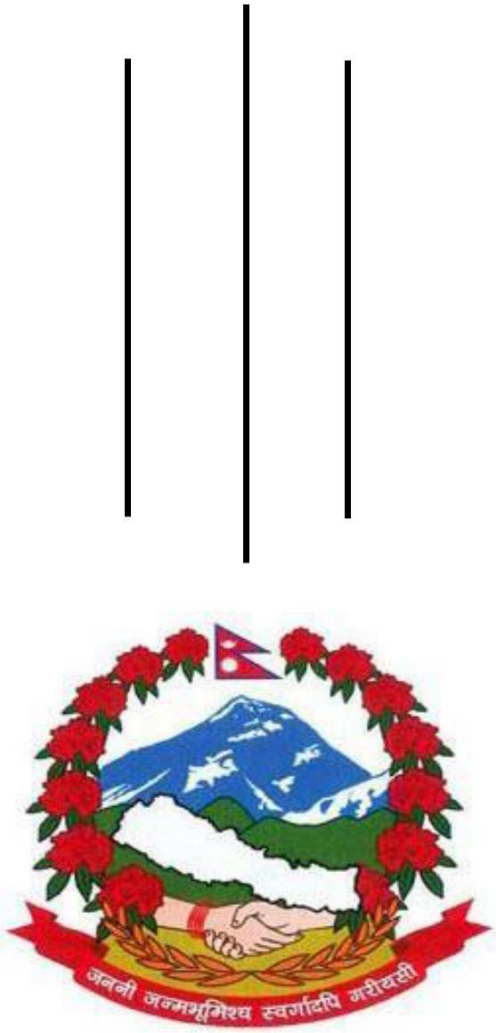
National Guideline for Good Practices

For

Pharmaceutical Microbiology Laboratories, 2075



Government of Nepal Ministry of Health

Department of Drug Administration

National Medicines Laboratory

Table of Contents

1. General requirements for the competence of Pharmaceutical Microbiology Laboratories

1.1 Scope

This document is applicable to national and commercial pharmaceutical microbiology laboratory.

Pharmaceutical microbiology laboratories may be involved in:

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

These guidelines relate to all microbiology laboratories involved in the above-mentioned testing activities, whether they are independent or a department or unit of a pharmaceutical manufacturing facility. These guidelines are based on and supplement the requirements described in National Guidelines on Good Laboratory Practice for Pharmaceutical Quality Control Laboratories, 2075.

1.2 Normative References

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

- ISO/IEC 17000, *Conformity assessment — Vocabulary and general principles*
- ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories
- Drug Act 2035
- Drug Standard Regulation 2043
- National GMP Guidelines, 2072
- WHO good practices for pharmaceutical microbiology laboratories, WHO Technical Report Series, No. 961, 2011
- WHO good manufacturing practices for pharmaceutical products: main principles (WHO TRS 986)
- WHO good practices for pharmaceutical quality control laboratories (WHO TRS No. 957, 2010)
- WHO, HANDBOOK of GOOD LABORATORY PRACTICE (GLP);

2. Terms & Definitions

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.

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.

limit of detection

The lowest number of microorganisms that can be detected but in numbers that cannot be estimated accurately.

precision

The degree of agreement among individual results.

quantitation limit (limit of quantitation)

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

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, which has been established to be fit for its intended use in a measurement process.

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.

reference strains

Microorganisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin. 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 (adapted from ISO).

reproducibility

Reproducibility expresses precision between laboratories.

robustness (or ruggedness)

The ability of the procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions.

sensitivity

The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection .

specificity (selectivity)

The ability of the method to detect the required range of microorganisms that might be present in the test sample.

validation

Action of proving, in accordance with the principles of good practice quality guidelines and regulations (GxP), that any procedure, process, equipment (including the software or hardware used), material, activity or system actually and consistently leads to the expected results.

verification

The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with GxP principles.

working culture

A primary subculture from a reference stock.

3. Personnel

3.1 Microbiological testing should be performed and supervised by an experienced person, qualified in B. Pharmacy or B.Sc/MSc 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.

3.2 Current job descriptions for all personnel involved in tests and/ or calibrations, validations and verifications should be maintained.

The laboratory should also maintain records of all technical personnel, describing their qualifications, training and experience.

3.3 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, regulatory and technological requirements and acceptability criteria.

