GOOD LABORATORY PRACTICE MANUAL PREPARED BY RAEIN-ARICA & PARTNERS

UNDER THE IMPLEMENTATION OF THE NATIONAL BIOSAFETY FRAMEWORK PROJECT FOR SWAZILAND

REF:

SIGNED:

DATE:

LIST OF ACRONYMS & ABBREVIATIONS USED

ACRONYM/ ABBREVIATION	
BSC	Biological Safety Cabinets
GLP	Good Laboratory Practice
OECD	Organization for Economic Co-operation and Development
PI	Principal Investigator
PPCE	Personal Protection and Clothing Equipment
QAU	Quality Assurance Unit
MSDS	Material Safety Data Sheet
SEA	Swaziland Environment Authority
SOPs	Standard Operating Procedures
UNISWA	University of Swaziland

DEFINITION OF TERMS USED

Good Laboratory Practice: A quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

Proficiency test: An inter-laboratory comparison, used to monitor laboratories' performance on an ongoing basis, to determine the performance of individual laboratories for specific tests versus a reference value.

Quality Assurance Programme: A defined system, including personnel, which is independent of study conduct and is designed to assure laboratory management of the compliance with the Principles of Good Laboratory Practice.

Quality Control: Measures of systematic checking to ensure the quality and accuracy of the work performed and reported, and to eliminate errors.

Raw data: Any original observations and activities of a study recorded on laboratory worksheets, records, memoranda, notes. This includes all data necessary for the construction of the report of the study. This may include handwritten notes, photographs, microfiche copies, computer print-outs, magnetic media, dictated observations, and electronically recorded data from automated instruments.

Standard Operating Procedures: Documented procedures which describe how to perform tests or activities normally not specified in detail in study plans or test guidelines.

Table of Contents

1. SCOPE

This Good Laboratory Practice (GLP) Manual applies to all users and activities carried out in the Molecular Biology Laboratory, Crop Production Centre, Department of Plant Production, Faculty of Agriculture & Consumer Science, University of Swaziland (UNISWA). The Manual covers all organisational processes and the conditions under which studies in the laboratory will be planned, performed, monitored, recorded and reported.

2. OBJECTIVE

The Manual sets out guidelines for Good Laboratory Practice to be followed by all users of the Molecular Biology Laboratory.

3. INTRODUCTION

The Organization for Economic Co-operation and Development (OECD) defines GLP as "a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported". GLP is aimed at providing tools to support sound management of laboratory processes and when applied properly, GLP can lead to:

- Improved laboratory and quality management,
- Enhanced efficiency of use of resources,
- Improved tracking of errors and overall quality control,
- Improved safety of personnel,
- Improved communication, and
- Improved quality and validity of test data.

4. GENERAL LABORATORY RULES

The following rules will apply:

- All laboratory personnel are responsible for laboratory safety and must conduct themselves in a manner which promotes and follows laboratory safety principles.
- Access to the laboratory will be restricted to authorized personnel only.
- Laboratory staff will be issued a badge in order to gain access through the badge reading system.
- Any visitors authorized into the laboratory will be issued a visitor's badge which will clearly indicate the following:
    - Name of visitor
    - Date and time of entry.
- Children are not allowed in the laboratory.
- Laboratory doors should be kept closed at all times.
- No animals are allowed in the laboratory.
- All personnel are responsible for maintaining the laboratory in a clean, tidy and safe condition at all times.
- It is the responsibility of all staff to always clean up after themselves for the safety of other laboratory users.
- Food and drink must not be stored in the laboratory refrigerators or freezers. No food must be warmed up in the laboratory microwaves.

