



OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

<p>Name of the diagnostic kit: BIONOTE® Rapid MERS-CoV Ag Test Kit Manufacturer: BioNote, Inc. OIE Approval number: 20160212 Date of Registration: May 2016</p>

Disease: Middle East Respiratory Syndrome

Pathogen Agent: Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Type of Assay: Immunochromatographic assay

Purpose of Assay: Certified by the OIE 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, 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 OIE 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 characteristics

Analytical sensitivity

BIONOTE® Rapid MERS-CoV Ag Test Kit detected up to 3.125 ng/ml of recombinant nucleocapsid antigen of MERS CoV.

Analytical specificity

Other coronaviruses such as bovine corona virus (vaccine and field strain), canine corona virus and feline corona virus did not react with this kit.

Repeatability data

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. CV values were all below 5%.

Diagnostic Characteristics

Threshold determination

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 10⁵ TCID₅₀ (50% Tissue Culture Infective Dose).

Diagnostic sensitivity (DSn) and specificity (DSp) estimates

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

Comparative performance

Summary		UpE and Orf1A rRT-PCR		Total
		POS	NEG	
BIONOTE Rapid MERS-CoV Ag Test Kit	POS	62	2	64
	NEG	4	521	525
Total		66	523	589

Reproducibility

The scope of this interlaboratory comparison was to determine the proficiency of the Real-Time PCR and the BIONOTE® Rapid MERS-CoV Ag Test Kit (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 BIONOTE Rapid MERS CoV Ag Test 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

Status: Abu Dhabi

Level of expertise : highly trained technician

Accreditation status : ISO 17025

2. King Faisal University Laboratory (KFU)

Location: Kingdom of Saudi Arabia

Status: Al-Hasa

Level of expertise : highly trained technician

Accreditation status : ISO 17025

3. Molecular Biology & Genetics laboratories (MBG)

Location: United Arab Emirates

Status: Dubai

Level of expertise : highly trained technician

Accreditation status : ISO 17025

[Materials]

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.

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 1 below.

Table 1. 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 1a 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 2 below.

Table 2. Real-Time PCR result

Sample No.	KFU, Saudi Arabia			MBG LAB		VLD- ADFCA		
	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	Uncertain**	>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	Uncertain**	>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	Uncertain**	>35	Negative	No Ct	No Ct
9	Negative	No Ct	39.95*	Uncertain**	>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 MGB lab the Ct value cut off is 35; any amplification beyond 35 is reported as inconclusive

Application

Laboratory in which the kit is in current use.

Laboratory name: Veterinary Laboratories Division, Abu Dhabi Food Control Authority

Location: Abu Dhabi

Status: National Laboratory

Accreditation status: ISO 17025 accredited

Purpose of test: Screening (see also the purpose of assay)

Status of test: Supplementary

References

Song D, Ha G, Serhan W, Eltahir Y, Yusof M, Hashem F, Elsayed E, Marzoug B, Abdelazim A, Al Muhairi S. 2015. Development and validation of a rapid immunochromatographic assay for detection of Middle East respiratory syndrome coronavirus antigen in dromedary camels. *J Clin Microbiol* 53:1178 –1182. doi:10.1128/JCM.03096-14.

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