1. OBJECTIVE

The purpose of this document is to outline operating procedures for agarose gel electrophoresis in order to protect laboratory personnel from potential risks of infections and other risks in the NUS Department of Medicine research laboratories

**The purpose of this SOP template is to outline the general procedures for performing agarose gel electrophoresis. It is the responsibility of the PI/users to customize the information to match his/her specific operations.*

2. SCOPE

This SOP is applicable to all staff and students who are conducting agarose gel electrophoresis in NUS Department of Medicine research laboratories.

3. RESPONSIBILITY AND ACCOUNTABILITY

3.1 Principal Investigators are responsible for making sure that staff are properly trained and equipment and facility are maintained in good working order.

3.2 All staff and students must be aware of the potential risks associated with toxic chemicals handling; must obtain the necessary training and work under supervision until proficient in the practices and techniques to work safely.

4. HAZARDS ASSOCIATED WITH GEL ELECTROPHORESIS

4.1 Chemical Hazards
Ethidium Bromide (EtBr) is a potent mutagen and should be handled with caution when mixed in the gel. Safer alternatives reagents are Sybr Safe, Gel Red, etc.

4.2 Electrical Hazards
The weighing balance, microwave, gel electrophoresis, UV transilluminator, etc can lead to the electrical hazard in the laboratory. Electrophoresis units operating at 100 volts can provide a lethal shock.

4.3 Thermal Hazards
Laboratory personnel may be exposed to thermal hazards when heating agarose solutions.

4.4 Ultraviolet Radiation Hazards
Ultraviolet (UV) light boxes are often used in visualizing EtBr gels and pose potential exposures to UV radiation.

5. SAFETY TRAINING

All personnel (staff and students) handling hazardous chemicals and biohazard materials are required to complete the relevant safety training Chemical Safety and Biosafety for BSL-2 Laboratories. Personnel working with body fluids and tissues from humans in laboratory setting are required to complete the Safe Handling of Human Tissue and Fluids vial IVLE @ https://ivle.nus.edu.sg/.

6. PERSONAL PROTECTIVE EQUIPMENT

Wear long sleeved lab coat, safety glasses/goggles, nitrile gloves (or glove material impermeable and resistant to the substance) and covered shoes. Wear insulated gloves if necessary.

7. SAFETY PRECAUTION

7.1 Pre-Operation

- Identified the location where ethidium bromide is used as "Designated Areas".
- The cytotoxic hazard symbol should be visible at the work area.
- Keep equipment clear of unintentional grounding points and conductors (e.g., sinks or other water sources, metal plates, aluminum foil, pipes or other electrical equipment).
- Inspect equipment to be used and ensure all are in proper working condition. Report any equipment deficiencies prior to use.

7.2 During Operation

- Make sure that the power is off before connecting the electrical leads.
- Connecting leads with only dried glove hands.
- Do not run equipment unattended.
- Never touch any part of the apparatus while the power is “ON”.
- If the electrophoresis buffer is spilled or is leaking from the gel tank, stop the run, turn off the equipment, clean up the bench top and inspect the device immediately before proceeding.
- Switch off all power and unplug the leads before opening the gel chamber lid or reaching inside the gel chamber.

8. PROCEDURE

8.1 Preparing Agarose Gel

- Prepare agarose gel according to instructions, typically in 0.7-2% agarose with TAE or TBE buffer. The volume of gel made will depend on the size of the casting tray.
- Prepare agarose using microwave. Do not over boil. Ensure that the vessel containing the agarose has NO LID. Do not walk away from the heating agarose.
- Ensure the agarose powder is fully dissolved in the buffer. Let the hot gel preps cool to 50°-60°C before adding Etbr or its alternatives. (Note: Do not boil the agarose gel with Etbr or its alternatives)

8.2 Pouring of Agarose Gel into Gel Tank

- Poured the melted solution into a gel tray and allowed to harden. The gel should be between 3-5 mm thick. Check that no air bubbles are under or between the teeth of the comb.
- When gel has set solid (20-30 min at room temp), Remove the comb vertically out of the gel.
- Place the agarose gel together with the gel tray into the electrophoresis chamber. Fill the reservoir with TAE or TBE buffer until the buffer covers the agarose gel.

8.3 Preparing and Loading DNA Samples

- Mix the samples of DNA with desired amount of 6x gel-loading buffer. The maximum amount of DNA that can be applied to a slot depends on the number of fragments in the sample and their sizes.
- Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette. Load ladder into slots on both the right and left sides of the gel. Avoid forming bubbles in the pipette tip when loading.

8.4 Running gel

- Ensure the terminals are connected the correct way (red to red, black to black) and the samples are at the negative end of the gel, running toward the positive end.
- Put a lid on the gel tank. Run the gel at an appropriate voltage (depends on size of gel). Ensure current is flowing.

8.5 Gel Documentation

- When the DNA samples or dyes have migrated a sufficient distance through the gel, turn off the electric current and remove the leads and lid from the gel tank.
- Transfer the gel to a small plastic box. Put the gel onto the UV transilluminator, close cabinet door, and switch on UV light. Adjust zoom and focus, then capture image.

9. DISPOSAL OF CYTOTOXIC WASTE

9.1 Cytotoxic Disposal Bag

9.2 Storage of Waste

- Liquid waste: Collected in carboy container with ‘Cytotoxic Waste’ labeled. The carboy is placed in a secondary container.

- Solid waste (Gels, contaminated gloves, tips, paper towels, etc.) should be collected in the bin labeled 'Cytotoxic Waste' with proper double purple bags.

Liquid waste

Solid waste

9.3 Disposal of Waste:

Both liquid and solid wastes are to be collected by the licensed waste contractor.

10. SPILL RESPONSE

If spillage occurs, inform the PI/supervisor/safety lead and spill responders immediately and refer to Spill Clean-Up Procedure: SOP-Medicine-01 Biological Spill Response and/or SOP-Medicine-03 Chemical Spill Disposal.

11. INCIDENT REPORTING

Accidents resulting in injuries must be reported to the PI and/or laboratory safety lead immediately after first aid is applied.

Seek medical attention when necessary at the University Health Centre or proceed to the Accident & Emergency units of National University Hospital after office hours.

All incidents or accidents have to be notified to OSHE within 24 hours via the online NUS Accident and Incident Management System (AIMS) @https://inetapps.nus.edu.sg/osh/portal/eServices/ehs360_aims.html. The AIMS report can be submitted by the injured staff/student, safety leads, his or her supervisor/representative if the staff or student is unfit/unable to do the initial report.

12. REFERENCES

a. NUS Laboratory Biorisk Management Manual (OSHE NUS/OSHE/M/01)
b. NUS Laboratory Chemical Safety Manual (OSHE NUS/OSHE/M/02)
c. SOP-Medicine-01 Biological Spill Response
d. SOP-Medicine-02 Biological Waste Disposal
e. SOP-Medicine-03 Chemical Spill Disposal
f. SOP-Medicine-04 Chemical Waste Disposal