RealStar® Filovirus Screen RT-PCR Kit 1.0

Here we present the evaluation of the RealStar® Filovirus Screen RT-PCR Kit 1.0 for the detection of Filovirus infections. The ongoing outbreak of Ebolavirus disease (EVD) in Guinea, Sierra Leone and Liberia clearly shows the need for rapid and reliable diagnostics of the infection and detection of the virus.

Ebola- and *Marburgvirus* are genera within the family *Filoviridae*. Genus *Marburgvirus* contains a single species termed *Marburg marburgvirus* (MARV). Genus *Ebolavirus* contains five species: *Bundibugyo ebolavirus* (BEBOV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SEBOV), *Taï Forest ebolavirus* (TAFV) and the *Zaire ebolavirus* (ZEBOV).

Due to the relatively unspecific signs and symptoms, direct detection of the virus RNA in patient samples is crucial to initiate proper health care measures. Several RT-PCR assays have been published, but the availability of the most recent sequence information required a revision of the protocols.

We developed two RT-PCR Kits, the **RealStar® Filovirus Screen RT-PCR Kit 1.0** and the **RealStar® Filovirus Type RT-PCR Kit 1.0**. The first is designed as a first-line assay to detect all known relevant Filovirus species and distinguish Marburgvirus and Ebolavirus to the genus level. The latter is a typing assay for positive confirmation of the screening result and for typing down to the species level. Both assays contain an internal control for reliable diagnostics.

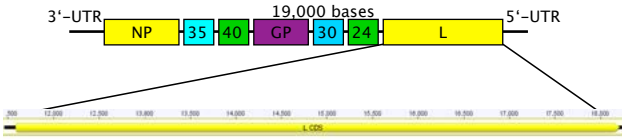

Figure 1 Oligonucleotide binding sites of the RealStar® Filovirus Screen RT-PCR Kit. The binding region within the L gene of the Filovirus genome is highly conserved and allows generic detection of all pathogenic species.

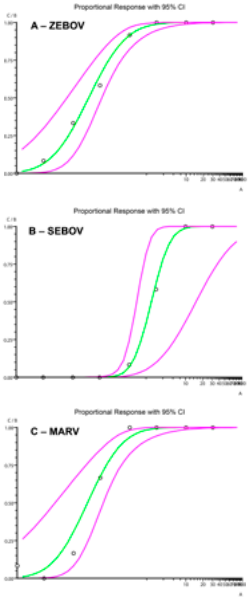

Figure 2 Probit analysis for the RealStar® Filovirus Screen RT-PCR Kit. The limit of detection for different targets was determined using *in-vitro* transcribed RNA (IVT) quantified by spectrophotometry. The IVTs were diluted in half-logarithmic steps (from 30 – 0.01 copies/µl) and tested in replicates (n=12) for positive amplification and detection.

ZEBOV RNA is detected down to a concentration of 1.39 copies/µl with a 95% probability (A). Cut-off for SEBOV detection is 6.75 (B) and for MARV 1.16 copies/µl eluate (C). The X-axis shows the concentration of RNA and the Y-axis the proportion of responders.

Table 1 Comparison of the RealStar® Filovirus Screen RT-PCR Kit 1.0 with other RT-PCR assays. Cell-culture supernatant was serially diluted in negative plasma and afterwards the RNA was extracted using the QIAamp Viral RNA kit. Extracted RNA was tested in replicates with different RT-PCR methods.

	Marburgvirus Leiden								
	uv	–1	–2	–3	–4	–5	–6	–7	–8
RealStar® Filovirus Screen Kit 1.0	3/3	3/3	3/3	3/3	3/3	1/3	0/3	0/3	0/3
Panning L-Gen Screen [1]	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Gibb EBOV GP [2]	2/2	2/2	2/2	2/2	0/2	0/2	0/2	0/2	0/2
Gibb MARV GP [3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Marburgvirus Leiden								
	uv	–1	–2	–3	–4	–5	–6	–7	–8
RealStar® Filovirus Screen Kit 1.0	3/3	3/3	3/3	3/3	3/3	3/3	0/3	1/3	0/3
Panning L-Gen Screen [1]	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
Gibb EBOV GP [2]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gibb MARV GP [3]	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	Zaire ebolavirus 2014/Gueckedou-C05								
	uv	–1	–2	–3	–4	–5	–6	–7	–8
RealStar® Filovirus Screen Kit 1.0	3/3	3/3	3/3	3/3	3/3	1/3	0/3	0/3	0/3
Panning L-Gen Screen [1]	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Gibb EBOV GP [2]	2/2	2/2	2/2	2/2	0/2	0/2	0/2	0/2	0/2
Gibb MARV GP [3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Sudan ebolavirus Gulu								
	uv	–1	–2	–3	–4	–5	–6	–7	–8
RealStar® Filovirus Screen Kit 1.0	3/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Panning L-Gen Screen [1]	3/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Gibb EBOV GP [2]	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Gibb MARV GP [3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The RealStar® Filovirus Screen RT-PCR Kit 1.0 is a valuable tool to assist clinicians in diagnostics, patient management and epidemiology. It is sensitive and specific. The assay includes an Internal Control, which ensures high diagnostic reliability. It is easy to handle and therefore offers molecular diagnostic laboratories a rapid and convenient way to perform Filovirus diagnostics. It has proven the suitability for application in the field during an outbreak situation.

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[2] Gibb, T. R., Norwood, D. A., Woollen, N., and Henchal, E. A. (2001a). Development and evaluation of a fluorogenic 5' nuclease assay to detect and differentiate between Ebolavirus subtypes Zaire and Sudan. J. Clin. Microbiol. *39,* 4125 – 4130.

[3] Gibb, T. R., Norwood, D. A., Woollen, N., and Henchal, E. A. (2001b). Development and evaluation of a fluorogenic 5'-nuclease assay to identify Marburg virus. Mol. Cell. Probes *15,* 259 – 266.

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