WHO Emergency Use Assessment and Listing for Ebola Virus Disease IVDs
PUBLIC REPORT

Product: ReEBOV™ Antigen Rapid Test Kit

EUAL Number: EA 0011-011-00

Abstract

Emergency Use Assessment and Listing of In Vitro Diagnostics Procedure

WHO has developed an Emergency Use Assessment and Listing (EUAL) procedure to expedite the availability of in vitro diagnostics (IVDs) needed in public health emergency situations. This EUAL procedure will generate WHO recommendations in order to provide advice to procurement agencies and Member States on the acceptability of a specific IVD in the context of a public health emergency, based on a minimum set of available quality, safety, and performance data and an agreed plan for further evaluation.

As such, the WHO EUAL procedure for IVDs consists of:

- a desktop review of selected manufacturing and quality management system documentation;
- the review of any existing documentary evidence of safety and performance; and
- a limited performance evaluation of relevant performance characteristics of the product.

ReEBOV™ Antigen Rapid Test Kit with product code 13966, manufactured by Corgenix Medical Corporation (Broomfield, USA) was listed as eligible for WHO procurement on 19 February 2015.

Assay Principle: ReEBOV™ Antigen Rapid Test Kit is an immunochromatographic dipstick immunoassay for the qualitative detection of Ebola virus (EBOV) VP40 antigen.

Intended use: The ReEBOV™ Antigen Rapid Test is for the presumptive detection of Ebola Zaire virus disease in whole blood, plasma or serum from individuals with signs and symptoms of Ebola virus infection in affected areas in conjunction with relevant epidemiological risk factors. Where possible, the results should be confirmed by further testing using an approved Ebola virus nucleic acid test (NAT).

Intended user: Professional use only.

Method: Capillary or venous whole blood, serum or plasma (EDTA and citrate) is added to the sample pad end of the dipstick. The dipstick is then inserted into a tube that contains sample buffer which initiates the flow of specimen along the test strip. Specific antibodies towards EBOV VP antigen (anti-EBOV VP40) conjugated to gold nanoparticles are striped on the nitrocellulose test strip to capture EBOV VP40 antigen that may be present in the
specimen. EBOV VP40 antigen present in the specimen will form an immune-complex with anti-EBOV VP40/gold nanoparticles. When the mixture containing the immune-complex moves over the reagent pads, these complexes are captured by anti-EBOV VP40 in the test line region. The deposition of gold conjugate generates a pink-red coloured test line which corresponds with the concentration of EBOV VP40 antigen in the test specimen. Excess gold-conjugate is captured by immobilized anti-EBOV in the control line which indicates a valid result. Interpret the test results 15 minutes after the test strip has been added to the tube with sample buffer. Do not interpret the results after 25 minutes.

Each 50 test kit of ReEBOV™ Antigen Rapid Test Kit consists of:
- 2 re-sealable foil pouch each with 25 test strips with desiccant and humidity indicator;
- 2 bottles x 0.25 mL positive control and 2 bottles x 0.25 mL negative control;
- 2 x 7ml sample buffer dropper bottles;
- 2 packets x 25 each 5ml polypropylene round-bottom tubes with caps;
- Disposable test tube rack;
- Instructions for use;
- Visual aid.

Other accessories and materials required for the ReEBOV™ Antigen Rapid Test Kit:
- ReEBOV™ Antigen Accessory Kit (product code 14036) consisting of 200 lancets, 200 alcohol swabs and 200 cotton wool pad for collection of capillary (finger stick) whole blood.
- Personal protective equipment (gloves, gowns, face shields, boots)¹;
- Precision pipettes and tips, capable of delivering between 10µl and 100µl for use;
- Deionized water

Storage temperature: 2 to 8 °C.
Stability: 6 months.

Caveats for use of ReEBOV™ Antigen Rapid Test Kit
- Optimally, specimen collection and the test procedure should be undertaken in a facility such as a primary health care, clinic or hospital with a Class II biosafety cabinet or “glove box” to minimise risk of infection.
- Control specimens should be handled carefully. Use extreme caution when removing tear off metal cap. Procedure can cause sharp edges to occur which can lead to compromising the integrity of the PPE. Use a hemostat or other appropriate instrument for removing metal cap. Properly discard metal cap seal in appropriate sharps container. Subsequent removal of the lid for each use should minimise the risk of aerosol formation, wherever possible.

¹WHO Rapid Advice Guideline “Personal protective equipment in the context of filovirus disease outbreak response”
http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1
Control specimens should be run each time a new test pouch is opened.
To prevent potential contact with infected specimen, the test strips loaded with patient specimen should not be removed from the tube. The result is read whilst in the tube.

**WHO EUAL Assessment**

Corgenix Medical Corporation submitted an expression of interest for WHO emergency quality assessment of ReEBOV™ Antigen Rapid Test Kit on 18 October 2014.

