Diagnostic testing for Ebola and Marburg virus diseases

Interim guidance

20 December 2024

World Health Organization

Key recommendations

- The goal of diagnostic testing for Ebola and Marburg virus diseases is to identify cases to provide timely and appropriate care and to stop disease transmission.
- All individuals meeting the case definition for Ebola or Marburg virus diseases should be tested.
- The recommended sample type for testing for orthoebolaviruses and orthomarburgviruses is whole blood or plasma for living patients, and oral swab for deceased individuals.
- Laboratory confirmation of *Orthoebolavirus* and *Orthomarburgvirus* infections and further species identification should be done using nucleic acid amplification testing (NAAT).
- If a suspected case tests negative (living patient) and the blood was drawn less than 72 hours after symptom onset, a second test should be performed with blood drawn more than 72 hours after symptom onset.
- All manipulations in laboratory settings of samples originating from suspected, probable or confirmed cases of Ebola and Marburg virus diseases should be conducted with appropriate biosafety measures according to a risk-based approach.
- Whole or partial genome sequencing can be used to characterize viruses and complement epidemiologic investigations. Member States are strongly encouraged to share genetic sequence data (GSD) in publicly accessible databases.
- Member States are required to immediately notify the World Health Organization (WHO) under the International Health Regulations (IHR) 2005 of positive laboratory results.

Introduction

The viruses belonging to O*rthoebolavirus* and O*rthomarburgvirus* genera are single stranded negative sense RNA viruses within the *Filoviridae* family. Six O*rthoebolavirus* species have been characterized to date, with one virus per species including Bombali virus (BOMV), Bundibugyo virus (BDBV), Reston virus (RESTV), Sudan virus (SUDV), Tai Forest virus (TAFV), and Ebola virus (EBOV) (*1*). Only one species of *Orthomarburgvirus* has been identified, which includes two viruses, Marburg virus (MARV) and Ravn virus (RAVV) (*2*). A summary of the classification according to the International Committee on Taxonomy of Viruses (ICTV) is shown in Table 1 below (*3*).

BDBV, SUDV, TAFV, EBOV, MARV and RAVV have been associated with outbreaks in humans. These viruses are transmitted to humans from wild animals and spread in the human population through human-to-human transmission. To date, one case of human disease with TAFV has been described. BOMV and RESTV have not been associated with human disease to date.

Family	Genus	Species	Virus name	Disease name
Filoviridae	*Orthoebolavirus*	*Orthoebolavirus bombaliense*	Bombali virus (BOMV)	Not known to cause human disease to date
		Orthoebolavirus bundibugyoense	Bundibugyo virus (BDBV)	Bundibugyo virus disease (BVD)
		Orthoebolavirus restonense	Reston virus (RESTV)	Not known to cause human disease to date
		Orthoebolavirus sudanense	Sudan virus (SUDV)	Sudan virus disease (SVD)
		Orthoebolavirus taiense	Taï Forest virus (TAFV)	Taï Forest virus disease (TVD)
		Orthoebolavirus zairense	Ebola virus (EBOV)	Ebola virus disease (EVD)
	Orthomarburgvirus	*Orthomarburgvirus marburgense*	Marburg virus (MARV)	Marburg virus disease (MVD)
			Ravn virus (RAVV)	Marburg virus disease (MVD)

Table 1 Virus classification according to ICTV, 2024[1].

Disease caused by infection with an orthoebolavirus may be referred to by the specific disease names as described above in the table, or by the generic term "Ebola disease" (EBOD). Disease caused by infection with either of the orthomarburgviruses is referred to as Marburg virus disease (MVD). For more information on disease names, see the International Classification of Diseases 11th Revision (*4*)

The incubation period for EBOD and MVD is most frequently 3-10 days but can range from 2 to 21 days (*5*). *Orthoebolavirus* and *Orthomarburgvirus* infections typically present with symptoms that can include fever, headache, lethargy, anorexia/loss of appetite, aching muscles or joints, stomach pain, difficulty swallowing,

[1] Note that there has been a recent change in filovirus nomenclature, more information can be found through the International Committee on Taxonomy of Viruses (ICTV) (*3*).

vomiting, difficulty breathing, diarrhoea and hiccups. As the disease advances, patients may experience internal or external bleeding and organ failure. Suggested case definitions can be found in the Ebola and Marburg virus outbreak toolbox (*6*). Case fatality rates of previous outbreaks vary from 24 to 90% (*7, 8*) but improve with early diagnosis, optimized supportive care and therapeutics (*9, 10*).

