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

BIONOTE

BIONOTE® Rapid MERS-CoV Ag Test Kit

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

World Organisation for Animal Health
Founded as OIE

■ Purpose of

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 diluent tube	0.3 ml/vial x 25	0.3 ml/vial x1 00
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 2~3 ml 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 100 µl of assay diluent and 100 µl 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 diluent 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) There has been no assessment of animal-side testing under a range of extreme temperature conditions.
5) 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

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 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.

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

Repeatability

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.
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
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]

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 300 µl and stored in 2 ml 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 2 ml vials containing 300 µl 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 la 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.

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	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	Inconclusive**	> 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	Inconclusive**	> 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	Inconclusive**	> 35	Negative	No Ct	No Ct
9	Negative	No Ct	39.95*	Inconclusive**	> 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 KFU.*
*** For KFU 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

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		Total
		Positive	Negative	
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.

References

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.
Lau, Susanna Kar-Pui, et al. "Evaluation of a Rapid Immunochromatographic Middle East Respiratory Syndrome Coronavirus Antigen Detection Assay." Infectious Microbes & Diseases 4.4 (2022): 175-177.
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)
https://www.cdc.gov/coronavirus/mers/lab/lab-testing.html

Acknowledgement

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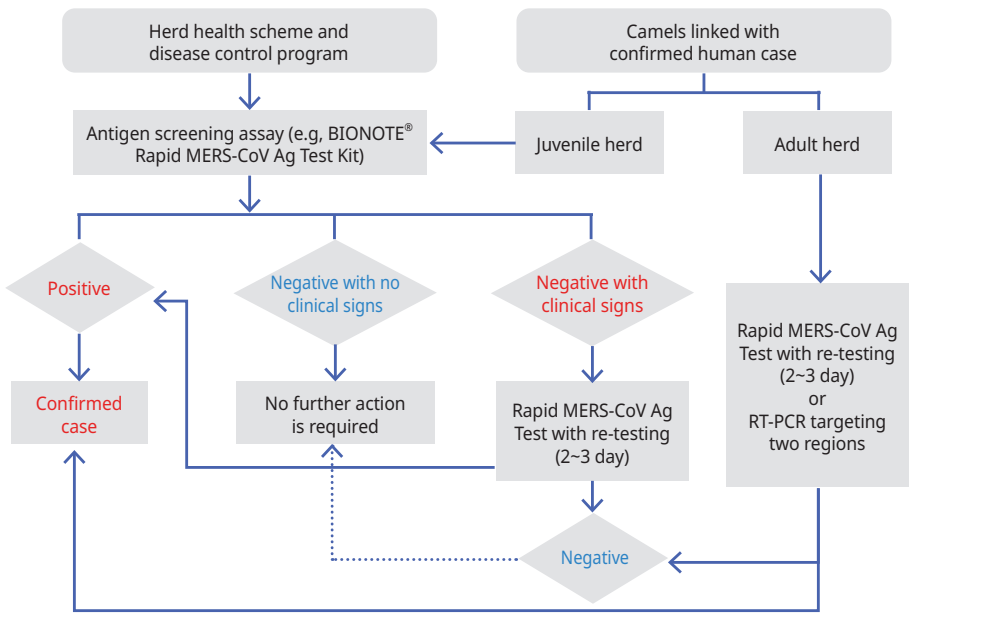
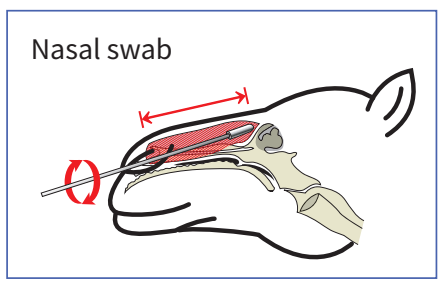
Diagnostic Algorithm for MERS CoV



* 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



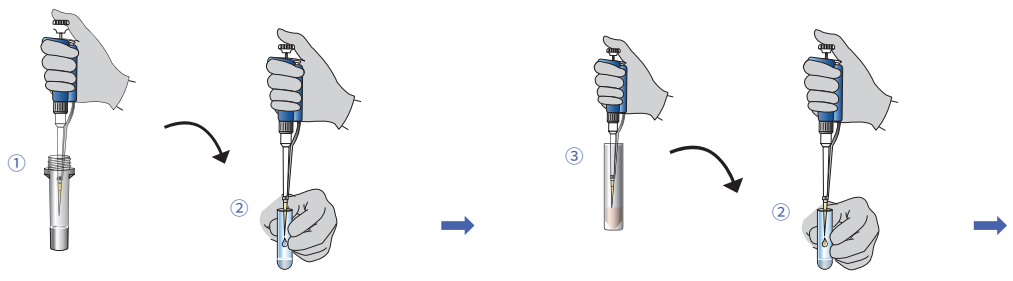
* Insert the swab until the level of the nasal turbinate.

* 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.

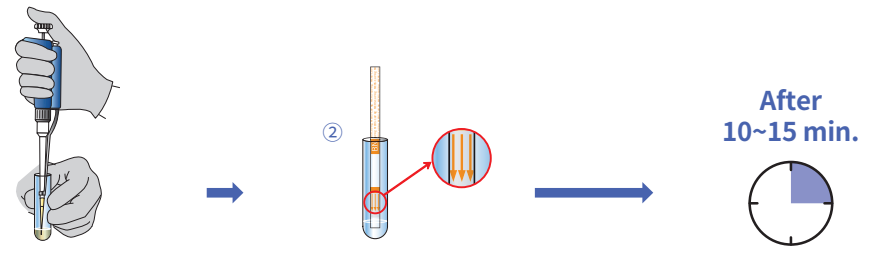
Test Procedure

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



Dispense 100 µl of assay diluents into a test tube.

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

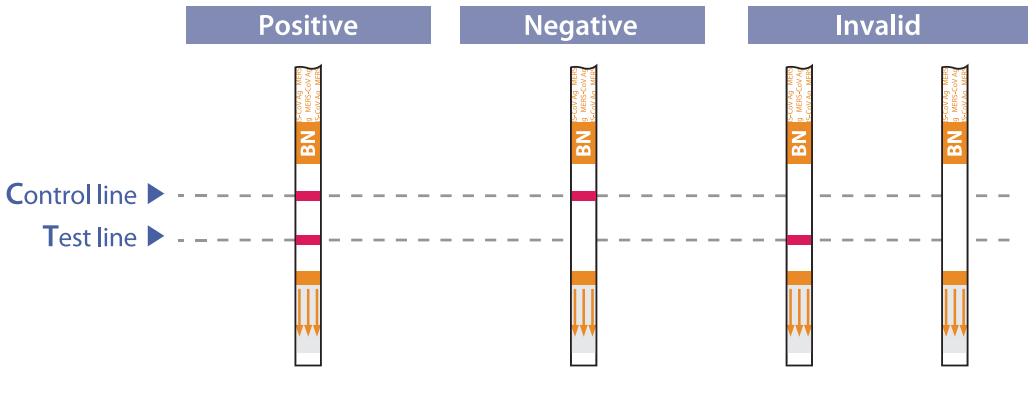


Mix well.

Place the test strip into the test tube.

Read the result.

Interpretation



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Manufactured by BIONOTE
BioNote, Inc.
22, Samsung 1-ro 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