

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) - Version 2

Summary of Changes in Version 2

This is an updated version of the interim guidance document issued by the Centers for Disease Control and Prevention (CDC) on June 7, 2013. CDC has revised the interim guidance based on comments received from public health partners, healthcare providers, professional organizations, and others. CDC will continue to update the document as necessary to incorporate new information that increases our understanding of MERS-CoV. Updates:

1. Modified the title of the document to reflect additional testing guidelines
2. Expanded the “Specimen Type and Priority” section to better describe what specimens are preferred for testing
3. Expanded the “Blood Components – Serum” section to better describe available testing options based on time between symptom onset and serum collection
4. Revised “Summary of MERS-CoV rRT-PCR Testing Guidelines for Respiratory Specimens” that describes reporting MERS-CoV test results and testing for other respiratory pathogens

Before collecting and handling specimens for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) testing, determine whether the person meets the current definition for a “patient under investigation” (PUI) for MERS-CoV infection prepared by the Centers for Disease Control and Prevention (CDC). See <http://www.cdc.gov/coronavirus/mers/case-def.html>.

Specimen Type and Priority

To date, little is known about pathogenic potential and transmission dynamics of MERS-CoV. To increase the likelihood of detecting infection, CDC recommends collecting multiple specimens from different sites at different times after symptom onset, if possible.

Points to consider when determining which specimen types to collect from a patient under investigation for MERS include:

1. The number of days between specimen collection and symptom onset
2. Symptoms at the time of specimen collection

Additional points to consider:

1. Maintain proper infection control when collecting specimens
2. Use approved collection methods and equipment when collecting specimens
3. Handle, store, and ship specimens following appropriate protocols

Lower respiratory specimens are preferred, but collecting nasopharyngeal and oropharyngeal (NP/OP) specimens, as well as stool and serum, are strongly recommended depending upon the length of time between symptom onset and specimen collection. For example, if symptom onset for a PUI with ongoing lower respiratory tract infection was 14 or more days ago, a single serum specimen for serologic testing (see *Section II. Blood Components – Serum*) in addition to a lower respiratory specimen and an NP/OP specimen (see *Section I. Respiratory Specimens*) are recommended.

Respiratory specimens should be collected as soon as possible after symptoms begin – ideally within 7 days and before antiviral medications are administered. However, if more than a week has passed since symptom onset and the patient is still symptomatic, respiratory samples should still be collected, especially lower respiratory specimens since respiratory viruses can still be detected by rRT-PCR.

General Guidelines

For short periods (≤ 72 hours), most specimens should be held at 2-8°C rather than frozen. For delays exceeding 72 hours, freeze specimens at -70°C as soon as possible after collection (with exceptions noted below). Label each specimen container with the patient's ID number, specimen type and the date the sample was collected.

I. Respiratory Specimens

A. Lower respiratory tract

Bronchoalveolar lavage, tracheal aspirate, pleural fluid

Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C up to 72 hours; if exceeding 72 hours, freeze at -70°C and ship on dry ice.

Sputum

Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C up to 72 hours; if exceeding 72 hours, freeze at -70°C and ship on dry ice.

B. Upper respiratory tract

Nasopharyngeal AND oropharyngeal swabs (NP/OP swabs)

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media. NP/OP specimens can be combined, placing both swabs in the same vial. Refrigerate specimen at 2-8°C up to 72 hours; if exceeding 72 hours, freeze at -70°C and ship on dry ice.

Nasopharyngeal swabs -- Insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nasopharyngeal areas.

Oropharyngeal swabs -- Swab the posterior pharynx, avoiding the tongue.

Nasopharyngeal wash/aspirate or nasal aspirates

Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C up to 72 hours; if exceeding 72 hours, freeze at -70°C and ship on dry ice.

II. Blood Components

Serum (for serologic testing)

For serum antibody testing: Serum specimens should be collected during the acute stage of the disease, preferably during the first week after onset of illness, and again during convalescence, ≥ 3 weeks after the acute sample was collected. However, since we do not want to delay detection at this time, a single serum sample collected 14 or more days after symptom onset may be beneficial. Serologic testing is currently available at CDC upon request and approval. Please be aware that the MERS-CoV serologic test is for research/surveillance purposes and not for diagnostic purposes – it is a tool developed in response to the MERS-CoV outbreak. Contact CDC's Emergency Operations Center (EOC) (770-488-7100) for consultation and approval if serologic testing is being considered.

Serum (for rRT-PCR testing)

For rRT-PCR testing (i.e., detection of the virus and not antibodies), a single serum specimen collected optimally during the first week after symptom onset, preferably within 3-4 days, after symptom onset, may be also be beneficial.

NOTE: These time frames are based on SARS-CoV studies. The kinetics of MERS-CoV are not well understood and may differ from SARS-CoV. Once additional data become available, these recommendations will be updated as needed.

