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WOAH Procedure for Registration of Diagnostic Kits
Validation Studies Abstract

Name of the diagnostic kit: BIONOTE® Rapid MERS-CoV Ag Test Kit

Manufacturer: BioNote, Inc.

Procedure /Approval number: 20160212

Date of Registration: May 2016

Date of Renewal: May 2023

Disease: Middle East Respiratory Syndrome

Pathogen Agent: Middle East Respiratory Syndrome (MERS)

Type of Assay: Immunochromatographic assay

Purpose of Assay: Certified by WOAH fit for the qualitative detection of Middle East Respiratory Syndrome Coronavirus antigens from nasal swabs in dromedary camels for the following purposes:

- Detection of MERS CoV infected herds (herd test) with acutely infected animals with high virus loads;
- When used as a supplemental test, to estimate prevalence of infection to facilitate risk analysis s, e.g. surveys, herd health schemes and disease control programs

Species and Specimen: Nasal swabs in dromedary camels

1. Information on the kit

Please refer to the kit insert available on the WOAH Registry web page or contact manufacturer at:
Website link: www.bionote.co.kr
Email address: bionote@bionote.co.kr

2. Summary of validation studies

Analytical specificity

Conclusion: The BRM kit does not have cross-reactivity with camel coronaviruses (DcCoV UAE-HKU23), COVID-19 (SARS-CoV-2), and other coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, RbCoV HKU14, Ty-Bat CoV HKU4).

Table 1 Analytical Specificity

Viruses			BRM kit result
Alpha coronavirus		Human coronavirus 229E (HCoV-229E)	Negative
		Human coronavirus NL63 (HCoV-NL63)	Negative
Beta coronavirus	**Embecovirus**	Human coronavirus OC43 (HCoV-OC43)	Negative
		Rabbit coronavirus HKU14 (RbCoV HKU14)	Negative
		Dromedary camel coronavirus UAE-HKU23 (DcCoV UAE-HKU23)	Negative
	Sarbecovirus	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Negative
	Merbecovirus	Middle East respiratory syndrome coronavirus (MERS-CoV)	Positive
		Tylonycteris bat coronavirus HKU4 (Ty-Bat CoV HKU4)	Negative

Analytical sensitivity

Conclusion:
Experiment 1. BIONOTE® Rapid MERS-CoV Ag Test Kit (BRM kit) detected up to 3.125 ng/ml of recombinant nucleocapsid antigen of MERS CoV.

Experiment 2. Negative camel nasal swabs, collected from Central Veterinary Research Laboratory (CVRL) in Dubai, UAE, and MERS-CoV Culture Fluid were used for the Limit of detection test. The MERS-CoV Culture Fluid was diluted into 2-fold steps and tested simultaneously with the the UpE and Orf1b real-time RT-PCR (Corman et al. (2012)). In experiments performed using MERS-CoV Culture Fluid, BRM kit can detect up to $1.63x10^2$ $TCID_{50}$/mL, corresponding to an UpE CT value of 32.51 and ORF1b CT value of 34.93 according to molecular analysis performed concurrently.

Repeatability

Within run variation was assessed using quadruplicates of 5 inhouse samples (one strong, one medium, one weak and two negative samples) in four runs by one operator. Between run variation was assessed using triplicates of 5 inhouse samples in 30 runs by 3 operators on separate days. Batch-to-batch variation was assessed using 5 inhouse samples by 1 operator on one day.

Conclusion: CV values were all below 5% in the Within run, Between run, and batch-to-batch variation.

Diagnostic Characteristics

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Conclusion***:*** BIONOTE® Rapid MERS-CoV Ag Test Kit is a qualitative test. The presence of the purple line on both the control(C) and test(T) position is considered to be the threshold determination. The test sample is positive when two lines (C line and T line both) appear and negative when only the C line appears. Lines consist of immuneo-reaction of the gold conjugate and target analytes. Gold conjugate consist of colloidal gold and MERS CoV antibody. The threshold is determined by the analytical sensitivity as 10^5 $TCID_{50}$ (50% Tissue Culture Infective Dose).