3.4 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, media preparation, serial dilutions, and basic techniques in identification, with acceptability determined using objective criteria where relevant. Personnel may only perform tests on samples either if they are recognized as competent to do so, or if they do so under adequate supervision. Competence should be monitored continuously with provision for retraining where necessary.

Where a method or technique is not in regular use, the competency of the personnel to perform the test should be verified before testing is undertaken. In some cases it is acceptable to relate competence to a general technique or instrument being used rather than to particular methods.

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

3.6 Personnel should be trained in safe handling of microorganisms.

3.7 Only the minimum number of personnel required should be present in clean areas; this is particularly important during aseptic processes. As far as possible, inspections and controls should be conducted from outside such areas.

3.8 Staff who have been engaged in the processing of animal-tissue materials or of cultures of microorganisms other than those used in the current manufacturing process should not enter sterile-product areas unless rigorous and clearly defined decontamination procedures have been followed.

4. Environment

4.1 Premises

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

4.1.2 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, contamination and cross-contamination. 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 (e.g. sterile media versus reference organisms or incubated cultures), separate storage locations may be necessary.

4.1.3 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.

4.1.4 There should be separate air supply to laboratories and production areas. Separate air-handling units and other provisions, including temperature and humidity controls where required, should be in place for microbiological laboratories. The air supplied to the laboratory should be of appropriate quality and should not be a source of contamination.

4.1.5 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.

4.1.6 Laboratory activities, such as sample preparation, media and equipment preparation and enumeration of microorganisms, should be segregated., false-positive results and false-negative results. Sterility testing should always be performed in a dedicated area. Culture handling and microbial limit test area should be segregated.

4.1.7 Consideration should be given to designing appropriate classified areas for the operations to be performed within the microbiology laboratory. The classification should be based on the criticality of the product and the operation being carried out in the area. Sterility testing should be performed under the same class as used for sterile/aseptic manufacturing operations. Zonal classifications should be as follows:

Zone	Grade	Class	ISO
Sample receipt	Unclassified		
Media Preparation	D	100000	7
Autoclave loading	D	100000	7
Decontamination	Unclassified	…….	……
Autoclave unloading, inside the sterility testing area	Grade B	100	5
Autoclave unloading, inside the area other than the sterility testing area	Grade D	100000	7
Microbial Limit Test (MLT)	Grade A	100	5
Background to MLT area	At least Grade C	At least 10000	7
Microbial Limit Test (MLT)	Grade A	100	5
Sterility Testing	Grade A	100	5
Background to sterility testing area	Graded B	100	5
Enumeration	Grade D	100000	7
Incubator	Grade D	100000	7
Endotoxin test	Grade A	100	5
Background to endotoxin test area	At least Grade C	At least 10000	7

4.1.8 In general, laboratory equipment should not routinely be moved between areas of different cleanliness class, 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.

4.2 Environmental monitoring in the laboratory

Where necessary and appropriate an environmental monitoring programme should be in place which covers environmental microbiological monitoring and temperature and pressure differentials. Alert and action limits should be defined. Trending of environmental monitoring results should be carried out.

4.2.1 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

— replicate organism detection and counting (RODAC) plates, swabs or flexible films;

— operators' glove prints.

Microbial environmental monitoring of the sterility test zone should be performed during every work session under operational (dynamic) conditions and for the microbial limit test and endotoxin testing area, the frequency should be defined to ensure the bioburden is within specified limit. There should be written specifications, including appropriate alert and action limits for microbial contamination. Limits for microbiological environmental monitoring are as per National GMP Code, 2072.

Limit for microbiogical environmental monitoring:

Grade	Air Sample Cfu/m^3	Settle plates 90mm diam. Cfu/4hrs	Contact plates 55mm diam. cfu/plate	Glove points forefingers cfu/glove*
A	<1	<10	<1	<1
B	10	5	5	5
C	100	50	25	------
D	200	100	50	------.

*optional for other tests except sterility testing

4.2.2 Cleaning, disinfection and hygiene

There should be a documented cleaning and disinfection program. Results of environmental monitoring should be considered where relevant. There should be a procedure for dealing with spillages. Adequate hand-washing and hand-disinfection facilities should be available.

4.2.3 Sterility test facilities

4.2.3.1 Sterility test facilities have specific environmental requirements to ensure the integrity of tests carried out. Sterility testing should be carried out and specifies requirements

for sterility testing. This section details the clean-room requirements for a sterility test facility.

