INSPQ INSTITUT NATIONAL DE SANTÉ PUBLIQUE DU QUÉBEC

Ebola Virus Disease (EVD): Practical Guide for Managing Laboratory Analysis Requisitions for Patients with Suspected EVD

LABORATOIRE DE SANTÉ PUBLIQUE DU QUÉBEC

Version 3.0; updated September 19, 2014

In this Guide:

Given the epidemiological situation concerning Ebola in 2014,[1,2] Quebec's clinical laboratories will have to review their procedures for managing samples potentially containing the Ebola virus, an infectious agent in Risk Group 4 (RG4). The Laboratoire de santé publique du Québec (LSPQ) receives requests for support in researching these agents. All requisitions for analysis of RG4 agents involve the application of the *Transportation of Dangerous Goods Regulations* (TDG), including infectious substances.

The *Plan québécois des urgences infectieuses - Maladies à surveillance extrême*[3] [Quebec plan for infectious emergencies - diseases to monitor extremely closely] is currently under review. The purpose of this guide is to offer information on managing requests for analysis from patients suspected of Ebola virus disease (EVD). The management of tests for confirmed cases will be addressed separately.

The incubation period for the Ebola virus can vary from 2 to 21 days, with an average of 8 days. Direct contact with blood, secretions, organs or other body fluids from infected individuals or animals, live or dead, is considered the principal mode of transmission.[4,5] Evidence of airborne transmission between humans is weak,[6] but transmission has been documented in animal models.[7] The risk of transmission via laboratory aerisolization seems low as a result, but the precautionary principle requires us to take this potential mode of transmission into consideration. There is no risk of transmission during the incubation period before the occurrence of fever, and the risk remains low in the early symptomatic stage.

Indirect contact through exposure to objects contaminated by blood, such as needles or other sharps is well described and is a possible cause of laboratory transmission of EVD.[8-10]

As a result, laboratory activities associated with a risk of transmission include:

- Splattering of infected materials (e.g. blood, CSF, feces, urine or other biological fluids) on broken skin or mucous membranes;
- Injury while handling infected materials;
- Procedures generating aerosols during the handling of specimens.

Québec

Assessment Criteria for Suspected Patients and Definition of a Patient with Confirmed Ebola Virus Disease as of September 19, 2014

SUSPECTED CASE

A. Clinical criteria

Sudden-onset fever lasting at least 24 hours (≥ 38.5°C) with :

- **non-specific flu-like syndrome (e.g. arthralgia, myalgia, fatigue, headaches, cough)**

OR

- **a syndrome compatible with EVD:**
- cutaneomucosal symptoms (conjunctivitis, macular exanthema, dysphagia, coughing); or
- gastrointestinal symptoms (diarrhea, vomiting, abdominal pain); or
- neurological symptoms (confusion, coma, agitation, epilepsy); or
- hemorrhagic symptoms (oozing at vein puncture sites, gingival bleeding, hematemesis, melena, bloody feces, skin hemorrhages, epistaxis).

AND

B. Epidemiological criteria

Scenario 1

Patient with a history of travel to an area at risk* within 21 days preceding the onset of fever AND for whom exposure without appropriate protection, as defined below, cannot be ruled out:

Exposure to an infected case or highly suspected of EVD

- Direct contact with a person (living or deceased) infected, or strongly suspected of being infected, with the virus (e.g. having provided care to; shared the same room or lived under the same roof as; had unprotected sexual relations with; or had contact with the cadaver of during funeral rites);showing hemorrhagic symptoms or with the body of a deceased person infected or strongly suspected of being infected with the Ebola virus;
- Indirect contacts through objects, surfaces, clothing or bedding contaminated by a person (living or deceased) infected, or strongly suspected of being infected, with the virus;

Exposure to medical care or clinical specimens

- Admission for another health problem, exposure to IM/IV injections or visit to a hospital that received patients infected with the Ebola virus.
- Admission for another health problem, exposure to IM/IV injections or visit to a hospital that received patients infected with the Ebola virus.