1. **Product dossier assessment**
Corgenix Medical Corporation submitted documentation in support of safety and performance for ReEBOV™ Antigen Rapid Test Kit as per the “Invitation to Manufacturers of Ebola Virus In Vitro Diagnostics to Submit an Expression of Interest (EOI) for Emergency Assessment by WHO”.\(^2\) The information submitted in the product application was reviewed by WHO staff and external experts (reviewers) appointed by WHO. The findings of the reviews were reported in accordance with “Emergency Quality Assessment Mechanism of In Vitro Diagnostics for Ebola Virus Protocol for the Review of Documentary Evidence of Safety, Quality and Performance” (document number WHO PQDx_0188 v0.2).

| Safety and performance documentation for Emergency Use Assessment and Listing conclusion: acceptable pending final approval of the instructions for use to reflect test limitations. |

2. **Review of quality management documentation**
To establish the eligibility for WHO procurement, Corgenix Medical Corporation was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation, it was established that sufficient information was provided by Corgenix Medical Corporation to fulfil the requirements described in the “Invitation to manufacturers of Ebola Virus In Vitro Diagnostics to submit an Expression of Interest (EOI) for emergency assessment by WHO”.

| Quality management documentation for Emergency Use Assessment and Listing conclusion: acceptable. |

3. **Laboratory evaluation**

\(^2\) Invitation to manufacturers of Ebola virus in vitro diagnostics to submit an Expression of Interest (EOI) for emergency assessment by WHO. Accessed on 24 November 2014 at http://www.who.int/diagnostics_laboratory/141002_revised_invitation_to_mx_of_ebola_virus_diagnostics_rc.pdf?ua=1
The limit of detection of ReEBOV™ Antigen Rapid Test Kit was validated using cell culture supernatant containing infectious Makona strain of Zaire Ebola virus spiked into whole blood from a healthy donor at Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany). The dilution series was tested on ReEBOV™ Antigen Rapid Test Kit and the benchmark assay; RealStar Filovirus Screen RT-PCR Kit 1.0 (altona Diagnostics GmbH). By the process of extrapolation, the limit of detection for ReEBOV™ Antigen Rapid Test Kit was 2.11E+08 RNA copies/ml.

ReEBOV™ Antigen Rapid Test Kit was evaluated independently by WHO-supported European Union and African Union field laboratories at Hastings and Prince of Wales (Sierra Leone) using 147 consecutive fresh venous whole blood, and 146 frozen plasma specimens.

<table>
<thead>
<tr>
<th>Ebola status based on altona Diagnostics GmbH RealStar® Filovirus Screen RT-PCR Kit 1.0</th>
<th>Fresh venous whole blood</th>
<th>Frozen plasma*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Hastings laboratory</td>
<td>15</td>
<td>97</td>
<td>0</td>
</tr>
<tr>
<td>Prince of Wales laboratory</td>
<td>8</td>
<td>27</td>
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</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>124</td>
<td>74</td>
</tr>
</tbody>
</table>

*The frozen plasma specimens were collected between December 2014 and February 2015, they were stored at -20°C in one of the laboratories and thawed only once.

The sensitivity of ReEBOV™ Antigen Rapid Test Kit was compared to the benchmark assay; RealStar Filovirus Screen RT-PCR Kit 1.0 (altona Diagnostics GmbH). The sensitivity (95% CI) of ReEBOV™ Antigen Rapid Test Kit was 91.8% (84.5, 96.8) in comparison with the benchmark assay. While the specificity (95% CI) of ReEBOV™ Antigen Rapid Test Kit was 84.6% (78.8, 89.4) in comparison with the benchmark assay.

**Laboratory evaluation for Emergency Use Assessment and Listing conclusion:** Acceptable as a screening assay in suspected symptomatic Ebola virus disease patients during the current Ebola virus outbreak in West Africa.

**WHO Emergency Use Assessment and Listing Decision**

Based on the review of the manufacturer’s submitted data, as well as data generated by WHO from the limited field laboratory evaluation in West African population and at the Bernhard Nocht Institute for Tropical Medicine, Hamburg, the ReEBOV™ Antigen Rapid Test Kit and associated controls, are eligible for WHO procurement. Due to the relatively lower sensitivity and specificity of antigen tests compared with RT-PCR, the ReEBOV™ Antigen Rapid Test is for the presumptive detection of Ebola Zaire virus disease in individuals with signs and symptoms of Ebola virus infection. Where possible, the results should be
confirmed by testing a new blood sample using an approved Ebola virus nucleic acid test (NAT). A final interpretation of the results should be made in conjunction with epidemiological and patient clinical parameters to make a final clinical judgement.

Ebola-specific safety precautions should be taken when using the rapid test.

Post market surveillance to monitor the performance of ReEBOV™ Antigen Rapid Test Kit in comparison with supplemental NAT is highly recommended.

**Scope and duration of procurement eligibility**

ReEBOV™ Antigen Rapid Test Kit with product code 13966, manufactured by Corgenix Medical Corporation is considered to be eligible for WHO procurement. The assay may be used to test symptomatic individuals for Ebola virus disease. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Corgenix Medical Corporation must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Corgenix Medical Corporation is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days. Furthermore, WHO will continue to monitor the performance of the assay in the field.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality and performance comes to WHO’s attention during post-market surveillance activities.