DEPARTMENT OF MICROBIOLOGY

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Micro/SOP/NO.SERO/027	Equipment maintenance and calibration	1.0
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Name	Designation	Function	Signature
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AMENDMENTS

Sl. No	Page No	Date of Amendment	Amendment made	Reason	Signature of Author	Signature of Approver

1.0 INTRODUCTION:

Preventive maintenance is the best measure to ensure that the equipment will function properly. Preventive maintenance schedules should be followed regularly for optimal performance of equipment. Ordinarily, maintenance schedules are set up as tasks to perform daily, weekly, monthly, every six months and yearly. Depending on such factors as work load, types of instrumentation and number of employees, a record or check-sheet of such activities can be adopted for any given laboratory to help the laboratory manager keep track of necessary tasks.

2.0 PURPOSE:

This SOP describes equipment maintenance and calibration for Microbiology Department.

3.0 SCOPE:

This SOP is applicable to Laboratory Staff in Microbiology Department.

4.0 CHECKLIST BEFORE DECIDING ON THE PURCHASE OF AN INSTRUMENT:

- The instrument specifications should fit the purpose.
- The specifications should conform to local conditions such as power supply, humidity and climate.
- The advice of other laboratories using a similar instrument should be sought.
- The prices of different brands with similar specifications should be compared, not forgetting prices of spare parts.
- Running costs should be compared.
- Availability of after-sale services, including maintenance should be assured.
- An operations and maintenance manual must be supplied.
- On delivery, an extra supply of commonly needed replacement parts (e.g. carbon brushes, fuses, bulbs, electrodes, heating elements) should be obtained.
- The equipment must be assessed for technical safety.

- The need for an operator from the laboratory to receive on-the-job training in operation, maintenance and minor repairs must be recognized by the supplier or a competent national agency.

5.0 OPERATION, MAINTENANCE AND CALIBRATION OF DIFFERENT EQUIPMENT:

Centrifuges –

Two types of centrifuges are currently used, mechanical and electrical. The major aspects in maintenance of different types of centrifuges used in laboratory routine are as follows:

- The centrifuges must be positioned exactly horizontally to prevent the instrument moving its place when out of balance during centrifugation. Check if the rubber buffers are in the buckets.
- It is critically important that the centrifuge load is balanced at all times. Therefore buckets loaded in matched pans and tubes should be arranged so that balanced tubes opposite each other in the centrifuge head. This is necessary to maintain the same forces of gravity in the opposite positions of the tubes. This arrangement involves placing a 'dummy' i.e., a tube filled with the appropriate volume of water corresponding to the weight of the volume, in the oppositely positioned test tube, when an odd number of specimens must be centrifuged.
- Haematocrit centrifuges need not be balanced before use, as the capillary tube samples are small. Capillaries should be plugged at one end to avoid spilling and loss of blood. The plugged end should always be placed against the sealing gasket.
- Turn the speed control slowly up and down.
- Stop the centrifuge immediately if it makes an abnormal noise.
- After use, the buckets should be inverted to drain dry.
- After any sample spillage, wipe and disinfect immediately.

- Clean the centrifuge at short intervals (preferably daily) because it is one of the most frequently used instruments.
- Check mounting and replace if necessary.
- Check brushes and bearings every 3 months. Replace if necessary.
- Check for corrosion and clean and repaint if necessary.
- Calibration: use a pre-calibrated tachometer to check centrifuge speed.

Refrigerators –

The following general advice may be helpful for maintenance:

- Refrigerators must be so placed that sufficient air can pass the condenser (at the back of the refrigerator) for exchange of heat and also to facilitate cleaning the condenser.
- The refrigerator door must seal perfectly to prevent warm outside air from entering the cool chamber.
- Calibration: Use a pre-calibrated thermometer to ensure accuracy of temperature check.

Daily Checks:
Check temperature daily. It should not exceed 12^0C. Application of battery driven mobile or stationary thermometers is recommended, preferably those including continuous printing or plotting of temperature measured when heat-sensitive reagents are stored for long periods.

Monthly Checks:

- Clean cool chamber and defrost the evaporator monthly.
- Clean refrigerator from outside.
- Clean condenser of dust.
- Clean door gasket.

Hot air oven –

Hot air ovens are mainly used for drying laboratory equipment and medical devices in dry air. Some hot ovens are also used for sterilization. Sterilization in dry air is only effective when the material is exposed for 60 minutes to 160°C or for 40 minutes to 180°C. It is important to remember that the timing of sterilization is sufficient when the holding period begins after the air in the oven has reached its expected temperature.

