *Good practices for pharmaceutical quality control laboratories (1)*.

During the inspections carried out when prequalifying laboratories, the inspectors had noticed that some of the texts of these guidelines might benefit from additional guidance, with a special focus on microbiology.

In light of the above, the Expert Committee recommended that the WHO Secretariat initiate the process of developing a new text on good practices for pharmaceutical microbiology laboratories.

On the basis of the above, the following text is proposed to cover this specific type of laboratory.

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INTRODUCTION AND SCOPE OF DOCUMENT

Pharmaceutical microbiology laboratories may be involved in:

- sterility testing;
- detection, isolation, enumeration and identification of microorganisms (bacteria, yest, and moulds) and their metabolites (including endotoxins) in different materials (e.g. starting materials, water, air), products, surfaces, garments and the environment;
- assay using microorganisms as part of the test system.

This guideline relates to all microbiology laboratories involved in the above outlined testing activities, whether they are independent or a department/unit of a pharmaceutical manufacturing facility.

This guideline is based on and supplements the requirements described in *Good practices for pharmaceutical quality control laboratories (1)*; General guidelines for the establishment, maintenance and distribution of chemical reference substances. Revision. *(2)*; *The International Pharmacopoeia.* Fourth edition *(3);* First Supplement to *The International Pharmacopoeia*. Fourth Edition *(4)*; and *General requirements for the competence of testing and calibration laboratories (5)*.

GLOSSARY

Calibration
The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (especially weighing), recording and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established (WHO).

Certified reference material
Reference material, characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty and a statement of metrological traceability (WHO).

False negative result
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.

False positive result
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.

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.

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.

Quantitation limit (*limit of quantitation*)
The lowest concentration of an analyte in a sample that may be determined with acceptable accuracy and precision (WHO).

Precision
The degree of agreement among individual results (WHO).

Reference cultures
Collective term for reference strain and reference stocks.

Reference material
Material sufficiently homogeneous and stable with respect to one or more specified properties, w has been established to be fit for its intended use in a measurement process (WHO).

Reference method
A method which has been validated as being fit for purpose, with which an alternative method may be compared.

Reference stocks
A set of separate identical cultures obtained by a single subculture from the reference strain (ISO 11133-1:2000).

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 recognized national or international collection.

Repeatability
Closeness of the agreement between the results of successive measurements of the same measure and under the same conditions of measurement (adjusted from ISO).

Reproducibility
Reproducibility expresses precision between laboratories (WHO)

Robustness (or ruggedness)
The ability of the procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions (WHO).

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

Specificity (*selectivity*)
The ability to measure unequivocally the desired analyte in the presence of components such as excipients and impurities that may also be expected to be present (WHO).

Validation
Action of proving, in accordance with the principles of GXP, that any procedure, process, equipment (including the software or hardware used), material, activity or system actually and consistently leads to the expected results (WHO).

Verification
The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with GXP principles (WHO.)

Working culture
A primary subculture from a reference stock (ISO 11133-1:2000).

1. PERSONNEL

1.1 Microbiological testing should be performed and supervised by an experienced person, qualified in microbiology or equivalent. Staff should have basic training in microbiology and relevant practical experience before being allowed to perform work covered by the scope of testing.

1.2 The laboratory should maintain current job descriptions for all personnel involved in tests and/or calibrations, validations and verifications. The laboratory should also maintain records of all technical personnel, describing their qualifications, training and experience.

1.3 If the laboratory includes opinions and interpretations of test results in reports, this should 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.

1.4 The laboratory management should 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 where relevant. 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. Ongoing competence should be monitored objectively with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance before testing is undertaken may be necessary. In some

cases it is acceptable to relate competence to a general technique or instrument used rather than to particular methods.

1.5 Personnel should be trained in necessary procedures for containment of microorganisms within the laboratory facility.

1.6 Personnel should be trained in safe handling of microorganisms.

2. ENVIRONMENT

2.1 Premises

2.1.1 Microbiology laboratories and certain support equipment (e.g. autoclaves, glassware) should be dedicated and separated from other areas.