Countermeasures, including vaccines and therapeutics, are specific to each *Orthoebolavirus* and *Orthomarburgvirus* species. Currently, licensed vaccines and therapeutics are only available for *Orthoebolavirus zairense*. Diagnostic assays may also be species or virus specific. It is therefore important to characterize the species and virus causing the outbreak to enable the deployment of appropriate countermeasures.

Purpose of this document

The scope of this guidance is to provide interim recommendations regarding the diagnosis of EBOD and MVD. The objectives are to describe the main steps that countries at-risk of EBOD and MVD outbreaks should undertake, and to describe the diagnostic strategy that should be implemented in-country in the event of a laboratory confirmed outbreak or isolated case. This guidance constitutes an update to the Laboratory diagnosis of Ebola virus disease, Interim guidance, 19 September 2014 (*11*). This update was necessary in order to reflect the importance of appropriate diagnosis in light of available countermeasures, to expand the scope of the recommendations to include MVD as well as EBOD, and to incorporate the latest understanding and best practices for the diagnosis of EBOD and MVD.

This document provides interim guidance for laboratories, clinicians, health workers, public health officials, and other stakeholders involved in the diagnosis and care of patients with suspected or confirmed EBOD or MVD, from specimen collection through to diagnostic analysis and reporting.

Indications for testing

There are two main situations in which diagnostic testing for EBOD and MVD is indicated:

- The primary indication for testing is to ascertain the diagnosis for any individual (including living patients or deceased individuals) meeting the case definition for a suspected case and to inform deployment of public health interventions and countermeasures.
- Secondly, testing is indicated prior to discharging a confirmed patient from a treatment centre, with two negative tests, from blood samples taken at least 48 hours apart[2].

All individuals meeting the case definition for EBOD or MVD should be tested. Case definitions for EBOD and MVD include both clinical and epidemiological elements. It may not be possible to clinically distinguish them from other diseases such as malaria. It is therefore important to consider other potential differential diagnoses and co-infections. If a suspected case tests negative (living patient) and the blood was drawn less

[2] Bodily fluids other than blood, for example semen, may still contain detectable RNA after it is no longer detected in the blood. Patients with no RNA detected in the blood should be discharged, provided they are clinically well. All recovered patients should be able to access a care programme that will support them after discharge, including testing of relevant body fluids and counselling on safer sex practices and hygiene.

than 72 hours after symptom onset, a second test should be performed with blood drawn more than 72 hours after symptom onset.

Diagnostic testing should not be performed on asymptomatic individuals, including asymptomatic contacts, as the likelihood of detecting RNA in an infected individual is very low prior to symptom onset. Contacts should be monitored for 21 days following their most recent exposure. Negative test results are not sufficient to stop monitoring of a contact prior to the end of the 21-day period.

Testing may also be conducted to support patient follow-up during the acute phase of illness, post discharge and to support research studies, notably on medical countermeasures. Serology may be used as a technique to identify individuals who had previous exposure to an *Orthoebolavirus* or an *Orthomarburgvirus*, to provide appropriate care if needed and to support epidemiological investigations.

Sample types, collection, transportation and storage considerations

Sample types

Required sample types vary depending on the analyses to be conducted at the laboratory. The recommended sample types for laboratory confirmation of *Orthoebolavirus* and *Orthomarburgvirus* infections are described in Table 2 below.

Purpose of testing	**Preferred assay type**	**Sample type**
Diagnostic or follow up testing - living patient	Nucleic acid amplification testing (NAAT)	EDTA-blood/plasma. *Oral or buccal swabbing of living patients is not recommended as likelihood of detecting RNA is lower in swabs than blood, due to viral load.* *Heel pricking of infants may be performed to obtain swabs of capillary blood if venous blood cannot be taken but may lead to a loss in sensitivity.* *Dry blood swabs may be used but may lead to a significant loss of sensitivity.*
Diagnostic testing - deceased individual	NAAT	Oral or buccal (inner cheek) swab, placed in viral transport medium (VTM). *The preferred swab type is nylon flocked swabs.* *Swabbing of the nasopharynx is not recommended as the likelihood of detecting virus is not well understood.* *Dry swabs may be used if the lab is equipped to resuspend them in nuclease-free water.*
Identify previous exposure - asymptomatic or convalescent individual who may have been	Serology	Whole blood/serum. *Serum is preferable but EDTA-plasma can be used.*

previously exposed within the current outbreak context or in the past		
Patient follow up during the acute phase of illness, post discharge or for research purposes	Multiple	Depending on clinical parameters and epidemiological investigations, EDTA-blood/plasma or serum, or other sample types such as cerebrospinal fluid (CSF), urine, breast milk, semen, amniotic fluid, placenta etc. may be collected.

