LABORATORIES ADMINISTRATION

Procedure-specific Biological Risk Assessment: ELISA | EIA (Virology & Immunology)

The Maryland Department of Health – Laboratories Administration's Laboratory Risk Assessment is intended to guide laboratory staff through the risk assessment process for the work they regularly perform. Throughout this process, a mindset of "what COULD go wrong" should be maintained. The goal of the risk assessment process is to identify and minimize all potential risks that may adversely affect 1) the health and safety of laboratory staff, 2) the health and safety of non-laboratory staff, 3) the health and safety of the general public, and 4) the quality of work being performed. For additional information on biosafety and the risk assessment process, please refer to the Biosafety Risk Assessment SOPM.

This risk assessment is developed for the enzyme-linked immunosorbent assay (ELISA) and the enzyme immunoassay (EIA). ELISA/EIA are plate-based assay technique designed to detect and quantify specific antibodies produced against infectious pathogens. In the assay, antigen of interest (e.g. Zika, Hepatitis, etc.) is immobilized by direct adsorption to the assay plate or by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection). Maryland Department of Health Laboratories Administration routinely use ELISA/EIA to confirm presence of specific pathogens in patient's specimen. The washer and plate reader used in this assay are provided by Bio-Tek and the pipettes are purchased from Gibson/Rainin/MLA.

This risk assessment addresses enhanced precautions, including personal protective equipment (PPE), for handling specimens from patients who may be at risk of having infection from various pathogens. Additional potential hazards and mitigations should be added by your laboratory depending on the pathogen and according to the testing performed.

Laboratory Unit	
Date of Assessment	
Name of Organism(s)/Agent(s)	List of organisms are in Appendix A

Laboratory Risk Management Worksheet: ELISA-EIA

Laboratory Section and/or Procedure: Procedure-specific Risk Assessment for ELISA-EIA				**Date Prepared:** **Date of revision:**	
Prepared by:			**Title/Position:**		
Phase of Testing | Procedure | Division	**Risk Component | Potential Hazards | Errors**	**Initial Risk Level**	**Control Measures**	**Implementation / Additional information**	**Responsible staff and When Will it be Done**
Pre-analytical Receiving clinical specimens and or isolates Accessioning	Aerosols, surface contamination (bench top), broken package, broken specimen tubes, leakage of specimen tubes - may cause exposure to blood, bloodborne pathogens (BBP's), toxins and or infectious pathogens. Sharps hazard - broken/cracked tubes. Chemical hazard - 70% ethanol and or 10% bleach - cause skin irritation, ingested or inhaled causes burns	High	● PPE: lab coat, single gloves, and safety glasses ● Accessioning must be performed in BSC for respiratory specimens tested for Influenza and Bordetella pertussis and should be performed in BSC for other clinical specimens ● Double bag leaking and/or broken specimen container with absorbent material in a biohazard bag prior to disposing in sharps container and autoclave ● For dropped specimen, treat the scenario as a spill - refer to spill response section below for details	Risk level after control measures for accessioning performed on bench top: Medium to high depending on the pathogen. All staff are trained and competent in the procedures described in the Sample Accessioning SOP and follow established laboratory practices. ● Disinfect work surface and change gloves after handling leaking/broken specimen ● After appropriate holding time, discard double bagged broken/leading specimens in a sharps container ● After completion of work, disinfect work-stations (BSC and bench-top) with 10% bleach solution followed by 70% ethanol	Laboratory Supervisor, Laboratory Manager and Division Chief

Pre-analytical Transfer of specimen and or samples to Virology and Immunology Accessioning	**Aerosol, splash, and spill**: Aerosols, spills, splashes, breakage or dropped specimen container - may cause exposure to blood, blood-borne pathogens (BBP's), and or infectious agents. **Sharps hazard -** broken/cracked tubes. **Chemical hazard** - 70% ethanol and or 10% bleach - cause skin irritation, if ingested or inhaled cause burns	High	For transport of specimen between Accessioning and V&I Division ● PPE: lab coat, gloves, and safety glasses ● Specimen must be secured in leak proof, durable and clearly labelled container(s) during transport ● A cart must be used to transport multiple containers to testing areas ● For dropped specimen tubes/bacterial culture plates, treat the scenario as a spill - refer to spill response section for details	Risk level after control measures: Low. ● Weekly decontaminate container(s), racks, and cart by spraying with 70% ethanol ● Autoclave containers, if necessary and as appropriate	Laboratory Supervisor, Laboratory Manager and Division Chief
Pre-analytical Triage, transfer of specimen between testing areas, storage Virology and Immunology	**Aerosol, splash, and spill**: Aerosols, surface contamination, breakage, accidental spill or dropped specimen - may cause exposure to blood, bloodborne pathogens (BBP's), toxins and or infectious agents. **Sharps hazard** : broken/cracked tubes. **Chemical hazard** : 70% ethanol and or 10% bleach – can cause skin irritation, harmful if ingested or inhaled, and can cause burn	High	● PPE: lab coat, gloves, and safety glasses ● Use separate container to sort/transfer rack containing specimen. Container should be shatter and leak proof, durable and clearly labelled ● Use of cart for specimen transport between testing areas is highly recommended ● For dropped or broken specimen tubes and accidental spill, treat the scenario as a spill - refer to spill response section for details	Risk level after control measures: Low. All staff are trained and competent in the procedures described in the ELISA-EIA SOP and follow established laboratory practices. ● Leaked specimen tubes should be handled in a BSC - use appropriate disinfectant to wipe exterior of specimen tube, wrap the tube with absorbent material and put in a biohazard bag. ● Disinfect the area/container where leaked tube was placed. ● After completion of work process, container(s), racks, and cart should be decontaminated with appropriate disinfectant.	Laboratory Supervisor, Laboratory Manager and Division Chief

