

Appendix 3: MERS-CoV Laboratory testing information

Samples suitable for testing

Respiratory samples - Upper respiratory tract

1. Nasopharyngeal swab and/or oropharyngeal swab
 - nasopharyngeal: insert a swab into each nostril parallel to the palate, leave the swab in place for a few seconds to absorb secretions
 - oropharyngeal: swab the tonsillar beds, avoiding the tongue
 - place swabs back into the accompanying transport media
2. Nasal wash/aspirates
 - collect 2-3 mL into a sterile, leak-proof, screw-top dry sterile container

Respiratory samples – Lower respiratory tract

1. Bronchoalveolar lavage, tracheal aspirate, pleural fluid
 - collect 2-3 mL into a sterile, leak-proof, screw-top sputum collection cup or dry sterile container
2. Sputum
 - patient should rinse his/her mouth with water before collection
 - expectorate deep cough sputum directly into a sterile, leak-proof, screw-top dry sterile container

There is now increasing evidence that lower respiratory tract specimens contain the highest viral loads, therefore, lower respiratory tract specimens should be collected where possible. Repeat testing (especially of lower respiratory tract specimens) in compatible cases should be performed if initial results are negative.

Serology

Serum should be collected during the acute phase of the illness (preferably within the first 7 days of symptom onset), stored, and tested in parallel with a convalescent serum collected 3 or more weeks after acute sample collection. If no acute sample was collected, a single serum sample collected 14 or more days after symptom onset may be tested.

Immunofluorescence and neutralization serology tests are used. Similar to NAT, a two stage approach using a screening followed by a confirmatory test can be employed. For screening purposes, an enzyme-immunosorbent assay (ELISA) against recombinant N protein can be used, followed by confirmatory testing using a whole virus indirect fluorescent antibody (IFA) test or microneutralization. Given that all serological tests developed so far have only been validated against a small number of convalescent sera from MERS-CoV cases, positive serological test results in the absence of nucleic acid testing (NAT) or sequencing are considered probable cases only.

Stool

2 – 5 grams of stool (formed or liquid) is collected in a sterile, leak-proof, screw-top dry sterile container.

Handling of specimens in the laboratory

Laboratory staff should handle specimens under PC2 conditions in accordance with AS/NZS 2243.3:2010 Safety in Laboratories Part 3: Microbiological Safety and Containment. Specimens should be transported in accordance with current regulatory requirements.

MERS-CoV testing

NAT using reverse-transcriptase polymerase chain reaction (RT-PCR) is the method of choice for detection of MERS-CoV. Currently, four targets are used for testing:-

- upstream region of the E protein (upE) gene
- open reading frames (ORF) 1a (ORF1a)
- ORF1b
- MERS-CoV specific nucleocapsid (N) protein gene

An algorithm using a screening assay, followed by confirmatory testing is recommended. For screening purposes, assays targeting the upE gene are appropriate. Confirmatory testing can be performed using an assay targeting the ORF1a (comparable sensitivity to upE gene), ORF1b (which is less sensitive than ORF1a or upE) or N gene. It is recommended that positive screening tests be reported to communicable diseases agencies whilst awaiting confirmatory testing.

Where available, RdRp gene (for the broad detection of β -coronavirus clade C) and/or N gene sequencing may also be considered for MERS-CoV confirmation. As the primers for the RdRp sequencing assay is highly conserved, it is not recommended that this assay be used alone for MERS-CoV confirmation, as false positive results may occur from cross-reactions with other β -coronaviruses. Further information about laboratory testing is available at:

- [Institute of Virology, Bonn](http://www.virology-bonn.de/index.php?id=40) (<http://www.virology-bonn.de/index.php?id=40>)
- [The US Food and Drug Administration](http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM355572.pdf) (<http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM355572.pdf>).

Testing algorithms may also need to be revised pending further information about the virus, and the number of specimens received in the laboratory for testing.

Viral culture is generally not performed for routine diagnosis, and should only be attempted in laboratories with appropriate experience and containment facilities. MERS-CoV replication has been previously observed on Vero and LLC-MK2 cells within 5 days of inoculation