2.1.2 Microbiology laboratories should be designed to suit the operations to be carried out in them. There should be sufficient space for all activities to avoid mix-ups, There should be adequate suitable space for samples, reference organisms, media (if necessary, with cooling), testing and records. Due to the nature of some materials, separate storage locations may be necessary, e.g. biological indicators, reference organisms and media.

2.1.3 Laboratories should be appropriately designed and should take into account the suitability of construction materials to enable appropriate cleaning, disinfection and minimize the risks of contamination.

2.1.4 There should be separate air supply to laboratories and production areas. Separate air-handling units and other provisions, including temperature and humidity where required, are needed for microbiological laboratories. The quality of the air supplied to the laboratory should be appropriate to the tests being carried out and their quality should not be a source of contamination.

2.1.5 Access to the microbiological laboratory should be restricted to authorized personnel. Personnel should be made aware of:

– the appropriate entry and exit procedures including gowning;
– the intended use of a particular area;
– the restrictions imposed on working within such areas;
– the reasons for imposing such restrictions; and
– the appropriate containment levels.

2.1.6 Laboratory activities, such as sample preparation, media and equipment preparation and enumeration of microorganisms, should be segregated by space or at least time, so as to minimize risks of cross-contamination and false positives. Where non-dedicated arras are used, risk management principles should be applied. Sterility testing should always be performed in a dedicated area.

2.1.7 Operations should be carried out preferably in the following zones:

Zone	Installation grade	Proposed
Sample receipt	Unclassified	Unclassified
Media prep	Grade D	ISO 8 & <200 cfu[1]/m^2
Autoclave loading	Grade D	ISO 8 & <200 cfu/m^2
Autoclave unloading	Grade B	ISO 5 (turbulent) & <50 cfu/m^2
Sterility testing – UDAF	Grade A	ISO 5 (UDAF) & <1 cfu/m^2
Sterility testing – background	Grade B	ISO 5 (turbulent) & <50 cfu/m^2
Incubator	Grade D	ISO 8 & <200 cfu/m^2
Enumeration	Grade D	ISO 8 & <200 cfu/m^2
Decontamination	Grade D	ISO 8 & <200 cfu/m^2

Note from Secretariat:
Feedback on the usefulness of this table either within the text or as Annex E is requested.

2.1.8 In general laboratory equipment should not routinely be moved between areas to avoid accidental cross-contamination. Laboratory equipment used in the microbiology laboratory should not be used outside the microbiology area, unless there are specific precautions in place to prevent cross-contamination.

2.2 Environmental monitoring in the laboratory

2.2.1 Where appropriate an environmental monitoring programme should be in place which covers, for example, use of air settlement plates and surface swabbing, temperature and pressure differentials. Alert and action limits should be defined. Trending of environmental monitoring results shall be carried out.

2.3 Cleaning, disinfection and hygiene

2.3.1 There should be a documented cleaning and disinfection programme. Results of environmental monitoring should be considered where relevant.

There should be a procedure for dealing with spillages.

2.3.4 Adequate hand-washing and hand sanitization facilities should be available.

[1] Colony-forming units (**CFU** or **cfu**).

2.4 Sterility test facilities

2.4.1 Sterility test facilities have specific environmental requirements to ensure the integrity of tests carried out. WHO good manufacturing practices (GMP) for sterile pharmaceutical products *(6)* requires that sterility testing should be carried out and specifies requirements for sterility testing. This section details the clean-room requirements for a sterility test facility.

2.4.2 Sterility testing should be performed under aseptic conditions, which should be equivalent to air quality standards applicable to that required for the aseptic manufacture of pharmaceutical products. The premises, services and equipment should be subject to the qualification process of DQ, IQ, OQ and PQ.

2.4.3 The sterility testing should be carried out within a Grade A unidirectional airflow protected zone or a biosafety cabinet (if warranted), which should be located within a clean room with a Grade B background. Alternatively the testing can be carried out within a barrier isolator, located in a controlled environment. Care shall be taken with the design of the facility layout, and room airflow patterns, that the unidirectional airflow patterns are not disrupted.

2.4.4 The clean-room classification and air-handling equipment should be requalified at least annually by a competent person or contractor.