Exposure to an infected animal or one highly suspected of being infected with Ebola virus

- Handling in a laboratory of Ebola virus strains or clinical specimens (e.g. blood, urine, stool, tissue, cultures) that may contain the Ebola virus;
- Working in a laboratory that handles bats or non-human primates from an area at risk* of Ebola;
- Contact with the blood or other body fluids (e.g. urine, feces) of an animal infected or strongly suspected of being infected with the virus;
- Direct contact with bats or non-human primates in or from an area at risk*;
- Exposure to a cave infested with bats in an Ebola-endemic area;
- Handling (butchering, drying, smoking) or consumption of meat (raw or undercooked) obtained by hunting (particularly non-human primates and bats) in an area at risk*;

OR

Scenario 2

Patient with no history of travel to an area at risk AND for whom has been documented:

- Close contact with a patient confirmed to have EVD within 21 days prior to the onset of the disease;

OR

- Sexual relations with a patient confirmed to have EVD within 13 weeks following the onset of symptoms.

*At risk area: The list of West African countries experiencing outbreaks of EVD since March 2014 is updated by the *ministère de la Santé et des Services sociaux* at the following website : http://www.msss.gouv.qc.ca/professionnels/ebola/index.php

CONFIRMED CASE

A confirmed patient is defined as anyone with laboratory confirmation** of infection with the Ebola virus from the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC).

**Laboratory confirmation : i) presence of the RNA of the Ebola virus detected by RT-PCR, ii) presence of the Ebola virus detected by culture, iii) detection of a viral antigen using ELISA, iv) presence of Ebola-specific IgM or rising titers of IgG.

Communications

The attending physician, in consultation with the on-duty microbiologist or infectious disease specialist will determine the level of suspicion for EVD based on the clinical and epidemiological data. **Consult the document *Ebola Virus Disease: Prevention and Control Measures for Hospitals*** produced by the *Comité sur les infections nosocomiales du Québec* (CINQ).[11]

- Report the case to the Agency's regional *Direction de santé* publique using the regular reporting process for infectious emergencies.
  - The on-duty public health specialist will advise his or her director (or any other relevant doctor or manager, depending on the internal procedures specific to each region).
  - The Direction de santé publique will advise the MSSS (following the usual procedures) and submit the Ebola form required by PHAC for a patient suspected of EVD.
  - If the situation justifies it, the Quebec National Director of Public Health will advise the Public Health Agency of Canada (PHAC). The Agency's emergency measures coordinator will be informed of the situation by his Direction as needed.
- Advise the LSPQ's Scientific Director.
- The LSPQ's Scientific Director will organize a teleconference involving :
  - the on-duty public health specialist at the ASSS;
  - the regional public health director or a representative;
  - the attending physician or a consultant and;
  - the public health protection director at the MSSS or a replacement.

to determine whether it is a patient suspected of EVD requiring specific analyses for the Ebola virus and to decide whether to activate the emergency response plan.[3,12,13]

Plan a meeting or teleconference involving the major managers and a representative from the hospital departments (e.g. IPAC, occupational health, infectious diseases, emergency, intensive care, laboratories, communications and media relations).

Sample Collection and Laboratory Tests

When EVD is suspected, it is recommended **to limit requisitions to minimum testing,** i.e. basic tests necessary for clinical management, excluding other pathologies and testing to confirm the diagnosis. **No viral cultures are allowed outside a containment level 4 facility (CL4). No cell cultures are to be undertaken, such as cultures for *C. difficile* on a Vero cell line or to find SLTs (Shiga-like toxins) on a cell line.**

Preliminary exclusion testing

Basic minimum testing and preliminary exclusion testing (e.g. CBC, blood sugar, malaria blood smear, etc.) are intended to quickly identify an acute condition threatening the health of the patient (e.g. uncontrolled diabetes) and rule out the possibility of certain infectious diseases such as malaria, typhoid fever, septicemia.

Only samples essential to the adequate clinical management of the patient are to be taken.