Table 2 Summary of indications for testing, preferred assay types for each indication and sample types to be collected.

Sample collection, storage considerations and transportation

Samples should be collected in accordance with the indication for testing and assay type to be conducted. All samples collected should be regarded as potentially infectious and handled with extreme caution (see section on Biological risk management of this guidance document), and only by those trained in the collection of filovirus samples. More information can be found in the interim guidance on How to safely collect samples by phlebotomy from patients suspected to be infected with Ebola or Marburg and How to safely collect oral swabs from deceased patients suspected to be infected with Ebola or Marburg (*12, 13*).

Samples should be transferred to the laboratory accompanied by the laboratory information sheets, including appropriate chain of custody forms to track samples, and a copy of the case investigation form (CIF). An example of a CIF can be found in the Ebola and Marburg virus outbreak toolbox (*6*).

Correct handling and storage of samples during transportation is essential for biosafety and for accurate diagnostic testing. More information on sample storage conditions can be found in Table 3.

Samples should be appropriately triple packaged and transported to the nearest laboratory with demonstrated capability as soon as possible after collection, and preferably within 24 hours. Transport of samples should comply with national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used (*14*).

For international transport, samples from suspected, probable or confirmed cases of *Orthoebolavirus* and *Orthomarburgvirus* infection, including clinical samples, viral isolates and cultures should be transported as Category A, UN2814 “Infectious Substance, Affecting Humans”. International shipping requires a dangerous goods certified shipper. For information on infectious substances shipping requirements, please see the WHO Guidance on regulations for the transport of infectious substances 2023-2024 (*15*).

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. Whole blood or EDTA-blood should not be frozen.*
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.*

Table 3 Considerations for sample storage conditions.

Laboratory testing methods and algorithm

A unique identifying number should be allocated upon arrival at the laboratory for tracking purposes. Testing for the presence of orthoebolaviruses or orthomarburgviruses should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures (see section on Biological risk management of this guidance document).

Sample inactivation for diagnostic workflow

Validated virus inactivation methods must be used before samples that may contain orthoebolaviruses or orthomarburgviruses can be manipulated outside the containment area (which may be a class III biosafety cabinet or glove box as described in the section on Biological risk management). Examples of inactivation methods include incubation with 50-70% guanidine thiocynate plus ethanol, heat treatment at 60°C for 15 min or use of detergent.

NAAT for orthoebolaviruses and orthomarburgviruses

In the context of an outbreak caused by an *Orthoebolavirus* or an *Orthomarburgvirus*, the method of choice for the detection of viral RNA is NAAT. This usually refers to real time reverse transcription polymerase chain reaction (RT-PCR) but isothermal amplification or other molecular techniques may also be used if available and appropriately validated. It is imperative to use a diagnostic technique with high sensitivity and specificity in the diagnosis of EBOD and MVD due to the implications of the result on both the patient and outbreak response. NAAT is at present the preferred technique as it is relatively fast (<6 hours to perform), is conducted on inactivated virus and without virus propagation, lowering biological risk and infrastructure requirements,

and can be set up to be high-throughput. It is also the only documented method that allows for early confirmation of infection, as viral RNA can be detected 48 to 72 hours after symptom onset.

At the beginning of an outbreak, testing should enable the identification of the *Orthoebolavirus* or *Orthomarburgvirus* species involved. However, to facilitate scale up of testing capacity it is acceptable to use an assay that identifies the genus only, differentiating between *Orthoebolavirus* and *Orthomarburgvirus*, once the species has already been identified. The possibility of simultaneous outbreaks involving different species should be considered. Therefore, additional species differentiation should be done if cases are not known to be epidemiologically linked to confirmed cases, for the first cases in a new outbreak and for importation or cross border events.

Depending on the assays available and their targets, several consecutive steps may be required, with for example the use of a pan-filovirus assay, and then an assay enabling the identification of the genus and/or of the species involved. The different types of assays that may be used are described in Table 4.