2.4.5 Air supplied to Grade A and B zones should be via terminal HEPA filters.

2.4.6 Appropriate airflow alarms and pressure differentials and indication instruments should be provided (*Ref:* GMP: Heating, ventilation and air-conditioning systems for non-sterile pharmaceutical dosage forms *(6)*; and GMP for sterile pharmaceutical products *(6)*.

2.4.7 Room pressure readings should be taken and recorded from externally mounted gauges unless a validated continuous monitoring system is installed. As a minimum, readings should be taken prior to operator entry to the test suite. Pressure gauges should be labelled to indicate the area served and the acceptable specification.

2.4.8 Entry to the clean room should be via a system of airlocks and change room where operators are required to don suitable clean-room garments. The final change room should be the same grade as the room it serves. Change rooms should be of adequate size for ease of changing. There should be clear demarcation of the different zones.

2.4.9 Garments for the sterility test operator should comply with the principles of section 10 of WHO GMP for sterile pharmaceutical products *(6)*. Operators should be trained and certified in gowning procedures with training records maintained.

2.4.10 The premises fittings and finishes should comply with Section 11 of WHO GMP for sterile pharmaceutical products *(6)*.

2.4.11 Environmental microbiological monitoring should reflect the facility used (room or isolator) and include a combination of air and surface sampling methods appropriate to the facility, such as:

– active air sampling;
– settle (exposure) plates;
– surface contact (RODAC) plates, swabs or flexible films;
– operators' gloved hand plates.

Microbial environmental monitoring of the sterility test zone should be performed during every work session under operational (dynamic) conditions.

There should be written specifications, including appropriate alert and action limits for microbial and non-viable contamination. Limits for microbiological and non-viable environmental monitoring are given in the WHO GMP for sterile pharmaceutical products *(6)*.

2.4.12 The sterility test environment should be periodically requalified and include non-viable and viable sampling in addition to verification of HEPA integrity and room air flows. Non-viable monitoring should be repeated periodically, normally every month. However, alternative frequency of the monitoring may be justified based on quality risk management. Mapping locations for sample points for routine monitoring should be documented, as well as exposure duration, and frequency of all types of microbiological environmental monitoring should be specified in written procedures.

3. VALIDATION OF TEST METHODS

3.1 Standard (pharmacopoeial) test methods are considered to be validated. However, the specific test method to be used by a specific laboratory for testing of a specific product needs to be shown to be suitable for use in recovering bacteria, yeast and mould in the presence of the specific product. The laboratory needs to demonstrate that the performance criteria of the standard test method can be met by the laboratory prior to introducing the test for routine purposes (method verification) and that the specific test method for the specific product is suitable (test method suitability).

3.2 Non-standard test methods with no formal endorsement or peer-reviewed validation data should be validated before use. Validation comprises determining the limit of detection, repeatability and reproducibility, and control mechanisms (e.g. positive and negative controls).

3.3 Quantitative microbiological test methods should be validated by quantitatively determining in assays the sensitivity, repeatability, reproducibility and the limit of quantification within a defined variability. Potentially inhibitory effects from the sample should be taken into account when testing different types of samples. The results should be evaluated

with appropriate statistical methods, e.g. as described in the national, regional or international pharmacopoeias.

4. EQUIPMENT

Each item of equipment, instrument or other device used for testing, verification and calibration should be uniquely identified.

As part of its quality system, a laboratory should have a documented programme for the maintenance, calibration and monitoring of its equipment.

4.1 Maintenance of equipment

4.1.1 Maintenance of essential equipment should be carried out in accordance with a procedure at pre-determined intervals. Detailed records should be kept. (Examples of maintenance of equipment and intervals Appendix D.)

4.2 Qualification

4.2.1 For qualificaton of equipment see Chapters 8 and 12 in *Good practices for pharmaceutical quality control laboratories (1).*

4.3 Calibration and monitoring

4.3.1 The date of calibration and servicing and the date when recalibration is due should be clearly indicated on a label attached to the instrument at predetermined intervals and demonstrated to conform to predefined acceptance criteria..

4.3.2 The laboratory must establish a programme for the calibration, requalification and monitoring 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, Appendix B and Appendix C.)