HANDLING OF SAMPLES

All samples from suspected cases must be handled in a Class 2 biosafety cabinet using CL3 biosecurity practices by technologists with up-to-date training.[14-19]

A. Collection of samples	▪ Samples must be taken by experienced staff wearing appropriate personal protective equipment (PPE) as recommended by the local infection prevention and control team (IPAC) for healthcare staff (at minimum: long-sleeved gown, respiratory protection, certified safety goggles or visor, waterproof gloves and overshoes). Refer to11: http://www.inspq.qc.ca/pdf/publications/1890_Ebola_Prevention_Control_Hospitals.pdf ▪ Avoid using glass containers and place disposable objects in resistant autoclavable containers. ▪ Take blood samples with caution. Dispose of materials used for collection immediately after use in a container suitable for sharp objects. Replace these containers on a daily basis; sterilize them in an autoclave or incinerate them. ▪ Clean the external surfaces of each sampling container with a disinfectant. ▪ Identify samples and place them in a hermetically sealed, leakproof bag labelled “biorisk – decontaminate in biosafety cabinet.” ▪ Insert the bag identified as “biorisk – decontaminate in a biosafety cabinet” and the requisitions for analysis in a second hermetically sealed bag.
B. Notification	The relevant laboratory staff must be notified that specimens potentially containing an agent causing EVD are to be sent.

C. Handling of samples	▪ The double-bagged specimen must be sent to the laboratory in a hard leakproof container, clearly labelled as containing samples that may be contaminated with the Ebola virus.
	▪ The container must be disinfected before leaving the care unit (according to "additional precautions" practices).
	▪ The container must be transported by messenger and physically handed over person to person.
	▪ Never use automated transportation (e.g. no pneumatics or conveyor belts).
	▪ No PPE is necessary for the messenger handling the transportation.
	▪ A single drop-off point should be defined in each physical facility.
	▪ Samples must be handled separately from others and tracking measures must be in place. The requisition for analysis must clearly indicate that the sample may be contaminated with the Ebola virus.
	▪ When serum samples are to be sent to the LSPQ or the NML in Winnipeg, use gel tubes with no anticoagulant ("yellow" or "gold" tubes or SSTs: separating serum tubes) and send the tubes without opening them after centrifuging.
	▪ Institutions with point-of-care testing (POCT) equipment for biochemistry and hematology such as the ABL80 FLEX radiometer for arterial gases, the Abaxis Piccolo Xpress for biochemistry, the Stago S4 coagulation analyzer or vet scan HM2 for hematology (veterinary equipment) must:
	  ▪ Operate them in a dedicated BSC or within the patient confinement area;
	  ▪ Ensure appropriate training has been provided to technical operating staff;
	  ▪ Wear technician protective equipment as described below.
	  ▪ POCT equipment such as i-Stat or the equivalent can be used in an isolation room with the same type of protective equipment described below.
	▪ Insure adequate cleaning of POCT equipments when removing them from the isolation room.

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<td rowspan="2">D. Opening containers in certified biological safety cabinets (BSC)</td>
<td>Personal protective equipment for technicians</td>
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Water-repellent disposable gown with ties at the rear and elastics wrists over a lab coat or smock and overshoes.
Double pairs of nitrile gloves covering the sleeves.
Certified safety glasses or visor.
High-power filtering mask (N95 or superior capacity). Biochemical and
Refer to the procedures set out in Appendix 1 for putting on and removing PPE.

Primary contamination using a certified BSC (class II type A or B)

An absorbent cloth with a waterproof backing soaked with disinfectant is placed on the BSC work surface, and a waste container must be within the cabinet.
Visually inspect the each specimen container to ensure its integrity before removal from the plastic bag.
Remove the specimens from the plastic bags labelled as biorisks.
Decontaminate the exterior of the containers.
As necessary, separate the samples into aliquot portions in polypropylene tubes with leak-proof screw cap.
All centrifuging must be carried out, optimally within a BSC, in a centrifuge with sealed safety buckets, and must respect waiting times following completion.

If the centrifuge is outside the BSC, buckets must be prepared and opened within the BSC.
If the centrifuge is within the BSC, do not perform other handling activities during centrifugation as the ensuing turbulence can compromise the integrity of the laminar flow.