Assay target	**Purpose**
Pan-filovirus NAAT	To confirm if the infection is caused by a filovirus affecting humans (*Orthoebolavirus* or *Orthomarburgvirus)*
Orthoebolavirus genus specific NAAT	To confirm if the infection is caused by any *Orthoebolavirus* species
Orthomarburgvirus genus specific NAAT	To confirm if the infection is caused by *Orthomarburgvirus marburgense (Marburg virus or Ravn virus)*
Orthoebolavirus species specific NAAT	To confirm if the infection is caused by *Orthoebolavirus bundibugyoense*, *sudanense*, *taiense* or *zairense* species

Table 4 Assay targets and purposes for diagnostic testing.

Few commercial testing kits are currently available, among which six obtained WHO Emergency Use and Assessment Listing (EUAL) status in response to the 2014 to 2016 EVD outbreak, of which one is pan-filovirus, one is pan-ebolavirus and four are specific for *Orthoebolavirus zairense* (*16*). Note that while the EUAL carried out up to 2016 remains valid, kits that have been commercialised since the close of the EUAL were not assessed and may also be viable. Note also that some available commercial kits are designated as research use only (RUO).

There are a number of primer and probe sequence sets for RT-PCR assays for orthoebolaviruses and orthomarburgviruses that have been published and can be used for in-house development of assays in laboratories with appropriate capacities.

Before an assay is utilized to test human clinical samples within a laboratory, it should be validated and/or evaluated within the laboratory including reagents, consumables and equipment used by appropriately trained staff.

Quality control

Inclusion of quality control materials can assist in controlling for any assay issues. Controls should provide information about sample quality, nucleic acid quality, and process quality. NAAT positive and negative controls should be included on every run. Positive control material for NAAT assays can be ordered from specialized initiatives, if not included in the assay kit (*17*). The positive control should be included at a low but

easily detectable concentration. In addition, NAAT assays are extremely sensitive so efforts should be made to monitor contamination events by including negative extraction control(s).

Sample integrity, extraction, positive and inhibition controls can be helpful in distinguishing a false negative or positive from a true negative or positive or an inconclusive result. If any of the assay controls fail, testing should be repeated.

Reagents should be stored according to manufacturer recommendations. Standard operating procedures (SOPs) should be in place for all processes carried out in the laboratory.

Additional techniques for detection and characterization

The following tests are additional techniques that may be used for detection and characterization of orthoebolaviruses and orthomarburgviruses but are not recommended for routine diagnostic purposes.

Serology: Serological tests detect antibodies (IgM or IgG) produced following exposure to orthoebolaviruses or orthomarburgviruses. Antibody detection from plasma or serum should not be used alone for diagnosis. However, IgM detection from recent acutely ill patients or IgG in paired serum samples, collected at least 21 days apart, with the first being collected during the first week of illness, can aid diagnosis if tested samples yield inconclusive results. Recent vaccination may interfere with results. Serological tests are important tools in identifying individuals who have experienced prior infection or have been vaccinated and may also play a role in epidemiological investigations or research.

Detection of viral antigen. Antigen detection rapid diagnostic tests (Ag-RDTs) may be available for some virus species. They can be beneficial tools when used for post-mortem diagnosis during an ongoing outbreak, to detect cases and reduce burden on teams carrying out safe and dignified burials. However, current data show lower sensitivity of Ag-RDTs compared with NAAT, and for some tests there is a lack of data on their performance outside laboratory conditions. For this reason, results should be interpreted with caution, considering clinical and epidemiological factors. Ag-RDTs are not currently recommended to confirm new outbreaks of *Orthoebolavirus or Orthomarburgvirus* infections, including in countries where there is not an ongoing outbreak, this should be done by NAAT.

Sequencing. Genetic sequence data (GSD) also provide valuable information to help understand the origins, epidemiology and characteristics of viruses, for example whether cases arise from a single introduction or if they are linked to multiple zoonotic spillover events or viral persistence in a person who recovered. A sequencing strategy should be defined based on the epidemiological situation and available resources, facilitating integration of GSD within surveillance. WHO strongly encourages countries and laboratories to share GSD, including non-human raw data whenever possible, in a timely manner through available publicly accessible databases. GSD can be generated using Sanger or next-generation sequencing methods.

Other advanced techniques can be used for phenotypic characterization, such as electron microscopy and viral culture. As these methods are not recommended as part of routine diagnosis, the specific details for these methodologies are not covered in this document.

- *Electron microscopy.* Electron microscopy can be used to evaluate the sample for a potential *Orthoebolavirus* or *Orthomarburgvirus*, but with the availability of molecular assays and the high technical skills and facility required for electron microscopy, this method is not routinely used for the diagnosis of orthoebolaviruses and orthomarburgviruses.

