**OIE Procedure for Registration of Diagnostic Kits**

**Abstract sheet**

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

**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 $10^5$ TCID$_{50}$ (50% Tissue Culture Infective Dose).

**Diagnostic sensitivity (DSn) and specificity (DSp) estimates**

<table>
<thead>
<tr>
<th>Test method under evaluation</th>
<th>Target Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>(66)</td>
</tr>
<tr>
<td>DSn</td>
<td>(93.9%)</td>
</tr>
<tr>
<td>CI</td>
<td>(85.20-98.32%)</td>
</tr>
<tr>
<td><strong>Diagnostic specificity</strong></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>(523)</td>
</tr>
<tr>
<td>DSp</td>
<td>(99.6%)</td>
</tr>
<tr>
<td>CI</td>
<td>(98.63-99.95%)</td>
</tr>
</tbody>
</table>

**Comparative performance**

<table>
<thead>
<tr>
<th>Summary</th>
<th>UpE and Orf1A rRT-PCR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td><strong>BIONOTE Rapid MERS-CoV Ag Test Kit</strong></td>
<td>POS</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>NEG</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>66</td>
<td>523</td>
</tr>
</tbody>
</table>

**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
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 OrfLa gene is targeted. KFU, (Saudi Arabia) real-time PCR results are based on UPE and CDC MERS-CoV 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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>KFU, Saudi Arabia</th>
<th>MBG LAB</th>
<th>VLD- ADFCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Targeted Results (Original)</td>
<td>CT Value UPE</td>
<td>CT Value N2</td>
</tr>
<tr>
<td>1</td>
<td>Positive</td>
<td>21.33</td>
<td>16.65</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>16.01</td>
<td>15.97</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>No Ct</td>
<td>No Ct</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>19.95</td>
<td>18.16</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>25.9</td>
<td>19.03</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>No Ct</td>
<td>No Ct</td>
</tr>
<tr>
<td>7</td>
<td>Positive</td>
<td>20.06</td>
<td>19.86</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
<td>No Ct</td>
<td>No Ct</td>
</tr>
<tr>
<td>9</td>
<td>Negative</td>
<td>39.95*</td>
<td>Uncertain**</td>
</tr>
<tr>
<td>10</td>
<td>Positive</td>
<td>22.16</td>
<td>18.95</td>
</tr>
</tbody>
</table>

* 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

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, the director of Veterinary Laboratories Division, Animal Wealth Sector, ADFCA, UAE for continuous support for this work.