Interim Biosafety Guidelines for Laboratories Handling Specimens from Patients Under Investigation for Ebola Virus Disease

Laboratories receiving specimens from patients under investigation for Ebola Virus Disease (EVD) must be aware that improper handling of these specimens poses serious risk to the health of laboratory personnel. The minimum requirement for handling these types of clinical specimens is Containment Level 2 (CL2)[1]. Due to the nature of EVD, special operational practices are highly recommended and are outlined below.

*No virus culture should be attempted outside of a Containment Level 4 laboratory that has been certified by the Public Health Agency of Canada.

Laboratory Handling and Transporting Specimens from Patients Under Investigation for EVD

Laboratory personnel handling these types of clinical specimens are recommended to don the following personal protective equipment (PPE);

- double gloves;
- fluid-resistant, impermeable laboratory gown, over the lab coat;
- either a combination of approved particulate respirators (e.g., N95, or N100) and eye protection (*e.g.* goggles/face shields/shroud), or powered air purifying respirators (PAPRs).

Specimens from patients under investigation for Ebola virus disease should not be manipulated on an open bench.

Activities with the potential to create infectious aerosols(e.g., pipetting, centrifugation, aspiration, slide preparation) should be carried out in a certified Biological Safety Cabinet (BSC)[1], [2], [3], in a minimum CL 2 laboratory.

Blood cultures should be prepared in a closed system. When this is not possible, manipulations should be undertaken in a certified BSC in a CL 2 laboratory with the use of appropriate PPE as identified above.

Sub-culturing of blood cultures has the potential to generate aerosols and should be done only when essential to patient care. This should be done in a certified BSC with the use of additional PPE as identified above, and the decision to subculture should be predicated on the clinical status of the patient and based on an on-going risk assessment.

Sample separation (*e.g.* blood, serum) should be undertaken using sealed centrifuge cups or a sealed centrifuge head that are unloaded in a certified BSC.

Blood smears: Malaria should be ruled out from travellers returning with a fever. Only thin Blood smears should be done (no thick smears) and repeated as necessary (e.g., if the first thin Blood smear is negative). It is recommended that dipstick tests from patients that are under investigation for EVD, should be performed only on inactivated blood. All manipulations should be undertaken in a certified BSC with the use of appropriate PPE as identified above. After air drying in the BSC, thin Blood smears should be fixed with absolute methanol (for 5 minutes) followed by 10% buffered formalin (for 15 minutes). All reagents should be sterilized prior to disposal.

PCR, if available on-site, may be considered a safer option, as routine extraction procedures are sufficient to inactivate the virus. Inactivation manipulations should be undertaken in a certified BSC in a CL 2 laboratory with the use of appropriate PPE as identified above.

Automated analyzers may be used after performing a local risk assessment for the potential for aerosol generation. If ports or vents are present on the system that may generate aerosols, it is recommended that the machine be contained, either in a BSC, plexiglass or flexible film cover, or through the use of HEPA's. After use, analyzers should be disinfected as recommended by the manufacturer or with a 500 parts per million solution of sodium hypochlorite (1:100 dilution of household bleach: 1/4 cup to 1 gallon water) after use.

Additional Operational Considerations

- Minimize activities that may generate aerosols whenever possible (e.g. mixing samples by pipetting, centrifugation);
- Restrict the use of glass or sharps wherever possible, and ensure staff are well trained in routine practices[4], including biosafety;
- Clearly pre-label tubes prior to the collection of patient specimens, and segregate samples when handling in the laboratory;
- Testing requisitions should be clearly labelled as Ebola Suspect, and containers should be labelled as such on the exterior of the container;
- Ensure that all samples are securely stored and accessed only by authorized personnel;
- Phlebotomy should be performed by trained staff that are proficient in collecting blood;

- Minimize unnecessary activities and personnel in the area during sample processing whenever possible;
- Designated personnel, laboratory areas and equipment should be utilized for testing.

Occupational Health

Potential exposures to these specimens must be reported immediately according to your institution's policy and procedures.

Decontamination

- Specimen containers to be transported for further/confirmatory testing to be surface decontaminated using an effective disinfectant prior to packaging. A list of effective disinfectants can be found within the Pathogen Safety Data Sheet for EVD[5]:

  Example of effective disinfectants:

  - Ebola virus is susceptible to 3% acetic acid, 1% glutaraldehyde, alcohol-based products, and dilutions (1:10-1:100 for ≥ 10 minutes) of 5.25% household bleach (sodium hypochlorite), and calcium hypochlorite (bleach powder)[2],[3],[6],[7],[8]. The WHO recommendations for cleaning up spills of blood or body fluids suggest flooding the area with a 1:10 dilutions of 5.25% household bleach for 10 minutes for surfaces that can tolerate stronger bleach solutions (e.g., cement, metal)[8]. For surfaces that may corrode or discolour, they recommend careful cleaning to remove visible stains followed by contact with a 1:100 dilution of 5.25% household bleach for more than 10 minutes[8].