Analytical Preparation of specimen for testing ELISA-EIA testing Virology and Immunology	**Specimen preparation - Aerosol, splash, and spill:** Aerosols, accidental spill, specimen centrifugation, specimen transfer to microfuge tubes (if applicable), specimen inactivation (if applicable), and specimen dilution (if applicable). **ELISA-EIA assay - Aerosol, splash, and spill:** Aerosols, accidental spill, dropped specimen, dropped plates/strips, specimen addition to coated plate/strips, washes, addition of STOP solution, plate reading, removal and disposal of plate/strips. **Chemical hazard**: 70% ethanol, LopHene and or 10% bleach – can cause skin irritation, harmful if ingested or inhaled, and can cause burn	High	● PPE when manipulating arbovirus: lab coat, gloves, and safety glasses. Sleeves and double gloves while handling arbovirus under BSC prior to inactivation. PPE post inactivation and other specimen transfer and testing – lab coat, gloves, and safety glasses ● All procedures involving vortex and centrifuge shall be conducted under BSC. In exceptional cases, if work is conducted on bench top, specimen tubes should be placed in centrifuge buckets to contain/limit aerosol risk ● Use of pipette tips with barrier filters is highly recommended during specimen manipulation ● After ELISA wash steps, residual buffer should be removed by wrapping absorbent material around the plate or strips and gentle tapping on absorbent material to reduce aerosols ● ELISA plates/strips should be carried in a secondary container to and from the plate reader to avoid spills ● Dispose all pipette tips and other disposables in clearly marked biohazard waste container ● Disinfect benchtop areas where testing is performed with appropriate disinfectant after test completion ● For dropped or broken specimen tubes and accidental spill, treat the scenario as a spill - refer to spill response section for details	Risk level after control measures for samples not processed in BSC: Medium. Risk level for samples processed in BSC: Low All staff are trained and competent in the procedures described in ELISA-EIA SOP and follow established laboratory practices. ● Pathogens tested for EIA/ELISA are highly infectious. Pathogens with known aerosol transmission route (arboviruses, measles, mumps, etc.) must be manipulated in a BSC prior to inactivation. Inactivated samples (e.g. Arboviruses) can be manipulated on benchtop. Measles and mumps samples will need to be manipulated in a BSC for IgM – ELISA testing as these samples are not inactivated. ● Transmission route of pathogen varies and therefore specimen must be manipulated following standard BSL-2 practices ● Standard BSL-2 PPE should be worn at all times. No exposed skin while working with pathogens ● If BSC is used, keep materials on sides to ensure proper air flow and limit traffic around BSC ● Bring necessary materials into the BSC or on bench top prior to beginning work to prevent airflow disruption and or accidental spills ● All materials including small instruments must be surface decontaminated prior to removal from BSC ● ELISA kit contains chemicals that	Laboratory Supervisor, Laboratory Manager and Division Chief

				may be reactive with certain disinfectants. Please refer to MSDS or package insert of each assay kit prior to use ● Disinfectants such as sodium hypochlorite should be used to clean spills	
Analytical Specimen retention Virology and Immunology	Specimen retention - loss of samples; Waste disposal - contamination of waste container surfaces.	Medium	● Use appropriate laboratory protocol for laboratory sample retention, storage and inventory ● Disinfect outside of waste containers prior to removal from the laboratory - 10% bleach disinfectant, followed by wiping down all surfaces with 70% ethanol.	Risk level after control measures: Low. ● Autoclave all PPE used in specimen handling waste and testing	

Spill Clean-Up:
Inside a Biological Safety Cabinet (BSC) (Reference Laboratories Administration Safety Manual 2.6.1 Biological Spills inside a BSC)

Response: Remain calm. Alert co-workers in the immediate area. Remove contaminated PPE, turn exposed area inward and put item in biohazard waste container. Inform Supervisor and Safety & Security Officer (SSO) call 443-681-3792 immediately. Have a biological spill kit ready prior to beginning of clean-up process.