Table 2a Relative diagnostic sensitivity (DSn) and specificity (DSp) estimates

Test method under evaluation		Target Species
Diagnostic sensitivity	N DSn CI	*(66)* *(93.9%)* *(85.20-98.32%)*
Diagnostic specificity	N DSp CI	*(523)* *(99.6%)* *(98.63-99.95%)*

Table 2b 2x2 table for relative DSn and DSp

Summary		UpE and Orf1A rRT-PCR		Total
		POS	NEG	
BRM kit	*POS*	*62*	*2*	*64*
	NEG	*4*	*521*	*525*
Total		*66*	*523*	*589*

Reproducibility

Analytical reproducibility

Reproducibility was assessed at three sites using a blinded coded reference panel. The panels were tested using three different lots in 21 runs at 3 different sites by an operator each day for three days. Each site ran positive and negative reference panels for each day of testing.

Conclusion: The CVs of the between site assay reproducibility is 3~11%.

Diagnostic reproducibility

The scope of this interlaboratory comparison was to determine the reproducibility of the Real-Time PCR and the BRM kit to detect MERS-CoV in real nasal swab samples collected in transport media in three participating laboratories.

[Test Date]: October 2015

[Test site] Three laboratories participated in the International Inter-laboratory Comparison on the BRM kit. (Participants also tested samples by Real Time PCR and results are shown for information only.)

1. Abu Dhabi Food Control Authority (ADFCA)

Location: United Arab Emirates
City: Abu Dhabi
Level of expertise : highly trained technician
Accreditation status : ISO 17025

2. King Faisal University Laboratory (KFU)

Location: Kingdom of Saudi Arabia
City: Al-Hasa
Level of expertise : highly trained technician
Accreditation status : ISO 17025

3. Molecular Biology & Genetics laboratories (MBG)

Location: United Arab Emirates
City: Dubai
Level of expertise : highly trained technician
Accreditation status : ISO 17025

[Materials]

1. Test panel information

The panel consisted of 6 positive and 4 negative samples. Samples were prepared from samples with known history. Samples were aliquoted in portions of 300µl and stored in 2ml vials. Test samples were prepared from nasal swabs from MERS positive and negative camels.

2. Shipping conditions

The samples were dispatched to the participants on the month of October 2015. Each participant received one box containing the test materials (Ten 2ml vials containing 300µl of each sample).
Samples were frozen and shipped with dry ice to the laboratories.

[Result]

BIONOTE® Rapid MERS-CoV Ag Test Kit

Samples were analyzed by each lab using BRM kit and Real-Time PCR. BRM kit results of three participants are illustrated in Table 3 below.

Table 3. BRM kit results of three participants

Sample No.	Targeted Results (Original)	KFU, Saudi Arabia	MBG LAB	VLD- ADFCA
1	Positive	Positive	Positive	Positive
2	Positive	Positive	Positive	Positive
3	Negative	Negative	Negative	Negative
4	Positive	Positive	Weak Positive	Positive
5	Positive	Positive	Weak Positive	Positive
6	Negative	Negative	Negative	Negative
7	Positive	Positive	Positive	Positive
8	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative
10	Positive	Positive	Positive	Positive

Real-Time PCR test

Samples were also analyzed by the 3 participants using real time PCR. ADFCA (Abu Dhabi, UAE) real-time PCR results are based on UPE and Roche MERS-CoV qPCR kit in which the Orf Ia gene is targeted. KFU, (Saudi Arabia) real-time PCR results are based on UPE and CDC MERS-Co V qPCR kit in which the N2 gene is targeted. MBG, (Dubai, UAE) real-time PCR results are based on 2nd Derivative Max Analysis. Qualitative and quantitative Real-Time PCR results of each participant are given in table 4 below.

It was concluded that the "No CT value" result was clearly negative. When CT values exceeded 35, interpretations were different for each laboratory, but when other PCRs were performed, interpretations were made along with the results. Because according to CDC, MERS CoV-positive samples must test positive for two separate genetic targets (e.g. upE and N2 or N2 and N3 or upE and N3, etc.), both targets must be positive to be interpreted as positive.