Use gauze soaked in 70% ethanol between the gloved hand and the stopper to ensure it opens without dispersing or propagating aerosols.
Disinfect the BSC with an appropriate virucide disinfectant according to local recommendations. As an example, PHAC's Material Safety Data Sheet for infectious agents describes the sensitivity of the virus to numerous disinfectants (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ebola-eng.php).
Dispose of single-use materials in a leak-proof container.

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<td>E. Distribution of biological specimens to analysis sectors</td>
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Notify analytical sectors that a specimen potentially containing Ebola virus is on its way.
Specimens must be handled in the holder and transported in a hermetically sealed container if they remain within a CL2.
If laboratory sectors are physically distinct and a specimen must be transferred through a public area (e.g. a microbiology sector separated from the biochemistry sector by a public zone), transport procedures outlined in section C should be applied.
No procedure, such as opening blood collection tubes under vacuum or aliquot preparation, should be carried out outside a BSC.

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F. Special characteristics of sectors	**Biochemical and hematological automated analyzers**
	
	Automated analyzers can be used based on the principles below: Conduct an assessment of the risk of generating aerosols based on the devices used. Treatments to inactivate the virus, as proposed further on, may be used before analysis to limit viral loads.
	
	▪ According to information obtained from the Centers for Diseases Control and Prevention as well as from manufacturers of most automated systems, the manufacturer-installed safety features and decontamination protocols appropriate for enveloped viruses such as HIV, influenza, hepatitis C, or Ebola should be used to ensure additional protection and safety (Annex 2).
	▪ In the absence of equipment break-down or spill, decontamination with a special procedure is not required after testing of specimens from patients suspected of EVD.
	▪ The specimen must be traceable and duly labelled at all times.
	▪ For automated analyzers using open tubes, the tubes under vacuum are opened with a gauze soaked in 70% ethanol between the gloved hand and the stopper to avoid dispersion or propagation of aerosols. If necessary, aliquot portions can be prepared in polypropylene tubes with leak-proof crew cap. If a polypropylene tube with an aliquot portion cannot be used, the proper tube must be covered until inserted in the automated analyzer.
	▪ Tube handling is performed by a technician wearing the PPE described previously (section D).
	
	BIOCHEMISTRY
	
	No urinalysis is to be performed unless it can be done in a BSC using a manual urine test strip.
	
	Triton X-100 treatment at a final concentration of 0.25% reduces viral load without altering most common biochemistry tests (chemistry and enzymes) with conventional reagents in a fluid environment. However, it is preferable that each laboratory confirm the validity of the results with its own analyzers following this treatment. Handle specimens following treatment as though potentially infectious.
	
	▪ For biochemical analyses, (plasma from a green tube or serum from a yellow-gold tube centrifuged in a safe environment) carry out inactivation with Triton X-100 in a BSC:
	  ▪ Prepare a 10% Triton X-100 solution, adding 10 ml to 90 ml of deionized water; heat to homogenize.
	  ▪ Transfer 1 ml of plasma using a single-use transfer pipette to a plastic tube with a screw top.
	  ▪ Using a micropipette, add 26 µl of the diluted Triton X-100 solution (10%) to the serum.
	  ▪ Screw on the cap and turn the tube over three times.
	  ▪ Let it sit for 60 minutes at room temperature.
	  ▪ Clean the exterior of the micropipette with bleach diluted to 5000 ppm (dilution of 1:10 domestic 5% bleach).
	  ▪ Alternatively, if Triton X-100 is not available, plasma can be inactivated at 60°C for one hour. However, only the following tests will be unaffected: Na, K, Mg, urea, creatinine, urate, total bilirubin, glucose and C-reactive protein. For osmolality or lactate tests, most enzymes undergo significant losses in activity.[20] Once again, it is preferable that each laboratory confirm the validity of the results after this treatment using its own analyzers.
	
	HEMATOLOGY
	
	▪ Blood smears are not infectious once fixed in solvents.
	▪ Malaria diagnosis :

- Thin smear is recommended for malaria diagnosis.
- Malaria smears are prepared in the BSC following the usual procedures after fixing the slide in methanol for 30 minutes.
- A thick smear is not recommended because of difficulties in virus inactivation.
- Rapid malaria antigen detection tests can be useful as a complement to microscopy and are carried out under a BSC or in the isolated patient's room.

