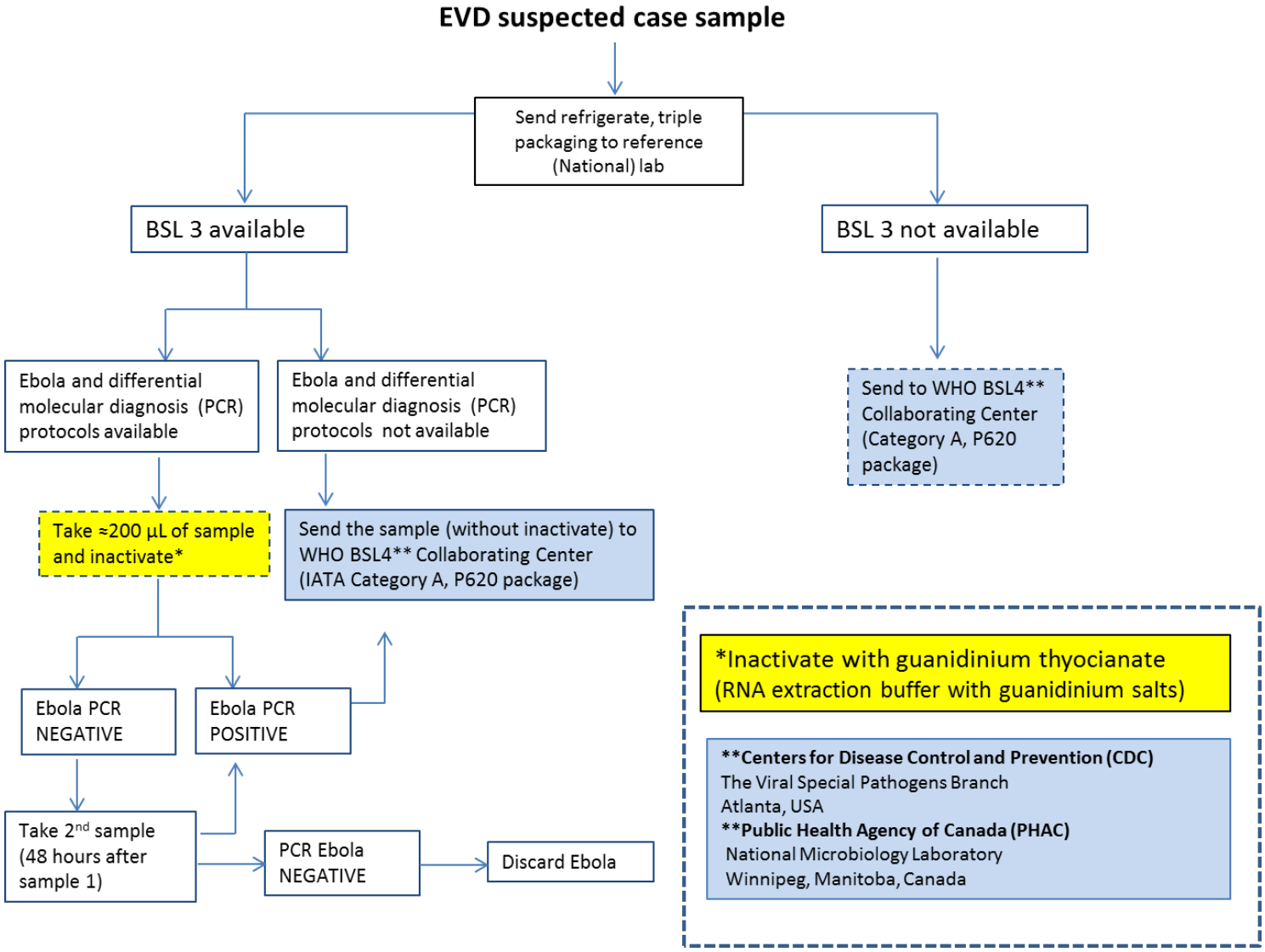
Algorithm for handling of samples from suspected Ebola Virus Disease (EVD)[1]

