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LETTERS TO THE EDITOR

A highly specific rapid antigen detection assay for on-site diagnosis of MERS

Dear Editors,

Gadsby et al. in this Journal, recently described the clinical utility of molecular diagnostics in identifying *Legionella* infection in patients with acute LRTI.[1] We developed a rapid antigen detection assay for on-site diagnosis of MERS, which can be finished in 30 min.

Since its first appearance in 2012, MERS has affected >25 countries in four continents with >1300 cases and a high fatality rate of >30%.[2] Detection of MERS coronavirus (MERS-CoV) and its antibodies in dromedaries implied that these animals are probably its reservoir.[3] Rapid laboratory diagnosis is essential for commencement of infection control measures. Although MERS-CoV can be cultured and antibody detection methods available, laboratory diagnosis of MERS-CoV infections is mainly achieved by real-time quantitative RT-PCR (qRT-PCR).[4] Recently, we have described the development of a monoclonal antibody (MAb)-based capture ELISA for MERS-CoV N protein (NP) detection and its use in field study for dromedaries.[5,6] However, both real-time qRT-PCR and NP capture ELISA take at least several hours to complete and require specific equipments in a clinical laboratory. In this study, we developed a MAb-based rapid NP detection assay for MERS.

Cloning and purification of $(His)_6$-tagged recombinant NP (rNP) of MERS-CoV was performed as described previously,[6] using a MERS-CoV strain (D789.1/14) isolated from a patient in Dubai. Production and selection of specific MAbs against the MERS-CoV NP was performed as described previously.[6] The rapid MERS-CoV NP detection assay, in the format of a lateral flow immunoassay (LFIA), was developed using a similar approach as described previously,[7] using LFIA test strip (Millipore) and two highly specific MAbs against the MERS-CoV rNP (MD3E9 for coating and MD8B6 for conjugating). Testing is carried out by mixing 380 μl of each sample with 20 μl lysis buffer for 10 s at room temperature. Then, 80 μl of the pre-treated sample was transferred into the sample well of the test card slowly at room temperature. Results are read visually in 30 min. Result is considered negative when the control line is positive but the test line negative, and considered positive when both the control and test lines are positive (Fig. 1). If the control line is negative, the test is considered invalid.

Initial assessment of the detection limit was performed using a human nasopharyngeal aspirate (NPA) spiked with various concentrations of MERS-CoV. First round of evaluation was performed using 56 human NPAs positive for various respiratory viruses and 10 human NPAs spiked with $10^{5.2}$ $TCID_{50}$/ml MERS-CoV. All human NPAs were collected from the clinical microbiology laboratory in Queen Mary Hospital, Hong Kong. Second round of evaluation was performed using 81 dromedary nasal swabs/tonsils collected during necropsies at the Central Veterinary Research Laboratory in Dubai. These dromedary respiratory samples were tested by real-time qRT-PCR and the rapid MERS-CoV assay. All tests were performed in duplicate.

Initial assessment showed that the rapid MERS-CoV assay was consistently positive for NPA spiked with $\geq 10^{4.2}$ $TCID_{50}$/ml of MERS-CoV (Table 1). Evaluation using human NPAs showed that all 56 NPAs positive for various respiratory viruses were negative for the rapid assay and all the 10 NPAs spiked with $10^{5.2}$ $TCID_{50}$/ml of MERS-CoV were positive for the rapid assay. Further evaluation using dromedary nasal swabs/tonsils showed that all 65 samples negative by real-time qRT-PCR were also negative for by the rapid assay, indicating a specificity of 100%. For the 16 nasal swabs/tonsils positive by

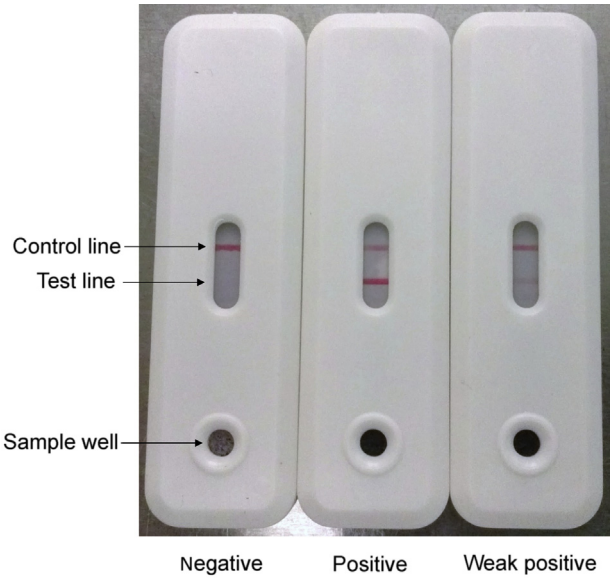

Figure 1 Rapid MERS-CoV assay showing negative, positive and weakly positive results.

