MERS-CoV: General considerations for Laboratory Diagnosis[1]

6 June 2015

Introduction:

The confirmation of a probable or suspicious case of infection by Middle East respiratory syndrome - coronavirus (MERS-CoV) (based on clinical and epidemiological criteria), can only be accomplished through laboratory testing. However, other viral respiratory pathogens (including, but not limited to: influenza, respiratory syncitial virus, other common human beta and alphacoronavirus) and bacterial (*Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Legionella pneumophila*) should be considered within the diagnostic algorithm.

Sample collection and proper shipment:

Samples should be collected by trained personnel and in consideration of all biosafety instructions and the personal protective equipment appropriate for respiratory viruses, according to the World Health Organization (WHO) guidelines for infection control and bio-risk management.[2]

Although the probability of detection is high during the first 7 days after the onset of symptoms, the viral genetic material has been detected in lower respiratory tract samples up to 14 days after the onset of the acute phase. Taking into account that the transmission mechanisms have not been clearly established, taking samples from a confirmed patient at least every 2-4 days until 2 consecutive negative results are obtained should be consider.

Since the highest viral load has been demonstrated in the lower respiratory tract, recommended samples include sputum, bronchoalveolar lavage and tracheal aspirate (when possible according to medical criteria). However, when it is not possible to take a better sample, those of the upper respiratory tract are also useful. In general, taking a nasopharyngeal swab combined with an oropharyngeal swab is recommended (swabs should be placed and transported in the same tube with viral transport medium). Although sampling of asymptomatic contacts on routine basis is not recommended, if it is considered necessary according to the guidelines adopted by the country, upper respiratory samples could be considered.

Serum samples for determination of antibodies can be collected to complement the diagnosis and eventually as part of the surveillance process. However, ensure paired samples with at least one week difference between the first (taken in the acute phase) and the second. A single sample could have diagnostic value only if it has been taken at a minimum 14 days after initiated the symptoms.

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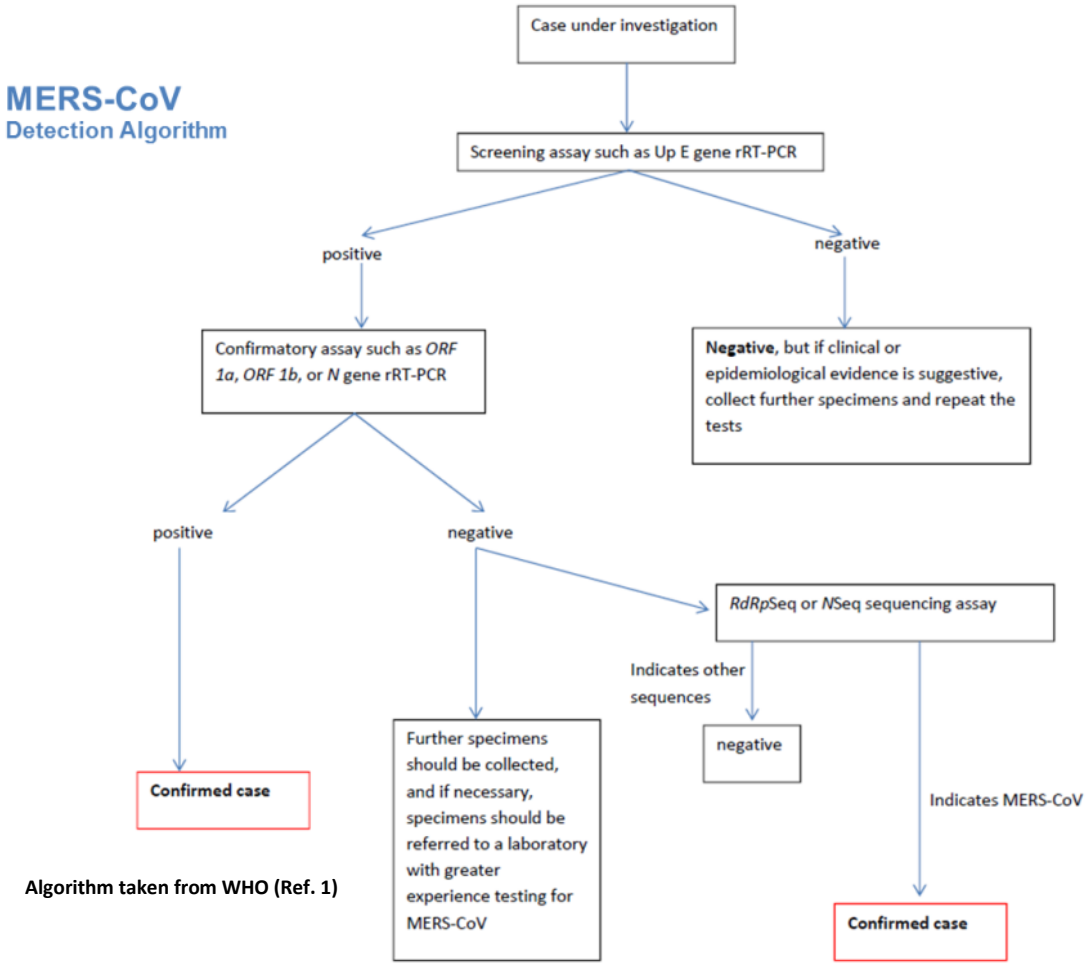
[1] The following recommendations have been adopted from the document ***WHO Interim Recommendations, Laboratory Testing for Middle East Respiratory Syndrome Coronavirus, September 2014*** (1) and are subject to further modifications in the light of advances in knowledge about the disease and the etiologic agent.