4.2.3.2 Sterility testing should be performed under aseptic conditions, which should be equivalent to air quality standards required for the aseptic manufacture of pharmaceutical products. The premises, services and equipment should be subject to the appropriate qualification process.

4.2.3.3 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. Care should be taken with the design of the facility layout and room airflow patterns, to ensure that the unidirectional airflow patterns are not disrupted.

4.2.3.4 The clean-room classification and air-handling equipment of the sterility test facilities should be requalified at least annually by a competent person or contractor. The environment should comply with the non-viable and viable limits, and verification of high efficiency particulate air (HEPA) filter integrity and room airflows should be performed. 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.

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

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

4.2.3.7 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 entry of the operator to the test suite. Pressure gauges should be labeled to indicate the area served and the acceptable specification.

4.2.3.8 Entry to the clean room should be via a system of airlocks and a change room where operators are required to don suitable clean-room garments. The final change room should be under "at rest" conditions of 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.

4.2.3.9 Outdoor clothing should not be brought into changing rooms leading to Grade B and C rooms. For every worker in a Grade A/B area, clean sterile (sterilized or adequately sanitized) protective garments should be provided at each work session. Gloves should be regularly disinfected during operations. Masks and gloves should be changed at

least every working session. Operators working in Grade A and B areas should wear sanitized goggles.

4.2.3.10 Wrist-watches, cosmetics and jewellery should not be worn in clean areas.

4.2.3.11 The clothing required for each grade is as follows:

• Grade D. The hair and, where relevant, beard and moustache should be covered. Protective clothing and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination from outside the clean area.

• Grade C. The hair and, where relevant, beard and moustache should be covered. A one-piece jumpsuit, gathered at the wrists and with a high neck, and appropriate shoes or overshoes should be worn. The clothing should shed virtually no fibres or particulate matter.

• Grades A and B. Entry of personnel into Grade A areas should be minimized. Headgear should totally enclose the hair and, where relevant, beard and moustache. A one-piece jumpsuit, gathered at the wrists and with a high neck, should be worn. The headgear should be tucked into the neck of the suit. A facemask should be worn to prevent the shedding of droplets. Sterilized, non-powdered gloves of appropriate material and sterilized or disinfected footwear should be worn. Trouser-bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and should retain particles shed by the body.

4.2.3.12 Clothing used in clean areas should be laundered or cleaned in such a way that it does not gather additional particulate contaminants that can later be shed. Separate laundry facilities for such clothing are desirable. If fibres are damaged by inappropriate cleaning or sterilization, there may be an increased risk of shedding particles. Washing and sterilization operations should follow standard operating procedures. Operators should be trained and certified in gowning procedures with training records maintained.

4.2.3.13 All premises should as far as possible be designed to avoid the unnecessary entry of supervisory or control personnel. Grade A and B areas should be designed so that all operations can be observed from outside.

4.2.3.14 In clean areas all exposed surfaces should be smooth, impervious and unbroken to minimize the shedding or accumulation of particles or microorganisms and to permit the repeated application of cleaning agents and disinfectants, where used.

4.2.3.15 To reduce the accumulation of dust and to facilitate cleaning, there should be no uncleanable recesses and a minimum of projecting ledges, shelves, cupboards and equipment. Doors should be carefully designed to avoid uncleanable recesses; sliding doors may be undesirable for this reason. Swing doors should open to the high-

pressure side and be provided with self-closers. Exceptions are permitted based on egress and site environmental, health and safety containment requirements.

4.2.3.16 False ceilings should be sealed to prevent contamination from the void space above them.

4.2.3.17 Pipes and ducts and other utilities should be installed so that they do not create recesses, unsealed openings and surfaces that are difficult to clean. Sanitary pipes and fittings should be used and threaded pipe connections should be avoided.

4.2.3.18 Sinks and drains should be avoided wherever possible and should be excluded from Grade A and B areas where aseptic operations are carried out. Where installed they should be designed, located and maintained so as to minimize the risks of microbial contamination; they should be fitted with effective, easily cleanable traps and with air breaks to prevent backflow. Any floor channels should be open and easily cleanable and be connected to drains outside the area in a manner that prevents the ingress of microbial contaminants.