Children and adults Collect 1 tube (5-10 mL) of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and separate sera into sterile tube container. The minimum amount of serum required for testing is 200 μ L. Refrigerate the specimen at 2-8°C and ship on ice-pack; freezing and shipment on dry ice is permissible.

Infants A minimum of 1 mL of whole blood is needed for testing of pediatric patients. If possible, collect 1 mL in an EDTA tube and in a serum separator tube. If only 1 mL can be obtained, use a serum separator tube.

EDTA blood (plasma)

Collect 1 tube (10 mL) of heparinized (green-top) or EDTA (purple-top) blood. Refrigerate specimen at 2-8°C and ship on ice-pack; do not freeze.

III. Stool

Collect 2-5 grams of stool specimen (formed or liquid) in sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C up to 72 hours; if exceeding 72 hours, freeze at -70°C and ship on dry ice.

IV. Shipping

Specimens from suspected MERS cases must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at http://www.iata.org/whatwedo/cargo/dgr/Pages/infectious_substances.aspx. Shipments from outside of the United States may require an importation permit that can be obtained from CDC.

Specimens should be stored and shipped at the temperatures indicated above. If samples are unable to be shipped within 72 hours of collection, they should be stored at -70°C and shipped on dry ice. When shipping frozen specimen from long distances or from international locations, it is best to use a combination of dry ice and frozen gel ice-packs. The gel ice-packs will remain frozen for a day or two after the dry ice has dissipated.

All specimens must be pre-packed to prevent breakage and spillage. Specimen containers should be sealed with Parafilm® and placed in ziplock bags. Place enough absorbent material to absorb the entire contents of the Secondary Container (containing Primary Container) and separate the Primary Containers (containing specimen) to prevent breakage. Send specimens with cold packs or other refrigerant blocks that are self-contained, not actual wet ice. This prevents leaking and the appearance of a spill. When large numbers of specimens are being shipped, they should be organized in a sequential manner in boxes with separate compartments for each specimen.

CDC recommends **against** the following:

- Do not place any dry ice in the "Primary Container" or "Secondary Container", foam envelopes,

- ziplock bags, cryovial boxes, or hermetically sealed containers.
- Do not place Primary Containers sideways or upside down in ziplock bags.
 - Do not use red top Secondary Containers for Category A Infectious Substances.
 - Do not place any paperwork in the Secondary Containers or ziplock bags, so as not to damage the paperwork.
 - Do not use biohazard/autoclave bags to prepack your materials due to the inadequate seal of these bags.

For additional information, consultation, or the CDC shipping address, contact the CDC Emergency Operations Center (EOC) at 770-488-7100. Specimens should be shipped for overnight delivery - if Saturday delivery is planned, special arrangements must be made with the shipping company.

Summary of MERS-CoV rRT-PCR Testing Guidelines for Respiratory Specimens

Many state health department laboratories are approved for MERS-CoV testing using the CDC rRT-PCR assay. Contact your local/state health department to notify them of the PUI and to request MERS-CoV testing. If your state health department is unable to test, contact CDC's EOC at 770-488-7100.

Testing for MERS-CoV and other respiratory pathogens can be done simultaneously. Virus isolation in cell culture and initial characterization of viral agents recovered in cultures of MERS-CoV specimens are NOT recommended at this time. However, if done, these activities must be performed in a BSL-3 facility using BSL-3 work practices.

Test for MERS-CoV

The laboratory must follow the protocol for the CDC rRT-PCR assay. "NEGATIVE" test results should be reported through the CDC Laboratory Response Network (LRN) within 24 hours. When a "PRESUMPTIVE POSITIVE" or "EQUIVOCAL" test result is obtained, CDC must be contacted immediately as per the assay protocol, and the result must also be reported to the LRN within 6 hours. Confirmation of a "PRESUMPTIVE POSITIVE" result by CDC is required, however this should not delay the local investigation and response, including the contact investigation.

Test for Other Respiratory Pathogens

Testing for common respiratory pathogens by molecular or antigen detection methods (**not by viral culture**) is **strongly recommended**. Common respiratory pathogens include 1) influenza A, influenza B, respiratory syncytial virus, human metapneumovirus, human parainfluenza viruses, adenovirus, human rhinovirus and other respiratory viruses; 2) *Streptococcus pneumoniae*, *Chlamydia pneumophila*, and other pathogens that cause severe lower respiratory infections. Clinical presentation, epidemiologic and surveillance information, and season should be considered when selecting which pathogens to test for. A few MERS-CoV cases have had other respiratory pathogens detected, so identification of a respiratory pathogen prior to MERS-CoV testing should not preclude testing for MERS-CoV, especially if MERS is strongly suspected. If your laboratory does not have molecular or antigen testing capability for respiratory pathogens, contact your state laboratory for assistance.

For more information,
visit <http://www.cdc.gov/coronavirus/mers/guidelines-clinical-specimens.html>