

Laboratory testing for Middle East respiratory syndrome coronavirus (MERS-CoV)

Interim guidance

Updated June 2015

[WHO/MERS/LAB/15.1](http://www.who.int/csr/disease/coronavirus_infections/en/)



1. Introduction

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in laboratory testing for Middle East respiratory syndrome coronavirus (MERS-CoV). WHO publishes regular updates on the current status of the MERS-CoV event at: http://www.who.int/csr/disease/coronavirus_infections/en/.

The first version of this guidance was published in December 2012. Since then our understanding of the virus and the disease it causes in humans and animals has increased significantly. This guidance was updated in September 2013 to incorporate new information on diagnostic assays, and was revised again in September 2014 following an international meeting of laboratory experts in Lyon, France. This current document is an update of the 2014 version.

WHO continues to monitor developments related to this virus and will revise these recommendations when necessary. Unless revisions are made earlier, this document will expire on 31 December 2015.

These recommendations cover laboratory testing for MERS-CoV in humans. Related to animal health, the World Organisation for Animal Health (OIE) has convened an ad hoc group on camel diseases and trade in camel products. This group has prioritised the gathering of more information on MERS-CoV in camels. Several veterinary laboratories are performing reverse-transcription PCR (RT-PCR) testing on camel samples, and the OIE is establishing a project for laboratory network in camel-rearing countries. Further information on the situation on MERS-CoV in animals is available on the OIE website at <http://www.oie.int/en/for-the-media/press-releases/detail/article/update-august-2014-questions-answers-on-middle-east-respiratory-syndrome-coronavirus-mers-cov/>.

2. Indications for testing

WHO recommends that clinicians, epidemiologists and laboratory scientists consult the revised case definitions for reportable cases of MERS-CoV at http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/ to determine which patients should be tested. The case definitions are regularly reviewed as new information becomes available. Testing for other respiratory pathogens using routinely available laboratory procedures (as recommended in local management guidelines for community-acquired pneumonia) should also be performed, but should not delay testing for MERS-CoV. Examples of other aetiologies include *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Legionella pneumophila*, influenza viruses, adenoviruses, rhinoviruses, enteroviruses and respiratory syncytial virus.

3. Specimen collection and shipment

Whenever specimens are collected from cases under investigation, infection control guidelines must be followed. WHO has produced guidance on infection prevention and control of epidemic and pandemic prone acute respiratory infections in health care, available at http://www.who.int/csr/bioriskreduction/infection_control/publication/en/.

Key points:

- All health-care workers who collect specimens from patients suspected or confirmed to be infected with MERS-CoV must wear appropriate personal protective equipment (PPE); and
- All those involved in collection and transporting specimens should be trained in safe handling practices and spill decontamination procedures.

Dipeptidyl peptidase 4 (DPP4) was recently identified as the cellular receptor for MERS-CoV (1). The receptor is expressed on non-ciliated bronchial epithelial cells (1), explaining the observation that lower respiratory tract specimens such as bronchoalveolar lavage, sputum and tracheal aspirates contain the highest viral loads (2, 3, 4, 5). These specimens should be collected whenever possible. A report of a case series of MERS-CoV infections detected in Saudi Arabia (6) also demonstrated the value of upper respiratory tract specimens such as nasopharyngeal / oropharyngeal swabs for detecting the virus, however care should be taken to ensure that nasopharyngeal swabs are taken from the nasopharynx and not just from the nostril. Details on how to collect nasopharyngeal swabs can be found at http://www.who.int/csr/resources/publications/surveillance/CDS_EPR_ARO_2006_1.pdf.

When collecting nasopharyngeal and oropharyngeal specimens, swabs specifically designed for collecting specimens for virology must be used. These swab kits should contain virus transport medium. The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load. **Both upper and lower respiratory tract specimens should be collected whenever possible.**

MERS-CoV nucleic acid has been detected by RT-PCR in serum (7, 8), thus collecting serum for virus detection may be useful, particularly if lower respiratory tract samples are not available. Serum samples should also be stored for antibody detection (9, 10, 11).

MERS-CoV has been detected in urine and faeces but at levels below those found in the lower respiratory tract (2).

In RT-PCR confirmed cases, repeat sequential sampling for RT-PCR testing is strongly encouraged in the respiratory tract (upper and lower) and multiple other body

compartments (e.g. serum, urine and stool) since it will add to current knowledge about the duration of virus shedding and can guide decision making for infection prevention and control measures.