4.2.3.19 Changing rooms should be designed as airlocks and used to provide physical separation of the different stages of changing and so minimize microbial and particulate contamination of protective clothing. They should be flushed effectively with filtered air. The final stage of the changing room should, in the at-rest state, be the same grade as the area into which it leads. The use of separate changing rooms for entering and leaving clean areas is sometimes desirable. In general hand-washing facilities should be provided only in the first stage of the changing rooms. There should not be a change of more than one grade between airlocks or passages and changing rooms, i.e. a Grade D passage can lead to a Grade C airlock, which leads to a Grade B changing room, which leads to a Grade B clean room. Changing rooms should be of a sufficient size to allow for ease of changing. Changing rooms should be equipped with mirrors so that personnel can confirm the correct fit of garments before leaving the changing room.

4.2.3.20 Airlock doors should not be opened simultaneously. An interlocking system and a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

4.2.3.21 A filtered air supply should be used to maintain a positive pressure and an airflow relative to surrounding areas of a lower grade under all operational conditions; it should flush the area effectively. Adjacent rooms of different grades should have a pressure differential of approximately 10–15 Pascals (guidance value). Particular attention should be paid to the protection of the zone of greatest risk, i.e. the immediate environment to which the product and the cleaned components in contact with it are exposed. The recommendations regarding air supplies and pressure differentials may need to be modified where it becomes necessary to contain certain materials, e.g.

pathogenic, highly toxic, radioactive or live viral or bacterial materials or products. The decontamination of the facilities and the treatment of air leaving a clean area may be necessary for some operations.

4.2.3.22 It should be demonstrated that airflow patterns do not present a contamination risk; for example, care should be taken to ensure that particles from a particle-generating person, operation or machine are not conveyed to a zone of higher product risk.

4.2.3.23 A warning system should be operated to indicate failure in the air supply. Indicators of pressure differentials should be fitted between areas where this difference is important, and the pressure differentials should be regularly recorded and failure alarmed.

4.2.3.24 Consideration should be given to restricting unnecessary access to critical filling areas, e.g. Grade A filling zones, by means of a physical barrier.

5. Microbial Limit test facilities:

5.1 Microbial limit test should be performed in dedicated zone as defined in section 4.1.7.

5.2 Microbial limit test should be performed under Grade A unidirectional airflow protected zone surrounded by at least Grade C background.

5.3 The air quality should be monitored regularly and requalified at least annually by a competent person or contractor.

5.4 Entry to the microbial limit test facilities should be via a system of airlock/s and a change room where operators are required to don suitable clean room garments.

5.5 There should be separate facilities for personnel and material entry.

6. Endotoxin test:

6.1 Endotoxin test should be performed in zone as defined in section 4.1.7.

6.2 Endotoxin test should be performed under Grade A unidirectional airflow protected zone surrounded by at least Grade C background.

6.2.1 The air quality should be monitored regularly and requalified at least annually by a competent person or contractor.

6.2.2 Entry to the endotoxin facilities should be via a system of airlock/s and a change room where operators are required to don suitable clean room garments.

6.2.3 There should be separate facilities for personnel and material entry.

6.2.4 Other requirements for clean room is as per sterility test facilities (for eg, piping, ducting false ceiling etc.)

7. Validation of test methods

7.1 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 should demonstrate that the performance criteria of the standard test method can be met by the laboratory before introducing the test for routine purposes (method verification) and that the specific test method for the specific product is suitable (test method suitability including positive and negative controls).

7.2 Test methods not based on compendial or other recognized references should be validated before use. The validation should comprise, where appropriate, determining accuracy, precision, specificity, limit of detection, limit of quantitation, linearity and robustness. Potentially inhibitory effects from the sample should be taken into account when testing different types of sample.

The results should be evaluated with appropriate statistical methods, e.g. as described in the national, regional or international pharmacopoeias.

8. 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 qualification, calibration, performance verification, maintenance and a system for monitoring the use of its equipment.

8.1 Maintenance of equipment

Maintenance of essential equipment should be carried out at predetermined intervals in accordance with a documented procedure. Detailed records should be kept.

8.2 Qualification

For qualification of equipment see sections 4.8 and 5.3 in National Guidelines for Good Laboratory Practices for Pharmaceutical Quality Control Laboratories.

8.3 **Calibration, performance verification and monitoring of use**

8.3.1 The date of calibration and servicing and the date when recalibration is due should be clearly indicated on a label attached to the instrument.

8.3.2 The frequency of 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 should be shorter than the time the equipment has been found to take to drift outside acceptable limits. The performance of the equipment should conform to predefined acceptance criteria.