[2] Novel Coronavirus: Interim Recommendations for Laboratory Biorisk Management. http://www.who.int/csr/disease/coronavirus_infections/NovelCoronavirus_InterimRecommendationsLaboratoryBiorisk_190213/en/index.html

Samples should be kept refrigerated (4-8°C) and sent to the laboratory (central, national or reference) where they will be processed within the first 24-72 hours after being taken. If they cannot be sent within this period, freezing is recommended at -70 /-80°C until shipment, guaranteeing the cold chain. The sending of suspicious samples to reference laboratories or collaborating centers outside the country and by air must ensure compliance with all international standards (IATA) for **Biological Substances Category B.**

Algorithm for the molecular detection of MERS CoV:

Samples should be processed and handled only by trained professionals and in consideration of all biosecurity instructions and the personal protective equipment appropriate for respiratory viruses, under BSL-2 conditions and according to the WHO guidelines for infection control and bio-risk management[1,3]



Algorithm taken from WHO (Ref. 1)

[3] Viral isolation can only attempted under BSL-3 or BSL-4 biosafety conditions

The confirmation of a case of MERS-CoV infection is based on the detection by PCR (rRT-PCR) of at least two (2) regions of the genome, or amplification of a genetic target followed by sequencing of a different nucleotide genetic segment.

There are three (3) protocols published for the molecular detection of MERS-CoV. The diagnostic algorithm must start with a highly sensitive assay (amplification of the adjacent region - upstream – of the E protein gene, upE) followed by amplification of the open reading frame 1a (ORF 1a); the amplification of other genetic targets including the ORF 1b, and the nucleocapsid gene (N) can also be considered for confirmation.

On the other hand, diagnostic confirmation can also be complemented by sequencing of a region of the RNA polymerase (RdRp) or from gene N.

Serological testing for antibody determination:

Different assays and platforms have been developed and published for the detection of antibodies against MERS-CoV, including ELISA, IFA, protein microarrays, and neutralization. However, serological assays require careful interpretation and they should be carried out only under two circumstances:

1 - To define a case of MERS-CoV which should be reported under the International Health Regulations: If access to molecular methods is not possible, a case can be confirmed by serology only if a screening test (ELISA, IFA) produces a positive result in a sample of acute phase, and there is evidence of seroconversion (increase of up to 4 fold the titers) in a second sample (taken at least 14 days after the first) with a specific test of neutralization. A single sample with a positive result (with both, screening and neutralization tests) is considered **probable**.

2 - For serological surveys in a population or investigation of previous exposure: For serological surveys normally only one single sample is available. Therefore, the interpretation will be the same for probable case (positive result for both, screening and neutralization assays) and only indicates past infection. It will not be possible to determine the time of infection.

Reagents:

As the primer and probe sequences for the rRT- PCR assays for MERS-CoV have been published (2, 3, 4) laboratories can order these from their usual suppliers. Additional information could be consulted in:

- http://www.virology-bonn.de/index.php?id=40

The United States Centers for Disease Control and Prevention (CDC) have produced a diagnostic kit for detection of MERS-CoV by rRT-PCR and will make it available on a limited basis. Please contact the Pan American Health Organization (PAHO), WHO Regional Office for the Americas for detailed information.

At least two commercial kits for detecting MERS-CoV by PCR are available. Further information on these kits is available at:

- http://www.altona-diagnostics.com/index.php/brealstar-mers-cov-rt-pcr-kitb-101.html
- http://www.fast-trackdiagnostics.com/products/50/ftd_hcov-emc/

Commercial kits for serological testing are currently being developed and are expected to be available in the near future.

PAHO/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.

References

1. WHO Interim recommendations: Laboratory Testing for Middle East Respiratory Syndrome Coronavirus, September 2014
   http://www.who.int/csr/disease/coronavirus_infections/WHO_interim_recommendations_lab_detection_MERSCoV_092014.pdf?ua=1

2. Corman VM et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Eurosurveillance 2012; 17:20285
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3. Corman VM et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Eurosurveillance 2012; 17:20334
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4. Novel Coronavirus 2012 Real-Time RT-PCR Assay, Centers for Disease Control and Prevention. available at:
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