March 1, 2016

Dear Public Health Laboratory Directors and Biosafety Officials:

Enclosed is a risk assessment template developed to assist you with preparing for Zika virus testing in your facility.

Each facility must perform a risk assessment for Zika virus testing based on their situation and facility needs. The APHL risk assessment template serves as a guideline for the receipt and testing of clinical specimens for Zika virus based on current information from the Centers for Disease Control and Prevention (CDC).

There are a few issues to consider before proceeding with the risk assessment and sample studies:

- Clinical specimens may come from patients who have the potential for infection or co-infection with other viruses such as chikungunya, which the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition guidelines recommend be handled in a Biosafety Level 3 (BSL-3) environment. The primary reason to use a BSL-3 versus BSL-2 laboratory environment is to reduce the risk of aerosol transmission of a pathogen. It is important to note that there has been very few reported cases of chikungunya transmission via aerosol. The only documented cases of aerosol transmission appear in a 1980 article that does not specify the nature of the work nor the specifics of the laboratory facilities.[i] If a laboratory receives specimens for Zika virus testing that may contain chikungunya, the appropriate laboratory biosafety level and precautions for handling these specimens should be based on each laboratory's capacity and its own risk assessment that reflects both the work and the conditions of the laboratory.
- Both Zika and chikungunya viruses are arboviruses whose primary route of transmission is through the blood. Evidence indicates that Zika is spread to people primarily through the bite of an infected *Aedes* species mosquito. Zika can also be transmitted from mother to child during pregnancy and at birth and through blood transfusion and sexual contact. Thus, it is critical that laboratories mitigate the risk of percutaneous exposure to Zika.
- Following risk assessments, some laboratories have determined that it is appropriate to work with specimens that may contain chikungunya in BSL-2 conditions, using a Biological Safety Cabinet (BSC) with additional precautions including heat inactivation. Chikungunya virus is susceptible to heat inactivation at 56 degrees Celsius for 120 minutes.[ii] Other laboratories have chosen to work in BSL-2 conditions with enhanced Personal Protective Equipment (PPE), such as disposable back-closing gown, double gloves, disposable sleeve covers and a disposable full-face shield.
- Until the association between Zika virus infection and congenital microcephaly is better characterized, pregnancy (or chance of pregnancy) should be considered. The involvement of pregnant workers or those who may become pregnant should be an informed decision made by the individual in consultation with their medical provider and the occupational health physician.

Refer to the CDC website for current information regarding Zika virus and further guidelines for laboratory work: http://www.cdc.gov/zika/index.html.

For any Zika related questions, please contact the APHL Emergency Operations Center (EOC) at eoc@aphl.org.

Regards,

Brit James Hart, MS
Manager, Biosafety & Biosecurity, Public Health Preparedness & Response

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[i] Scherer, W. F., Eddy, G. A., & Monath, T. P. (1980). Laboratory safety for arboviruses and certain other viruses of vertebrates. *American Journal of Tropical Medicine and Hygiene, 29*(6), 1359-1381.

[ii] Huang, Y. S., Hsu, W., Higgs, S., & Vanlandingham, D. L. (2015). Temperature Tolerance and Inactivation of Chikungunya Virus. *Vector-Borne and Zoonotic Diseases, 15*(11), 674-677.

Template for Public Health Laboratory Risk Assessment for Zika Virus Testing

Important Note:

Please review the APHL cover letter prior to using this template which is designed to assist laboratories in the development of their risk assessment for the testing process for Zika virus. The APHL template may not be an all-encompassing plan as each facility will have their laboratory specific risk assessment procedures. Risk assessments should be performed by technical supervisors, laboratory technicians who perform work with the agent, and safety and quality specialists to maximize efficacy.

