GENERAL PROCEDURES FOR INACTIVATION OF POTENTIALLY INFECTIOUS SAMPLES WITH EBOLA VIRUS[1]

20 May 2026

Purpose of the inactivation

Once collected and handled safely (see document: *How to safely collect blood samples from persons suspected to be infected with Ebola virus*. WHO, 2026), clinical samples from susped cases of infection with highly pathogenic viral agents can be used to carry out:

- Specific etiological diagnosis (virological diagnosis)
- Evaluation and follow-up to the patient

However, Ebola virus has been classified risk group 4 pathogens, which means that **viral isolation in cells (or any protocol with viable virus) should only be carried out in an equivalent level of biosafety (BSL-4).**

For this reason, diagnostic testing (molecular detection by RT-PCR) and biochemical and hematological determinations (to follow-up and management of the patient) should be performed only after the **inactivation of the sample** in a **BSL-3 laboratory** to subsequently allow safe handling in a BSL-2 environment.

Inactivation **should be** carried out in a **BSL-3** containment at the National Reference Laboratory, guaranteeing the adequate use of personal and environmental protective measures (management of biological risk and good laboratory practices) and according to the recommendations below.

The recommendations proposed below are general and have been compiled from various international publications. However, these recommendations can be subject to later modifications in accordance to the advances in the knowledge of the disease and the etiologic agent.

> *Each health institution **should** have its own biosafety manual, aligned with quality policies and good laboratory practices. Furthermore, a **local** risk assessment should be performed **before** initiating any process.*
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> ***Contact with the samples should always be restricted and unnecessary manipulation should be avoided.***

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

Considerations about the sample

- Samples should be used only in order to analyze the minimum necessary for the etiological diagnosis and management of the patient.
- The type of sample recommended for the virological diagnostic is whole blood (5mL, preferably in a collection tube with EDTA); however, serum or plasma can also be used for the diagnosis[2].
- For the follow-up and monitoring of biochemical and hematological markers of the patient, a second sample of whole blood, serum, or plasma should be taken, according to the test to be carried out and the analyte to determine (tube with EDTA, sodium citrate, sodium fluoride, heparin or dry tube).
- Oral swab is indicated only for *post-mortem* cases or in situations where the blood sample is impossible to obtain. It should be collected in universal viral transportation media (VTM), only by trained personnel. The sensitivity of detection by laboratory techniques in this type of sample is low.
- Once collected, the sample should be transported to the laboratory with the appropriate precautions: secondary container within the hospital or health facility and triple packaging for land transport toward laboratories outside the establishment (see document: *Recommendations for proper packaging and shipping by land, of samples potentially infectious with Ebola virus. PAHO/WHO, 2026*).
- Laboratory staff should be previously notified about the shipment of the sample.
- Sample **should never be left unattended**.

Considerations about the laboratory

- Minimize the number of people involved with the management of the sample. All nonessential staff should evacuate the area where the sample will be processed.
- The inactivation and initial manipulation of the sample should be carried out in a BSL-3 environment, for which the laboratory should have at least one verified and annually certified class II biosafety cabinet or, preferably, class III biosafety cabinet (see *Manual on Maintenance for Laboratory Equipment, PAHO/WHO, 2005*). Furthermore, the installations should have a sink with water supply, a refrigerator and a water bath or heating block (for the inactivation of the sample by heat; see below).
- Before inactivation, samples **should only be opened inside biosafety cabinets** (class II or III) **of the BSL-3 containment laboratory**. If a centrifugation process is required, it should be done in equipment with closed buckets[3].

[2] The sample for virological diagnosis (**viral detection at the PAHO/WHO Collaborating Center Lab**) should be send without inactivating. However, in special circumstances sending inactivated samples may be considered (category B or exempt), previous consultation with the PAHO regional office and the Collaborating Centre.

- Laboratory staff that handles the samples should wear all the personal protective equipment (PPE) recommended for the sampling, including goggles with lateral protection, laboratory cap, N95 mask (for aerosol-generating processes), waterproof cover of shoes and gowns (aprons) (disposable, insofar as possible)[4].
- The sample should be inactivated and handled **only** by professionals fully trained and qualified for the management of potentially infectious specimens with high-risk pathogens.
- For biochemical and hematological tests, the use of equipment or closed analytical systems is highly recommended (to minimize contact with the sample).
- Upon leaving the laboratory, ensure removal of all the PPE and place them in a biological risk bag in order to continue the regular sterilization processes.
- **DO NOT ATTEMPT VIRAL ISOLATION.**

INACTIVATION OF SAMPLES

The following methods to be performed **under BSL-3** conditions, are appropriate **to reduce viral infectiousness** and thus permit processing of the samples even in BSL-2 conditions, **only for molecular detection** and **some limited tests for monitoring of patient**, using adequate precautions and complete PPE[5].

