Laboratory Guidelines for the Detection and Diagnosis of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection

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Context and general considerations

Coronaviruses are a group of highly diverse RNA viruses in the Coronaviridae family that are divided in 4 genera: alpha, beta, gamma and delta, and cause disease varying from mild to severe in human and animals (1-3). There are endemic human coronavirus as alphacoronaviruses 229E and NL63 and betacoronaviruses OC43 and HKU1 that can cause influenza-like illness or pneumonia in humans. However, three zoonotic coronaviruses have emerged causing severe disease in humans: Severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and the virus causing COVID-19 (SARS-CoV-2).

The MERS-CoV virus was first detected in 2012 and since then more than 2,600 confirmed cases have been reported in 27 countries, the majority corresponding to cases identified in Saudi Arabia. Updated monthly, information on MERS cases can be found at: http://www.emro.who.int/health-topics/mers-cov/mers-outbreaks.html. Based on the situation as of December 2022, WHO does not recommend the application of any travel or trade restrictions or entry screening related to MERS-CoV (2). However, PAHO/WHO recommends that all Member States strengthen the surveillance of acute respiratory infections, including MERS-CoV, and investigate in detail unusual events or patterns.

Typical presentation of MERS is fever, cough and shortness of breath. Pneumonia is a common finding, but MERS patients may not always develop this condition. Gastrointestinal symptoms, including diarrhoea, have also been reported among MERS patients. Up to 35% of cases reported to WHO have died (2).

In patients with typical symptoms, a history of travel to affected countries, of direct or indirect contact with dromedary camels (including the consumption of raw or undercooked animal products), or of visit to a health facility within 2–14 days of symptom onset, should prompt health workers to suspect a MERS-CoV infection; to evaluate and, if necessary, reinforce infection prevention and control measures; and to initiate sampling and response according to national guidelines. However, confirmation of a suspected case of MERS-CoV infection can only be done through laboratory testing. Other viral (including, but not limited to, influenza, Respiratory Syncytial Virus, SARS-CoV-2 and other coronaviruses) and bacterial (Streptococcus pneumoniae, Haemophilus influenzae type b, Legionella pneumophila) respiratory pathogens should also be considered within the diagnostic algorithm.

In the presence of a suspected or confirmed MERS patient, it is essential that health personnel and authorities implement timely infection prevention and control measures indicated for the health care setting (3). Although zoonotic transmission of MERS-CoV by direct or indirect contact with infected dromedary camels has been the most frequent mode of transmission, person-to-person transmission is possible and has led to extensive outbreaks in healthcare facilities in Saudi Arabia, the United Arab Emirates and the Republic of Korea (2). For example, transmission originating from a patient from the Arabian

1 The following recommendations have been adapted from the WHO document: Laboratory testing for Middle East respiratory syndrome coronavirus – revised (ref. 1) and are subject to subsequent modifications based on advances in our knowledge of the disease and its etiological agent.
Peninsula caused a large outbreak in the Republic of Korea in 2015 in which 186 people were infected (39 died) in 16 health facilities (4, 5). More than 80% of the cases were epidemiologically linked to five superspreading events in healthcare facilities. Guidance on infection prevention and control during health care for probable or confirmed cases of MERS-CoV infection updated in October 2019 can be found at: https://apps.who.int/iris/handle/10665/174652 (3). Outside the health care setting, there has been no sustained human-to-human transmission documented anywhere in the world.

PAHO publishes these Guidelines aware that national follow-up and laboratory confirmation capabilities are an essential component of an investigation and of the response to suspected MERS cases. The context and general considerations reported in this section also highlight the need to frame laboratory actions in a multidisciplinary and integrated response. A toolbox that collects products and tools for MERS outbreak management is continually updated by WHO and can be found at https://www.who.int/emergencies/outbreak-toolkit/disease-outbreak-toolboxes/mers-outbreak-toolbox (6).

Sample collection and proper shipment

Sample collection
Samples should be collected by trained personnel and considering all biosafety instructions including the use of personal protective equipment appropriate for standard, contact, and airborne precautions. In particular, personnel should use proper hand hygiene, gown, respirator (N95 or FFP2), eye (goggle) or facial (face shield) protection, and gloves.

Respiratory samples
Although the probability of detection is high during the first 7 days after symptom onset, viral genetic material has been detected in samples from the lower respiratory tract up to 14 days after symptom onset.

Since the highest viral load has been demonstrated in the lower respiratory tract, recommended samples include sputum, bronchoalveolar lavage, and tracheal aspirate (whenever possible, mainly from hospitalized patients and according to medical judgment). However, samples from the upper respiratory tract are also useful for diagnosis, when those from the lower tract are not available. For upper respiratory tract sampling, it is generally recommended to collect combined nasopharyngeal and oropharyngeal swabs (swabs should be transported in the same tube with viral transport medium).

Although routine sampling of asymptomatic contacts is not recommended, if deemed necessary according to national guidelines, upper respiratory tract specimens (nasopharyngeal and oropharyngeal swabs) are preferred for collection.

In general, flocked swabs made with synthetic materials (including nylon, Dacron or polyester) should be used; cotton swabs should be avoided. Protocols for the in-house production of viral transport media are available upon request to PAHO Regional Office (laboratoryresponse@paho.org). Additionally, sterile saline or nucleic acid preserving solution (eg, DNA/RNA shield) might be used if transport medium is not available (see below for sample transport considerations).
Serum samples
Serum samples for determination of antibodies can be collected to complement the diagnosis and eventually as part of surveillance processes (see Serological tests section). However, paired samples must be collected: the first during the acute phase and the second at least one week after the first. A single sample could have diagnostic value only if it was collected at least 14 days after the onset of symptoms.