There has been limited experience handling specimens potentially contaminated with Zika virus in a clinical laboratory using current specimen handling procedures and automated instrumentation. **This risk assessment addresses enhanced precautions, including personal protective equipment (PPE), for handling specimens from patients who may be at risk of having a Zika virus infection. Additional potential hazards and mitigations should be added as determined by your laboratory and according to the testing performed.**

Laboratory Unit/Section	
Date of Assessment	
Names of Assessors	
Name of Organism/Agent	**Zika Virus**
Activity or Procedure	

Specimen Receipt and Transport within the Laboratory

Procedure	Potential Hazard(s)	Control/Protection	Additional Information
A. Package receipt and transfer of packages to testing area	Leaking Package	• Place leaking package in plastic bag and transfer to a Biological Safety Cabinet (BSC) • PPE: nitrile/latex gloves, lab coat, safety glasses	• Immediately contact Safety Officer • Disinfect exterior of sealed plastic bag prior to transfer to testing area • Disinfect area where leaking package was placed
	Unexpected delivery	• Immediately transfer to BSC • Deliver specimen in original category B packaging to testing area	• Notify key staff of expected package delivery • All category B Packages opened with safety blades in certified class II BSC with safety precautions
B. Transport of Specimens between testing areas	Breakage of the specimen container	• Specimens should be transported in a clearly labeled, durable, shatter and leak-proof transport container directly to the specimen handling area of the laboratory	• Decontaminate all surfaces of transport container prior to reuse

C. Preparation of Specimens for testing	Aerosolization, Splash/Splatter	• Use only trained personnel with experience in infectious disease testing, minimize the number of workers handling specimens if possible • Work inside a certified class II BSC with the sash at the appropriate level • Minimize unnecessary movements while working in the BSC. Follow acceptable BSC practices • Use enhanced BSL-2 practices that include the following PPE: fluid resistant back-closing gown, double gloves and safety glasses, goggles or disposable full-face plastic shield • Limit the traffic around the BSC. • In the BSC, work over a decontamination solution moistened plastic backed absorbent pad • Use only pipette tips with barrier filters • Have a dedicated rigid waste container in the BSC • If making aliquot tubes: Wipe outside of primary and aliquot tubes before removing from BSC – Go to "Step D"	• No exposed skin inside the BSC • Bring all necessary material into the BSC before starting to work. • Including decontamination with an intermediate level disinfectant • Minimize use of sharps. Dispose of all pipette tips and sharps in the dedicated container in the BSC • Specimens, equipment, and all materials must be decontaminated before removing from BSC • DO NOT set up any viral cultures

For PCR Testing: Specimen Processing

D. Viral RNA extraction for PCR based testing	Accidental exposure (droplets)	• Perform in BSC • Use BSL-2 practices that include the following PPE: fluid resistant back-closing gown, double gloves, glasses, goggles or full face shield, (eyes and mucous membranes covered). • Vortex inside BSC. Ensure microcentrifuge tube (with o-ring seal) is tightly sealed	• Prevent contact with skin, eyes and clothing • Wash exposed skin with soap and water immediately. Remove all contaminated clothing or shoes • After RNA extraction, specimen is no longer infectious and may be handled using BSL-2 practices
	Lysis Buffer reactivity	• Do not use bleach or acids as a decontaminant for spills • Most RNA extraction kits use guanidinium isothiocyanate. Do not add bleach or acidic solutions to solutions containing guanidinium isothiocyanate, as reactive compounds and toxic gases are formed	• If accidental mixture of guanidinium isothiocyanate and bleach/acid occurs, remove PPE, exit lab as soon as possible and notify Safety Officer and supervisor. Post Do Not Enter sign on lab. Do not enter BSL-2 for at least 1 hour
E. Vortexing and Centrifuging	Aerosolizing	• Vortex inside the BSC. Ensure microcentrifuge tube (with o-ring seal) is tightly sealed • Transfer samples to buckets inside BSC • Move buckets for centrifuging on the benchtop • Load and unload buckets in the BSC	• Specimens containers, equipment, and all materials must be decontaminated before removing from BSC

F. After specimen inactivation (if applicable) and before removal of specimen from the BSC	Accidental transfer of contaminated material from the BSC	• Wipe all tubes with disinfectant before removing from BSC • Place remaining specimen in re-sealable plastic bag (or box) • Disinfect exterior of bag before removing from BSC • Change gloves • Store specimen(s) in refrigerator inside BSL-2 suite	• Disinfectant containers and work surfaces • Dedicated waste bag for gloves and other waste in the BSC
G. Post RNA extraction BSC Decontamination	Contamination of BSC surfaces	• Wipe the inside of the BSC with disinfectant • Remove all PPE and discard into medical waste stream	• 10% bleach disinfectant is used: contact time = 10 minutes followed by wiping down all surfaces in the BSC with 70% alcohol and allow to air dry
H. Waste autoclaving	External contamination of waste containers	• Disinfect outside of waste containers before removal from BSC (prior to disinfecting BSC)	• 10% bleach disinfectant is used: contact time = 10 minutes • Autoclave all PPE used in specimen handling waste and testing