To confirm clearance of the virus, respiratory samples should continue to be collected until there are two consecutive negative results in clinically recovered persons. The frequency of specimen collection will depend on local circumstances but should be at least every 2-4 days. If the release of a patient from an isolation ward requires consecutive negative RT-PCR results, specimens can be collected daily.

For antibody detection, paired serum samples are required for confirmation of infection but single samples may also be used to identify probable cases. Paired serum samples should ideally be collected 14-21 days apart, with the first sample taken during the first week of illness. If only a single sample can be collected, it should be done at least 14 days after the onset of symptoms. Table 1 lists the specimens that can be collected from symptomatic patients along with the appropriate storage and transportation requirements. Routine sampling of exposed asymptomatic contacts of cases should be considered in health care settings with high exposure risks, such as settings using nebulizers.

Table 1. Specimens to be collected from symptomatic patients and asymptomatic contacts

Patient	Test	Type of sample	Timing	Storage and transportation	Remarks
Symptomatic	RT-PCR	Lower respiratory tract - sputum - aspirate - lavage	Collect on presentation. To confirm clearance of the virus, sample collection to be repeated until the results are negative on 2 sequential samples.	If the specimen will reach the laboratory in less than 72 hours, store and ship at 4°C. If the specimen will reach the laboratory in more than 72 hours, store at -80°C and ship on dry ice or liquid nitrogen.	Follow international regulations and triple package system for transportation.
		Upper respiratory tract - nasopharyngeal and oropharyngeal swabs - nasopharyngeal wash/nasopharyngeal aspirate Serum for virus detection (particularly if lower respiratory tract specimens are not available.) For monitoring the distribution of virus in the body: other sample types, stool, urine			
Symptomatic	Serology	Serum for serological testing.	Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-3 weeks later. If only a single serum sample can be collected, this should occur at least 14 days after onset of symptoms for determination of a probable case.	As above.	As above.
Asymptomatic Contact (particularly in health-care centre associated outbreaks or other situations of high-intensity contact)	PCR	Nasopharyngeal and oropharyngeal swabs; sputum if possible.	Within 14 days of last documented contact.	As above.	As above.
	Serology	Serum	Baseline serum taken within 14 days of last documented contact and convalescent serum taken 2-3 weeks later. If only a single sample is possible, collect at least 14 days after last documented contact	As above.	As above.

Specimens should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation is essential. Specimens which can be delivered promptly to the laboratory can be stored and shipped at 4°C. When there is likely to be a delay of more than 72 hours in the laboratory receiving respiratory tract specimens, it is strongly recommended that the specimens be frozen to -80°C and shipped on dry ice. It is important to avoid repeated freezing and thawing of specimens. Serum should be separated from whole blood and can be stored and shipped at 4°C if the specimen will reach the laboratory in less than 72 hours, or frozen and shipped on dry ice or liquid nitrogen if storage and transportation will take longer. The storage of respiratory and serum specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations.

Transport of specimens within national borders should comply with applicable national regulations.

International transport of MERS-CoV specimens should follow applicable international regulations, as described in WHO's *Guidance on regulations for the Transport of Infectious Substances 2015-2016* (applicable from 1 January 2015) available at http://www.who.int/ihr/publications/who_hse_ihr_2015.2/en/.

4. Laboratory biosafety

Any testing for the presence of MERS-CoV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on the laboratory biosafety should be followed in all circumstances.

Key points:

- Each laboratory should conduct a risk assessment to ensure it is competent to safely perform this testing
- When handling and processing specimens, including blood for serological testing, laboratory practices and procedures that are basic to good microbiological techniques (GMT) should be followed.
- The handling and processing of specimens from cases with suspected or confirmed MERS-CoV infection intended for additional laboratory tests such as haematology or blood gas analysis should follow local guidelines for processing potentially infectious material.
- Non-culture diagnostic laboratory work including RT-PCR analysis on clinical specimens from patients who are suspected or confirmed to be infected with MERS-CoV should be conducted adopting practices and procedures described for basic laboratory – Biosafety Level 2 (BSL-2) in the WHO Laboratory Biosafety Manual, 3rd edition http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/.
- Initial processing of all specimens including those for RT-PCR analysis should take place in a class II or class III biosafety cabinet with current certification.

- All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets.
- Appropriate PPE should be worn by all laboratory staff handling these specimens
- Handling of live virus (such as when performing neutralization assays) should be performed only in laboratories capable of meeting additional essential containment requirement including practices recommended for Biosafety Level 3 (BSL-3) laboratories in the WHO Laboratory Biosafety Manual.