- Eating, drinking, smoking, handling of contact lenses, or applying cosmetics are not permitted in the laboratory,
- Hands must always be washed after handling chemical materials, removing gloves, and before leaving the laboratory.
- Mouth pipetting is forbidden
- Care must be taken to perform all procedures carefully to minimize the creation of splashes or aerosols.
- No high risk activities are to be performed after hours. Working after hours should only be done if it is unavoidable and only on SOP's for which risk assessments deem the risk to be low and manageable.
- All equipment breakages must be reported to the Laboratory Manager. Equipment must be decontaminated before repair is commissioned.
- It is the responsibility of all personnel to study the user guide and SOPs for each equipment item.
- Fume cupboards and extractor hoods should not be used as storage cupboards.
- The procedure shown in Figure1 is to be used on finishing work for the day in the laboratory.

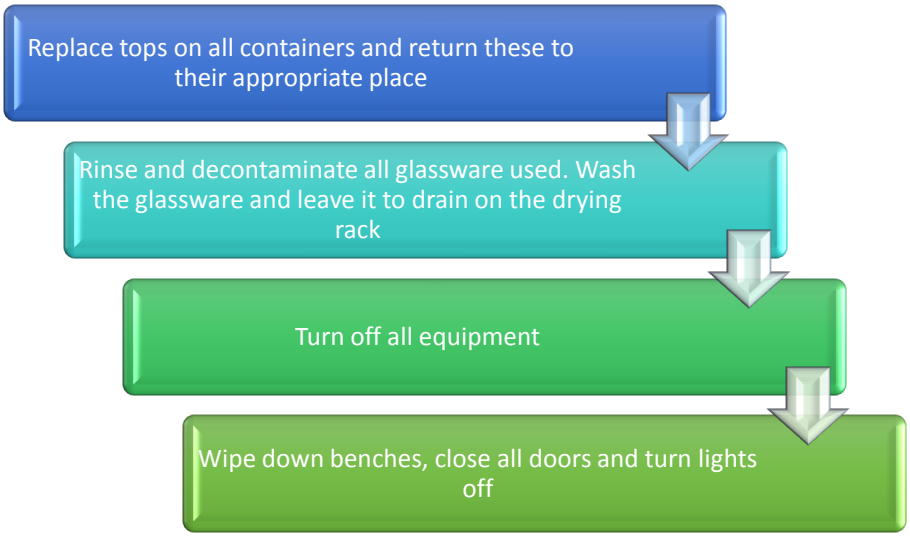

Figure 1 End of day procedure to be used in the laboratory

- Measures to reduce contamination must be applied at all times. These include:
  - Unidirectional workflow to be followed and maintained.
  - Different laboratory coats and equipment must be dedicated and used in each work area. These can be colour coded for ease of distinction.
  - No equipment should be moved from the sample receiving and preparation areas of the laboratory to the Pre-PCR and PCR rooms. This also applies to racks, tubes etc.

- Backward flow of reagents is also not allowed.
- Work areas are to be kept free from physical hazards that might cause spillages or breakages including items that may overhang the edge of benches.

5. HEALTH & SAFETY

Laboratory safety is mandatory and is based on prevention, hazard and incident management.

- All personnel working in the laboratory shall be responsible for maintaining the health and safety provisions in this Manual in a manner that will safeguard people, equipment and the facility.
- All personnel are thus expected to
  - Attend Laboratory Safety Training provided by the University or other agencies
  - Adhere to procedures and laboratory practices outlined in this Manual and other related documents pertaining to safety, housekeeping
  - Adhere to requirements for use of PPCE as appropriate;
  - Report all accidents, near misses, and potential chemical exposures to the Study Director and Laboratory Manager in writing using the established Incident Report Form
  - Review and acknowledge understanding of SOPs including procedures for working with particularly hazardous substances in the Laboratory.

- Laboratory coats must be worn at all times in the laboratory.
- Laboratory coats must be changed and laundered frequently.
- Lab coats must be kept hung on individual coat hooks and not draped on chairs or other furniture.
- Lab coats are not to be worn outside the laboratory.
- All lab coats are to be labelled with the user's name.
- It is the responsibility of all personnel to ensure that lab coats are changed at least weekly and kept clean.
- The laboratory shall also keep additional laboratory coats for visitors.
- Gowns for visitors must be kept clean and folded in a cupboard to be taken out only as needed. They must not be kept on hooks when not needed.
- Eye protection and appropriate gloves must be worn when working with chemicals.
- Reuse of disposable gloves is not allowed.
- Gloves must be removed before handling phones, stationery, accessing any bins, storage cupboards for clean glassware and equipment and any access to the storage units.
- Used gloves must be discarded with contaminated laboratory waste.
- The procedure described in Box 1 must be used to safely remove and discard used gloves.

