WHO Emergency Use Assessment and Listing Procedure for EVD IVDs
PUBLIC REPORT

Product: Liferiver™ - Ebola Virus (EBOV) Real Time RT-PCR Kit
Number: EA 0009-009-00

Abstract
In order to respond to the urgent need for quality-assured in vitro diagnostic tests in the ongoing Ebola Virus Disease (EVD) outbreak in West Africa, WHO has established a WHO Emergency Use Assessment and Listing (EUAL) Procedure of In Vitro Diagnostics (IVDs) for EVD. It consists of review of existing evidence of safety and performance; desktop review of selected manufacturing and quality management systems documentation and limited laboratory evaluation of the product.

Liferiver™ - Ebola Virus (EBOV) Real Time RT-PCR Kit manufactured by Shanghai ZJ BioTech Co., Ltd., N°20 Building, 528 Ruiqing Road, Zhangjiang High-tech Industrial East District, 201203 Shanghai, People’s Republic of China was listed as eligible for WHO procurement 27 April 2015.

Liferiver™ - Ebola Virus (EBOV) Real Time RT-PCR Kit is an in vitro diagnostic test, based on real-time PCR technology intended for the detection of all highly pathogenic members of Ebolavirus: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV) and Bundibugyo ebolavirus (BDBV) in blood, serum, plasma (non-heparin anticoagulant

The test is based on real-time RT-PCR technology, utilizing reverse transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes. Probes specific for Ebola virus RNA are labelled with the fluorophore FAM. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore VIC.

The test consists of three processes:
- Reverse transcription of target RNA to cDNA
- PCR amplification of target cDNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

Liferiver™ - Ebola Virus (EBOV) Real Time RT-PCR Kit is validated to be used with QIAamp® Viral RNA Mini Kit (QIAGEN), QIAmp DSP virus Spin kit and Liferiver™ RNA isolation kit to extract the viral RNA.

Liferiver™ - Ebola Virus (EBOV) Real Time RT-PCR Kit was developed and validated to be used with the following real-time PCR instruments:
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- CFX96 system/Dx real-time system (Bio-Rad)
- SLAN®-96

Liferiver™ - EBOV Real Time RT-PCR Kit consists of:
1. EBOV Super Mix
2. RT-PCR Enzyme Mix
3. Internal Control (IC)
4. EBOV Negative Control
5. EBOV Positive Control

**Test Kit Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>EBOV Super Mix</th>
<th>RT-PCR Enzyme mix</th>
<th>Internal Control</th>
<th>Positive Control</th>
<th>Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Vials</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Volume [μl/Vial]</td>
<td>513</td>
<td>27</td>
<td>30</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

EBOV Super Mix and RT-PCR Enzyme mix contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection (Filovirus specific RNA and Internal Control) in one reaction setup.

**Background information**


**1. Product dossier assessment**

Shanghai ZJ Bio-Tech Co., Ltd. submitted documentation in support of safety and performance for Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR 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”¹. 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 “WHO Emergency Use Assessment and Listing Procedure 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 assessment conclusion: acceptable.

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¹ 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
2. Review of quality management documentation

To establish the eligibility for WHO procurement, Shanghai ZJ Bio-Tech Co., Ltd. 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 Shanghai ZJ Bio-Tech Co., Ltd. to fulfill 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 assessment conclusion: acceptable.

3. Laboratory evaluation

A limited analytical evaluation of the Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR Kit was conducted by the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg, Germany which is a WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research. The limit of detection (LOD) of the assay was verified and compared to the RealStar® Filovirus Screen RT-PCR Kit 1.0 (altona Diagnostics GmbH) using simulated specimens generated by spiking cell culture supernatants containing infectious Ebola virus strain Makona into whole blood of a healthy donor. The evaluation of the Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR Kit was performed on the Roche LightCycler 480 instrument.

The 95% limit of detection of the assay was found to be 23.9 copies/reaction 95% CI (13.4-405.9 RNA copies/reaction).

Laboratory evaluation conclusion: acceptable.

Based on the review of the submitted documentation and the results of the laboratory evaluation, a recommendation was made to consider Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR Kit as eligible for WHO procurement.

Scope and duration of procurement eligibility

Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR Kit with manufactured by Shanghai ZJ Bio-Tech Co., Ltd. is considered to be eligible for WHO procurement. The assay may be used to test symptomatic individuals for EVD. 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, Shanghai ZJ Bio-Tech Co., Ltd. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Shanghai ZJ Bio-Tech Co., Ltd 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.

**Commitment to WHO**

As a requirement to listing, the manufacturer is required to participate in the WHO collaborative study for the assessment of the suitability of an interim standard for Ebola virus nucleic acid amplification tests.