

WHO Emergency Quality Assessment Mechanism for EVD IVDs PUBLIC REPORT

Product: RealStar® Filovirus Screen RT-PCR Kit 1.0
Number: EA 0002-002-00

Abstract

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

RealStar® Filovirus Screen RT-PCR Kit 1.0 with product code 441013 manufactured by **altona Diagnostics GmbH**, Mörkenstraße 12, 22767 Hamburg, Germany (CE marked regulatory version) was listed as eligible for WHO procurement on 25 November 2014.

RealStar® Filovirus Screen RT-PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the qualitative detection of filovirus specific RNA in human plasma (EDTA) using the QIAamp® Viral RNA Mini Kit (QIAGEN) for RNA extraction. The assay is designed to detect all filovirus species which are relevant human pathogens and Reston virus. In addition, it includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents or the kit.

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. Probes specific for Marburg virus RNA are labelled with a fluorophore with the same characteristics as Cy5. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore JOE. Using probes linked to distinguishable dyes enables the parallel detection and discrimination of Ebola- and Marburg virus specific RNA as well as the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:

- 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

RealStar® Filovirus Screen RT-PCR Kit 1.0 is validated to be used with QIAamp® Viral RNA Mini Kit (QIAGEN) to extract the viral RNA.

RealStar® Filovirus Screen RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- Versant® kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)
- CFX96 system/Dx real-time system (Bio-Rad)

RealStar® Filovirus Screen RT-PCR Kit 1.0 consists of:

1. Two Master reagents (Master A and Master B)
2. Template Internal Control (IC)
3. Two Positive Controls: Positive Control Target Ebola and Positive Control Target Marburg
4. PCR grade water

Test Kit Components

Lid Colour	Blue	Purple	Green	Red	Orange	White
Component	Master A	Master B	Internal Control	Positive Control Target Ebola	Positive Control Target Marburg	PCR grade Water
Number of Vials	8	8	1	1	1	1
Volume [µl/Vial]	60	180	1000	250	250	500

Master A and Master B reagents 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

altona Diagnostics GmbH submitted an expression of interest for WHO emergency quality assessment of RealStar® Filovirus Screen RT-PCR Kit 1.0 on 16 October 2014.

1. Product dossier assessment

altona Diagnostics GmbH submitted documentation in support of safety and performance for RealStar® Filovirus Screen RT-PCR Kit 1.0 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 “Emergency Quality Assessment Mechanism of In

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_ebolavirus_diagnostics_rc.pdf?ua=1

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.

Based on the review of the submitted documentation, a recommendation was made to consider RealStar® Filovirus Screen RT-PCR Kit 1.0 as eligible for WHO procurement.

2. Review of quality management documentation

To establish the eligibility for WHO procurement, Altona Diagnostics GmbH 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 Altona Diagnostics GmbH 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.

Based on the review of the submitted documentation, a recommendation was made to consider RealStar® Filovirus Screen RT-PCR Kit 1.0 as eligible for WHO procurement.

3. Laboratory evaluation

Given the quality and extent of the data submitted as part of the product dossier to support the claims for its intended use, RealStar® Filovirus Screen RT-PCR Kit 1.0 assay will not be required to undergo additional laboratory evaluation for the purpose of this quality assessment.

The assay was evaluated independently 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 sensitivity of the RealStar® Filovirus Screen RT-PCR Kit 1.0 was compared to published assays, cell culture supernatant of EBOV Mayinga, EBOV 2014/Gueckedou-C05, MARV Leiden 2008, and SUDV Gulu serially diluted in negative plasma in the biosafety level 4 laboratory. The supernatants were inactivated and the RNA was extracted. The eluates were tested in replicates with the RealStar Filovirus Screen RT-PCR Kit 1.0, the pan-filovirus assay published by Panning et al. 2007², and an EBOV/SUDV-specific assay published by Gibb et al. 2001³ using in-house protocols used for routine testing. The RealStar Filovirus Screen RT-PCR Kit 1.0 was able to detect virus RNA in comparable or lower concentrations compared to the published reference assays. Additionally, no cross-reactivity with RNA of other human pathogenic viruses including

²Panning M., et al., Diagnostic reverse-transcription polymerase chain reaction kit for filoviruses based on the strain collections of all European biosafety level 4 laboratories. *J Infect Dis.* 2007 Nov 15; 196 Suppl 2:S199-204.

³Gibb TR., et al., Development and evaluation of a fluorogenic 5' nuclease assay to detect and differentiate between Ebola virus subtypes Zaire and Sudan. *J Clin Microbiol.* 2001 Nov; 39(11):4125-30.

haemorrhagic fever viruses, e.g. Lassa virus, Yellow fever virus, Rift-Valley fever virus, and Crimean Congo haemorrhagic fever virus was observed.

Laboratory evaluation conclusion: acceptable.

Based on the review of the submitted data, a recommendation was made to consider RealStar® Filovirus Screen RT-PCR Kit 1.0 as eligible for WHO procurement.

Scope and duration of procurement eligibility

RealStar® Filovirus Screen RT-PCR Kit 1.0 with product code 441013 manufactured by Altona Diagnostics GmbH 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, Altona Diagnostics GmbH must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Altona Diagnostics GmbH 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.