- *Viral culture*. Virus isolation is not recommended as a routine diagnostic procedure because it involves propagation of virus, requiring appropriate experience and maximum containment facilities, and takes days to perform.

External quality assurance

For each outbreak of EBOD or MVD, the first samples tested (positive and negative) should be sent to an international reference laboratory for inter-laboratory comparison as part of external quality assurance implementation. The list of WHO Collaborating Centres (CC) for Viral Haemorrhagic Fever (VHF) can be found in Annex 1 List of WHO Collaborating Centres for Viral Haemorrhagic Fever (VHF).

Interpretation of laboratory results

Confirmation of an *Orthoebolavirus* and an *Orthomarburgvirus* infection should consider clinical and epidemiological information. Positive detection using a pan-filovirus assay followed by confirmation of species via NAAT and/or sequencing, or positive detection using species specific NAAT in suspected cases, indicates confirmation of an *Orthoebolavirus* or *Orthomarburgvirus* infection. Note that multiple factors could contribute to false-negative NAAT results, such as poor quality of sample, incorrect handling or shipping, or technical reasons inherent to the test, *e.g.* RNA extraction failure.

When the clinical presentation and epidemiology suggest an infection with EBOD or MVD, but NAAT results are negative, serological IgM and IgG testing may be useful to further investigate prior infection for epidemiological purposes, although serological results should be cautiously interpreted, considering vaccination status.

Definitions of acute confirmed cases, confirmed convalescent cases, non-cases and equivocal cases using laboratory results from EDTA-blood/plasma and serum testing are described in Table 5 below.

Phase	Test results	Type of case
Symptomatic	NAAT (+) NAAT (-) (or not done), and IgM (+)	Acute confirmed case
	NAAT (-) (after 72 hours following symptom onset)	Non-case
Post-symptomatic	NAAT (-) (or not done) IgM (+) and IgG (+) NAAT (-) (or not done) IgM (-) and IgG (+) only if sampled more than 3 months after symptom onset and with clinical and epidemiologic findings compatible with an *Orthoebolavirus* and an *Orthomarburgvirus* infection	Confirmed convalescent case
	NAAT (-) or not done during symptomatic phase, IgM (-) and IgG (+) only if tested less than 3 months since symptom onset and with clinical and epidemiology findings compatible with an *Orthoebolavirus* and an *Orthomarburgvirus* infection	Equivocal

Table 5 Definitions of acute confirmed case, confirmed convalescent case, non-case and equivocal according to test results from EDTA-blood/plasma and serum, and presence of symptoms.

When using RT-PCR, a cycle quantification (Cq) value is associated with each positive sample (also referred to as cycle threshold or Ct value). A Cq value is the number of amplification cycles required for the sample to be detected as positive. Cq values vary across platforms and assays used, and between different sample types. There is an inverse relationship between Cq values and amount of virus in a patient sample and so Cq values may serve as proxy for viral load estimates with a low Cq value representing a high viral load and vice versa. However, many factors may impact the observed Cq value, such as the type of sample taken, the sample quality, the conditions in which the sample was stored, volumes used in different steps of the procedure, platforms and reagents used for extraction and RT-PCR, efficiency of the extraction and/or RT-PCR process and potential contamination of the sample with disinfectant and other inhibitors. Therefore, Cq values should be interpreted with significant caution.

All test results should be interpreted in line with the manufacturer's recommendations.

Differential diagnosis and clinical testing

Individuals meeting the case definition for EBOD and MVD who have negative laboratory results for orthoebolaviruses and orthomarburgviruses should be tested for other pathogens. Differential diagnoses may vary depending on diseases endemic in the specific country context, and possibility of infection with other pathogens should be considered. Differential diagnosis could include malaria, sepsis-causing bacteria including leptospirosis, rickettsia and typhoid, and other viral haemorrhagic diseases, which may include dengue fever, yellow fever, Rift Valley fever, Lassa fever and Crimean-Congo haemorrhagic fever.

If all differential diagnostic tests are negative, the investigation may benefit from additional laboratory analysis, through use of advanced characterization techniques including metagenomic sequencing. If capability is not available in country, shipment to international referral laboratories may be considered.

Investigations of co-infections should also be considered, according to the epidemiological setting (for example for malaria), in patients testing positive for orthoebolaviruses and orthomarburgviruses, to provide appropriate care.

