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MERS-CoV diagnosis: An update

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Summary Diagnosis of MERS-Cov still a major concern in most of daignostic laboratories. To date the Real-time Polymerase Chain reaction (RT-PCR) is the mainstay for diagnosis of MERS-CoV. RT-PCR has limitations, including a long turnaround time and lack of common measurements and correlations with Viral Load (VL). It is recommended to screen for MERS-CoV using RT-PCR of the upstream of envelope gene (upE) followed by confirmation of the presence of one of the following genes; open reading frame 1A, 1B genes or nucleocapsid (N) gene. Scientists are looking to implement viral sequencing on all negative samples by RT-PCR and they beleive that can be exposed to another level of testing using sequencing of the RNA-dependent RNA polymerase (RdRp) gene or N gene and in this case a positive result is diagnostic. It is also very important to maintain a contineous and random sequencing for MERS-Cov samples to be able to pick early viral mutations. Serological assays still not widely or routinely performed, and a lot of studies looking to implement such method in routine patient's testings.
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Three years have passed since the discovery of the newly emerging coronavirus namely Middle East Respiratory Syndrome Coronavirus (MERS-Cov) and many questions are still unanswered. We will summarize the latest diagnostic testing for confirmation of a case with MERS-CoV infection raising some questions that are still unanswered.

Sampling

Detecting the virus in respiratory tract samples remains the gold standard in diagnosing MERS-CoV infection. Several samples can be obtained from the respiratory system that can be used for diagnosing MERS-CoV infection. These include tracheal aspirates, nasopharyngeal swabs, bronchoalveolar

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lavage and sputum. Tracheal aspirates and bronchoalveolar lavage samples (lower respiratory samples) yielded significantly higher viral copies than nasopharyngeal and sputum samples [1]. Analyzing whole blood and plasma also yielded positive viral genome [2]. The kinetics of viral presence in blood is not well understood. The observation of lymphopenia in severe cases and the presence of the virus receptor on naïve and memory T lymphocytes [3] warrant the study of viral replication inside T lymphocytes.

PCR

To date the Real-time Polymerase Chain reaction (RT-PCR) is the mainstay for diagnosis of MERS-CoV. RT-PCR has limitations, including a long turnaround time (chiefly due to transportation of virus and test preparation), and lack of common measurements and correlations with Viral Load (VL). Most laboratories are determining only cycle threshold (C_t) values (which are inversely related to virus load) as a predictor for VL, disease progression and as a cut off marker in order to diagnose cases. Yet, few studies have evaluated whether cycle threshold (C_t) values are associated with clinical severity [4].

It is recommended to screen for MERS-CoV using RT-PCR of the upstream of envelope gene (upE) followed by confirmation of the presence of one of the following genes; open reading frame 1A, 1B genes or nucleocapsid (N) gene [5]. Negative samples by RT-PCR can be exposed to another level of testing using sequencing of the RNA-dependent RNA polymerase (RdRp) gene or N gene [5] and in this case a positive result is diagnostic. Commercially available kits utilizing this algorithm were tested and found to be highly sensitive and specific [6].

Quality of reported results should be maintained at all times. Part of the quality programs available is the external proficiency testing. This method was applied locally during the Korean outbreak [7] and later at a wider level [8].

Sequencing and molecular data

In an attempt to uncover the nature of the first isolate of the virus, Zaki et al. [9] used random primers to sequence the virus RNA. The sequence revealed several open reading frames common to betacoronaviruses, such as 1ab, which encodes many enzymatic products, the spike (S) protein, the small-envelope (E) protein, the matrix (M) protein, and the nucleocapsid (N) protein.

Seong et al. [10] also sequenced MERS-CoV viral genomes from four Korean patients during the May—July 2015 outbreak. There was no evidence of substantial evolutionary change in the virus compared to Middle Eastern sequences. Borucki et al. [11] used deep sequencing on nasal samples of three camels that were infected with MERS-CoV and found only 5 mutations in the consensus sequences. Yet, any mutations could potentially influence the virus phenotype and impact the detection of the virus by molecular assays.