For Serology Testing

Procedure	Potential Hazard(s)	Control/Protection	Additional Information
A. Unpacking submitted virus serology samples	Contamination of packaging	• PPE: back-closing disposable gown, gloves, and disposable full-face shield • Outer package is opened on the counter, inner transport container is transferred to the BSC in the same room • Accession samples according to protocol	

B. Preparation of Serum Specimens for Testing	Aerosolization, splash/splatter	• Minimize the number of workers handling the specimens • Work inside a certified class II BSC with the sash at the appropriate level • Minimize unnecessary movements while working in the BSC. Follow acceptable BSC practices • Use BSL-2 practices that include the following PPE: fluid resistant back-closing gown, double gloves and safety glasses, goggles or disposable full-face plastic shield • Limit the traffic around the BSC. • Lay down a fresh pad in the BSC • Process the sample and aliquot of sample (serum) for testing • If sample may contain Chikungunya virus, consider heat inactivation at 56 degrees Celsius for 120 minutes	• If possible, only bring one set of newly labeled tubes (serum) for a single patient into the BSC • Use only extended pipette tips with barrier filters • Have dedicated rigid waste container in the BSC for tips and tubes • Change gloves between specimens • No exposed skin inside the BSC • Use a dedicated waste bag for gloves within the BSC • Bring all necessary material into the BSC before starting to work • Minimize use of sharps Dispose of all pipette tips and sharps in the dedicated container in the BSC • Specimen containers, equipment, and all materials must be decontaminated before removing from BSC • DO NOT set up any viral cultures

C. Specimen Transfer	Accidental Exposure, breakage of specimen transport containers	• Place aliquots for testing inside a plexi-glass transport container • Spray the outside of the container with 10% bleach • Place the transport container within a 4 degree refrigerator until ready for testing • Transport final serum samples in a biosafety transport container	
D. Serum Dilution	Aerosolization, splatter/splash	• PPE: disposable gown, double gloves, disposable sleeves, and disposable full-face shield • Dilute patient serum by dispensing serum into wash buffer in a screw cap tube in the BSC if heat inactivation is not used • Mix sample (either with capped tube and vortex or pipette)	• Use only extended pipette tips with barrier filters • Change tips in between specimens • Have dedicated waste container for tips and tubes
E. Addition of Sample to Coated Plate	Aerosolization, splatter/splash	• PPE: disposable gown, gloves, and disposable face shield • Cover the plate and incubate	• Use only extended pipette tips with barrier filters • Change tips/gloves in between specimens • Have dedicated waste container for tips and tubes

F. Washing of Plates	Contaminated waste area	• PPE: disposable gown, gloves, and disposable full face shield • Use carrier or holder to transport plate over to automated plate washer • Use plastic plate covers when transporting plates to reduce aerosols • If feasible, consider washing plate in BSC, if not feasible wash plate in low traffic area of laboratory, with appropriate PPE protecting mucus membranes and/or utilizing engineering controls, such as a plastic barrier/shield on plate washer • Waste is transferred to satellite container at previously agreed upon time periods	
G. Waste Autoclaving	External contamination of waste containers	• Disinfect outside of waste containers before removal from BSC (prior to disinfecting BSC)	• 10% bleach disinfectant is used: contact time = 10 minutes • Autoclave all PPE used in specimen handling waste and testing
H. Retention of remaining original Specimens.	Loss of sample	• Residual specimen retained as control material for validation and proficiency test materials, etc. • Use appropriate laboratory protocol for laboratory sample inventory	