Use of hot air ovens:

- Set up the thermostat at the required temperature prior to sterilization.
- If there is a fan, check if it is working.
- Allow to continue heating for an additional 60 minutes after the temperature reaches the pre-set degree.
- Switch off the heat when the time is up.
- Wait until the temperature falls to 40°C before opening the door.
- Calibration: Use a pre-calibrated thermometer to ensure accuracy of temperature checks.

Autoclave –

Bacteria cannot survive in an autoclave environment; however, sterilization does not necessarily kill viruses. It should be borne in mind that autoclaves need careful handling and must be regularly inspected. They can be hazardous and can seriously injure a person with hot steam accidentally escaping from the instrument.

The main factors influencing perfect steam sterilization are

- saturated steam
- temperature
- time

Use of Autoclaves:

- Prepare the material for autoclaving with indicator paper.
- Fill the bottom of the autoclave with demineralized water up to the support.
- Before placing the material in the autoclave, ensure that there is an adequate amount of water. Close the lid and make sure that the rubber washer is in its groove. Screw down the clamps firm.
- Open the air outlet valve.
- Turn on the heating (electric element). Do not leave the autoclave unattended.
- Close the outlet valve when a constant jet of steam starts to escape. Reduce the heat as not to heat too quickly.
- Once the expected temperature is reached, reduce the heat to maintain the temperature.
- Do not touch drainage tap, outlet or safety valve while heating under pressure.
- When the time required for sterilization has passed, turn off the heat completely.
- When the temperature falls below 100°C, open the outlet valve slowly. Do not leave the outlet valve unopened for too long.
- Never unscrew the lid clamps or open the lid except after the whistling sound stops.
- Leave the sterilized material to cool before its removal from the autoclave.
- Check the autoclave tape (used in the preparation of the material to be sterilized) which must have turned black and the covering paper brown (not yellow or black).

- Calibration: Use a pre-calibrated pressure gauge to check accuracy of pressure in the autoclave.

Adequate sterilization by autoclaves and dry ovens should be monitored by the weekly use of a biological (spore suspension) or chemical indicator.

Sl. No	ITEMS	AUTOCLAVE	HOLDING TIME
1.	Glassware/media/buffers etc. • Special media not containing glucose • Pipettes • Test tubes • Measuring cylinders • Reagent bottles etc	121°C at 15 psi	15 minutes
2.	Plasticware syringes • Microtips • Troughs • Cryovials • Eppendorfs, etc.	121°C at 15 psi	30 – 45 minutes
3.	Decontamination of lab waste	121°C at 15 psi	45 minutes

Incubators –

Incubators should be subjected to continuous recording of temperature. However, if it is not possible, the temperature must be recorded every day and before opening of the

incubator.

- The incubator should have a fan to maintain uniform temperature.
- Incubators are used for bacterial culture by laboratories working in microbiology.
- The incubator must maintain a constant temperature of 37 +/- 1°C.
- Temperature should be daily recorded in incubators.

Like all laboratory instruments, incubators must be cleaned and disinfect routinely at short intervals (at least every fortnight) and after spilling of infectious material.

- The actual temperature must correspond to the thermometer control when the instrument is used.
- Calibration: Use a pre-calibrated thermometer to ensure accuracy of temperature checks.

Water bath –

It is important that the water bath maintains a constant temperature within a narrow range (+/- 0.5°C) when used for kinetic measurements such as determination of enzyme activities or clotting assays. Inadequate adjustment and insufficient stabilization of the temperature will strongly affect the results of kinetic measurements.

Use of water baths:

- The level of water in the water bath must be above the level of the solution to be incubated.
- Open containers, vials or tubes must be incubated in a water bath with an open lid to avoid contamination and dilution of the incubated material by condensed water.
- The water bath must be refilled regularly to prevent growth of algae and bacteria.
- The variety of designs for these devices is so numerous that it is difficult to discuss the subject systematically. It must be noted, however, that the replacement of broken conventional glass pipettes can be very costly, and that there may be other economic advantages in using mechanical pipettes.

Pipettes –

- Each pipette, whether manual, semi-automated or automated, must be tested periodically to determine if it is delivering the correct volume.
- Pipettes are used for volumetric measurements of liquids. They may be made of glass or plastic and may have different volumes, ranging from 2 mL to 100 mL. They may be calibrated or non-calibrated. Glass pipettes are either calibrated to contain (In) or to deliver (Ex).
- Mechanical pipettes are preferably used to pipette small volumes (4 µl to 1000 µl); they have a higher precision and are less subject to errors in pipetting than glass pipettes. Volumes less than 500 µl should be pipette with mechanical pipettes. However, mechanical micropipettes can only be recommended where a reliable supply of standardized disposable tips can be guaranteed. They are usually of air displacement (indirect), or direct displacement design. To avoid contamination during pipetting samples, most designs use a disposable tip which discarded after each delivery.
- The practice of washing and reusing disposable tips is to be discouraged, as any cleaning procedure will change the "wettability" of the plastic. In addition, drying even to only slightly elevated temperatures may distort the tip. This will prevent a good pneumatic seal with the pipette body and change the volume of liquid to be pipetted.
- After each use, the mechanical pipettes must be kept in an upright position and thoroughly cleaned at periodic intervals. Pipettes are precise and important basic instruments of the laboratory, and as such, need to be calibrated at least every 3 – 6 months. Several methods of pipette calibration are available.