5. Algorithm for detecting MERS-CoV by PCR and sequencing

Routine confirmation of cases of MERS-CoV infection is based on detection of unique sequences of virus RNA by real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. See Section 6 below for information on serological testing for MERS-CoV.

Virus isolation is not recommended as a routine diagnostic procedure.

Individual WHO Member States will decide which, if any, of their laboratories should perform MERS-CoV diagnostic tests. Each rRT-PCR run should include both external and internal controls, and laboratories should participate in external quality assessment schemes whenever possible.

Three rRT-PCR assays for routine detection of MERS-CoV have been developed and their details published. Currently described tests include an assay targeting upstream of the E protein gene (upE) (12) and assays targeting the open reading frame 1b (ORF 1b) (12) and the open reading frame 1a (ORF 1a) (13). The assay for the upE target is considered highly sensitive and is recommended for screening, with the ORF 1a assay considered of equal sensitivity. The ORF 1b assay is considered less sensitive than the ORF 1a assay. The United States Centers for Disease Control and Prevention (US CDC) has developed rRT-PCR assays targeting the MERS-CoV nucleocapsid (N) protein gene, which can complement upE and ORF 1a assays for screening and confirmation (14). To date, these rRT-PCR assays have shown no cross-reactivity with other respiratory viruses including human coronaviruses.

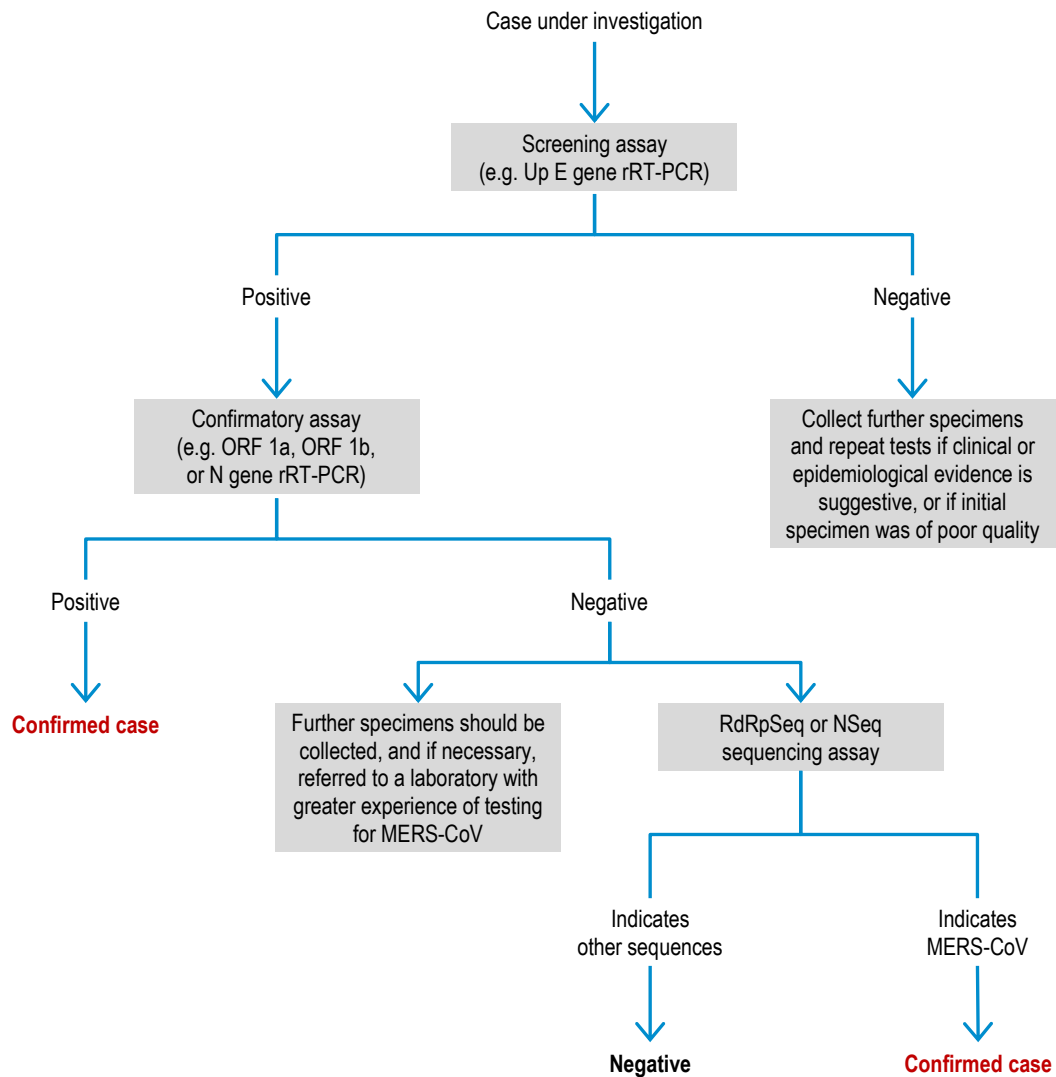
Two target sites on the MERS-CoV genome suitable for sequencing to aid confirmation have been identified. These are in the RNA-dependent RNA polymerase (RdRp) and (N) genes (13).