BOX 1: PROCEDURE FOR REMOVING AND DISCARDING USED GLOVES

1. Slowly remove the first glove by unravelling it from the fingers taking care not to flick the glove off at the end.
2. While holding the dirty glove with the other gloved hand carefully insert a finger under the remaining glove at the wrist taking care not to touch the outside of the glove.
3. Carefully unravel the second glove over the first one holding it from the inside ("clean") part of the glove.
4. Discard immediately into a bio-waste bin. Never put gloves into general waste even if you think they are not contaminated.
5. Immediately wash your hands

- Hands must be disinfected prior to putting on fresh gloves.
- Care must be taken not to grasp contaminated areas to avoid contaminating gown sleeves.
- Care must be taken to wash hands thoroughly after handling any contaminated material, chemical, or waste.
- Where the procedure poses risk of impact or splashing, UV, bright light, laser, etc., protective eye wear must be worn.
- Other Personal Protection Clothing and Equipment (PPCE) must be worn where the risk assessment determines necessity.
- Comfortable, sturdy closed shoes must be worn at all times in the laboratory.
- Shoes worn in the lab must be made from leather or a water proof synthetic material.
- It is the responsibility of all personnel to know where the accident and safety equipment are located as follows:
    - Emergency shower: Located along the back wall of the laboratory.
    - First Aid Kit
    - Eye wash station:
    - Chemical response spill kits:
    - Fire extinguishers:
    - Emergency telephone:

- In each area, a dedicated laboratory coat (following the established colour coding system) must be used.
    - Sample receiving: Khaki
    - Sample processing: Blue
    - Pre-PCR: Red
    - PCR Room: White
    - Post-PCR: Green

- Unidirectional flow of work must be maintained at all times (as shown in Figure 2).

Figure 2: Direction of work flow

- Equipment and laboratory coats must not be moved between work stations.
- Disposable gloves must be changed when moving between workstations.
- Doors and windows must be kept shut at all times.
- All staff members are to read and sign all Standard Operating Procedures (SOPs) in place in the laboratory.
- SOPs should be reviewed and updated periodically as appropriate.
- For procedures such as grinding seeds treated with phyto-pharmaceuticals, a mask must be worn to reduce the risk of inhalation.

For all research to be conducted in the laboratory, conditions shown in Box 2 shall hold.

BOX 2: CONDITIONS TO BE MET FOR ALL RESEARCH STUDIES TO BE CARRIED OUT IN THE LAB

- **Study Director:** For each study to be carried out in laboratory, a Study Director who shall be responsible for oversight of all aspects of the research study. The PI will be responsible for technical conduct of the study, data analysis and interpretation, documentation and reporting of the results.
- **A Quality Assurance Unit:** The laboratory will be audited by a Quality Assurance Unit (QAU). This will comprise an inspector to be designated by UNISWA or the Swaziland Environment Authority (SEA). The QAU shall also be responsible for all aspects of GLP inspection and reporting to UNISWA and regulatory agencies.
- **Laboratory personnel:** The laboratory must be manned by suitably qualified staff who should also be able to follow directions and conduct all activities related to the functions of the lab.
- **Standard operating procedures:** All activities to be conducted in the laboratory shall be performed in accordance with standard operating procedures (SOPs) as approved by the Laboratory Manager. SOPs will be made available to all personnel working in the lab.
- **Control and test material and samples:** All reagents and solutions must be clearly labelled with information on origin, identity, concentration, storage conditions, and expiration date.
- **Equipment:** The laboratory will be equipped with appropriate analytical instruments to meet the range of activities that will be carried out. All equipment will be regularly maintained and calibrated.