- Leave the BSC turned on
- Don new PPE
- Use tongs or forceps for sharp objects, and dispose items into a sharps container
- Work carefully, starting at the edges and working towards the center, cover the area with absorbent materials
- Select appropriate disinfectant and pour material onto the spill area. A germicidal disinfectant such as a 0.04 % solution LopHene or a fresh 1:10 dilution of sodium hypochlorite (household bleach) should be used in wiping down all accessible cabinet surfaces
- Allow at least 30 minutes of contact time with the germicide before removing items from the cabinet. NOTE: spills that contain organic material, such as blood or feces, will interfere with the microbicidal activity of many agents by acting as physical barrier. As a result, these spills may require preliminary wiping in order to reduce the organic load prior to disinfection
- If the catch basin is contaminated contact the SSO for further instructions on decontamination process
- If the spill overflows into the interior of the cabinet, inform the immediate supervisor and the SSO. This requires a Biological Safety Cabinet certification technician to perform the decontamination
- All items within the cabinet should be placed in autoclave cans for transport to the autoclave. Items that cannot be autoclaved, such as viable cultures to be retained, should be wiped carefully with the disinfectant
- Run the cabinet for 15 minutes after cleanup is completed, and before routine use is resumed

- Discard clean-up materials and PPE, into biohazard waste bag or container

Spills Outside a BSC (Reference Laboratories Administration Safety Manual 2.6.2 Biological Spills in the laboratory)

Response: Remain calm. Alert co-workers in the immediate area. Remove contaminated PPE, turn exposed area inward and put item in biohazard waste container. Wash hands with disinfectant soap and evacuate. Inform Supervisor or Division Chief, Principle Investigator and SSO immediately. Call 3911 if necessary to reach key personnel. Allow 30 minutes for any aerosol resulting from the spill to settle before beginning the clean-up process. SSO, or designee, and Lab Director will determine if BSL-2 Open Lab will need to be shut down. Have a biological spill kit ready prior to beginning of clean-up process.

- SSO will assist lab employee with clean-up
- Don appropriate PPE
- Assess splash and spill area
- Select appropriate disinfectant and pour material onto the spill area. A germicidal disinfectant such as a 0.04 % solution LopHene or a fresh 1:10 dilution of sodium hypochlorite (household bleach) should be used in wiping down all accessible cabinet surfaces
- Flood the spill area with additional disinfectant and/or wipe down the area with new paper towels soaked with disinfectant.
- Allow at least 30 minutes of contact time
- Use tongs or forceps for sharp objects, and dispose items into a sharps container
- Work carefully, starting at the edges and working towards the center, cover the area with absorbent materials
- Continue to disinfect bench tops, bench legs, chairs, equipment floor in the splash area with additional disinfectant and/or wipe down the area with new paper towels soaked with disinfectant
- Discard clean-up materials and PPE, into biohazard waste bag or container

Risk Management Worksheet

To be completed by laboratory staff during and/or after they perform work with control measures in place	**Yes**	**No**
1. Are the planned control measures sufficient and effective in minimizing the level of risk?		
2. Have there been any changes to the planned control measures?		
3. Are any changes and/or additional control measures required in the future?		
Details:		

Risk Management Worksheet Authorization (to be signed by Unit Supervisor and Division Manager/Chief)	Review completed by:	Position/Title:
	Signature:	Date:
	Review completed by:	Position/Title:
	Signature:	Date:

Appendix A

Pathogen(s) tested by ELISA-EIA (See examples below)
Chlamydia / Chlamydia trachomatis (Lymphogranuloma venereum (LGV))
Epstein Barr Virus
Hepatitis A, B, C
Herpes Simplex Virus
Legionella pneumophila
Measles
Mumps Virus
Mycoplasma pneumoniae
Rubella
Syphilis (Treponema pallidum)
Toxoplasma gondii
Varicella Zoster Virus (VZV)/Chickenpox

