 NUS National University of Singapore	Department of Medicine		Doc. No:	SOP-Medicine-19
	Standard Operating Procedure		Rev No:	002
Title: ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)			Page:	1 of 4

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1. OBJECTIVE

The purpose of this document is to outline operating procedures for the safe working with Enzyme-Linked Immunosorbent Assay (ELISA) in order to protect laboratory personnel and students 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 ELISA. 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 laboratory personnel and students who are conducting ELISA in the NUS Department of Medicine research laboratories.

3. RESPONSIBILITIES


- 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 ELISA; must obtain the necessary training and work under supervision until proficient in the practices and techniques to work safely.

4. POTENTIAL HAZARDS

- 4.1 All specimens of cells, tissues or body fluids from human are potential sources of infection. Potential laboratory hazards associated with human samples/cells include blood borne pathogens e.g. Hepatitis B virus, HIV, etc. Know the risks associated with the biohazards before starting work.
- 4.2 Most buffers and media used are not hazards at the concentrations used, however all chemicals should be considered potentially hazardous. Know the risks associated with each reagent by reading the Safety Data Sheets (SDS) before you start work.

5. TRAINING REQUIREMENTS

Complete the relevant safety training Chemical Safety, Biosafety for BSL-2 Laboratories and Safe Handling of Human Tissue and Fluids via NUS LumiNUS @ <https://luminus.nus.edu.sg/>

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6. IMMUNIZATION

Immunization for hepatitis B is required for all personnel handling human samples.

7. ENGINEERING CONTROL

The biosafety cabinet is the most important equipment to provide containment of infectious splashes or aerosols.

8. PERSONAL PROTECTIVE EQUIPMENT


All personnel are required to wear the following personal protective equipment during the ELISA work: long sleeved lab coat, nitrile gloves, safety glasses and covered shoes.

9. SAFETY PRECAUTION

- Read the SDS for the materials used in the ELISA. The SDS contains information about the dangers when working with a particular substance, required protective measures, as well as required actions in case of emergencies.
- Do not eat, drink, smoke, handle contact lenses, apply cosmetics, or store food for human consumption in the laboratory. Oral pipetting of any substance is prohibited.
- Wash hands after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.
- Decontaminate all work surfaces before and after the experiments, and immediately after any spill or splash of potentially infectious material with an appropriate disinfectant. Clean laboratory equipment routinely, even if it is not contaminated.
- Use biosafety cabinet when handling human or other biohazard samples.

10. PROCEDURE

- Microtiter plate is coated with capture antibody.
- Antigen such as serum, plasma is added to the solid surface of the plate. Incubate and wash.
- The plate is incubated with blocking buffer. Incubate and wash.
- The wells are then incubated with specific “detecting” antibody and washed to remove unbound antibody.
 - When using the direct detection method, a single primary antibody is used that is conjugated with enzyme.
 - With indirect detection, the samples are firstly incubated with a primary antibody and then a secondary antibody that is conjugated with enzyme.

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- The plate is developed by adding an enzymatic substrate to produce signal which indicates the quantity of antigen in the sample.
- The antigen is detected. Color-producing (chromogenic), light-producing (chemiluminescent) or fluorescent detection methods can all be used.
- Refer to ELISA kit-specific protocol for detail instructions.

11. WASTE DISPOSAL

11.1 Biohazard Solid Waste

- Solid biohazard waste such as culture flasks, centrifuge tubes, contaminated gloves, tissues, etc. should be double bagged (yellow bag) in the disposal bin with biohazard label. When the bag is 2/3 full, tie both biohazard waste bags tightly. Label bag with lab location, PI name and contact number. The biohazard waste will be collected by the licensed service provider.

11.2 Biohazard Liquid Waste

- Biohazard liquid waste must be treated with appropriate disinfectant - either inactivate for at least 1 hour in freshly prepared Presept solution (5,000 ppm) or appropriate disinfectant prior to disposal with an excess of water.

11.3 Chemical Liquid Waste

- If the ELISA assay produces hazardous chemical waste, the waste should be collected in the compatible waste container. Chemical liquid waste should be collected in the compatible waste container. NUS Hazardous Waste Label and GHS label must be placed on the container upon the start of accumulation. Wastes are collected by the licensed waste contractor. It is good practice to dispose chemical waste within 90 days from date of generation.


12. 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.

13. 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.

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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.

14. 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

15. REVISION HISTORY

Date Revised	Version No.	Author	Summary of Revisions
29-09-2020	002	Chow Wai Lyn Adeline	Update of (5) Training Requirements- NUS online training portal, and (8) Personal Protective Equipment