> For differential diagnosis with other microorganisms, it is recommended to perform molecular tests (molecular detection by RT-PCR), with inactivated sample, **ONLY** if these protocols are **ALREADY standardized** in the National Laboratory (central or reference). Otherwise, **do not attempt to handle the sample**. In any case, sample(s) **should** be shipped (Category A, triple packaging) to a PAHO/WHO Collaborating Center (please see PAHO/WHO guidelines).

Inactivation for specific etiological diagnosis by molecular tests (RT-PCR)

- For molecular diagnosis of any pathogen, the inactivation processes should ensure a total loss of infectiousness while conserving the integrity of the nucleic acids.

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[3] Procedures that have probability of producing aerosols or splatters (for example centrifugation) should be avoided and only be carried out **if they are strictly necessary**. They should never be carried out outside a biosafety cabin.

[4] If laboratory staff is accidentally exposed to the infectious material (for example, through puncture, cuts or abrasions in the hands) the part affected should be wash immediately with abundant soap and water and apply a disinfectant solution.

[5] The virological confirmation (definitive) of infection **should** be carried out in one of the PAHO/WHO Collaborating Centers, by at least two different diagnostic platforms and with samples without inactivation.

- For the Ebola virus, inactivation with guanidine salts (50-70%) followed by ethanol is recommended. Most RNA extraction kits (viral or total) provide lysis solutions with required natural characteristics. In any case, the manufacturer's instructions and the standardized protocols of each laboratory must be followed, as well as the established personal protection measures.
- The viral transport medium where the oral swab is preserved can be inactivated and processed for molecular detection as described above. Please make sure you carefully remove the swab in a bag for infectious waste for disposal (incineration).
- Complete autopsy in fatal cases under suspicion of infection by Ebola virus, is **contraindicated** in order to avoid any contact with organs. However, a skin sample for *post mortem* confirmation of the case can be taken and immediately fixed at 10% buffered formalin (or 2.5% glutaraldehyde), with enough time that makes it possible to completely penetrate the sample. This sample will be useful for molecular detection with adequate protocols for extraction from fixed and paraffin embedded tissues.

Inactivation for clinical assays

- Inactivation by heat at 60°C during 15 min for serum samples or other organic fluids is recommended. The heating does not significantly affect the estimates of electrolytes (sodium, potassium, magnesium) as well as urea, urates, creatinine, bilirubin, glucose, and C-reactive protein. However, studies have demonstrated that enzymes such as the alkaline phosphatase and transaminases are inactivated or in any case its determination is altered. This temperature can also affect serological tests (determination of antibodies).
- The treatment of serum or other organic fluids with 10 ml of 10% Triton X-100 by ml of liquid during 1 hour is also recommended to reduce viral titers. However, since it is a detergent, tests where the cellular preservation is necessary will be altered.
- The slides for **thick blood** film should be fixed in 10% buffered formalin during 15 min and subsequently be washed (at least 3 times) with distilled water pH 7.0 before carrying out the staining.
- The blood smears should initially be fixed by 5 min in methanol and subsequently 15 min in 10% buffered formalin, followed by 3 washings with distilled water pH 7.0 before carrying out the staining. Optionally, fixation with methanol can be extended to 30 min, followed by dry heat (95^{o}C) during 1 hour.
- Serum samples for ELISA based determinations can be inactivated with final concentrations of 0.2% of sodium dodecyl sulphate (SDS) / 0.1% Tween 20 and heat treatment at 60^{o}C 15 min.

If the sample was taken and processed under a different clinical suspicion, and subsequently suspected of Ebola virus infection, the sample should be sent immediately and under the appropriate packaging conditions (IATA, Category A) to the PAHO/WHO Collaborating Center. All surfaces (biosafety cabinets, laboratory tables, equipment, etc.) where the sample has been handled should be disinfected with 0.5% hypochlorite. (see document: *Infection prevention and control guideline for Ebola and Marburg diseases. WHO, 2026*: https://www.who.int/publications/i/item/9789240111332.)
All laboratory staff that have had contact with the sample should be considered as contact.