4.3.3 *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 shall be of an appropriate quality to achieve the accuracy required (e.g. liquid-in-glass thermometers, thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves).
(b) Calibration of devices shall be traceable to national or international standards for temperature.

4.3.4 *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. The operating temperature of this type of equipment shall be monitored and records retained.

4.3.5 *Autoclaves, including media preparators*

(a) Autoclaves should be capable of meeting specified time and temperature tolerances; monitoring pressure alone is 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 revalidation should consider the suitability of come-up and come-down times as well as time at sterilization temperature.
(c) Clear operating instructions should be provided based on the heating profiles determined for typical uses during validation/revalidation. 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 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.

Laboratories should have a separate autoclave for decontamination. However, one autoclave may be 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.

4.3.6 *Weights and balances*

Weights and balances shall be calibrated traceably at regular intervals (according to their intended use) using appropriate standard weights traceable to certified standard weights.

4.3.7 *Volumetric equipment*

(a) Microbiology laboratories should carry out initial verification of volumetric equipment (automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable pipettes) 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 of the suitability of the equipment, it is recommended that random checks on accuracy are carried out. If the supplier does not have a recognized quality system, laboratories should check each batch of equipment for suitability.

4.3.8 *Other equipment*

Conductivity meters, oxygen meters, pH meters and other similar instruments should be verified regularly or before each use. The buffers used for verification 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.

When centrifuges are used in test procedures, an assessment of the rotations per minute (RPM) shall be made. Where it is critical, the centrifuge shall be calibrated.

5. REAGENTS AND CULTURE MEDIA

Laboratories should ensure that the quality of reagents and media used is appropriate for the test concerned.

5.1 Reagents

5.1.1 Laboratories should verify the suitability of each batch of reagents critical for the test, initially and during its shelf-life, if necessary using positive and negative control organisms which are traceable to recognized national or international culture collections.

5.2 Media

5.2.1 Media may be prepared in-house or purchased either partially or fully prepared. Vendors of purchased media should be approved and qualified. The qualified vendor may certify some of the quality parameters listed subsequently. Growth promotion should be done on all media on every batch by the user.

5.2.2 The suitable performance of culture media, diluents and other suspension fluids should be checked, where relevant, with regard to:

– recovery or survival maintenance of target organisms. Recovery of approx. 10-100 colony-forming units (**CFU** or **cfu**) should be demonstrated;
– inhibition or suppression of non-target organisms;
– biochemical (differential and diagnostic) properties; and
– physical properties (e.g. pH, volume and sterility).

Quantitative procedures for evaluation of recovery or survival are preferred

5.2.3 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.

5.2.4 Water of a suitable microbiological quality and free from bactericidal, inhibitory or interfering substances, should be used for preparation unless the test method specifies otherwise.

5.2.5 Media containing antimetabolites or inhibitors should be prepared using dedicated glassware, as carry-over of these agents into other media could inhibit the growth and detection of microorganisms present in the sample under test. If dedicated glassware is not used, washing procedures for glassware should be validated.

5.2.6 Repartition of media after sterilization should be performed under unidirectional airflow (UDAF) to minimize potential for environmental contamination. This should be considered a

minimum requirement for media to be used in relation to sterile product testing. This includes the cooling of media as container lids will need to be removed during cooling to prevent build-up of condensation.

5.2.7 Plated media which is to be irradiated requires the addition of an antioxidant and free radical scavenger to provide protection from the effects of the irradiation process. The irradiated media should be validated by performing quantitative growth promotion testing on both irradiated and non-irradiated media.

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

5.2.9 Batches of media should be identifiable and is conformance with quality specifications documented. For purchased media the user laboratory should ensure that it will be notified by the manufacturer of any changes to the quality specification.

5.2.10 Where the manufacturer of media procured ready to use or partially complete is covered by a recognized quality system, checks by the user laboratory of conformance of supplies with the specification defined through initial validation may be applied in accordance with the certificate of conformance. In other circumstances, adequate testing would be necessary on every batch received.

5.3 Labelling

5.3.1 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.