6. PERSONNEL AND RESPONSIBILITIES

6.1. Laboratory staffing

- The Laboratory will be managed by a Manager who will be trained in a relevant field to at least MSc. level.
- The Manager will work closely with a Laboratory Technician who must be trained to at least BSc level in a relevant field.
- Each Research Project/Study to be conducted in the Laboratory will be led by a Study Director.
- Staffing arrangements are shown in Figure 3.

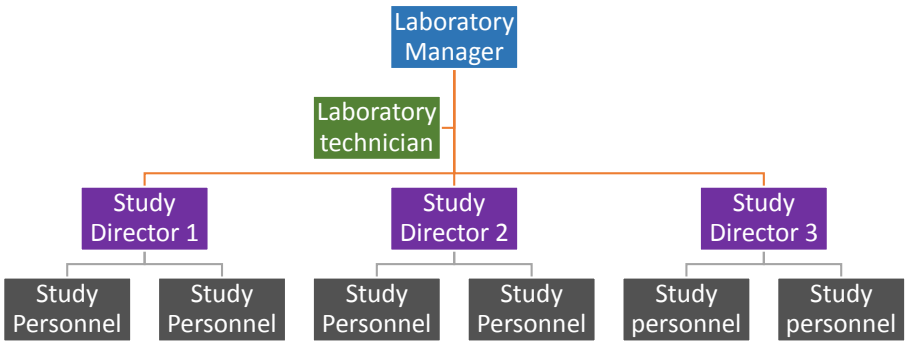

Figure 3: Organogram of staffing arrangements for conducting research in the Laboratory

*The Laboratory Manager may also act as a Study Director or be part of staff personnel in some projects but care must be taken to ensure he/she does not become overwhelmed with responsibilities.
*Study personnel may include post-graduate research students attached to any department within UNISWA, provided they have been trained as described in the relevant section.

6.2. General responsibilities of staff

- All personnel working in the laboratory should be aware of GLP Manual and ensure they comply with all its provisions at all times.
- Personnel are responsible for the quality of their data and must maintain high standards and integrity at all times.
- All personnel are responsible for **recording raw data** promptly, accurately and in compliance with GLP.
- Staff are to ensure compliance with Laboratory SOPs at all times.

6.2.1. Laboratory Manager

- The University shall designate a Laboratory Manager who will be responsible for day to day management of all aspects of the laboratory. The specific responsibilities of the Laboratory Manager will include:
    - Compliance to the provisions of this GLP Manual and all established SOPs by all personnel working in the laboratory at all times.
    - Ensuring that the laboratory is provided with suitably qualified and trained personnel, suitable and well maintained equipment, materials and reagents at all times.

- Maintenance of current and up to date records of the qualifications, training, relevant experience and job descriptions for all personnel working within the laboratory.
- Ensuring that all personnel clearly understand the functions they are required to perform, and where required, provide training for these functions.
- Maintaining copies of all protocols and study plans, including any amendments.
- Ensuring sufficient personnel for the timely and proper conduct of the work.
- Ensuring that laboratory records are archived and maintained.
- Ensuring that Health and Safety precautions within the laboratory are applied according to national regulations.
- Ensuring that a Quality Audit Programme is in place.
- Ensuring that **Quality Control (QC)** is implemented within the laboratory, including membership to external **proficiency schemes.**

6.2.2. Laboratory technician

- Assisting the Laboratory Manager in overall laboratory work planning
- Receiving and preparing samples referred for analysis
- Working with the Laboratory Manager to maintain the Inventory Control Register and ensuring that the laboratory always has adequate supplies
- Working with laboratory personnel to ensure proper maintenance of equipment and maintaining service records
- Working with the Laboratory Manager to ensure that laboratory personnel are complying with all requirements of this Manual.
- Ensure that general cleaning staff are maintaining standards to the requirements of this Manual.
- Responding to general enquiries related to the laboratory
- The Laboratory Technician will also be a trained First Aider.