20 May 2026

Given that the initial manifestations of Ebola Virus Disease (EVD) can be very unspecific, the only way to establish the etiology of a suspected case will be through a laboratory test. However, specimens obtained from patients suspected of EVD represent a high biological risk. Therefore, in order to perform diagnostic assays (molecular detection by RT-PCR) or biochemical and hematological determinations (for clinical monitoring and patient management), specimens must undergo an **inactivation process** in **a Biosafety Level 3 (BSL-3) laboratory**. Once inactivated, specimens may be safely handled in Biosafety Level 2 (BSL-2) laboratories. Consequently, countries that do not have access to BSL-3 laboratories must ensure that specimens are shipped to a PAHO/WHO Collaborating Center (PAHO/WHO CC), in compliance with current regulations for the transport of Category A infectious substances, in accordance with the International Air Transport Association (IATA) regulations (Guidance on Regulations for the Transport of Infectious Substances 2025–2026: https://iris.who.int/items/b13811ad-8711-424a-9a7e-6dd6d997c448).

Although preliminary detection using molecular techniques may be performed in BSL-2 laboratories once the specimen has been inactivated, final confirmation of the first cases identified in a country or territory should be conducted by a PAHO/WHO CC. Furthermore, because Ebola virus is classified as a Risk Group 4 pathogen, any procedure involving the manipulation of viable virus, such as viral isolation, strictly requires Biosafety Level 4 (BSL-4) facilities.

[1] Both the algorithm and the recommendations in this document can be subject to later modifications in accordance to the advances in the knowledge of the disease and the etiologic agent.


EVD suspected case sample
Send refrigerate, triple packaging to reference (National) lab
BSL 3 available
BSL 3 not available
Ebola and differential molecular diagnosis (PCR) protocols available
Ebola and differential molecular diagnosis (PCR) protocols not available
Send to WHO BSL4** Collaborating Center (Category A, P620 package)
Take ≈200 µL of sample and inactivate*
Send the sample (without inactivate) to WHO BSL4** Collaborating Center (IATA Category A, P620 package)
Ebola PCR NEGATIVE
Ebola PCR POSITIVE
Take 2nd sample (48 hours after sample 1)
PCR Ebola NEGATIVE
Discard Ebola
*Inactivate with guanidinium thyocianate (RNA extraction buffer with guanidinium salts)
**Centers for Disease Control and Prevention (CDC)
The Viral Special Pathogens Branch
Atlanta, USA
**Public Health Agency of Canada (PHAC)
National Microbiology Laboratory
Winnipeg, Manitoba, Canada

Sample selection, collection, and shipment

Type of sample:

- Viral detection is only recommended in symptomatic patients. **Sample should not be collected from healthy contacts**.
- Once the symptoms have begun, viremia reaches its highest peak around day 6 and can be detected until (approximately) day 15. However, samples collected during days 1-2 after the onset of symptoms can be negative even in infected individuals. For this reason, a second sample should be collected at least 48 hours later, according to the infection dynamic.
- The recommended sample for virological diagnostic is **whole blood** (5 ml, in plastic tube with EDTA is preferred); however, serum or plasma can also be used for the diagnosis.
- The oral swab is recommended only for post-mortem cases or in situations where the blood sample is impossible to obtain. It should be collected in viral transportation universal media, and should be take only by trained personnel. The sensitivity of laboratory tests in this type of sample is low.
- The collection of the sample should be carried out only by trained personnel, guaranteeing the adequate use of all personal protective equipment. (See document: *Recommendations for the safe collection and proper management of samples potentially infected with Ebola virus.* PAHO/WHO, 2026)

Conservation of the sample:

- The sample can be kept under refrigeration (2–8°C) for up to a week. However, shipping the sample to the reference laboratory during the first 48 hours after collection is recommended (Table 1).
- DO NOT STORE NON-INACTIVATED BIOLOGICAL SAMPLES UNDER BSL2 CONDITIONS ANY LONGER THAN NECESSARY BEFORE SHIPMENT.