Further details of these assays, including bench protocols, are available at the website of the Institute of Virology, Bonn, Germany: <http://www.virology-bonn.de/index.php?id=40>

Further information on the assays developed by US CDC is available from Dean D. Erdman (dde1@cdc.gov).

Figure 1 shows a testing algorithm for investigation of suspected cases of MERS-CoV by rRT-PCR.

Figure 1. Algorithm for testing cases under investigation for MERS-CoV by rRT-PCR



Laboratories with limited experience in testing for MERS-CoV are encouraged to have their initial test results confirmed by laboratories with greater experience handling specimens containing this virus. WHO can assist Member States to identify laboratories able to provide this support. Additionally, laboratories may wish to check their own positive results by repeating the nucleic acid extraction and retesting the sample.

When there are discordant results with two rRT-PCR assays targeting unique sites on the MERS-CoV genome, the patient should be resampled. In addition, sequencing of an amplicon generated from an appropriate rRT-PCR assay such as for RdRp or N genes (13) should be attempted to confirm the test result. In addition to providing confirmation of the presence of the virus, sequence data, when analysed in real time, can also provide valuable information for understanding the origins of the virus and how it is spreading, hence, it is recommended to sequence MERS-CoV nucleic acid from as many positive specimens as possible. Full genome sequencing and sequencing of the spike protein gene directly from clinical samples is particularly desirable, but should be carried out by facilities with experience in this area.

Four endemic human coronaviruses (HCoVs) are known causes of respiratory tract infections of mild to moderate

severity. These are the betacoronaviruses HCoV-OC43 and HCoV-HKU1 and the alphacoronaviruses HCoV-229E and HCoV-NL63. Commercial multiplex PCR assays for respiratory pathogens may detect these viruses. It is important that positive results for these viruses should not be confused with MERS-CoV.

A series of negative results should not absolutely rule out the possibility of MERS-CoV infection. A number of factors could lead to false-negative results, including:

- poor quality of the specimen
- the specimen was collected late or very early in the illness
- the specimen was not handled and shipped appropriately
- technical reasons inherent in the test, e.g. virus mutation or PCR inhibition.

If a negative result is obtained from patients with a high index of suspicion for MERS-CoV infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract if possible, should be collected and tested. Laboratories may also consider sending one or more negative specimens to outside laboratories for confirmation.

To consider a case as laboratory-confirmed by RT-PCR, one of the following conditions must be met:

- A positive RT-PCR result for at least two different specific targets on the MERS-CoV genome using a validated assay; OR
- One positive RT-PCR result for a specific target on the MERS-CoV genome and MERS-CoV sequence confirmation from a separate viral genomic target (13).

A case with a positive RT-PCR result for a single specific target without further testing but with a history of potential exposure and consistent clinical signs is considered a **probable case**.

6. Serological testing for MERS-CoV

Details of two immunofluorescence assays to detect antibodies to MERS-CoV have been published (13), and these assays, along with a serum neutralization test, were used in a 2 to 3 stage procedure to screen contacts in Germany and determine population seroprevalences in KSA (9, 10, 11, 12). An assay for detection of MERS-CoV antibodies using protein microarray technology has also been developed and the details have been published (15,16) suggesting that it is highly specific. US CDC has developed a two-stage approach for detecting antibodies to MERS-CoV, based on a screening test using a recombinant nucleocapsid (N) protein-based indirect enzyme-linked immunosorbent assay (ELISA), followed by a confirmatory test using a whole-virus indirect fluorescent antibody (IFA) test or microneutralization test. A neutralisation test based on retroviral pseudoparticles has also been published (17) which also demonstrates high levels of specificity to MERS-CoV.