5.4 Media resuscitation

5.4.1 Organism resuscitation is required where test methodologies may produce sublethally injured cells. For example exposure to:

– injurious effects of processing, e.g. heat;
– antimicrobial agents;
– preservatives;
– extremes of osmotic pressure; and
– extremes of pH.

5.4.2 Media resuscitation may be achieved by:

– exposure to a liquid media like a simple salt solution at room temperature for 2 hours;
– exposure to a solid repair medium for 4-6 hours.

6. REFERENCE MATERIALS AND REFERENCE CULTURES

6.1 International standards and pharmacopoeial reference substances

6.1.1 Reference materials and certified reference and certified reference materials are used in a microbiological laboratory to qualify, verify and calibrate equipment. Whenever possible these reference materials should be used in appropriate matrices. International standards and pharmacopoeial reference substances are employed to:

-determine potency or content;
-validate methods;
-enable comparison of methods.

If possible reference materials should be used in appropriate matrices.

6.2 Reference cultures

6.2.1 Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating ongoing 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.

6.2.2 Reference strains may be subcultured once to provide reference stocks. Purity and biochemical checks should be made in parallel as appropriate. Usually not more than 5 generations from the original reference strain can be subcultured if defined by a standard method or laboratories can provide documentary evidence that there has been no change in any relevant property. 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 A on preparation of working stocks). If reference stocks have been thawed, they must not be refrozen and reused.

6.2.3 Working stocks should not normally be subcultured. Usually not more than 5 generations from the original reference strain can be subcultured if 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 subcultured to replace reference stocks. Commercial derivatives of reference strains may only be used as working cultures.

Note from Secretariat:

Feedback is sought about the number of subcultres being permitted for reference strains and for working stocks. Please kindly comment, if current version is acceptable.

7. SAMPLING

For general principles reference is made to *Good practices for pharmaceutical quality control laboratories (1).*

7.1 Where testing laboratories are responsible for primary sampling to obtain test items, it is strongly recommended that this sampling be covered by a quality assurance system and should be subject to regular audits.

7.2 Transport and storage should be under conditions that maintain the integrity of the sample (e.g. chilled or frozen where appropriate). Testing of the samples should be performed as soon as possible after sampling. There should be demonstration that the storage conditions, time and temperature, will not affect the accuracy of the testing result. The storage conditions should be monitored and records kept. The responsibility for transport, storage between sampling and arrival at the testing laboratory shall be clearly documented.

7.3 Sampling should only be performed by trained personnel. It should be carried out aseptically using sterile equipment. Appropriate precautions should be taken to ensure that sample integrity is maintained through the use of sterile sealed containers for the collection of samples where appropriate. It may be necessary to monitor environmental conditions for instance air contamination and temperature at the sampling site. Time of sampling should be recorded.

8. SAMPLE HANDLING AND IDENTIFICATION

8.1 The laboratory should have procedures that cover the delivery and receipt 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.

8.2 The laboratory should record all relevant information, e.g.:

– date and, where relevant, the time of receipt;
– condition of the sample on receipt and, when necessary, temperature; and
– characteristics of the sampling operation (sampling date, sampling conditions, etc.).

8.3 Samples awaiting test shall be stored under suitable conditions to minimize changes to any microbial population present. Storage conditions should be validated, defined and recorded.

8.4 The packaging and labels of samples may be highly contaminated and should be handled and stored with care so as to avoid any spread of contamination. Disinfection processes applied to the outer container should not affect the integrity of the sample. It should be noted that alcohol is not sporicidal.

8.5 Subsampling by the laboratory immediately prior to testing may be required as part of the test method. It may be appropriate that it is performed according to national or international

standards, where they exist, or by validated in-house methods. Subsampling procedures should be designed to collect a representative sample.

8.6 There should be a written procedure for the retention and disposal of samples. If sample integrity can be maintained it may be appropriate that samples are stored until the test results are obtained, or longer if required. Laboratory sample portions that are known to be contaminated should be decontaminated prior to being discarded (see 11.1).

9. DISPOSAL OF CONTAMINATED WASTE

9.1 The procedures for the disposal of contaminated materials should be designed to minimize the possibility of contaminating the test environment or materials. It is a matter of good laboratory management and should conform to national/international environmental or health and safety regulations.

