ONE STEP Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Antigen Test

BIONOTE

BIONOTE® Rapid MERS-CoV Ag Test Kit

Validated and certified by the OIE as fit for the purposes defined in the kit insert provided with this kit. Registration number 20160212

■ OIE Statement

The validation of data for the BIONOTE® Rapid MERS-CoV Ag Test Kit has been certified in May 2016 by the OIE, based on expert review, as 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

■ Principles

BIONOTE® Rapid MERS-CoV Ag Test Kit is an immunochromatographic assay for the qualitative detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) antigens from nasal swabs in dromedary camels. This assay is intended for rapid screening in laboratory. This test should be conducted by trained technician, wearing appropriate personal protective equipment(PPE).

BIONOTE® Rapid MERS-CoV Ag Test Kit has two letters on the surface of the strips indicating test line (T) and control line (C). Test line and control line in the result window are not visible before applying any samples. The control line is a reference line which indicates the test is performing properly. The control line has to appear every time when the test is performed. If MERS-CoV is present in the sample, a purple test line would appear. The highly selective antibodies to MERS-CoV are used as capture and detector in the assay. These antibodies are capable of detecting MERS-CoV antigens directly, with a high accuracy.

■ Materials provided

Reagent	25 Tests/Kit	100 Tests/Kit
MERS-CoV Ag Test Strip	25	100
Assay diluents tube	0.3ml/vial x25	0.3ml/vial x100
Disposable swab	25	100
Disposal bag	25	100
Instructions for use	1	1

■ Materials required, but not provided

1) Timer or clock
2) Test tube
3) PPE(Personal Protective Equipment)
4) Transport media

■ Precautions

1) The test kit is for dromedary camel use only. Do not use for other animals.
2) The test strip is sensitive to humidity as well as heat. Perform the test immediately after removing the test strip from the foil pouch.
3) Do not reuse the test components.
4) Do not touch the membrane in the result window of test strip.
5) Do not use the test kit beyond the stated expiration date marked on the package label.
6) Do not use the test kit if the pouch is damaged or the seal is broken.
7) Do not mix components from different lot numbers because each lot is quality control tested as a standard batch unit.
8) All samples should be handled as being potentially infectious. Wear protective gloves while handling samples. Wash hands thoroughly afterwards.
9) Decontaminate and dispose of all samples, reaction kits and potentially contaminated materials safely in accordance with your biohazard waste disposal regulation.

■ Laboratory bio-safety

1) Any testing for the presence of MERS-CoV should be performed in laboratories by staff or technician trained in the relevant technical and safety procedures.
2) Appropriate PPE should be worn by all laboratory staff handling these specimens.
3) The handling and processing of specimens from cases with suspected or confirmed MERS-CoV infection intended for additional laboratory tests should follow local guidelines for processing potentially infectious material.

■ Storage and Stability

1) Store the test kit at 2~40°C. **DO NOT FREEZE.**
2) Do not store the test kit in the direct sunlight.
3) The test kit is stable within the expiration date that marked on the package label.

■ Collection and Preparation of Sample

Nasal swab samples of dromedary camel should be used.

[Before specimen collection]

Whenever specimens are collected from cases under investigation, infection control guidelines must be followed.

- All health-care workers who collect specimens from dromedary camels suspected or confirmed to be infected with MERS-CoV must wear appropriate personal protective equipment (PPE).
- All those involved in collection and transporting specimens should be trained in safe handling practices and spill decontamination procedures.

[Collection of nasal swab specimens]

Collect the nasal swab specimens using sterile swab. Insert the swab through the nostril which presents more secretion under visual inspection. Insert the swab until the level of the nasal turbinate. Rotate and swab a few times on the respiratory epithelium of the nasal turbinate. The swab specimen should be placed immediately into sterile tubes containing 2-3ml of viral transport media and transported from the field to the lab.