Sample shipment
Respiratory samples should be kept refrigerated (4-8 °C) and sent to the laboratory where they will be processed within the 24-72 hours of collection. If samples cannot be sent within this period, freezing at -70 °C (or less) is recommended until samples are shipped (ensuring the cold chain is maintained).

Shipment of suspected samples should comply with national regulations and use at a minimum a basic triple packaging system (7). Additionally, shipments to reference laboratories or collaborating centers outside of the country must ensure compliance with all international standards for Biological Substances, Category B (7).

Laboratory testing

Biosafety
Samples should only be handled and processed by trained professionals, after a local risk assessment considering all biosafety indications and appropriate personal protective equipment for respiratory viruses in general, and for MERS-CoV in particular (1, 8). Viral isolation or culture is not recommended for routine diagnosis and should only be performed in biosafety level (BSL) 3 or 4 facilities.

Molecular methods
Routine confirmation of MERS cases is based on the detection of viral nucleic acid (RNA) using real-time RT-PCR assays with confirmation by nucleotide sequencing when necessary and as available (see Algorithm for molecular detection).

RNA extraction
RNA can be extracted from samples mentioned above using any standard extraction protocols or kits. Additionally, sputum samples require liquification prior to molecular extraction. In general, the sample lysis step in RNA extraction inactivates any viruses that may be present. In the case of MERS-CoV, inactivation has been verified for some protocols and commercial kits (9). Thus, lysed samples are generally considered non-infectious.

Algorithm for molecular detection of MERS-CoV
Confirmation of a case of MERS-CoV infection is based on:

- the detection by RT-PCR of at least two regions of the genome, or
- the amplification of a genetic target followed by nucleotide sequencing of a different genetic segment.

Three protocols for the molecular detection of MERS-CoV have been published. Currently described tests are an assay targeting upstream of the E protein gene (upE) (10) and assays targeting the open reading frame 1b (ORF 1b) (10) and the open reading frame 1a (ORF 1a) (11). The assay for the upE target is considered highly sensitive and is recommended for screening. The ORF 1a assay is considered equally
sensitive to the upE assay while the ORF 1b assay is considered less sensitive. The ORF 1a or ORF 1b assays can be used for confirmation (Figure 1).

An alternative algorithm that includes two RT-PCR assays targeting the nucleocapsid protein gene (N2 and N3) of MERS-CoV has also been described. The N2 assay can be used for screening together with the upE assay, while the N3 assay can be used for confirmation (12, 13). To date, these RT-PCR assays have shown no cross-reactivity with other respiratory viruses including human coronaviruses and were suitable to detect all known MERS-CoV strains in humans and dromedary camels.

Several methods for MERS-CoV sequencing have been published (11).

![Figure 1. Laboratory algorithm for cases under investigation for MERS. Adapted from WHO algorithm (1).](image-url)
Serological testing for antibody detection

Different tests and platforms have been developed and published for the detection of antibodies directed to MERS-CoV, including ELISA, immunofluorescence (IF), protein microarrays, and neutralization. However, serological tests require careful interpretation and their performance is indicated only under three circumstances:

1) To define a case of MERS to be reported under the International Health Regulations

If access to molecular methods is not possible, a case can be confirmed by serology only if there is evidence of seroconversion in paired samples using a screening test (e.g., ELISA, IF) and this is confirmed with a specific neutralization test. Paired samples consist of one sample taken during the acute phase and a second sample taken at least 14 days after the first. Evidence of a 4-fold or greater increase in neutralizing antibody titers is needed for confirmation.

A symptomatic patient with a single sample with a positive result on screening and neutralization tests is considered a probable case (or suspected case, depending on the case definitions of the surveillance system).

2) As part of an outbreak investigation

When investigations of outbreaks or contacts of confirmed MERS cases, serology can provide additional useful information. It is recommended that serum samples are collected from contacts as early as possible after the contact and that a second serum sample is collected 3-4 weeks after the last contact. Sera may be tested by a screening serological test (e.g., ELISA or IFA) and positive screening results need confirmation with neutralization tests. In symptomatic cases, appropriate respiratory samples should also be collected for molecular testing.

3) For population-based serosurveys and investigations of past exposures

Usually, in serosurveys, only a single specimen is available from each person. Thus, the same criteria for interpretation would apply as for asymptomatic contacts of cases mentioned above, i.e., a positive result for at least one screening assay (e.g., ELISA or IFA) plus a positive result for a neutralization assay would indicate a past infection. With a single specimen it is not possible to determine the time of infection.

Reactivos

PAHO/WHO recommends the implementation of the reference protocols described in the section Algorithm for molecular detection of MERS-CoV. Since the primers and probes for the reference RT-PCR tests for MERS-CoV have been published (10-13), laboratories can request their synthesis from their usual suppliers. Positive controls for the upE and ORF 1a specific RT-PCR assays can be requested on the European Virus Archive portal: https://www.european-virus-archive.com/search/node/MERS-CoV.

Several commercial kits for detection of MERS-CoV by RT-PCR are available. PAHO/WHO does not recommend the use of any particular product and urges countries and laboratories to carry out their own verifications/validations for the selection of commercial kits according to their circumstances and relevance.
References