10. QUALITY ASSURANCE OF RESULTS/QUALITY CONTROL OF PERFORMANCE

10.1 Internal quality control

10.1.1 The laboratory should have a system of internal quality assurance/control to ensure the consistency of results day to day and their conformity with defined criteria.

11. TESTING PROCEDURES

11.1 Sterility testing

11.1.1 Test procedures can be found in the national, regional and international pharmacopoeias.

11.1.2 Typical standards applied by the pharmaceutical industry are as follows:

– microbial limit testing/total bioburden – 1000 g for bacteria and 100 g for yeasts and moulds; and
– environmental monitoring – 15 organisms for TVC on Tryptone Soy Agar (TSA) and 5 organisms for yeasts and moulds on Schwartz Differential Agar (SDA).

12. TEST REPORTS

12.1 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. In the raw data the result should not be given as zero for a defined unit unless it is a regulatory requirement. A reported value of "0" may be used for data entry and calculations or trend analysis in electronic databases

12.2 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 microorganisms within the sample) have to be made clear to the client.

REFERENCES

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

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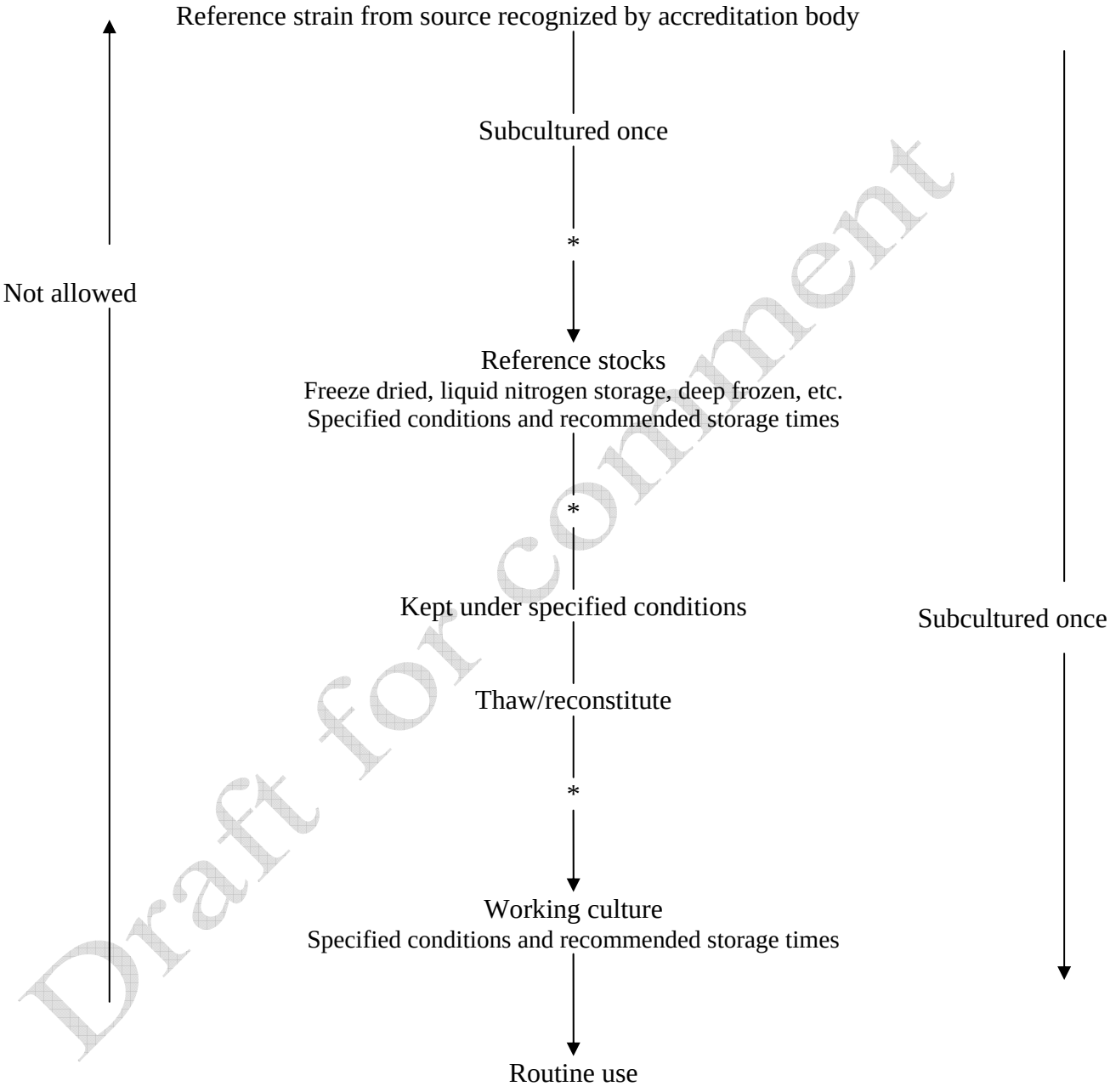
[Note from Secretariat: As portions of the main text of the report have been shifted the references will be put in correct order in the final version of the document.]