[Sample transport and storage]

1) Specimens should reach the laboratory as soon as possible after collection. For short periods(≤ 72 hours) of transport, store and ship the specimen at 4°C or below. For long periods(>72 hours) of transport, store at -80°C or below and ship on dry ice or liquid nitrogen.
   It is important to avoid repeated freezing and thawing of specimens. The storage of specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations.
2) When shipping frozen sample from long distances or from international locations, it is best to use a combination of dry ice and frozen gel ice-packs. The gel ice-packs will remain frozen for one or two days after the dry ice has dissipated.
3) Each specimen container should be labelled with the patient identifier, specimen type, and the sample collection date.
4) All samples must be pre-packed to prevent breakage and spillage. Sample containers should be sealed with Parafilm® and placed in ziplock bags. Place enough absorbent material to absorb the entire contents of the Secondary Container (containing Primary Container) and separate the Primary Containers (containing specimen) to prevent breakage. Send specimens with cold packs or other refrigerant blocks that are self-contained, not actual wet ice. This prevents leaking and the appearance of a spill. When large numbers of specimens are being shipped, they should be organized in a sequential manner in boxes with separate compartments for each specimen.

***Transport media**

Saline	EMEM+1%BSA
PBS	EMEM+0.5%BSA
PBS+0.5%BSA	Trypticase soy Broth+0.5%BSA
PBS+0.5%Gelatin	Trypticase soy Broth+0.5%Gelatin

■ Procedure of the Test

*Refer to the back page.

[Sample Extraction]

Nasal swab samples in transport media should be extracted as following method.

1) Allow test strip and the sample to room temperature(15~40°C) prior to testing.
2) Add 100µl of assay diluents and 100µl of extracted samples into a test tube, and mix well.

[Reaction with Test Strip]

1) Remove the test strip from the foil pouch prior to use.
2) Place the test strip into the assay diluents tube with the arrows on the test strip pointing down. Do not handle or move the test strip until the test is complete and ready for reading.
3) Read result at 10 ~ 15 minutes. Some positive results may appear soon.

[Disposal of waste]

After a test is done, add all wastes into a disposal bag(provided) and dispose of it in accordance with your biohazard waste disposal regulation.

■ Interpretation of the Result

*Refer to the back page.

1) **Negative result:** Only one control("C") band appears.
2) **Positive result:** Test("T") band and control("C") band appear.
3) **Invalid:** Control("C") fails to appear.

***** If the control band is not visible within the result window after performing the test, the result is considered invalid. It is recommended that the sample be re-tested using a new test kit.

■ Limitations of the Test

1) MERS-CoV shedding start during 1 -2 dpi, as detect by the presence of infectious virus and viral RNA by qPCR in nasal swab samples. Infectious virus shedding is detected <7 DPI, and shedding of viral RNA is detected <35 DPI in nasal swab samples. So false negative reaction by BIONOTE® Rapid MERS-CoV Ag Test Kit might be detected from 8 dpi due to low detection limit.
2) Although the BIONOTE® Rapid MERS-CoV Ag Test Kit is very accurate in detecting MERS-CoV antigen, a low incidence of false results can occur. Other clinical and/or laboratory tests might be required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should be diagnosed by veterinarian after all clinical and laboratory findings have been evaluated.
3) The reading window may show a light pink background coloration; this will not affect the accuracy of the results.
4) BioNote and its distributors cannot be held responsible for the consequences of misuse or misinterpretation of the results given by the test.

■ References

1. 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.
2. Interim Guidelines for MERS-CoV – Version 2 in CDC 01/10/14
3. "Danielle R Adney, Neeltje van Doremalen, Vienna R Brown, Trenton Bushmaker, Dana Scott, Emmie de Wit, Richard A Bowen, Vincent J Munster. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerging Infectious Diseases,www.cdc.gov/eid, Vol. 20, No. 12, December 2014"
4. Laboratory Testing for Middle East Respiratory Syndrome Coronavirus : WHO Interim guidance, June 2015

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 corona viruses 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 in house 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 in house samples in 30 runs by 3 operators on separate days. Batch-to-batch variation was assessed using 5 in house 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 $10^5 TCID_{50}$ (50% Tissue Culture Infective Dose).