COAGULATION

- Only necessary coagulation tests should be performed

Triton X-100 treatment at a final concentration of 0.25% reduces viral load without altering most common coagulation tests with conventional reagents in a fluid environment. However, it is preferable that each laboratory confirm the validity of the results with its own analyzers following this treatment. Handle specimens following treatment as though potentially infectious.

For coagulation tests on automated systems, if POCT is not available or generates abnormal results, carry out inactivation with Triton X-100 in a BSC:

- Prepare a 10% Triton X-100 solution, adding 10 ml to 90 ml of deionized water; heat to homogenize.
- Transfer 1 ml of plasma using a single-use transfer pipette to a plastic tube with a screw top.
- Using a micropipette, add 26 µl of the diluted Triton X-100 solution (10%) to the serum.
- Screw on the cap and turn the tube over three times.
- Let it sit for 60 minutes at room temperature.
- Clean the exterior of the micropipette with bleach diluted to 5000 ppm (dilution of 1:10 domestic 5% bleach).

TRANSFUSION MEDICINE

- If a patient suspected of EVD must be transfused, it is recommended to proceed with the procedure for extreme emergencies until results for Ebola diagnostic tests are known.
- Notify Hema-Quebec if an important volume of blood products (Group O Red Blood cells or group AB plasma) is required.

BACTERIOLOGY

Non-blood specimens (urine, sputum, etc.)

- These samples are preserved and worked on only after the results of the Ebola diagnostic test are known.

Blood cultures

- Bottles, preferably unbreakable, must be duly identified and recognizable in the incubator.
- If they prove positive, they should only be worked on after the results of the Ebola diagnostic test are known. However, if patient care requires, and following discussion with the microbiologist / infectious disease specialist, the bottle can be worked on within a certified BSC during subculturing and work with primary agar media. The Gram smear should be inactivated with methanol for 30 minutes.
- No cell culture of any kind is to be undertaken, such as cultures for *C. difficile* on a Vero cell line or to find SLTs (Shiga-like toxins) on a cell line.

POST-MORTEM EXAMS

- An autopsy should not be performed until test results eliminate EVD.

G. Waste management	▪ Samples and all soiled materials must be disposed of in a leak-proof biomedical container. ▪ Follow the procedures for your institution for incinerating (or decontaminating) non-anatomical infectious biomedical waste in accordance with current regulations.
H. Laboratory exposure	▪ Consult a medical microbiologist when laboratory exposure occurs. In the case of a spill, cleaning staff must wear the same PPE as recommended for technical staff. **SPILLS** ▪ The area must be evacuated and secured. ▪ Let aerosols settle for at least 30 minutes. ▪ Accidental spills of potentially contaminated materials should be covered with absorbent paper and then generously covered with disinfectant; allow to act for the proper time according to the type of disinfectant selected and its concentration before wiping up. After removing the material, the disinfection process must be repeated. ▪ Individuals who participate in cleaning must wear protective equipment. In accordance with regular procedures in response to laboratory spills, plan to provide these people with a motorized air purification respirator or another approved respirator (e.g. N95 or N100). Disposable gloves, waterproof gowns and eye protection equipment must be removed immediately after cleaning and placed in an autoclave bag and sterilized before being disposed of (5). ▪ Follow the disinfection protocol in effect in your institution. **IN CASE OF NEEDLE PRICK OR INJURY, ACCIDENTAL SPLASHING ON BROKEN SKIN OR MUCOUS MEMBRANE WITH INFECTED MATERIAL OR EXPOSURE TO AEROSOLS WITHOUT ADEQUATE PROTECTION** ▪ **Consult the document** *Ebola virus disease : Prevention and Control Measures for Hospitals* http://www.inspq.qc.ca/pdf/publications/1890_Ebola_Prevention_Control_Hospitals.pdf

Laboratory Diagnosis of EVD

Confirmation of the diagnosis depends on laboratory analyses conducted by the NML's Special Pathogens Program. Preliminary PCR analyses for Ebola are now performed by the LSPQ.

Isolation of Ebola virus, molecular detection testing, testing to detect IgG, IgM and the antigen for Ebola virus are offered by the NML.

