World Health Organization
REGIONAL OFFICE FOR Africa

JOB AIDS
for collection, storage and transport of specimens for laboratory confirmation of Middle East respiratory syndrome coronavirus (MERS-CoV)

Integrated Disease Surveillance Programme
Disease Prevention and Control Cluster

July 2013

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Introduction

This job aid deals with the procedures for collecting and processing respiratory specimens for laboratory confirmation of Middle East respiratory syndrome coronavirus (MERS-CoV). It is primarily for use by health-care providers and public health staff during investigation of suspected cases of MERS-CoV. It is not intended as an exhaustive manual on laboratory procedures.

The World Health Organization (WHO) continues to closely monitor the MERS-CoV situation worldwide and if changes that might affect the procedures contained in this job aid occur, WHO will issue an update to these procedures. Otherwise, this document will expire 12 months from the date of publication.

These recommendations have been prepared by WHO and reviewed by laboratory experts. Part of the review process involved examination of existing literature on MERS-CoV.

Background

Coronaviruses are a large family that includes viruses responsible for a range of illnesses in humans from the common cold to the severe acute respiratory syndrome (SARS). Viruses of this family also cause a number of animal diseases.

MERS-CoV is a particular strain of coronavirus that has not been previously identified in humans. Information on its transmission, severity and clinical impact is limited and only a small number of cases have been reported so far.

Although it is not known during which period of the illness exposure to it results in infection or the nature of exposure that leads to infection, in patients with established or strongly suspected exposure the incubation period of MERS-CoV as confirmed laboratory testing is generally less than one week. Evidence from patients exposed over a range of time suggests that only in a minority of cases does the incubation period exceed one week, and is less than two weeks.

All people confirmed with MERS-CoV infection had respiratory disease, most had pneumonia and half of them died. Complications during the course of illness include severe pneumonia with respiratory failure requiring mechanical ventilation, acute respiratory distress syndrome

(ARDS) with multi-organ failure, renal failure requiring dialysis, consumptive coagulopathy, and pericarditis. In a number of cases gastrointestinal symptoms were noted during the illness, including diarrhea.

Access to timely and accurate laboratory testing of samples from cases under investigation is an essential part of the surveillance for this emerging infection. Each country should have its own reliable testing facilities for primary detection or confirmatory testing of MERS-CoV or guaranteed access to such facilities in another country.

Following proper procedures during collection and processing of specimens is important and has implications on the results from laboratory testing. For example, false-negative results could originate from:

- poor quality specimens such as a respiratory tract specimen containing primarily oropharyngeal material
- specimens collected too late or too early in the illness
- improper handling or shipping of specimens

Appropriate handling of MERS-CoV *specimens is essential during all steps from collection to laboratory testing*

This document provides guidelines on the procedures for collecting, handling and transporting specimens from the ward to the reference laboratory whether inside or outside a country for MERS-CoV confirmation. It covers all the key components of this process:

- when to collect specimens
- what samples to collect
- how to prepare documentation
- what supplies are needed
- how to collect specimens
- how to store specimens
- how to pack specimens
- how to transport specimens

Collecting and handling specimens for MERS-CoV confirmation

Before collecting specimens for testing for MERS-CoV determine that the patient meets the current requirements defined for MERS-CoV by WHO (see http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html).

When to collect specimen

Respiratory tract specimens for the direct detection of nucleic acids can be collected at any time during the acute phase of the illness but the best time is the first week of the illness. Specimens should be collected at different times after symptom onset, if possible.

What samples to collect

A variety of specimens are suitable for diagnosis of MERS-CoV including samples from the respiratory tract and acute-phase and convalescent serum. This document deals with respiratory tract specimens.

Accumulating evidence suggests that specimens from nasopharyngeal swabs are not as sensitive as samples from the lower respiratory tract in detection of MERS-CoV infection. Head-to-head comparisons have not yet been made of the two approaches but in some patients and during the course of the same illness nasopharyngeal swab specimens gave negative results for MERS-CoV at one point while lower respiratory specimens gave positive results at another time. In addition, in several clusters, nasopharyngeal swabs from people strongly suspected to have MERS-CoV infection because of being directly exposed to it and having severe respiratory illness gave negative results while lower respiratory tract specimens from other people in the cluster gave positive results.

WHO now strongly recommends using specimens from the lower respiratory tract such as sputum, endotracheal aspirate and bronchoalveolar lavage samples, when possible, in addition to nasopharyngeal swabs for diagnostic polymerase chain reaction (PCR) until other information is available showing the best practice. To increase the chances of detecting infection, collecting specimens from different sites is recommended.