Testing to support clinical care

Daily monitoring of electrolyte levels should be done during the acute phase of illness for patients admitted to treatment facilities following positive test results for orthoebolaviruses and orthomarburgviruses. Haematology parameters should be tested on admission and as needed. For more information on patient management, refer to Optimised supportive care for Ebola virus disease: clinical management standard operating procedures (*9*).

In addition, it may be beneficial to perform repeat NAAT of patients during the course of their illness to support clinical management. However, Cq values should be interpreted with significant caution, as described above.

Biological risk management

All samples collected should be regarded as potentially infectious and handled with extreme caution. Use of adequate SOPs must be ensured. Personnel handling samples must be trained and assessed as competent in the safe and consistent execution of all tasks they are expected to carry out. These may include donning and doffing and use and selection of personal protective equipment (PPE), sample collection, sample receipt, sample inactivation and extraction, RT-PCR, waste disposal, spill decontamination procedures and full laboratory decontamination procedures in the event of closure or decommissioning of the facility.

Laboratory staff should be in good physical and mental health before undertaking work. Any minor wounds (e.g. cuts on the hands) should be covered before donning PPE. Major wounds should prohibit staff from handling samples. Laboratory personnel should wear appropriate PPE at each step of the diagnostic process.

It is recommended that all manipulations of samples originating from suspected or confirmed cases of EBOD or MVD be conducted according to a risk-based approach. Each laboratory should conduct a local risk assessment. National guidelines on laboratory biosafety should be followed under all circumstances.

Heightened control measures are recommended in addition to the core requirements while handling samples that have not been inactivated. For the purpose of clinical testing without virus propagation, these include the following:

- Samples from patients with suspected EBOD or MVD should be handled in a negative pressure Class III biosafety cabinet or glovebox, to perform sample inactivation and aliquoting. In the absence of Class III biosafety cabinet or glovebox, it is still possible to process samples, using a Class II biosafety cabinet and appropriate additional PPE[3].
- The outside surfaces of tubes containing inactivated samples should be wiped with disinfectant prior to removal from the biosafety cabinet or glovebox.
- Sample containers and laboratory surfaces should be appropriately disinfected using an effective disinfectant.

Additional control measures should be considered for specific procedures, for example for aerosol-generating procedures, according to the local risk assessment. For more information on biological risk management, see the Laboratory Biosafety Manual, 4th Edition, particularly the monograph entitled Outbreak preparedness and resilience (*18, 19*).

Disinfectants

Effective disinfectants include freshly made 0.5% (or 5000ppm) sodium hypochlorite solution and quaternary ammonium compounds. 70% ethanol may be used; however, rapid evaporation of alcohols reduces the exposure time and therefore their effectiveness. Note that for all use of disinfectants, an appropriate contact time should be observed. Some disinfectants may interfere with NAAT therefore care should be taken not to contaminate samples.

[3] Handling of samples that were inactivated using a validated protocol does not require a biosafety cabinet or glovebox.

Disposal of Waste

All waste that may contain orthoebolaviruses or orthomarburgviruses should be decontaminated at source before disposal by using an approved method, such as chemical disinfection and autoclaving, or chemical disinfection and incineration where an autoclave isn't available. Sharps containers must be used for sharp items, including pipette tips. All waste containers should be securely sealed.

Items containing guanidium thiocynate and chlorine should be kept separate as their mixing results in the forming of toxic hydrogen cyanide. Cartridges from automated or semi- automated testing platforms may require special conditions for disposal, for example incineration at high temperature to reduce risks associated with chemical components of the cartridges.

Occupational health

All laboratory personnel working with samples suspected or confirmed to contain orthoebolaviruses or orthomarburgviruses should immediately report any symptoms that meet the case definition to health authorities and the head of their laboratory.

Incidents or accidents involving potential or actual exposure to these viruses should also be immediately reported and any affected laboratory area/equipment appropriately decontaminated. Personnel who may have been exposed should seek medical advice as soon as possible. In case of laboratory exposure, medical countermeasures including vaccine administration may be considered, following national protocols.

There are two licensed vaccines for *Orthoebolavirus zairense*, the first is Ervebo, recommended for both outbreak response and preventive use, whereas the second is Zabdeno and Mvabea, a two-dose vaccine, which is not recommended for outbreak response but can be used preventively. National health authorities should conduct a risk assessment and consider whether immunization of health care workers, including laboratory personnel, who are at risk of exposure to individuals with or samples containing *Orthoebolavirus zairense* is required, either prior to, or in response to, an outbreak. For further recommendations on vaccine use, refer to the 2024 recommendations of the Strategic Advisory Group on Immunization (*20*).