Table 1. Considerations for sample storage conditions. Adapted from *Diagnostic testing for Ebola and Marburg virus diseases. Interim guidance, 20 December 2024, WHO.*

Sample type	Storage conditions
EDTA-blood/plasma or serum	≤ 24 hours: ambient temperature (up to 25° C)
	1 – 7 days: 2 – 8°C
	> 7 days: -20°C or lower
	> 60 days from collection: -70°C
	Before freezing (-20°C or -70C°C), EDTA-plasma and serum samples should be aliquoted into cryogenic tubes. Freeze-thaw cycles should be avoided as this may affect sample quality. Note that aliquoting of samples should only be done in an appropriately equipped laboratory.
Oral swabs in VTM or nuclease free water	≤ 24 hours: ambient temperature
	1 – 7 days: 2 – 8°C
	> 7 days: -20°C (or -70°C if available)
	> 60 days from collection: -70°C
	Before freezing (-20°C or -70C°C), VTM or nuclease free water suspension from samples should be aliquoted into cryogenic tubes. Freeze-thaw cycles should be avoided as this may affect sample quality. Note that aliquoting of samples should only be done in an appropriately equipped laboratory. Dry swabs that have not been resuspended in nuclease free water should not be frozen.

Shipment of the sample to the National Reference Laboratory and to the PAHO/WHO CC:

- According to the algorithm, the samples should be sent to the National Reference Laboratory, ensuring triple packaging and all pertinent biosafety measures (see document: *Recommendations for proper packaging and shipping by land, of samples potentially infectious with Ebola virus*. PAHO/WHO, 2026).
- For air transportation and shipment to the PAHO/WHO CC, IATA recommendations for infectious substances **Category A** should be strictly fulfilled (see document: *Guidance on Regulations for the Transport of Infectious Substances 2025–2026. WHO*: https://iris.who.int/items/b13811ad-8711-424a-9a7e-6dd6d997c448)
    - Triple packaging (certified box P620)
    - Certified shipper
    - Shipper's Dangerous Goods Declaration (DGD)
    - Air waybill
- In addition, the refrigerated condition of the sample should be guaranteed. In cases where dry ice is used, proper cooler P954 box (Styrofoam box) should be used as well as the corresponding

label (see document: *Guidance on Regulations for the Transport of Infectious Substances 2025–2026. WHO*: https://iris.who.int/items/b13811ad-8711-424a-9a7e-6dd6d997c448)

- Before collecting and shipping the sample, the PAHO/WHO CC should be contacted through the PAHO regional office. The PAHO/WHO CC will not receive samples without previous authorization.
- For the shipment of samples to the PAHO/WHO CC, an available transport company (Courier or civil airline) should be guaranteed. (See document: *Electronic bulletin EB 2014/57 of the International Civil Aeronautic Organization*, ICAO 2014).
- **Samples shipped to the PAHO/WHO CC should be shipped without inactivation**. Under special circumstances where the transportation of Category A infectious substances is impossible (and once all other possibilities have been exhausted), the shipment of an inactivated sample (category B or exempt) might be considered, after consulting with the PAHO/WHO CC and the PAHO regional office.

Reference documents

- Recommendations for the safe collection and proper management of samples potentially infected with Ebola virus. PAHO/WHO, 2026
- Recommendations for proper packaging and shipping by land, of samples potentially infectious with Ebola virus. PAHO/WHO, 2026
- Interim guidance for specimen collection, transport, testing and submission for patients with suspected infection with Ebola Virus Disease. Centers for Disease Control and Prevention. USA, 2014. https://stacks.cdc.gov/view/cdc/25734
- How to safely collect oral swabs (saliva) from deceased patients suspected to be infected with Ebola or Marburg. Interim guidance. WHO, 2017. https://iris.who.int/server/api/core/bitstreams/73d351ff-7c6b-49fa-b8c7-c1c3c93daa38/content
- How to safely collect blood samples by phlebotomy from patients suspected to be infected with Ebola or Marburg. Interim guidance. WHO, 2017. https://iris.who.int/server/api/core/bitstreams/502372ec-33fa-4ef5-ba12-371c24632eb5/content
- Guidance on Regulations for the Transport of Infectious Substances 2025–2026. WHO https://iris.who.int/items/b13811ad-8711-424a-9a7e-6dd6d997c448
- Diagnostic testing for Ebola and Marburg virus diseases. Interim guidance. 20 December 2024, WHO. https://iris.who.int/server/api/core/bitstreams/e209d826-5f3d-4ca8-b278-69254569e7ac/content