Patients strongly suspected to have MERS-CoV infection who in initial testing using a nasopharyngeal swab specimen show negative results should be retested using specimen from the lower respiratory tract. If specimens cannot be collected from the lower respiratory tract testing should be repeated using upper respiratory specimen but with oropharyngeal specimen as an addition.

How to prepare documentation

The information to be provided on the case investigation and laboratory request forms to accompany the specimens is stipulated in the *Integrated disease surveillance technical guidelines* or the *Influenza sentinel surveillance protocol*.

The field data collection form should include the general information about the patient, type of specimens and the date they were collected, contact information of the person completing the form etc. When possible, the data should describe the clinical presentation of the illness including the date of the onset of symptoms, occurrence of complications and natural history of the infection. It is also crucial that the health worker collecting the specimenss obtain details on the patient's clinical exposure history, contact with animals and recent travel to areas where the epidemic has been reported.

Each specimen container should be labeled with the patient's ID number and the specimen type and the date it was collected.

What supplies are needed

Before starting to handle specimens make sure that all the materials required for their efficient and safe collection and transport to the laboratory for testing are ready. The proposed list of essential materials should include:

- sterile latex or nitrile gloves
- protective clothing such as a gown, coat or apron
- masks
- eye visor, goggles or face shield
- chemical disinfectant such as hypochlorite
- triple packaging boxes

- leak-proof waste disposal container

For samples from the lower respiratory tract the following are required in addition to the essential items:

- sputum or mucus trap
- polyester-fibre-tipped applicator
- plastic vials
- tongue depressor
- sterile, leak-proof, screw-cap sputum collection cup or sterile dry container
- transfer pipettes

For samples from the upper respiratory tract add the following items to the list:

- Swabs — use only sterile dacron or rayon swabs with plastic shafts or flocked swabs if these are available. DO NOT use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate c viruses and inhibit some molecular assays.
- Viral transport medium for storing and shipping the swabs.

How to collect specimens

All specimens should be regarded as potentially infectious, and health workers who handle clinical specimens should adhere rigorously to standard precautions to minimize the possibility of exposure to pathogens. MERS-CoV specimens should be handled according to the appropriate biosafety practices to avoid laboratory-related infections and spread of disease.

Standard precautions should always be followed and barrier protection should be used by all staff collecting samples from patients. It is important to always remember that safety and decontamination measures protect the specimen collector and colleagues, laboratory personnel and the patient from risks associated with specimen collection.

Personal protective clothing must be worn when handling the specimens

Clinical samples from patients should be collected by trained personnel. It is essential that the people to be in charge of collecting specimens in the clinical wards and those to be responsible for the logistical aspects of sample handling from the ward to the laboratory be identified in advance.

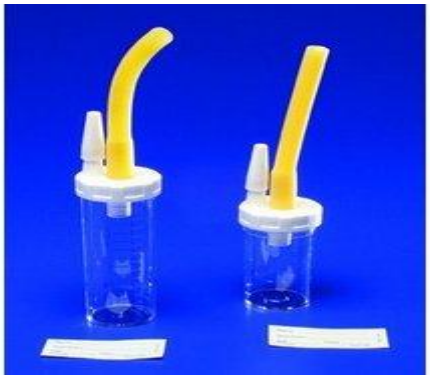
Lower respiratory tract specimens

Owing to shortages of staff with adequate technical skills and equipment, people from who lower respiratory tract specimens other than sputum may be collected might be limited to only those admitted in hospitals.

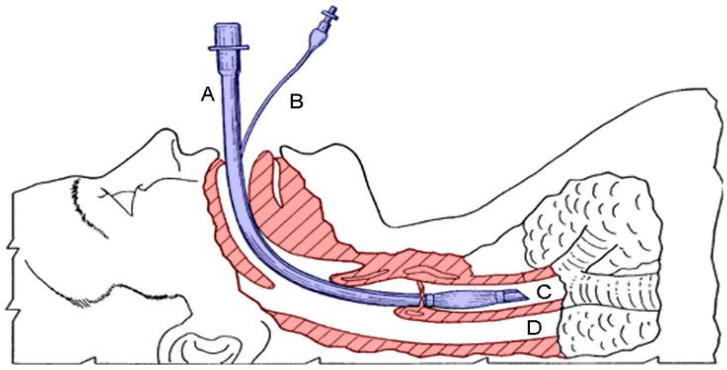
***Sputum*:** Collection of naturally produced sputum may pose special infection risks for health-care workers. The health worker collecting the specimen needs to ensure the material is from the lower respiratory tract. It is important to explain to the patient about the difference between sputum and oral secretions. The patient has first to rinse the mouth with water. Next he or she should take a deep breath and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or a sterile dry container.