6.2.3. Study Director

The Study Director is the single point of study control and has the responsibility for the oversight and overall conduct of the study and for the final report. Responsibilities of the Study Director include:

- Approving the study plan and any amendments to the study plan evidenced by signatures;
- Ensuring that the Quality Assurance personnel have a copy of the study plan and any amendments in a timely manner;
- Communicating effectively with the Quality Assurance personnel as required during the conduct of the study;
- Ensuring that study plans, amendments and SOPs are available to study personnel;
- Ensuring that the procedures specified in the study plan are followed,
- Assessing and documenting the impact of any deviations from the study plan on the quality and integrity of the study, and as appropriate taking corrective action.
- Acknowledging deviations from SOPs during the conduct of the study;

- Ensuring that all raw data generated are fully documented and recorded;
- Ensuring that computerised systems used in the study have been validated;
- Signing and dating the final report to indicate acceptance of responsibility for the validity of the data and to indicate the extent to which the study complies with the Principles of Good Laboratory Practice set out in this Manual.
- Ensuring correct recording of experimental data.
- Ensuring collating records of, and verifying, all experimental data, including observations of unforeseen events.
- Ensuring timely archiving of documentation, samples etc.

6.2.4. Study personnel

- All staff working in the laboratory should be aware of this GLP Manual.
- Laboratory personnel must be fully aware of the function they are required to perform.
- All personnel must record raw data promptly, accurately and in compliance with GLP principles.
- Laboratory personnel are responsible for the quality of their data and must maintain integrity at all times.
- All personnel must follow the instructions, protocols, study plans and established SOPs.

6.3. Staff training programme

All activities to be carried out in the laboratory, including GM detection, are complex and require highly skilled, well-trained and experienced personnel. All personnel involved must thus be trained and assessed to ensure competence before they can work in the laboratory. The training programme should include:

- Orientation for new members of staff,
- Biohazard and chemical waste management
- Basic laboratory safety
- An overview of tests run and procedures used in the lab
- Training on all procedures and methodology to be performed. This training will be conducted as follows:
    i. The new staff member is to observe the procedure whilst following an SOP. The trainer will give an explanation of the procedure and allow for questions.
    ii. The new staff member will then run the procedure under supervision by the trainer.
    iii. The new staff member will then run the procedure independently using pre-tested samples.

Other aspects of the training will include:

- Proper use and maintenance of the laboratory and equipment,
- All Standard Operating Procedures (SOPs) in place in the laboratory,
- Competency must continue to be assessed using an established **quality control programme.**
- Capacity of laboratory personnel will be strengthened through on-going programmes as well as external training programmes when such opportunities can be identified.

- The Laboratory shall maintain a summary record of the training, experience and job description for each individual engaged in research in the Laboratory.
- Records of all training courses related to implementation of work in the Laboratory will also be maintained.
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7. QUALITY CONTROL PROGRAMME

- The Laboratory Manager will be responsible for the development and implementation of a quality control programme which will be periodically reviewed.
- Elements of the quality control programme will include:
  i. A biannual internal parallel test in which all staff will perform a complete assay of the same sample with a comparison of the results across all staff.
  ii. An annual external **proficiency test** run with an external accredited laboratory.
  iii. On-going training for laboratory personnel

8. HOUSEKEEPING

In line with good housekeeping principles for laboratories, the following will apply:

- The designated laboratory technician will be responsible for oversight of good housekeeping practices at all times.
- A high standard of cleanliness must be maintained at all times
- Work areas must be kept tidy and free of clutter at all times.
- Disposable material will not be allowed to accumulate as it may result in obstruction and may also pose trip hazards
- Work benches and all other work surfaces must be regularly wiped down with water and soap to remove dust.
- Prior to commencing work, the bench should be wiped down with 1% bleach followed by 70% ethanol to inactivate pathogens and destroy nucleic acid residues.
- The cleaning agents must be prepared fresh daily.
- Centrifuges and thermocyclers must be wiped down with 1% bleach followed by 70% ethanol to prevent contamination.
- Spillages of samples, nucleic acids, reagents, etc. must be cleaned up immediately. Appropriate protective equipment and cleaning materials should be used.
- Any changes in laboratory practices, working habits, or materials that may result in potential hazards to housekeeping and/or maintenance staff shall be communicated with the Laboratory Manager and in writing to the managers of the housekeeping and maintenance staff.
- The floors should be uncluttered to allow ease of cleaning by the cleaning staff.
- Responsibilities for cleaning the laboratory are shown in Table 1.