pH meters –

A pH meter needs to be standardized before each run with a standard buffer of ph 7.0. However, in instances when the work is related to a pH range of less than 6.0, it is advisable to use a standard buffer of ph 4.0. The buffer solution should be checked monthly with another pH meter and discarded if the pH deviates by more than +/- 0.4 or if the buffer is contaminated with microorganisms.

Glass electrodes must be stored in buffer solutions at pH 4 to pH 8. The buffer solution must be regularly changed at short intervals. Glass electrodes which have been stored for longer periods must be soaked in 0.1 molar HCl for at least 4 hours. Thereafter they must be carefully washed with distilled water. The same procedure must be applied for dried-up electrodes. The life-span of properly maintained glass electrodes is about two years. Thereafter they should be replaced. Aging of an electrode is indicated when the constant electrode potential does not develop 20 seconds after insertion of the electrodes into the ion solution. Glass electrodes are sensitive to mechanical damage.

Calibration of pH Meters:

To obtain a precise measurement of pH, the pH meter must be calibrated with two different buffers at pH 4 and pH 7 every day (two-point calibration). For the calibration of a pH meter special buffer solutions must be used, the pH of which should be near the pH of the solution to be measured. Phosphate buffers and acetate buffers are preferable. Calibration measurements should be done using plastic containers.

Use of the pH Meter:

- Switch off the measuring circuit
- Wash the electrode with deionized water

- Standardize the electrode at the start of the day with two standard buffers (pH 7.0, pH 4.0)
- Transfer the electrode to a small beaker containing the standard buffer, pH 7.0
- Measure the pH of the standard buffer and adjust the buffer control knob to reading
- Wash the electrode and gently wipe with fibre-free material
- Transfer the electrode to another beaker containing the standard buffer, pH 4.0
- Read the pH of the standard buffer. It should be very close to expected pH value (pH 4.0)
- Use fresh standard buffer every day. Discard standard buffer if it is contaminated or cloudy

Measuring the pH of the test solution:

The following steps must be taken:-

- Wash the electrode with deionized water and transfer it to the test solution. With proper electrodes the potential establishes 5 to 20 seconds after insertion of the electrodes into the sample solution. Air bubbles at the electrodes must be avoided since they cause a drift of the electrode potential.
- Compensate for the temperature at each run. Measure the temperature and adjust the temperature dial to the reading.
- Do not read the pH before the reading is stable.
- Record the pH reading of the pH solution.
- Wash the electrode and store it in a container with storage solutions.

Balances –

Balances are used to measure weight and mass. The principle of weighing is based on attracting forces between separate masses. In daily life this is the attracting force between mass and earth. In laboratories, weighing is an essential step in preparing defined quantities of

reagents and reaction mixtures.

There are two main categories of balance

- Mechanical balances
- Electromagnetic balances (battery driven or connected to the mains)
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The following factors influence weighing and can cause errors in measurement

- Temperature
- Moisture (atmospheric humidity)
- Electrostatic effects
- Magnetism
- Gravitational forces
- Air

Maintenance of balances:

The following guidelines are worth remembering:-

- The balance should be placed on a solid vibration-free surface, free from dust and at even temperature, away from sunlight.
- The instrument must be placed in an exactly horizontal position.
- The balance should be zeroed prior to each use.
- Use the smallest possible vessel for weighing. Avoid weighing in vessels made of plastic, because they can become electrostatically charged. Use instead glass vessels or weighing paper, as applicable. The weighing vessel and the sample to be weighed should be at the ambient temperature. Never put your hand into the weighing chamber so as not to warm it up.
- Place the sample to be weighed in a weighing vessel in the middle of the weighing pan, to avoid corner-load error. Liquids or powders should never be directly weighed on the pan. The weight of the weighing vessel needs to be

determined prior to placing the substance to be weighed. A tweezer is useful as a substitute for hands in the weighing chamber.

- Use clean weighing vessels. Keep the working place, weighing chamber and weighing pan clean. To avoid any possible corroding effect of chemicals, any spillage must be cleaned immediately. Biological materials may be a source of infection. Disinfection can be done with 70% alcohol.
- After completing weighing return the balance to zero weight.
- Keep the working place at the balance as clean as possible.
- Weight of the material to be weighed should be within the range of the balance.