8.3.3 *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 should be of appropriate quality to achieve the accuracy required (e.g. liquid-in-glass thermometers, thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves).

8.3.3.1 Calibration of devices should be traceable to national or international standards for temperature.

8.3.4 *Incubators, water-baths and 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 should 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 should be checked and recorded after each significant repair or modification. The operating temperature of this type of equipment should be monitored and records retained. The use of the equipment should be considered when determining what temperature controls are required.

8.3.5 *Autoclaves, including media preparators*

8.3.5.1 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 should be verified.

8.3.5.2 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 any significant repair or modification (e.g. replacement of thermoregulatory probe or programmer, change to loading arrangements or operating cycle)

or where indicated by the results of quality control checks on media or risk assessment. Sufficient temperature sensors should be positioned within the load (e.g. in containers filled with liquid/medium) to enable location differences to be demonstrated.

8.3.5.3 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.

8.3.5.4 Monitoring may be achieved by one of the following:

— using a thermocouple and recorder to produce a chart or printout;

— 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 and 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.

8.3.6 **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.

8.3.7 **Volumetric equipment**

8.3.7.1 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, as appropriate, to ensure that the equipment is performing within the required specification. Initial 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.

8.3.7.2 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.

8.3.8 **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) should be made. Where it is critical, the centrifuge should be calibrated.

9. Reagents and culture media

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

9.1 **Reagents**

Laboratories should verify the suitability of prepared reagents critical for the test, initially and during its shelf-life.

9.2 **Media**

9.2.1 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 and, if appropriate, other suitable performance tests (see section 9.2.3) should be done on all media on every batch and on every shipment. Where the supplier of fully prepared media is qualified and provides growth promotion certification per batch of media and transportation conditions have been qualified, the user may rely on the manufacturer's certificate with periodic verification of his or her results.

9.2.2 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 50–200% (after inoculation of not more than 100 colony-forming units (CFU or cfu) should be demonstrated;

—inhibition or suppression of non-target organisms;

—biochemical (differential and diagnostic) properties; and

— other appropriate properties (e.g. pH, volume and sterility).

Quantitative procedures for evaluation of recovery or survival are preferred.

9.2.3 Raw materials (both commercial dehydrated formulations and individual constituents) and media should be stored under appropriate conditions recommended by the manufacturer, 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 color change should not be used.

9.2.4 Water of a suitable microbiological quality should be used for preparation unless the test method specifies otherwise. The limit for microbial limit and frequency of testing should be defined.

9.2.5 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.

9.2.6 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.

9.2.7 Plated media which is to be irradiated may require 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.

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

9.2.9 Batches of media should be identifiable and their 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.

9.2.10 Media should be prepared in accordance with any manufacturer’s instructions, taking into careful account specifications such as time and temperature for sterilization.

9.2.11 Microwave devices should not be used for the melting of media due to the inconsistent distribution of the heating process.

9.3 **Labelling**

9.3.1 Laboratories should 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.

9.3.2 Organism resuscitation

- — Organism resuscitation is required where test methodologies may
- — injurious effects of processing, e.g. heat;
- — antimicrobial agents;
- — preservatives;
- — extremes of osmotic pressure; and
- — extremes of pH.

9.3.3 Organism 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.

10. Reference materials and reference cultures

10.1 International standards and pharmacopoeial reference substances

10.1.1 Reference materials and certified reference materials are generally 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, for example, to:

—determine potency or content;

—validate methods;

—enable comparison of methods;

—perform positive controls; and

—perform growth promotion tests.

If possible reference materials should be used in appropriate matrices.

10.2 Reference cultures

10.2.1 Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods, for verifying the suitability of test methods and for assessing or 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.

10.2.2 Reference strains may be subcultured 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/ lyophilized or in 2-8degreec, provided that the adequate validation has been done ensure that the property has not been altered with reference to reference strains with documentary evidence.

Working cultures for routine use should be primary subcultures from the reference stock. If reference stocks have been thawed, they must not be refrozen and reused.

10.2.3 Working stocks should not be subcultured. Usually not more than five generations (or passages) 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. Commercial derivatives of reference strains may only be used as working cultures.

11. Sampling

For general principles reference is made to National guidelines for Good Laboratory Practice for Pharmaceutical Laboratory Practices, 2075.

11.1 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 it should be subject to regular audits.