Cleaning and decontamination of environment, laboratory equipment and PPE

- The application of disinfectants should be preceded by cleaning to avoid inactivation of disinfectants by organic matter.
- Abundant supply of disinfectant should be guaranteed, including 0.5% sodium hypochlorite, 70% (w/v) alcohol, among others.
- Accidental spilling of potentially contaminated material should be covered immediately by at least 30 min with a absorbent pad (or towel) saturated with 0.5% sodium hypochlorite, and then cleaned with absorbent material impregnated with 0.5% sodium hypochlorite. Waste should be placed in a biological risk bag for further destruction.
- The holders of the centrifuge (buckets) and rotors should be sterilized in autoclave or by immersion in 1% glutaraldehyde (in a sealed container) during 10 min.
- Any automated equipment should be decontaminated with 0.5% hypochlorite (repeated cycles of cleaning). If the manufacturers recommend an alternative procedure of decontamination, then it should be verified that it is adequate in order to inactivate agents such as Ebola virus; if it is known that the process is sufficient for the inactivation of hepatitis C or hepatitis B viruses, then it will be adequate for filoviruses.
- Any reusable PPE should initially be washed with water/detergent solution and subsequently soaked in 0.5% hypochlorite solution (minimum 30 min; leaving it overnight is highly recommended) for decontamination. The disposable equipment should be placed in leak proof biological risk bags, within covered containers for later destruction (incineration) (see document *Infection prevention and control guideline for Ebola and Marburg diseases. WHO, 2026: https://www.who.int/publications/i/item/9789240111332*).

Elimination of biological waste

Waste should be segregated in a place designated only for this purpose, allowing proper and safe handling.

- Sharp objects (for example, needles, syringes, glass items) and tubes that have been in contact with blood or body fluids, should be discarded in puncture resistant containers for further destruction (incineration).
- Infective solid waste, non-cut sharp items should be collected in leak proof biological risk bags and placed within covered containers.
- Solid waste should be sterilized by high pressure steam heat (in autoclave of sufficient size that permits the adequate steam flow and with physical or biological indicators that ensure effectiveness of the process) or directly incinerated (in conventional double chamber incinerator).
- Wastes such as stool, urine and vomit, or liquids from the washing, can be discarded directly in the drainage, toilet or latrine[6].
- The designated area for treatment and final disposal of the waste should have controlled access to avoid the entry of animals, untrained personnel, or children.
- **DO NOT STORE BIOLOGICAL SAMPLES WITHOUT INACTIVATION UNDER BSL-2 CONDITIONS.**

For the final disposal, previously sterilized or incinerated waste should be buried or taken to sanitary landfills authorized by the responsible health authority and according to the current legal standards.

[6] See document *Infection prevention and control guideline for Ebola and Marburg diseases. WHO, 2026:* *https://www.who.int/publications/i/item/9789240111332*

Reference documents

- Recommendations for the safe collection and proper management of potentially infected samples with Ebola virus. PAHO/WHO, 2026
- Recommendations for proper packaging and shipping by land, of samples potentially infectious with Ebola virus. PAHO/WHO, 2026
- Infection prevention and control guideline for Ebola and Marburg diseases. OMS, 2026: https://www.who.int/publications/i/item/9789240111332
- Diagnostic testing for Ebola and Marburg virus diseases. Interim guidance. 20 December 2024, OMS: https://iris.who.int/server/api/core/bitstreams/e209d826-5f3d-4ca8-b278-69254569e7ac/content
- 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
- Laboratory precautions for samples collected from patients with suspected viral haemorrhagic fevers. Commonwealth Department of Health and Aged Care. ISBN 06427356X Canberra, Australia 2001.
- Grolla A, Lucht A, Dick D, Strong JE, Feldmann H. Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. Bull Soc Pathol Exot, 2005. 98(3): 205-9
- Bhagat CI, Lewer M, Prins A, Beilby JP. Effects of heating plasma at 56 degrees C for 30 min and at 60 degrees C for 60 min on routine biochemistry analytes. Ann Clin Biochem, 2000. 37 (Pt 6):802-4.
- Smither SJ, Weller SA, Phelps A, Eastaugh L, Ngugi S, O'Brien LM, Steward J, Lonsdale SG, Lever MS. Buffer AVL Alone Does Not Inactivate Ebola Virus in a Representative Clinical Sample Type. J Clin Microbiol. 2015 Oct;53(10):3148-54.