Table 1: Responsibilities for laboratory cleanliness

Responsibilities of cleaning staff	Responsibilities of laboratory personnel
• Keeping workbenches, reagent shelves, equipment benches, all cabinets and laboratory sinks and draining boards clean and tidy • Providing a complete clean-up at the end of the working day • Keeping floors free from clutter	• Regular cleaning of floors and windows • Keeping hand washing facilities clean • Removal of all general waste • Washing walls as necessary. • Ensuring that the laboratory is free of pests.
• Keeping equipment benches and open shelves clean and tidy	

- Surfaces in the laboratory will be cleaned as follows:
    - Laboratory sinks and all stainless steel surfaces will be cleaned at least once a week using a non-scratch scourer.
    - Work benches will be cleaned with a damp cloth and detergent. A disinfectant detergent may also be used.
    - Floors will be wet mopped regularly. Dry sweeping is to be avoided to minimise raising and spread of dust.
- Cleaning staff will confirm cleaning on a daily cleaning record sheet

9. PROCEDURES

- Pipetting by mouth is strictly forbidden.
- No material must be placed in the mouth. Licking of labels is prohibited.
- Care must be taken to reduce formation of aerosols and droplets.
- All spills, accidents and other potential exposures must be reported to the Laboratory Manager.
- Records of all such incidents shall be maintained.
- Clean-up of all spills shall follow established SOPs.
- Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer.

10. HANDLING OF RAW DATA

The following shall apply to the handling of raw data.

- All laboratory personnel are to keep a laboratory notebook in which all raw data will be entered for each study.
- Raw data entries shall be done in indelible ink.
- No data is to be recorded on scraps of paper, paper towel or other material apart from the designated laboratory notebook.
- Amendments / changes to raw data entries should be made by drawing a single line through the data being changed, recording the corrected information and the date of change, and indicating a reason for the change.

11. INTEGRITY OF DATA IN AUTOMATED DATA SYSTEMS

- Access to automated data systems shall be limited through use of usernames and passwords.
- Personnel will not be allowed to share their login details with unauthorized people.
- Regular virus checks will be conducted to ensure safety of data.
- Regular data back-up will be implemented.

12. ARCHIVING

- All raw data, documentation, SOPs, protocols, final reports, and specimens shall be retained.
- An archiving system will be designed to facilitate orderly storage and expedient retrieval of all raw data, documentation, protocols, specimens etc.

Comment [AM1]: Suggestive time frame for archives?

Comment [P2]: We will get guidance from Chris based on his experience
Diana - Check is UNISWA has a policy regards this

13. FIRST AID

There are four main routes of exposure to chemical hazards in the lab as shown in Box 3.

BOX 3: ROUTES OF EXPOSURE TO CHEMICAL HAZARDS IN THE LAB
Inhalation: This can be minimised through use of fume hoods and masks.
Skin & eye contact: Can be avoided through use of lab coats, gloves, and goggles.
Ingestion: Can be prevented by avoiding eating or drinking in the lab or leaving the lab without removing gloves and washing hands.
Injection: Broken glass and needles must be discarded in sharps bins provided.