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%)

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. Lab A

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

2. Lab B

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

3. Lab C

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)	Lab A	Lab B	Lab C
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. Real-time PCR results of Lab A are based on UPE and CDC MERS-Co V qPCR kit in which the N2 gene is targeted. Real-time PCR results of Lab B are based on 2nd Derivative Max Analysis. Real-time PCR results of Lab C are based on UPE and Roche MERS-CoV qPCR kit in which the Orf la gene is targeted. Qualitative and quantitative Real-Time PCR results of each participant are given in table 2 below.

Table 2. Real-Time PCR result

	Lab A			Lab B		Lab C		
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	Positve	21.33	16.65	Positve	19.59	Positve	23.65	24.1
2	Positve	16.01	15.97	Positve	19.61	Positve	23.34	23.84
3	Negative	No Ct	No Ct	Uncertain**	>35	Negative	No Ct	No Ct
4	Positve	19.95	18.16	Positve	21.2	Positve	24.8	24.68
5	Positve	25.9	19.03	Positve	21.15	Positve	24.89	24.51
6	Negative	No Ct	No Ct	Uncertain**	>35	Negative	No Ct	No Ct
7	Positve	20.06	19.86	Positve	19.22	Positve	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	Positve	22.16	18.95	Positve	20.84	Positve	24	23.87

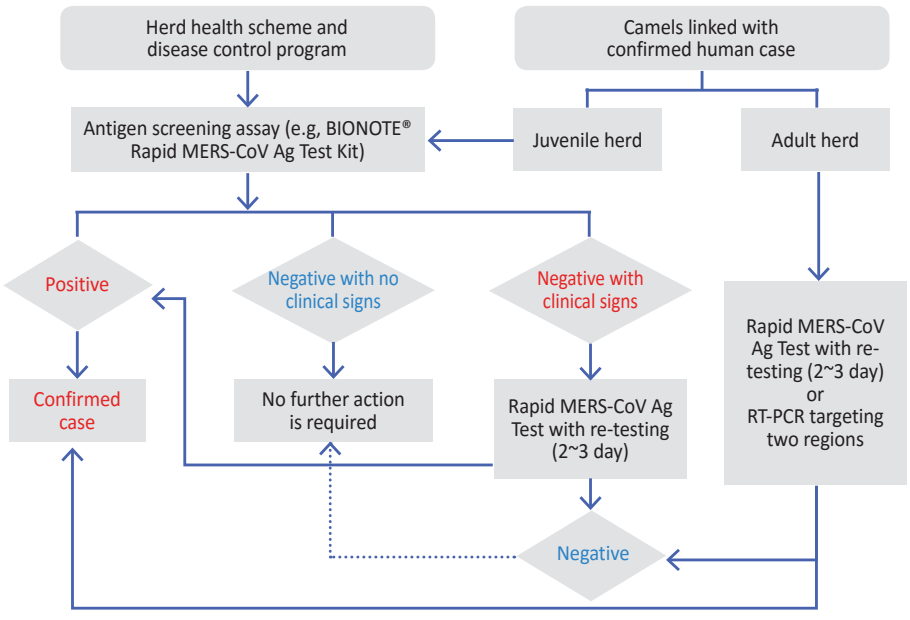
** Sample 9 gave an inconclusive Ct value of 39.95 in N2 qPCR, but no Ct in upE and therefore, it was considered as negative by lab A.*

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

Acknowledgement

Most of the validation studies were organised and performed by the Veterinary Laboratories Division of Abu Dhabi Food Control Authority (ADFCA), United Arab Emirates. BioNote thanks Dr. Salama Al Muhairi, the director of Veterinary Laboratories Division, Animal Wealth Sector, ADFCA, UAE for continuous support for this work.

Diagnostic Algorithm for MERS CoV

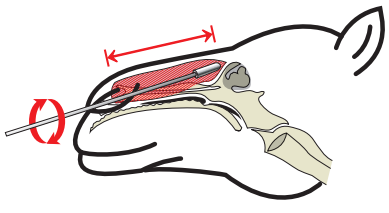


* If the test is negative and the animals are showing clinical sign, then further investigation is required. This could be explained due to having low virus titer below the detection limit of the rapid antigen test. In this case, further investigation will include re-testing of the negative camels at 2-3 days intervals to detect viral antigen as the viral antigen increases over time. 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.