In a patient suspected of EVD, if an initially negative PCR test result is obtained from a specimen collected less than 3 days after onset of symptoms, it is recommended to repeat testing after the third day, unless a definite alternative diagnosis has been established or EVD is no longer considered in the differential diagnosis.

Virus isolation and molecular detection require a minimum blood volume of 1.5 ml in an EDTA tube. Two tubes will be collected, one for the LSPQ and one for the NML.

Detection of IgG, IgM and the antigen for Ebola virus requires a serum or a pair of serums (preferred), collected in gel tubes with no anticoagulant ("yellow" or "gold" tubes or SSTs: S*eparating Serum Tubes*). A minimum volume of 1 ml is necessary.

In summary, two types of samples are required:

- Total blood volume in two (2) EDTA tubes; minimum volume required is 1.5 ml per tube.
- Single serum or paired serums in an SST; minimum volume required is 1 ml.

  Samples are stored and shipped refrigerated.

Samples must be packaged and prepared for separate shipments to the LSPQ and the NML based on the procedures for RG4 pathogens, as described in the *Transportation of Dangerous Goods Regulations* concerning Class A packages requiring an Emergency Response Assistance Plan (ERAP).
http://www.tc.gc.ca/eng/tdg/clear-part7-374.htm
This must be handled jointly with the LSPQ's ERAP team.

The EDTA tube intended for the LSPQ must be accompanied by requisition form LSPQ 221.

Samples intended for the NML must be accompanied by the duly completed *Requisition for Special Pathogens* form: https://www.nml-lnm.gc.ca/guide2/files/26-Requisition-form-Special%20Pathogens-ENG.pdf.

Shipping samples for EVD diagnosis

Shipment of samples suspected of containing RG4 pathogens must be entrusted to a person holding a training certificate on the transportation of dangerous goods (TDG) for shipment by plane, in accordance with the TMD Regulations. Staff training and certification is the responsibility of each employer and immediate supervisors.

A laboratory shipping a specimen for the detection of a RG4 virus must include an ERAP when shipping the package. An ERAP is required for transportation by road or air of all clinical samples or cultures that may contain the viruses set out in section 7.1.7 of the TDG Regulations, which can be consulted at the following address:
http://www.tc.gc.ca/eng/tdg/clear-part7-374.htm#sec71

A provincial team certified by Transport Canada for shipment of packages requiring an ERAP is in place at the LSPQ.[21] The team liaises with federal and provincial public health authorities. You must contact one of its members right away to obtain the ERAP number required for the shipment. The team will assist you throughout the process of shipping specimens to the NML in Winnipeg.

> To reach the ERAP team at the LSPQ, dial the pager number of the person on duty at the LSPQ: 514-720-8314

Alternatively, dial 514-457-2070 and ask to speak to a member of the ERAP team. Outside business hours, dial the same number, press "0" and ask the security guard to contact the person on duty at the LSPQ **for an emergency.**

- The LSPQ team will take you through the following procedures:
  - Packaging and labelling the samples in accordance with the transportation rules for class A specimens by a person holding a TDG certificate valid for shipment by road or air.

- Completing the "Shipper's declaration for dangerous goods / Déclaration de l'expéditeur TMD" form.
- Contacting a carrier recognized by the ERAP team at the LSPQ which holds the necessary authorizations to transport RG 4 specimens by road or air.

Contact Information for ERAP Team at the LSPQ

To reach the ERAP team at the LSPQ, dial the pager number: 514-720-8314

Or dial 514-457-2070, press 0, indicate that it is an emergency and ask for a member of the ERAP team.