First Aid responses to these are as follows:

- **Swallowing**
  - **i.** Administer/drink plenty of water or milk.
  - **ii.** **DO NOT induce vomiting**.
  - **iii.** If the patient is unconscious, **no drinks should be administered.**
- **Splashes:** Strip off affected clothing whilst taking care not to smear more of the chemical onto the patient. Shower/wash for at least 20 minutes. A shower is situated at the back of the laboratory.
- **Eye splashes**: Rinse with water/ eye rinse solution for 20 minutes.
- Basic First Aid Kits are located.
- All accidents, however small, should always be treated.
- All injuries sustained whilst working in the Laboratory, irrespective of severity, must be recorded in the Accident Report Book
- All incidents of any kind, even where no injury is sustained must be reported in the Incident Report Book.

Comment [AM3]: Location of these to b added

- In case of Accident in the Laboratory, the following procedure must be followed:
  - **i. Call for help: The Phone is located ------ Dial 0 and when through:**
    - State your name
    - Your location
    - The nature of the accident
    - The number of people involved

14. CLEANING AND TREATMENT OF EQUIPMENT

- Equipment such as racks must be washed thoroughly and soaked in 1% bleach overnight before being soaked in distilled water and dried prior to returning to work areas.
- Laboratory personnel are responsible for keeping equipment clean.
- Equipment such as centrifuges, water baths, incubators, refrigerators, deep freezers etc. shall be cleaned and, if necessary, decontaminated at regular pre-determined intervals or when the need arises.
- Equipment shall also be cleaned and decontaminated prior to servicing or being sent away for repair or disposal.

15. MAINTENANCE OF EQUIPMENT

- All equipment must be maintained in good working order at all times.
- Equipment breakage and/or malfunction must be reported immediately and logged into the appropriate register.
- A maintenance chart must be maintained for each work area.
- A service label indicating a service and maintenance plan will be attached to each piece of equipment.
- All service (routine and non-routine) must be documented.
- Where troubleshooting becomes necessary, the staff member who implements this must follow the established trouble shooting guide as described in SOP for the equipment.
- In such cases, the staff member must also document exactly what was done and sign the appropriate register.
- Use of major equipment such as Thermocyclers should be logged in on the Equipment Booking and Use Log Sheet.

16. STANDARD OPERATING PROCEDURES

The following SOPs will be developed and numbered as shown in Table 2.

Table 2 : Types of SOPs to be developed

Type of SOP	Purpose	Numbering Notation
Procedure SOPs	To provide Guidance on how specific procedures will be conducted in the	P + index number

	laboratory.	
Equipment SOP	To provide guidance on proper use of equipment in the laboratory. This will include a trouble shooting guide and responsibility (/ies) for maintenance	E + index number
Analysis SOP	To provide guidance on how specific analytical procedures will be conducted in the laboratory.	A + index number

A full list of SOPs will be developed. The List of SOPs will be updated as more SOPs are developed and or revised.

17. REAGENT PREPARATION, HANDLING AND STORAGE

- All reagents must be labelled in large, legible, firmly attached labels showing the contents of the container.
- Labels attached to incoming containers of hazardous chemicals must not be removed or defaced.
- Damaged labels must be immediately replaced with new labels that show the same identification, warnings, and source information.
- Substance-specific storage guidance provided in Material Safety Data Sheet (MSDS) documentation should be adhered to at all times.
- Deteriorated or outdated reagents and solutions should not be used and should be disposed as recommended.
- The following information must be included on reagent labels:
  - Reagent identity,
  - Concentration,
  - Source
  - Storage requirements such as environmental conditions (e.g. away from direct sunlight, refrigeration temperature, etc.),
  - Expiry date
  - ***Date opened.*** This can be critical for some chemicals

18. SAMPLE PREPARATION

18.1 Considerations for sample preparation

The sample used in the analysis must meet the following criteria:

- It must be homogenous and representative of the material received.
- As much as possible, the entire sample received must be homogenised to obtain the test sample.
- Care must be taken to avoid cross-contamination and degradation of DNA

Clean area: Positive air pressure blowing air out of rooms. The clean area is to be kept free of amplicon at all times.

A. **specimen processing area**
   - Receipt of specimens
   - Storage of specimens
   - Nucleic acid/ protein extraction
   - Direction of work must always be room Workflow to no template area.

B.

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Appendix 1