Photometer instruments (ELISA reader etc.) –

Most photometric instruments require calibration to ensure accuracy and linearity of their readings. This is usually accomplished using special calibration plates, available from the manufacturer. The plates consist of different wells, each capable of producing a different O.D. reading. Observed readings are compared to theoretical values and evaluated using confidence limits. Likewise, automated diluters must be calibrated. This is usually accomplished by diluting a standard colour solution and reading O.D. spectrophotometrically. Results must be within 10% of the expected limits. The manufacturer of any automated instrument can be contacted for details of these procedures. The annual maintenance contract should include the above mentioned check-ups and laboratory incharges should ensure the implementation.

Calendar of activities for maintenance and calibration of equipment –

Daily:

- Controls or calibrators added to each run.
- Recalibration of instruments if necessary; For ELISA readers, the proper filter is put in place.

- Waste containers, rinse sample ports, etc. are emptied with distilled water.
- All spills cleaned up.
- Reagent levels are checked.
- ELISA washers flushed.
- Biohazardous waste disposed.

Weekly:

- Optical components etc. are kept free from dust.
- Surfaces of instruments are cleaned. Fresh batches of reagents are prepared as needed.

Monthly:

- Electronic or optical checks are performed on all components. Many automated and semiautomated instruments have built-in programs for calibration.

Every 6 months:

- Filters are cleaned or charged, fluid lines and tubing for signs of deterioration are checked and replaced as needed. All pipettes are regressed as needed.

Yearly:

- All fluid lines and tubing of major instruments are charged. A service call for factory representative if possible is done. Pipettes are calibrated.

Function checks –

It is essential that laboratory personnel know and document that all equipment is in good condition each day of use. This can be accomplished by undertaking function checks, often referred to as calibration and validation.

Calibration:

The process which is applied to quantitative measuring or metering of equipment to assure its accurate operation throughout its measuring limits.

Validation:

The steps taken to confirm and record the proper operation of equipment at a given point of time in the range in which tests are performed.

Documentation:

The assurance that a piece of equipment is operating properly can be best judged by examining its performance over time. Records of performance parameters, therefore, are a vital element in the proper operation of laboratory equipment. Some suggested information is provided below:

- Name and serial number of instrument
- Elements to be checked and kind of data to be collected
- Frequency of checking
- Record of data
- Changes made to restore accuracy and precision, if any
- Signature with date of the person performing these tasks

Preventive maintenance –

Maintenance of equipment is an extremely important function in the microbiology laboratory. Unfortunately, this is often grossly neglected because of indifference on the part of laboratory workers and on the erroneous belief that it is too costly. The expense of such maintenance policies as inspection, lubrication and adjustment of instruments is insignificant when compared with the cost of emergency repairs, rebuilding or overhauling equipment, and the additional personnel time and materials involved in producing test results when equipment

is down.

Preventive maintenance is defined as a programme of scheduled inspections of equipment and instruments resulting in minor adjustments or repairs for the purpose of delaying or avoiding major repairs and emergency or premature replacements. It provides the following advantages over breakdown maintenance.

- Better quality results
- Identification of components showing excessive wear
- Greater safety
- Fewer interruptions in services
- Lower repair costs
- Less stand by equipment requirements

ELISA Reader:

Regular maintenance of the ELISA machine should be carried out according to the manufacturers' instructions and may vary between brands. The machine itself should not be opened.

Note - The filters must be protected from moisture and fungal growth. Keep silica gel packet in the filter box.

Plate washer:

Plate washers are critical in ELISA assay performance. The washer works on a simple principle and comprises of wash fluid, waste fluid reservoirs, pressure and vacuum pumps, a dispense manifold and a plate carrier.

The wash fluid is pressurized and a valve opens and allows the fluid through a manifold and into assay microwells. The waste fluid under vacuum is aspirated back through the manifold to the waste container. A number of cycles of dispense and aspiration comprises the washing of a plate. At the end of the wash procedure the wells are empty of the fluid.

After use care-

- Fill the rinse bottle with about 500 ml distilled water.
- Dispose off the unused wash buffer. Rinse with distilled water, a couple of times and leave about 500 ml in the wash bottle. Fix the cap tightly.
- After using the washer switch off power.

6.0 REFERENCES:

1. HIV Testing Manual: Laboratory Diagnosis, Biosafety and Quality Control. NACO document.

I have read this SOP and understood it completely and will try to implement in toto

Sl. No.	Name of the Staff	Signature of Staff with Date
1.	Lalhriathlui Khiangte	18.04.19
2.	Zairempuii	18.04.19
3.	Z.D. Lalremruati	18.4.19
4.	T. Lalvensangi	18/4/19.