11.2 Any disinfection processes used in obtaining the sample (e.g. disinfection of sample points) should not compromise the microbial level within the sample.

11.3 Transport and storage of samples 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.

11.4 For samples where a growth in the microbial population during transport and storage is possible it should be demonstrated 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 should be clearly documented.

11.5 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 example, air contamination and temperature, at the sampling site. Time of sampling should be recorded, if appropriate.

12. Sample handling and identification

12.1 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.

12.2 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 (including sampling date and sampling conditions).

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

12.4 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.

12.5 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.

12.6 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.

13. Disposal of contaminated waste

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.

14. Quality assurance of results and quality control of performance

14.1 Internal quality control

The laboratory should have a system of internal quality assurance or quality control (e.g. handling deviations, use of spiked samples, replicate testing and participation in proficiency testing, where appropriate) to ensure the consistency of results from day to day and their conformity with defined criteria.

15. Testing procedures

15.1 Testing should normally be performed according to procedures described in the national, regional and international pharmacopoeias or pharmacopoeia recognized by Drug Standard Regulation, 2043.

15.2 Alternative testing procedures may be used if they are appropriately validated and equivalence to official methods has been demonstrated.

16. Test reports

16.1 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

16.2 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

1. National Guidelines for Good Laboratory Practices for Pharmaceutical Quality Control Laboratories, 2075
2. National GMP Code, 2072
3. Drug Standard Regulation, 2043
4. Good Practices for pharmaceutical quality control laboratories. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty fourth report*. Geneva, World Health Organization. WHO Technical Report Series, No. 957, 2010, Annex 1.
5. General guidelines for the establishment, maintenance and distribution of chemical reference substances. Revision. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-first report*. Geneva, World Health Organization. WHO Technical Report Series, No. 943, 2007, Annex 3.
6. *The International Pharmacopoeia*, Fourth Edition. Geneva, World Health Organization, 2006. Also available on CD-ROM.
7. *The International Pharmacopoeia*, Fourth Edition, First Supplement. Geneva, World Health Organization, 2008. Also available on CD-ROM.
8. ISO/IEC 17025 (2017) *General requirements for the competence of testing and calibration laboratories*.
9. ISO 11133-1 (2000) *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 culture media in the laboratory*.
10. ISO 13843 (2000) *Water quality — Guidance on validation of microbiological methods.*
11. WHO good manufacturing practices: main principles for pharmaceutical products. In: *Quality assurance of pharmaceuticals. A compendium of guidelines and related materials. Volume 2, 2nd updated edition. Good manufacturing practices (GMP) and inspection.* Geneva, World Health Organization, 2007, and subsequent updates, including WHO GMP for sterile pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*. Geneva, World Health Organization. WHO Technical Report Series, No. 961, Annex 6, 2011; and GMP: Heating, ventilation and air-conditioning systems for non-sterile pharmaceutical dosage forms. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*.

Government of Nepal
Ministry of Health
Department of Drug Administration
National Medicines Laboratory

ATTENDANCE SHEET (OPENING / CLOSING MEETING)

Industry/Laboratory:		**Location:**		
Date :	**Opening Meeting Time:**	**Closing Meeting Time:**		
Type of Visit: *Assessment / 1st Surveillance / Re-Assessment / Supplementary Visit*				
Management Representative:		Address (email):		
			Signature	
S. N	**Name**	**Capacity/ Designation**	**Opening**	**Closing**

Government of Nepal
Ministry of health & population
Department of Drug Administration
National Medicines Laboratory

ASSESSOR'S OBSERVATIONS

Industry/Laboratory:		Location:
Date:	Section:	Audit Criteria:
Auditee:		

S. N	Cl No	OBSERVATION	REMARKS

Name of Assessor:

Signature

Government of Nepal
Ministry of health & population
Department of Drug Administration
National Medicines Laboratory
TESTING/RE-TESTING WITNESSED DURING ASSESSMENT

Industry/Laboratory:	Section:		Location:
Discipline of Testing:		Date(s)of Assessment	

	Test 1	***Test 2***	***Test 3***
Product / Material of Test			
Test Witnessed			
Test Method Specification used			
Range/Accuracy/Measurement Uncertainty			

1. Re-testing of Retained Sample

Sample ID			
Date of Earlier Testing			
Earlier Tested by			
Earlier Reported Results			
Results of Test Witnessed			
Test conducted by			

