Olf Statement
The validation of data for the BIONOTE® Rapid MERS-CoV Ag Test Kit has been confirmed in May 2016 by the OIE, based on accepted tests, 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 hands (test kit) with acutely infected animals with high viral 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 camel. This assay is intended for rapid screening in laboratory. The test should be conducted by trained technician, wearing appropriate personal protective equipment (PPE).

BIONOTE® Rapid MERS-CoV Ag Test Kit has two lines on the surface of the strips indicating test line (T) and control line (C). T line should appear only 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

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

Precautions
1. The test kit 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 cold pack.
3. Do not re-use 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 samples from different kit numbers because each kit 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 after handling samples.
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 2-8°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 of nasal swab specimens
Collect the nasal swab samples 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 2-5 ml of 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 period(s) 2 hours of transport, store the specimen at 4°C or below. For long period(s) >72 hours of transport, store at -20°C or below and ship on dry ice or liquid nitrogen.
2. It is important to avoid repeated freezing and thawing of specimens. The storage of specimens in domestic-freeze freezers should be avoided, owing to their wide temperature fluctuations.
3. If receiving frozen specimens 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 to two day after the dry ice has dissipated.
4. Each specimen should be labeled with the patient identifier, specimen type, and the sample collection date.
5. All samples must be pre-paired to prevent breaking and spillage. Sample containers should be labeled with the patients name, hospital ID, and date of collection.
6. In order to avoid misidentification of the contents, the Primary Container (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 avoids the appearance of a spill. When large numbers of the specimens are being shipped, they should be organized in a sequential manner in boxes with separate compartments for each specimen.

Procedure of the Test
1. Repeat to the back page. [Sample Examination]
2. Nasal swab samples in transport media should be extracted as following method.
3. Allow test strip and the sample to room temperature (23°C-25°C) prior to testing.
4. Add 100μl of assay diluted and 100μl of extracted samples into a test tube, and mix well.

Reproduction
The scope of this inter-laboratory 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 nasal swab samples collected in transport media in three participating laboratories.

Summary of validation studies

Analytical characteristics

Analytical sensitivity
BIONOTE® Rapid MERS-CoV Ag Test Kit detected up to 0.125 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 laboratory was assessed by using quadruplicate of 5 in house samples (one strong, one medium, on week and two negative samples) in four runs by one operator. Between laboratory was assessed by using triplicate of 5 in house samples in 10 runs by 3 operators on separate day. Batch-to-batch variation was assessed in 5 in house samples by 2 operators on one day, mean CV was 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. Line consists of microscopic reaction of the gold conjugate and target analyte. Gold conjugate consist of colloidal gold and MERS-CoV antibody. The threshold is determined by the analytical sensitivity as 10^7 TCID50/50 Tissue Culture Infectious Dose.

<table>
<thead>
<tr>
<th>Test method under evaluation</th>
<th>N</th>
<th>Dn</th>
<th>C</th>
<th>O</th>
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<tr>
<td>BIONOTE® Rapid MERS-CoV Ag Test Kit</td>
<td>POS</td>
<td>NEG</td>
<td>POS</td>
<td>NEG</td>
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<tr>
<td>Total</td>
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<td>525</td>
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Diagnostic sensitivity (DSn) and specificity (DSp) estimates

Comparison performance

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<tr>
<td>Inter-laboratory comparison</td>
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<tr>
<td>Total</td>
<td>82</td>
<td>525</td>
<td>525</td>
<td>609</td>
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</table>

| Test site | Location | Accreditation status : ISO 17025 | Level of expertise : highly trained technician | Accreditation status : ISO 17025 |
|-----------|----------|---------------------------------|----------------|$1,370$ |
| 2. Lab B | Abu Dhabi | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |
| 3. Lab C | Al Ain | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |

| Test site | Location | Accreditation status : ISO 17025 | Level of expertise : highly trained technician | Accreditation status : ISO 17025 |
|-----------|----------|---------------------------------|----------------|$1,370$ |
| 2. Lab A | Abu Dhabi | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |
| 3. Lab B | Al Ain | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |

| Test site | Location | Accreditation status : ISO 17025 | Level of expertise : highly trained technician | Accreditation status : ISO 17025 |
|-----------|----------|---------------------------------|----------------|$1,370$ |
| 2. Lab A | Abu Dhabi | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |
| 3. Lab B | Al Ain | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |

| Test site | Location | Accreditation status : ISO 17025 | Level of expertise : highly trained technician | Accreditation status : ISO 17025 |
|-----------|----------|---------------------------------|----------------|$1,370$ |
| 2. Lab A | Abu Dhabi | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |
| 3. Lab B | Al Ain | Level of expertise : highly trained technician | Accreditation status : ISO 17025 | $1,370$ |
Methods of Sample Collection

**Nasal Swab**

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

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

**Test Procedure**

1. **Dispense 100 µl of assay diluents into a test tube.**
2. **Add 10 µl of extracted sample from the transport media into the same test tube.**
3. **Mix well.**
4. **Place the test strip into the test tube.**

After 10-15 min.

Results are available.

**Interpretation**

- **Positive**
- **Negative**
- **Invalid**