Animals are in the incubation period at the time of antigen test. In this case, even PCR could give false negative results. Here, re-testing is needed using the rapid antigen test at 2~3 days interval.

Methods of Sample Collection

Nasal swab



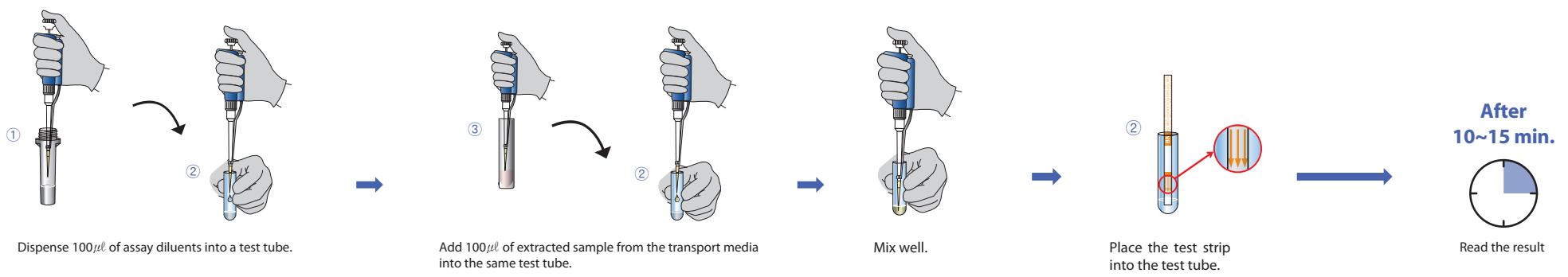
*Insert the swab until the level of the nasal turbinate.

*Rotate and swab a few times the area indicated in red in the picture.

* Insert the swab into transport media and mix the swab until the sample dissolves into the transport media.

Test Procedure

① Assay diluents tube	② Test tube (Not provided)	③ Transport media (Not provided)
		



Dispense 100㎕ of assay diluents into a test tube.

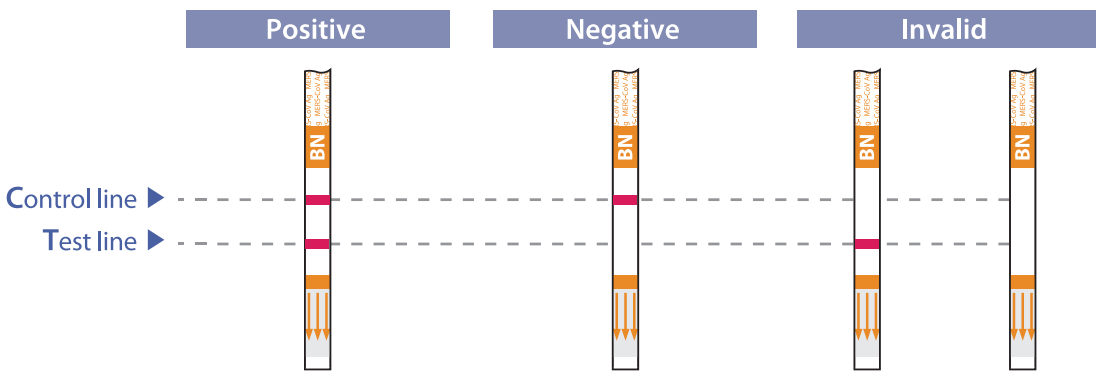
Add 100㎕ of extracted sample from the transport media into the same test tube.

Mix well.

Place the test strip into the test tube.

Read the result

Interpretation



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Manufactured by BIONOTE
BioNote, Inc.
22 Samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 18449, Republic of Korea
TEL: 82-31-211-0516 I FAX : 82-31-8003-0618 I www.bionote.co.kr