There are no licensed vaccines or therapeutics for pre- or post-exposure for other species of orthoebolaviruses nor for orthomarburgviruses, although research is being undertaken on experimental candidates.

Reporting of cases and test results

All test results should be generated and communicated to the referring physician and patient (or patient family) as fast as possible within 24-48 hours of sample collection to ensure timely public health measures and patient management can be implemented, should the case be confirmed.

International reporting requirements

States Parties to the International Health Regulations (IHR) are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 2 of the International Health Regulations (2005) (*21*) .

National reporting requirements

Individual patient results must be quickly communicated to healthcare providers who will inform patients (or patient family) as soon as possible. This should include whether the sample was positive or negative, and the Cq value where applicable. In the case of deceased individuals, the results should be communicated to national authorities responsible for safe and dignified burials.

In addition, information regarding the number of samples tested and the number of positive samples, should be communicated to national authorities responsible for outbreak response and surveillance, according to national protocols.

An anonymized database should be established to monitor laboratory tests. A line list of samples should also be in place, including the laboratory's unique identifying number to facilitate a link back to the patients. It is also recommended that the following additional indicators are tracked weekly:

- Average time from sample collection to result delivery.
- Average time from sample reception at the laboratory to result delivery.
- The positivity rate among tested samples and by sample type.
- Number of failed tests and reasons of failure (e.g. technical failures, procedural nonconformity, inhibition in samples).
- Average consumption of test kits.

Considerations for national testing strategies

Setting up a national strategy for testing

A country-level strategy for *Orthoebolavirus* and *Orthomarburgvirus* testing should be endorsed and implemented to ensure clear organization of laboratory capacities in country, and efficient sample transportation and information management. This can be undertaken while the country is not experiencing an active outbreak and updated as needed in the event of an outbreak in-country, or in a neighbouring country.

A laboratory should be designated as the national reference laboratory (NRL) for *Orthoebolavirus* and *Orthomarburgvirus* testing in each country at risk of outbreaks. If *Orthoebolavirus* and/or *Orthomarburgvirus* testing is not available in-country, samples can be shipped internationally to a reference laboratory. The diagnostic strategy adopted by the NRL for *Orthoebolavirus* and *Orthomarburgvirus* detection should be based on testing objectives and available capacities and resources.

Adapting the national strategy to respond to a disease outbreak

The national testing strategy for the diagnosis of EBOD and MVD should be determined by available capability and resources and should be considered carefully. The strategy should be based on a risk assessment of the situation and modified as needed.

A strategy based on testing in a central facility, such as the NRL, on an open[4] NAAT platform, offers the advantages of being performed in a more controlled environment using a more flexible platform, with ability

[4] An open NAAT platform refers to the use particularly of a thermocycler which is open-source and non-proprietary.

to perform differential diagnoses. If the time taken to refer samples to a central testing laboratory is prolonged, countries may consider strengthening in-country transportation systems, which could also support testing for other pathogens, and decentralization options.

Decentralization of testing capacities may be recommended in high risk or highly affected zones to decrease testing turnaround time. This can be done through deployment of rapid response mobile laboratories (RRMLs) or use of near patient testing where possible (*22*). In the event of an outbreak due to *Orthoebolavirus zairense*, the use of semi-automated, near-patient testing devices can be considered.

More information on the specific steps to be taken when developing a strategy are described in Annex 2 National testing strategies for EBOD and MVD diagnosis.

Global laboratory referral

WHO, through its regional and country offices, can assist Member States to access testing and inter-laboratory comparison through international referral of samples. Where appropriate and safely performed, inactivation of samples in the local laboratory may facilitate referral and ease logistical challenges. Additional details on WHO CCs for VHF can be found in Annex 1.

Process and methods for developing this guidance

The recommendations in this guidance have been prepared by the WHO secretariat, responsible for development of the document, in consultation with and reviewed by subject matter experts with experience handling and detecting orthoebolaviruses and orthomarburgviruses, diagnosing and treating EBOD and MVD, responding to outbreaks of VHF, and those with expertise in the development of diagnostic assays for these viruses.

The WHO Secretariat undertook a literature review of available information and best practices, and held preparatory conference calls with key experts from various countries to identity and list key questions in this area. The Secretariat also reviewed relevant guidance documents related to EBOD and MVD to ensure relevant updates are noted and aligned. Following this, draft recommendations were prepared by the secretariat, in consultation with these key experts.