2. Testing of RM/CRM

Reference Material			
Specified Value			
Results of Test Witnessed			

Remarks*:*

Deviations observed, if any			
Conclusion on the technical competence of the lab for the test witnessed			

(Enclose all supporting data sheets for tests witnessed)

(Signature & Name of Assessor)

Government of Nepal
Ministry of health & population
Department of Drug Administration
National Medicines Laboratory

CORRECTIVE ACTION ON NON-CONFORMITY

(Please use separate sheet for raising each Non-Conformity)

<table>
<tr><td colspan="2">Industry/Laboratory:</td><td colspan="2">Location:</td></tr>
<tr><td>Date:</td><td colspan="2">Section:</td><td>Activity Assessed:</td></tr>
<tr><td>NC No:</td><td colspan="3">Reference to Serial No. in Assessor Observation Sheet:</td></tr>
<tr><td colspan="4">NON-CONFORMITY RAISED:</td></tr>
<tr><td colspan="2">Ref to NML-GLP, Clause No:</td><td colspan="2">Type of NC: MAJOR / MINOR</td></tr>
<tr><td colspan="2">Signature & Name of Laboratory Representative</td><td colspan="2">Signature & Name of Assessor</td></tr>
<tr><td colspan="4">CORRECTIVE ACTION PROPOSED BY THE LABORATORY:

Signature of Laboratory Representative</td></tr>
<tr><td colspan="4">ASSESSOR’S COMMENTS ON CORRECTIVE ACTION PROPOSED BY THE LABORATORY:

Signature of Assessor</td></tr>
<tr><td colspan="4">REMARKS BY LEAD ASSESSOR, IF ANY:

Signature & Name of Lead Assessor</td></tr>
</table>

Government of Nepal
Ministry of health & population
Department of Drug Administration
National Medicines Laboratory

CONSOLIDATED NON-CONFORMITIES

Industry/Laboratory		Location:	
Date(s) of Visit:		Section:	
Audit Criteria:			
NML-GLP Check List Clause No.	NML-GLP Check List Requirement	No. of Non-Conformity raised during Assessment	
		MAJOR	MINOR
4	Management & Infrastructure		
4.1	Organization and Management		
4.2	Quality Management System		
4.3	Control of documentation		
4.4	Records		
4.5	Data-processing equipment		
4.6	Personnel		
4.7	Premises		
4.8	Equipment, instruments and other devices		
4.9	Contracts		
5	Materials, equipment, instruments and other devices		
5.1	Reagents		
5.2	Reference substances and reference materials		
5.3	Calibration, verification of performance and qualification of equipment, instruments and other devices		
5.4	Traceability		
6	Working procedures		
6.1	Incoming samples		
6.2	Analytical worksheet		
6.3	Validation of analytical procedures		

6.4	Testing		
6.5	Evaluation of test results		
6.6	Certificate of analysis		
6.7	Retained samples		
7	Safety		
7.1	General rules		

The non-conformities raised during the assessment are as a result of limited sampling and therefore it shall not be assumed that other non-conformities do not exist.

Signature & Name of Authorised representative of Laboratory

Signature & Name of Lead Assessor

Government of Nepal
Ministry of health & population
Department of Drug Administration
National Medicines Laboratory

SUMMARY OF THE ASSESSMENT

Industry/Laboratory:	**Location:**	
Laboratory Representative:	**Date(s) of Visit:**	
Type of Visit: *Assessment / 1st Surveillance / Re-Assessment / Supplementary Visit*		
Lead Assessor:	**Assessor 1:**	**Assessor 2:**
Date of earlier visit:	*Non-Conformities during earlier visit have/ have not been discharged.*	

ASSESSMENT SUMMARY:

Assessment Team Comment on compliance of laboratory to:

- **(a) Documentation:**
- **(b) Equipment Status & Adequacy:**
- **(c) Equipment Calibration and Traceability :**
- **(d) Personnel Adequacy and Competency:**
- **(e) Safety:**

Non-Conformities raised during the assessment	**MAJOR**	**MINOR**

COMMENTS OF ASSESSMENT TEAM:

Enclosures	**NML-GLP-F-1**	**NML-GLP-F-2**	**NML-GLP-F-3**	**NML-GLP-F-4**	**NML-GLP-F-5**	**Any other Supporting documents**
No. of Pages						

Acknowledgement by Authorised Representative of Laboratory & Date	Signature of Lead Assessor & Date