Biological Safety	
Item	**Response**
1. Indicate the biosafety level (BSL) established in this unit. (BSL-1, BSL-2, BSL-3, N/A)	BSL-2: Sample processing including extraction steps BSL-2: sample storage BSL-2: PCR and associated reagent preparation
2. Is there potential for aerosol generation?	*Yes, please indicate the task. (i.e., sonicating, vortexing, etc.)* Vortexing Pipetting Centrifuging Opening and closing collection tubes and microcentrifuge tubes
3. Equipment such as centrifuges, incubators, freezers involved in the use and storage of infectious materials have the biosafety label affixed?	Equipment needed (all to have biosafety label affixed): BSC Centrifuge 2 freezer/refrigerators (1 for specimen, 1 for reagents) Pipettes (dedicated to Zika testing) Heat block (maybe)
4. Buckets with safety caps/cups or aerosol tight rotor lids used when centrifuging infectious materials?	Yes for the Centrifuge
5. Is health monitoring performed in this Unit?	*If yes, please indicate frequency and the process.* [LAB TO COMPLETE]
6. Are vaccines recommended for work in this Unit?	*If yes, please indicate how employees are informed of the vaccines? What vaccines are recommended?* There is no commercially licensed vaccine available for Zika virus.
7. Are sharps used?	*If yes, please indicate the sharp (needle, blades, etc.) Does the sharp include safety device feature?* Pipette tips
8. Does work include a Biological Safety Cabinet?	*If yes, indicate if the BSC has been certified within the past year, the air vents are not blocked, and the sash is in place and operable?* [LAB TO COMPLETE]

Chemical Safety		
Item	**Yes**	**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. Materials 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 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	**Yes**	**No**
1. Laboratory staff aware of personal protective equipment (PPE) requirements for this laboratory		
2. Do staff receive annual PPE competency assessment?		
3. PPE Care:		
a. Appropriately stored in laboratory?		
b. Inspected prior to use and in good condition?		
c. Not worn in laboratory area?		
4. PPE Selected:		
a. Facial shields/splash guards?		
b. Disposable laboratory coats?		
c. Nitrile gloves?		
d. Respiratory protection?		
i. Users are enrolled in a respiratory protection program?		
e. Cryo or autoclave gloves?		
f. Over sleeves/booties/bonnet		
5. Closed-toe shoes that cover entire foot worn in laboratory?		
Comments:		

Emergency Preparedness		
Item	**Yes**	**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	**Yes**	**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	**Yes**	**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:		
3. Broken glass placed in appropriate receptacle?		
Comments:		

Engineering Controls		
Item	**Yes**	**No**
1. Laminar Flow Hoods		
2. Transport Containers		
3. Sharps Container		
Comments:		

At Risk Employees: ***Due to the possible association of Zika infection and birth defects (congenital microcephaly), pregnant women should have minimal involvement in work with Zika virus.***		
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.

Approved by:

	Printed Name	**Signature**	**Date**
Principal Investigator			
RO/ARO			
Section Manager			
Unit Manager			

Reviewed by:

Testing Personnel	**Printed Name**	**Signature**	**Date**

LABORATORY SPECIMEN(S) HANDLING LOG

One Form For Each Patient – Multiple Specimens Per Patient May Use Single Log Form

Receiver of Specimen(s):

1) Date Received:_____Time:_____Intake #: _____

2) Method of Delivery:

☐ FedEx/UPS ☐ Storeroom ☐ Department of Health Driver ☐ Messenger/Name: _____

3) Laboratory Number Call for Pick-Up:_____Time Picked Up By Laboratory: _____

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To Be Completed by Laboratory: Picked Up from Receiver By: _____

Purpose: ☐ Patient Clinical Test ☐ QC ☐ Training/Validation ☐_____Proficiency

Number of Specimens in Package:_____Sample Type(s): _____

Accession #: (1)_____(2) If Applicable: _____

Patient ID # on Submission Form:_____Submitter: _____

Function	Name of Responsible Technologist	Time
Delivered to Lab/BSL-2		
Accessioned By		
BSL-2 Processing		
RNA Extraction		
PCR Reaction Setup/Master Mix/Template		
ABI Run		
ABI Analysis		
Storage of Original Sample(s) Unit: Location:		
Storage of RNA Unit: Location:		
LIMS Result Verification and Report Verification of Report Received ☐		
Results Reported To: ☐ CDC ☐ _____ Specimen(s) Shipped To: ☐ CDC ☐ _____		
Other		
Other		

Questions pertaining to this template should be directed to emergency.preparedness@aphl.org.