Sophie Grenier ERAP Coordinator	Extension: 2372 Email address: sophie.grenier@inspq.qc.ca
Alexandre Chammat	Extension: 2278 Email address: alexandre.chammat@inspq.qc.ca
Bouchra Serhir	Extension: 2231 Email address: bouchra.serhir@inspq.qc.ca
Ida Pedro	Extension: 2218 Email address: ida.pedro@inspq.qc.ca
Outside business hours	Dial "0" to reach the team on duty

Contact Information for ERAP Team at the MSSS (reached by the LSPQ)

Director of Public Health	Email address: horacio.arruda@msss.gouv.qc.ca
Michel Savard	Email address: michel.savard@msss.gouv.qc.ca

Lists of suppliers of containers and placards

https://www.tc.gc.ca/eng/tdg/moc-infectious-suppliersab-140.html
http://www.tc.gc.ca/eng/tdg/training-distributors-243.htm

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Appendix 1 Procedure for putting on and removing personal protective equipment

The sequence for putting on personal protective equipment is as follows:

1. Wash your hands with an alcohol-based hand sanitizer or soap and water.
2. Put on overshoes.
3. Put on the water-repellent gown and tie at the neck and waist.
4. Put on the N95 and ensure a proper seal.
5. Put on the visor with the foam cushion on your forehead.
6. Put on the short nitrile gloves.
7. Put on the long nitrile gloves that cover the gown's sleeves.
8. Never touch your face with your gloves.
9. Limit the number of surfaces handled in the room.
10. Change gloves if they are torn or become strongly contaminated.

The sequence for removing personal protective equipment is as follows:

1. Take all precautions to avoid creating splashes.
2. Remove the first pair of gloves (long outer ones).
   a. Grasp the outer part of the glove with the opposite hand and carefully remove it.
   b. Hold on to the removed glove in the other still-gloved hand.
   c. Slide your fingers into the gloved hand and carefully remove the glove.
   d. Dispose of the gloves in the container provided for that purpose.
3. Remove overshoes and gown, untying it at the waist and then pulling at thorax level to undo the upper tie.
4. Remove the second pair of gloves (short inner ones).
5. Wash your hands with an alcohol-based hand sanitizer.
6. Remove the visor by the elastic band (the visor itself is contaminated!).
7. Wash your hands with an alcohol-based hand sanitizer.
8. Exit the room and close the door.
9. Remove the N95 by the elastics (the filter itself is contaminated!).
10. Wash your hands with an alcohol-based hand sanitizer or soap and water.

Appendix 2 Extracted from: How U.S. Clinical Laboratories Can Safely Manage Specimens from Persons under Investigation for Ebola Virus Disease

http://www.cdc.gov/vhf/ebola/hcp/safe-specimen-management.html

How U.S. Clinical Laboratories Can Safely Manage Specimens from Persons Under Investigation for Ebola Virus Disease

Who this is for: Laboratorians and other healthcare personnel handling specimens from patients under investigation (PUI) for Ebola virus disease (EVD)

What: CDC provides answers to frequently asked questions regarding the safe handling of specimens from PUI for EVD

How to use: This document should be used as a supplement to CDC's document, Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Persons Under Investigation for Ebola Virus Disease in the United States.

Routine Testing

CAN CLINICAL LABORATORIES SAFELY MANAGE ROUTINE TESTING OF SPECIMENS FROM A PUI FOR EVD?

Yes. Clinical laboratories can safely do routine laboratory testing such as traditional chemistry, hematology, or other laboratory testing used to support and treat patients by following and strictly adhering to CDC's recommendations and proper use of PPE.

Ebola virus is spread by direct contact with blood or body fluids from an infected individual. OSHA's bloodborne pathogens standard was put in place many years ago to protect laboratory personnel from any known and unknown infectious specimens that are present in blood or body fluids. By wearing appropriate PPE during specimen collection and utilizing PPE plus a certified Class II biosafety cabinet or Plexiglass splash guard when processing and testing specimens, laboratory personnel can safely conduct routine diagnostic tests on PUI for EVD or other potential infectious diseases.

For automated systems, the manufacturer-installed safety features and decontamination protocols appropriate for enveloped viruses such as HIV, influenza, or hepatitis C, should be used to ensure additional protection and safety.

U.S. hospitals or clinical laboratories that are concerned about a PUI for EVD should contact their relevant state public health authorities and CDC (770-488-7100) for consultation.

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Ebola Virus Disease (EVD): Practical Guide for Managing Laboratory Analysis Requisitions for Patients with Suspected EVD

This document is available in French in its entirety in electronic format (PDF) on the Institut national de santé publique du Québec website at: http://www.inspq.qc.ca.

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