APPENDIX A

GENERAL USE OF REFERENCE CULTURES



* Parallel 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 B

EXAMPLES OF CALIBRATION AND CALIBRATION CHECKS

This information is provided as an example and the frequency will be based on the need, type, previous performance and criticality of the equipment.

Type of equipment	Requirement	Suggested frequency
Reference thermometers (liquid-in-glass)	Full traceable recalibration Single point (e.g. ice-point check)	Annually Annually
Reference thermocouples	Full traceable recalibration 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	Annually
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 C

EXAMPLES OF EQUIPMENT QUALIFICATION AND MONITORING

This information is provided as an example and the frequency will be based on the need, type, previous performance and criticality 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
Sterilizing 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
Grade A areas used for sterility testing: • Safety cabinets • UDFs • Isolators	(a) Establish performance (b) Microbiological monitoring (c) Airflow monitoring (d) Test for integrity of HEPA filters	(a) Initially, every year and after repair/modification (b) Each use (c) 6-monthly (d) 6-monthly
Unidirectional cabinets	(a) Establish performance (b) Microbiological monitoring (c) Airflow monitoring (d) Test for integrity of HEPA filters	(a) Initially, and after repair/modification (b) Weekly (c) 6-monthly (d) 6-monthly
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-ionizers and reverse osmosis units	(a) Check conductivity (b) Check for microbial contamination	(a) 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	Based on risk assessment an adequate environmental monitoring programme should be established.

APPENDIX D

EXAMPLES OF MAINTENANCE OF EQUIPMENT

This information is provided as an example and the frequency will be based on the need, type and previous performance of the equipment and suppliers' manuals.

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 Unidirectional 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 descale	As required (e.g. every 3 months)
De-ionizers, 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 sterilize as appropriate	Each use
Spiral platers	(a) Service (b) Decontaminate, clean and sterilize	(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

APPENDIX E

EXAMPLES OF ZONES IN WHICH OPERATIONS COULD BE CARRIED OUT

The zones are designed as grade X, during the installation and monitoring can be carried out, e.g. through appropriate air supply.

Zone	**Installation grade**	**Proposed**
Sample receipt	Unclassified	Unclassified
Media prep	Unclassified	Unclassified
Autoclave loading	Unclassified	Unclassified
Autoclave unloading, inside the sterility testing microlaboratory	Grade B	ISO 5 (turbulent) & <50 cfu[1]/m^2
Sterility testing – UDAF	Grade A	ISO 5 (UDAF) & <1 cfu/m^2
Sterility testing – background to UDAF	Grade B	ISO 5 (turbulent) & <50 cfu/m^2
Sterility testing – Isolator	Grade A (NVP and microbiology only)	ISO 5 (UDAF) & <1 cfu/m^2
Sterility testing – background to Isolator	Unclassified	Unclassified
Incubator	Unclassified	Unclassified
Enumeration	Unclassified	Unclassified
Decontamination	Unclassified	Unclassified

"Unclassified" should have as a minimum a filter F9, some companies would categorize it as grade D.

Note from Secretariat:
Feedback on the usefulness of this table either within in the text or as Annex is requested.

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[1] Colony-forming units (**CFU** or **cfu**).