The WHO secretariat then convened an expert group comprising a multidisciplinary panel of virologists, scientists, public health officials, and clinicians with relevant experience with EBOD and MVD. The draft recommendations were circulated to this group for review and feedback. A first meeting was convened to discuss key questions and draft recommendations. Following this, the document and recommendations were revised and resubmitted to the expert group for review. A second meeting was then convened to finalise the recommendations and document. During the second meeting, all outstanding questions were discussed and consensus across all experts was reached. A final version was circulated to all experts involved for final review.

Document development and review benefited from consultations with WHO technical teams and experts in WHO headquarters, the WHO Regional Office for Africa and the WHO Office for the Eastern Mediterranean who have appropriate experience in the areas of laboratory testing, surveillance, clinical management, infection prevention and control, biosafety and biosecurity. These consultations provided support in drafting

recommendations and ensuring alignment with existing guidance and other technical areas. The final document was submitted and approved for WHO executive clearance by the WHO secretariat.

Plans for updating

This version of the guidance incorporates the latest understanding and considerations for diagnosis of EBOD and MVD. WHO closely monitors developments related to EBOD and MVD and will revise and publish updated recommendations as necessary. Otherwise, this interim guidance will expire one year after the date of publication.

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Declaration of interests

Non-WHO experts completed a confidentiality agreement and declaration of interest. Declarations of interest were reviewed, and no conflicts regarding the support of this guidance document were identified.

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Annex 1 List of WHO Collaborating Centres for Viral Haemorrhagic Fever (VHF)

1. WHO Collaborating Centre for Arboviruses and Viral Haemorrhagic Fevers, Centre International de Recherches Médicales de Franceville, Gabon
2. WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard-Nocht Institute for Tropical Medicine, Germany
3. WHO Collaborating Centre for Arboviral and Zoonotic diseases preparedness research and reference, Erasmus University Hospital, Netherlands
4. Centre collaborateur de l'OMS pour les Arbovirus et les Virus de Fièvres Hémorragique, Institut Pasteur de Dakar, Senegal
5. WHO Collaborating Centre for Virus Reference & Research (Special Pathogens), United Kingdom Health Security Agency, United Kingdom of Great Britain and Northern Ireland
6. WHO Collaborating Centre for Viral Hemorrhagic Fevers, Centers for Disease Control and Prevention, United States of America

Annex 2 National testing strategies for EBOD and MVD diagnosis

The following steps can be considered when developing the national laboratory strategy for EBOD and MVD diagnosis. Ideally, this should be done before there is an active outbreak.

Endorsing a national *Orthoebolavirus* and *Orthomarburgvirus* laboratory strategy and standard operating procedures

Step 1. Map and assess laboratory resources (equipment, reagents and consumables) in the country: laboratory testing sites, sample referral and transport pathways, equipment for the sample inactivation, nucleic acid extraction and amplification (e.g., glovebox, RNA extraction kits, thermocyclers, semi-automated platforms, RT-PCR kits, and other reagents and consumables, etc.).

Step 2. Designate or confirm the NRL for orthoebolaviruses and orthomarburgviruses and ensure that access to an international reference laboratory or WHO Collaborating Centre (CC) is available for inter-laboratory comparison and/or further characterization.

Step 3: Develop and endorse a national testing strategy based on NAAT for orthoebolaviruses and orthomarburgviruses and share with all relevant laboratories in country.

Step 4. Develop or update and adopt national SOPs for sample collection, handling transport and testing, data management and data sharing and distribute to all involved for implementation. Provide training on the SOPs to relevant staff if necessary.

Preparing for testing

Step 5. Assess the needs of each laboratory with regard to number of samples to test per week for surveillance; reagents, equipment (e.g., glovebox) and consumables required for the number of samples.

Step 6. Assess sample transport capacities in country and internationally, considering:

- possible routes of transportation
- availability of vehicles and fuel
- resources required e.g. Category A shipping material
- availability of certified shippers (or the need for training)
- designated VHF reference laboratory, such as a WHO CC, for sample referral and confirmatory testing
- the need to have a Material Transfer Agreement (MTA) ready.

Step 7. Assess training needs, considering carrying out simulation exercises to test readiness for outbreak response.

Step 8. Purchase any additional equipment, reagents and consumables needed and implement any trainings required. Requests can be made to WHO and/or other partner organizations for possible support.

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