Table 4. Real-Time PCR results of three participants

	KFU, Saudi Arabia			MBG LAB		VLD- ADFCA		
Sample No.	Real-Time PCR-Result	CT Value UPE	CT Value N2	Real-Time PCR-Result	2nd Derivative Max Analysis	PCR-Result	CT Value UPE	CT Value ORF1a
1	Positive	21.33	16.65	Positive	19.59	Positive	23.65	24.1
2	Positive	16.01	15.97	Positive	19.61	Positive	23.34	23.84
3	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
4	Positive	19.95	18.16	Positive	21.2	Positive	24.8	24.68
5	Positive	25.9	19.03	Positive	21.15	Positive	24.89	24.51
6	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
7	Positive	20.06	19.86	Positive	19.22	Positive	23.16	23.26
8	Negative	No Ct	No Ct	Inconclusive**	>35	Negative	No Ct	No Ct
9	Negative	No Ct	39.95*	Inconclusive**	>35	Negative	No Ct	No Ct
10	Positive	22.16	18.95	Positive	20.84	Positive	24	23.87

* *Sample 9 gave an inconclusive Ct value of 39.95 in N2 qPCR, but no Ct in upE and therefore, it was considered as negative by KFU.*

** *For MBG lab the Ct value cut off is 35; any amplification beyond 35 is reported as inconclusive.*

[Conclusion]

Interlaboratory comparison testing of the BRM kit with a panel consisting of 6 MERS positive and 4 MERS negative samples in 3 different laboratories showed 100% concordance of results for the BRM kit using KFU and VLD molecular assays as reference tests. Results from MGB assay were excluded because no negative results were produced in this assay.

Additional testing

Further testing of spiked samples of 12 positive and 18 negative camel nasal swab samples was performed by the BRM kit, MERS-CoV RT-PCR, MERS-CoV real-time PCR and DcCoV UAE-HKU23 real-time PCR . The relative specificity and sensitivity of the rapid MERS-CoV Ag test kit compared to the qPCR were 100% (18/18) and 91.7% (11/12), respectively (Lau, Susanna Kar-Pui, et al., 2022).

Table 5. 2x2 table

		MERS-CoV N Real-time PCR		
		Positive	**Negative**	**Total**
Rapid MERS-CoV Ag test kit	**Positive**	11	0	11
	Negative	1	18	19
	Total	12	18	30
Sensitivity		91.7%		
Specificity		100%		

Conclusion

The BRM kit is shown to be less sensitive than the real-time PCR assays. Samples with viral load below the detection limit of the BRM kit are likely to test negative in the BRM kit. It is a common observation that antigen tests can be markedly less sensitive than real-time PCR tests. MERS-CoV-2-infected camels can shed a low level of viral RNA for an extended period (several weeks). Nonetheless, infectious virus can only be detected mainly in the first week after infection (Adney et al., EID 2014).

In summary, the BRM kit can detect a positive sample with a high viral load and would be useful as a screening assay for a prompt identification of highly infectious camels, thereby allowing timely risk management (e.g. quarantine). As this antigen test might fail to detect some MERS-positive camels that have low viral load (e.g. those at early onset), a negative test result cannot completely exclude MERS-CoV infection. The BIONOTE test has an estimated **diagnostic window of 1~7 days (as opposed to the real-time PCR 1-35 days)**. Samples that are taken beyond this time point are likely to be negative in the Bionote test (see also detailed protocol for the sampling, storage and transport of specimens in kit information).

When using the BRM test kit, the diagnostic algorithm as provided in the instructions for use should be followed. If the test is negative and the animal is showing clinical signs, then further investigations are required. This could be explained due to having low virus titer below the detection limit of the rapid antigen test. In this case, further investigations will include re-testing of negative camels at 2-3 days intervals to detect viral antigen as the viral antigen is likely to increase shortly after infection. We set the monitoring interval as 2~3 days, because the rapid antigen test could detect MERS-CoV antigen in 7 days after onset of infection.

Reference

WOAH Terrestrial Manual (2021).

Song, Daesub, et al. "Development and validation of a rapid immunochromatographic assay for detection of Middle East respiratory syndrome coronavirus antigen in dromedary camels." Journal of clinical microbiology 53.4 (2015): 1178-1182.

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Corman, V. M., et al. "Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction." Eurosurveillance 17.39 (2012): 20285.

Adney, Danielle R., et al. "Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels." Emerging infectious diseases 20.12 (2014): 1999. (DOI: http://dx.doi.org/10.3201/eid2012.141280).

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