Assiri et al. [12] sequenced MERS-CoV spike gene in an outbreak in Taif. Phylogenetic analysis of their samples revealed a cluster located within the Hafr-Al-Batin clade and were closely related to viruses isolated from Riyadh during 2013 and 2014 outbreaks. They also identified five unique nucleotide substitutions in the spike protein [12]. Whereas, Kim et al. [13] found mutations in the receptor binding domain of the S protein in 12 out of 13 MERS-CoV isolates from Korea resulting in reduced affinity to the human receptor CD26.

These data suggest that sequencing of MERS-CoV is likely warranted for any new and subsequent outbreaks. Changes that may affect the virulence of the virus. In addition, it will be important to observe the location of new mutations in the virus genes and in order to attempt to predict effects on primer and probe binding sites, which may influence diagnostic ability of the currently available commercial kits.

Serology testing

As with other acute viruses, detection of antibodies and even antigens might lag sometime after the detection of viral genome by molecular methods. The kinetics of antigen production in the nasopharyngeal samples has not been studied so far. However, antibodies to the virus generally appear after 10 days of the illness onset. In severely ill patients requiring mechanical ventilation, antibodies were delayed further [14].

An ELISA capture assay to detect NP antigens of the MERS-CoV virus in nasopharyngeal samples was developed recently [15]. The assay was highly sensitive (detection of the MERS-CoV-NP of less than 1 ng/mL) and specific (specificity of 100%) for MERS-CoV and has the potential to be used in animals a well. Song et al. developed a rapid immunochromatographic assay for the detection of MERS-CoV nucleocapsid protein from camel nasal swabs with 93.9% sensitivity and 100% specificity compared to RT-rtPCR. The assay is very promising and worth

replication in both camel and humans. As yet, antigen detection assays are not widely available; yet this type of assays will ideally be valuable in ruling infection in, and out.

MERS-CoV is very similar to SARS, both are betacoronaviruses; and in both cases the S and N proteins are very immunogenic and good candidates for developing an immune assay for the detection of virus specific antibodies [16]. Several assays have been developed to detect antibodies to MERS-CoV; including neutralization assays, ELISA and IFA.

Perera et al. [17] developed a microneutralization assay to detect specific anti-MERS-Cov antibodies. They used serial dilution of serum which was incubated with Vero cells/MERS-CoV virus. After three days at 37 °C, they scored the antibody titers based on the virus CPE. In addition, they developed a MERS-CoV spike pseudoparticle neutralization assay [17]. They used HIV/MERS spike pseudoparticles to infect Vero E6 cells, after 2 days infected cells were lysed and the antibody that gave 90% luciferase reduction was assigned as ppNA antibody titer. Compared to virus neutralization assay, the pseudoparticle neutralization assay does not require BSL3 containment.

Indirect fluorescent antibody assay for the detection of antibodies to MERS-CoV was carried out using whole virus in Vero cells [18,19] or transfected Vero cells with spike or nucleocapsid proteins of MERS-CoV [18]. ELISA utilizing S1 protein is also available and been used for investigating epidemiology of the exposure to the virus [20]. So far, there are no studies comparing ELISA for the detection of anti-MERS-CoV antibodies with either IFA or neutralization assays.

Detection of anti-MERS-CoV antibodies using the different assays described above still requires confirmation by the neutralization assays. Other techniques are needed to support confirmation of the antibody specificity, as neutralization assay is not widely used. Western blotting has been used in SARS [21] and other viruses to confirm antibody specificity. There is a need for Western blotting assay in case of MERS-CoV to confirm antibody specificity. This can be in the form of genetically engineered specific MERS-CoV antigens blotted on membranes.

In summary, molecular techniques are the first line of assays to be used for confirmation of MERS-CoV infection. Tracheal aspirates and bronchoalveolar lavage samples (lower respiratory samples) are recommended for detecting the virus by RT-PCR or sequencing. Serology is valuable confirming cases suspected for MERS-CoV but the virus is not detected in respiratory samples.

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Competing interests

None declared.

Ethical approval

Not required.

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