Samples of 3–5 ml mucopurulent viscous material are considered to be specimens of good quantity and quality.

Endotracheal aspirate*:* Collection of endotracheal aspirate is a medical procedure in which aspirate samples are obtained from an intubated patient. Tracheal aspirate samples, which should be handled in a sterile fashion, can be collected into a endotracheal tube by suctioning into a sterile trap. This technique should be used by only well-trained health workers.

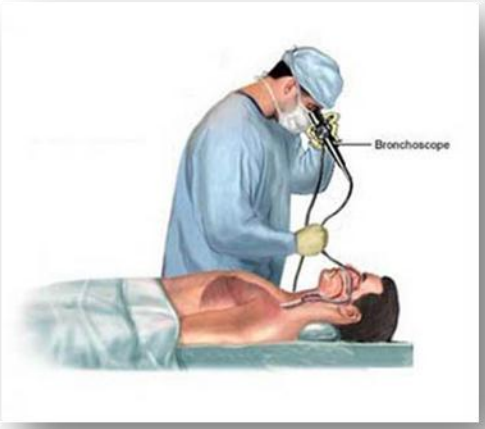


Sterile specimen traps may be used to collect tracheal aspirate



Only qualified staff should collect endotracheal aspirate samples

Bronchoalveolar lavage specimens: Bronchoalveolar lavage is a medical procedure in which a bronchoscope is passed through the mouth or the nose into the lungs and fluid squirted into a small part of the lung and then recollected for examination. Because of the invasive nature of this procedure only trained health professionals should use it to collect specimen.



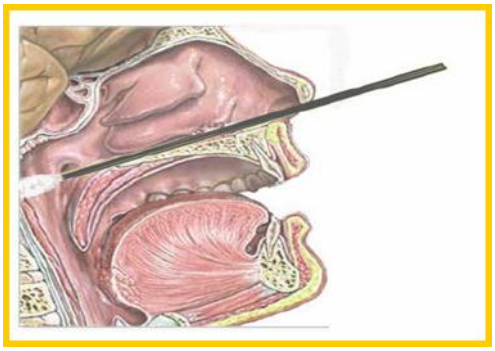
The bronchoalveolar lavage procedure is invasive and should be performed by only qualified personnel

For endotracheal aspirate or bronchoalveolar lavage procedures 2–3 ml samples should be collected into a sterile, leak-proof, screw-cap sputum collection cup or a sterile dry container.

Upper respiratory tract specimens

***Nasopharyngeal swab specimen*:** This procedure involves inserting a dry rayon or polyester swab into the nostril to reach the nasopharynx. A flexible shafted swab must be used; DO NOT use a rigid swab. The swab should be slid straight into the nostril with the patient's head held slightly back. The swab should be inserted touching the base of the nostril aiming towards the auditory pit and for about 5–6 cm in adults so that it reaches the posterior pharynx. The following steps should be followed to ensure that the sample collected is of good quality:

- The swab should be left in place for a few seconds.
- The swab should be removed slowly while being rotating slightly.
- The tip of swab should be put in a vial containing virus transport medium and then the applicator stick should be broken off.
- A second swab should be used for the other nostril and placed in a second tube. This can serve as the second sample for the patient.



A nasopharyngeal swab should be inserted to reach the nasopharynx

Nasopharyngeal sampling is an invasive process that can cause considerable distress to the patient.

How to store specimens

Lower respiratory tract specimens such as sputum, endotracheal aspirate and bronchoalveolar lavage samples should be placed in a centrifuge tube without a virus transport medium; however, the tip of a nasopharyngeal swab should be placed in a virus transport medium.

Specimens from either the lower or the upper respiratory tract should be stored as follows before transportation to the laboratory:

- Specimens should be kept on ice and transported to the laboratory as soon as possible.
- For periods 24 hours or shorter most specimens can be held at 2–8 °C rather than frozen;
- If there is a delay in testing of more than 24 hours freezing of the specimens should be considered using dry ice or storing them at -70 °C as soon as possible after collection.