Risk Assessment - Appendix B

Biological Safety Risk Assessment Checklist	
Item	**Response**
1. Indicate the biosafety level (BSL) established in each Unit and required PPE. (BSL-1, BSL-2, Enhanced BSL-2, BSL-3, N/A)	
2. Is there potential for aerosol generation?	E.g. Yes (Pipetting, Centrifugation, manipulation of specimen, dilution, opening/closing collection tubes and micro centrifuge tubes)
3. Equipment such as centrifuges, incubators, freezers involved in the use and storage of infectious materials have biosafety labels affixed?	E.g. No. Laboratories (open and closed) are labelled with biohazard warning label at each entrance
4. Buckets with safety caps/cups or aerosol tight rotor lids used when centrifuging infectious materials?	
5. Is health monitoring performed in each Unit?	
6. Are vaccines recommended for work in each Unit?	
7. Are sharps used?	
8. Does work include a Biological Safety Cabinet?	E.g. Only for endemic arbovirus, arbovirus triplex panel, measles and mumps virus IgM testing Class II BSCs are certified annually

Chemical Safety		
Item	**Response (Yes)**	**Response (No)**
1. Proper labeling: All containers labeled with the name of chemical?		
2. Fire Department Permit posted on the laboratory door?		
3. Updated chemical inventory?		
4. Material Safety Data Sheets/Safety Data Sheets accessible to staff?		
5. Incompatible chemicals segregated?		
6. Flammable liquids stored: rated chemical cabinets?		
7. Flammable liquids stored: stored in flammable-rated refrigerators/freezers?		
8. Excessive chemicals stored in chemical storage room?		
9. Compressed gas cylinders properly stored in laboratory?		
10. Chemicals stored at eye-level?		
11. Acids and bases stored:		
a. Cabinet?		
b. Labeled area?		
c. Free from metals?		
12. Chemical fume hoods:		
a. Certified within past year?		
b. Sash closed when not in use?		
c. Exhaust air not blocked by large equipment or containers?		
d. Used for hazardous/toxic or flammable procedures?		
Comments:		

Personal Protective Equipment		
Item	**Response (Yes)**	**Response (No)**
1. Laboratory staff aware of personal protective equipment (PPE) requirements for this laboratory		
2. Laboratory staff/employee aware of occupational health information?		
3. Does staff receive annual PPE competency assessments?		
4. PPE Care:		
a. Appropriately stored in laboratory?		
b. Inspected prior to use and in good condition?		
c. Worn in laboratory area?		
5. PPE Required :		
a. Facial shields/eye protection/splash guards?		
b. Disposable aprons, laboratory coats?		
c. Appropriate gloves?		
d. Double gloves required for work under the BSC		
e. Cryo or autoclave gloves?		
6. Closed-toe shoes that cover entire foot worn in laboratory?		
Comments:		

Emergency Preparedness		
Item	**Response (Yes)**	**Response (No)**
1. Emergency contact information posted?		
2. First aid kit maintained?		
3. Biological spill kit maintained?		
4. Staff aware of occupational injury procedures?		
Comments:		

Documentation and Training –		
Item	**Response (Yes)**	**Response (No)**
1. Employee(s) completed right-to-know training?		
2. Employee(s) completed unit-specific training?		
3. Employee(s) read and understand safety and health plans?		
4. Door sign up-to-date and posted?		
5. Laboratory microwaves and refrigerators labeled with “Not for Food or Drink – Biohazard”?		
Comments:		

Waste Management -		
Item	**Response (Yes)**	**Response (No)**
1. Chemical waste containers:		
a. Labeled with chemical name and percent of each chemical?		
b. Properly sealed?		
c. In good condition for transport?		
2. Biohazard waste containers?		
3. Broken glass placed in appropriate receptacle?		
4. Sharps container		
Comments:		

Engineering Controls		
Item	**Response (Yes)**	**Response (No)**
1. Laminar Flow Hoods		
2. Transport Containers		
Comments:		

I, the undersigned, have read and understand the biosafety risks associated with the procedure described in the SOP. I understand that I must follow the biosafety measures recommended in this risk assessment while conducting the procedure(s). Read & Signed by (At Risk Employees) (Laboratories Administration Safety Manual, section 12.0):		
Unit Name: Accessioning		
Name	**Signature**	**Date**
Unit Name: Virology and Immunology		
Name	**Signature**	**Date**

This risk assessment should be reviewed annually or after any major changes (e.g., new facility, new employees, new technology, new method, changes in information for organism/agent, etc.). Reviews have been carried out on the following dates. Minor changes should be recorded under Amendments. Major changes require a new risk assessment to be performed.

Prepared by:			
	Printed Name	**Signature**	**Date**

Reviewed by:			
	Printed Name	**Signature**	**Date**

Approved by:			
	Printed Name	**Signature**	**Date**

Revised by:			
Version	**Changes made**	**Printed Name & Signature**	**Date**
V1			

Reviewed by:			
Version	**Printed Name**	**Signature**	**Date**

Approved by:			
Version	**Printed Name**	**Signature**	**Date**