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Table 1 Evaluation of rapid MERS-CoV assay.

Evaluation	Rapid MERS-CoV assay results
Initial evaluation [Concentration of spiked MERS-CoV (log_{10} $TCID_{50}$/ml)]	
5.2	Positive
4.2	Positive
3.7	Positive
3.2	Negative
2.2	Negative
Evaluation using human NPAs	
NPAs positive for various respiratory viruses (n = 56)[a]	100% negative
NPAs spiked with MERS-CoV (n = 10)	100% positive
Evaluation using dromedary nasal swabs/tonsils	
RT-PCR positive (n = 16)	81% positive
RT-PCR negative (n = 65)	100% negative

[a] Include influenza A virus (n = 5), influenza B virus (n = 5), influenza C virus (n = 2), parainfluenza virus 1 (n = 5), parainfluenza virus 2 (n = 5), parainfluenza virus 3 (n = 5), parainfluenza virus 4 (n = 5), human rhinoviruses (n = 5), human metapneumovirus (n = 5), human adenovirus (n = 2), respiratory syncytial virus (n = 2), human bocavirus (n = 2), human CoV HKU1 (HCoV-HKU1) (n = 2), HCoV-OC43 (n = 2), HCoV-229E (n = 2) and HCoV-NL63 (n = 2).

real-time qRT-PCR, 13 were also positive by rapid assay, indicating a sensitivity of 81%. All the 13 rapid MERS-CoV assay-positive samples had viral loads of $\geq 7.36 \times 10^7$ copies/ml, whereas the three rapid MERS-CoV assay-negative samples had viral loads of $\leq 2.14 \times 10^7$ copies/ml.

We developed a rapid MERS-CoV assay with high specificity. Since the emergence of MERS, the diagnosis of almost all cases was confirmed by real-time qRT-PCR. However, its turn-around-time is about 4 h and the test requires expensive equipment, stringent laboratory set-up and personal attention to prevent cross contamination. As for the antigen capture ELISA that we developed recently, although the main equipments involved are less expensive ELISA plate washer and reader, it still requires technical expertise and 3 h to finish the test.[6] However, for the rapid MERS-CoV assay developed in the present study, it is extremely simple to perform by any health care worker with minimal training and can be finished in 30 min. Furthermore, the test does not require any specific equipment and the samples need only minimal preparation and pretreatment. In addition to being user-friendly and rapid, the test is highly specific. All human NPAs positive for other respiratory viruses, including all the four human CoVs, or dromedary nasal swabs/tonsils negative for MERS-CoV showed negative results using the rapid MERS-CoV test. In addition, results were also negative for dromedary respiratory/fecal samples spiked with our recently sequenced lineage A betacoronavirus found in dromedaries[8] (data not shown). All these confirm that this rapid MERS-CoV test has a very high specificity.

The present rapid MERS-CoV test can be used as a rapid screening test for MERS-CoV infection. Despite its high specificity, the rapid MERS-CoV test has a moderate sensitivity. Our results showed that the detection limit of this rapid MERS-CoV test is around $10^{3.7}$—$10^{4.2}$ $TCID_{50}$/ml of MERS-CoV, which is about 25—100 times less sensitive than the antigen capture ELISA with detection limit of about $10^{2.3}$. A moderately high sensitivity of 81% was confirmed using dromedary respiratory samples positive for MERS-CoV by real-time qRT-PCR. It is quite well-known that similar rapid tests for antigen detection in other viruses are less sensitive than ELISA or other antigen detection methods.[9] This is because a higher antigen load is required to for these rapid tests to produce a band visible to the naked eye. Since its sensitivity is just moderately high, this rapid MERS-CoV test can be used for initial rapid screening for suspected case of MERS. If the result is positive, the patient can be isolated with other cases of MERS; but if the result is negative, the patient should be isolated in single room and further tests such as real-time qRT-PCR or antigen capture ELISA should be performed.

Contributions

YC, HC, SKPL and PCYW conceived the study. UW, NX, SKPL and PCYW supervised the study. YC, KHC, CH, YK, SG and EYMW performed the experiments. KHC, EYMW, SJ and NGP collected the samples. YC, KHC, EYMW, SKPL and PCYW analyzed the data. PCYW wrote the manuscript. All authors corrected the manuscript.

Competing interests

No author has conflict of interest.

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High levels of susceptibility to measles, mumps and rubella (MMR) in HIV-infected individuals in Ireland

Dear Editors,

Recently in this Journal, Minakami et al.[1] and Yamada et al.[2] highlighted the consequences of low levels of immunity to rubella, a common vaccine preventable infection. Vaccine preventable infections continue to cause significant morbidity and mortality worldwide. In recent years, outbreaks of measles, mumps and rubella (MMR) have been observed even in highly vaccinated populations where coverage has fallen below the threshold required to contain spread.