There are two situations where laboratories may wish to conduct serological testing for MERS-CoV, namely:

- 1) serology in relation to defining a MERS-CoV case for reporting under the International Health Regulations (IHR);
- 2) serological surveys, including to retrospectively assess the extent of an outbreak.

1) Use of serology in relation to defining a MERS-CoV case for reporting under the International Health Regulations

Where a patient has evidence of **seroconversion** in at least one screening assay (e.g. ELISA, IFA) and confirmation by a neutralization assay in samples ideally taken at least 14 days apart, this patient can be considered a **confirmed case**, regardless of the results of PCR assays. Our understanding of the antibody response to MERS-CoV is still limited, however based on the experience with SARS patients and preliminary information on MERS patients, a seroconversion can be expected to result in a minimum 4-fold increase in titre.

When a symptomatic patient, without a positive RT-PCR test has a positive result for at least one screening assay (e.g. ELISA or IFA) plus a positive result for a neutralization assay in a single specimen, they are indicated as a **probable case**.

When a case is confirmed by RT-PCR, the taking of sequential serological samples, where possible, is

encouraged in order to add to knowledge on the kinetics of the antibody response. Routine testing of asymptomatic contacts of confirmed cases may be justified in circumstances such as the investigation of nosocomial outbreaks. An asymptomatic contact with positive serological test results on a single specimen does not meet the current WHO case definition for a confirmed or probable acute case.

2) Use of serology in relation to population-based serosurveys and investigations of past exposures

Usually only a single specimen is available from each person in the survey. The same criteria for interpretation applies 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 indicates a **past infection** (16). With a single specimen it is not possible to determine the time of infection.

7. Reagents

As the primer and probe sequences for the rRT-PCR assays for MERS-CoV have been published, laboratories can order these from their usual suppliers. Positive control material for the upE and ORF 1a specific rRT-PCR assays can be ordered from the European Virus Archive portal: http://www.european-virus-archive.com/Portal/produit.php?ref=1386&id_rubrique=9.

Member States requiring support for obtaining control material for the rRT-PCR assays can ask WHO for assistance.

US CDC has produced a diagnostic kit for detection of MERS-CoV by rRT-PCR and will make it available on a limited basis. Further information is available from Dean D. Erdman (dde1@cdc.gov).

At least two commercial kits for detecting MERS-CoV by PCR are available. Further information on the kit produced by Altona Diagnostics is available at <http://www.altona-diagnostics.com/realstar-mers-cov-rt-pcr-kit.html>.

Fast Track Diagnostics has also produced a kit and information is available at http://www.fast-trackdiagnostics.com/products/50/ftd_hcov-emc/.

Commercial kits for immunofluorescence and ELISA testing of human and camel serum have recently become available. See <http://euroimmun.org/index.php?id=35&uid=12280&L=1#c12280>.

To date, no comparative evaluation of available serological assays using a single set of sera to allow a fuller assessment of their performance has been done. In the meantime, laboratories planning to perform serological testing for MERS-CoV are encouraged to work with an international laboratory experienced in the performance of serological assays for this virus.

WHO does not endorse any particular product and laboratories are encouraged to make their own enquiries to determine which kit, if any, is appropriate to their particular circumstances and has obtained necessary regulatory approval.

8. Global laboratory networking

Timely and accurate laboratory testing of samples from cases under investigation is an essential part of the surveillance of this emerging infection. All countries should have access to reliable testing, either nationally or internationally, in laboratories willing to perform primary detection or confirmatory testing. WHO can assist Member States to access testing internationally should the need arise. Member States may wish to sign material transfer agreements (MTAs), covering such topics as ownership of clinical material and intellectual property rights, with international laboratories before shipping specimens.

9. Reporting cases and test results

Laboratories should follow national reporting requirements, but in general, in countries with no confirmed cases or only sporadic cases of MERS-CoV infection, the relevant public health authorities should be notified as soon as the laboratory receives a specimen, even before any testing is performed. All test results, whether positive or negative, should likewise be immediately reported to the authorities. If the infection becomes widespread, laboratories should notify public health authorities immediately of each new confirmed case, or positive screening test if there will be a delay in confirmatory testing. Laboratories should also periodically report the number of negative test results to the relevant public health authorities.

Member States are requested to notify WHO immediately of all confirmed cases and of positive screening results if there will be delay in confirmatory testing or if specimens are being sent internationally for confirmation. Details of the particular assays performed should be included with the notifications.

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