- Remove PPE in a manner that minimizes contamination of the skin and hair. It is recommended to avoid any contact between soiled items (*e.g.*, gloves, gowns, respirators) and any area of the face. Contaminated clothing and PPE are to be appropriately sterilized.
- Wash hands thoroughly immediately after the removal of PPE.

Disposal

All potentially contaminated liquid and solid materials should be appropriately sterilized before disposal, reuse or removal from the laboratory.

Spill Consideration

The area should be evacuated and secured. Let aerosols settle for a minimum of 30 minutes. Accidental spills of potentially contaminated material should be covered with absorbent paper towels, liberally covered with disinfectant, and then left to soak for 15 minutes before being wiped up. Following the removal of the initial material, the disinfection process should be repeated. Individuals attending to this task should wear protective attire. As per standard laboratory spill response procedures, PAPRs or other approved respirators (e.g., N95, N100) should be considered for those involved in the clean-up activity. Disposable gloves, impermeable gowns and protective eye wear are to be removed immediately after completion of the process, placed in an autoclave bag, and sterilized prior to disposal[5].

Transportation

Clinical samples from patients under investigation for Ebola virus disease should be shipped separately from other samples.

Laboratories should maintain a log of all individuals who have handled, decontaminated and transported these types of clinical specimens.

If transportation delays are expected, samples should be refrigerated or frozen at -70°C.

Within Hospital

Specimens should be placed in a durable, leak-proof secondary container for transport within a facility. To reduce the risk of breakage or leaks, do not use any pneumatic tube system for transporting suspected EVD specimens[3].

Shipping Samples to the National Microbiology Laboratory (NML)

Liaise with the provincial public health laboratory of your jurisdiction to coordinate with the NML Operations Center Director (OCD) at 1-866-262-8433. This number is staffed 24/7.

The NML OCD will work with the requesting provincial jurisdiction to activate the Emergency Response Assistance Plan (ERAP). If you require assistance with the shipping process, sample requirements, sample shipping conditions, the NML OCD will connect you with the appropriate subject matter experts.

Packaging, shipping and transport of specimens must comply with the requirements of the *Transportation of Dangerous Goods Regulations*, Transport Canada and the *Dangerous Goods Regulations*, International Air Transport Association.

- For shipments, patient/primary sample specimens should be shipped as (*UN2814, Category 6.2*), and ERAP must be activated.

Concurrent with a request for laboratory services for EVD or other viral haemorrhagic fevers, provinces and territories are requested to notify and provide a clinical history of the patient's illness to the Public Health Agency of Canada Health Portfolio

Operations Centre (HPOC) at 1-800-545-7661. Clarification of or further information may be requested from the patient's clinician in order to optimise the delivery of the requested laboratory service(s).

Contact Information

Please note that this information is based on currently available scientific evidence and is subject to review and change as new information becomes available. Further general biosafety information may be obtained from the Public Health Agency of Canada @ 1-800-545-7661.

References and Resources

1 Canadian Biosafety Standards and Guidelines (CBSG) (1st ed., 2013, Government of Canada) (http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php)

2 Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Haemorrhagic Fever. BDP/EPR/WHO, Geneva March 2008. http://www.internationalbiosafety.org/Organizations/fde5681c-ca94-4a20-827a-0716f524babc/Resources/Emerging%20Issues/WHO%20Infection%20Control%20Guidelines%20Haemorrhagic%20Fever.pdf

3 Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease. CDC. August 2014. http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html#Specimen Handling for Routine Laboratory Testing (not for Ebola Diagnosis)

4 Routine practices are a combination of universal precautions and body substance isolation. http://www.ccohs.ca/oshanswers/prevention/universa.html.

5 Pathogen Safety Date Sheet - Ebola Virus. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ebola-eng.php

6 Mitchell, S. W., & McCormick, J. B. (1984). Physicochemical inactivation of Lassa, Ebola, and Marburg viruses and effect on clinical laboratory analyses. Journal of Clinical Microbiology, 20(3), 486-489.

7 Elliott, L. H., McCormick, J. B., & Johnson, K. M. (1982). Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. Journal of Clinical Microbiology, 16(4), 704-708.

8 World Health Organization (2010). WHO best practices for injections and related procedures toolkit. March 2010. http://whqlibdoc.who.int/publications/2010/9789241599252_eng.pdf?ua=1

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