How to pack specimens

Triple packaging is required for infectious material. It comprises:

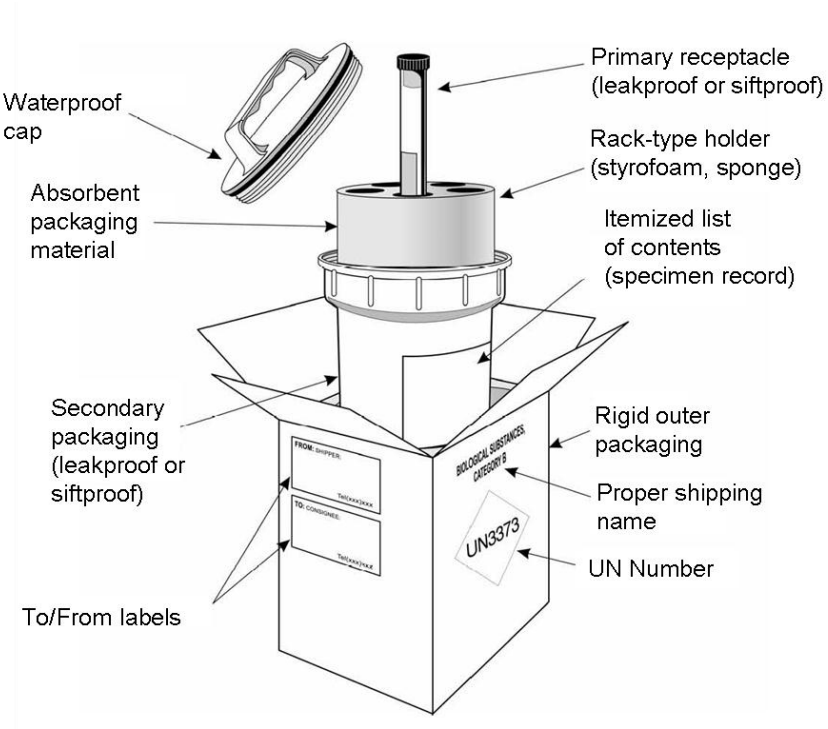
- Primary packaging that contains the specimens.
- Secondary packaging, which may have the capacity to hold several primary containers.
- Outer packaging that protects the inside containers during transportation.

All specimens must be packed protected from container breakage and spillage. Specimen containers should be leak-proof as should be secondary containers. Absorbent material should be enough to absorb the entire contents of the secondary container in case of spillage and to

separate the primary containers to prevent breakage. Specimens should be transported with cold packs or other self-contained refrigerant blocks not wet ice. This prevents leaking and the appearance of a spill. When large numbers of specimens are shipped, they should be arranged sequentially by number in boxes with separate a compartment for each.

Detailed packaging instructions for the international transport of infectious specimens are contained in the *WHO guidance on regulations for the transport of infectious substances 2013–2014* (applicable as from 1 January 2013).

Based on current knowledge, the characteristics of MERS-CoV do not warrant classification of its specimens for transportation purposes as infectious substance Category A. Consequently, and according to applicable international transport regulations, the WHO advice is that MERS-CoV specimens can be safely transported as "Biological Substance, Category B", assigned to UN 3373.



MERS-CoV *specimens may be transported in the packaging used for Category B biological materials. [Packing Instruction 650 (PI650)].*

Important things to note:

- Do not place dry ice, foam envelopes, Ziploc bags, cryovial boxes or hermetically sealed containers in the primary or secondary container.
- Do not place the primary containers sideways or upside down in Ziploc bags.
- Do not place any paperwork in the secondary container or Ziploc bags where it could get damaged.
- Do not use biohazard or autoclave bags to prepack your materials because their seal is inadequate.

How to transport specimens

Transporting specimens within national borders should comply with applicable national regulations. International transport of novel coronavirus specimens should follow applicable international regulations as described in the *WHO guidance on regulations for the transport of infectious substances 2013–2014 (*applicable as from 1 January 2013) available at http://www.who.int/ihr/publications/who_hse_ihr_20100801/en/index.html. Shippers should be aware that individual airlines might have policies that are stricter than applicable international transport regulations.

It is desirable to use commercially produced packaging materials if the specimens are to be sent outside the country. Maintenance of appropriate temperature during transportation is crucial. When the time between specimen collection and arrival at the laboratory is likely to exceed 24 hours, the specimens should be frozen and shipped using 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.

Diagnostic services for MERS-CoV are not often available in every country. If specimens are to be shipped internationally, it is essential to have the agreement of the receiving laboratory before their dispatch so that the laboratory can arrange for import permits and get the assays ready to run as soon as possible after the specimens arrive. WHO will help countries to contact a reference laboratory if this is needed.

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