Validation of the RealStar® Dengue Type RT-PCR Kit 1.0 for Differentiation of Dengue Virus Serotypes 1–4.

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Introduction

Dengue virus (DENV; family *Flaviviridae*) is the causative agent of dengue fever. The virus is transmitted mainly by *Aedes* mosquitoes. Dengue infections can range from a mild flu-like syndrome to a severe dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS). Dengue infections are a permanent problem in tropical and sub-tropical countries. The spread of dengue has increased dramatically around the world in recent decades and a large worldwide outbreak took place in 2015 [1]. There are four Dengue serotypes that can cause Dengue infections in humans (DENV-1, DENV-2, DENV-3 and DENV-4). The first infection leads to a long-lasting protection to that serotype. However, a second infection with a different serotype leads to a higher risk of developing dengue hemorrhagic fever [2]. For this reason, it is important to have a diagnostic test that differentiates between the four different Dengue virus serotypes.

Materials and Methods

altona Diagnostics GmbH developed the RealStar® Dengue Type RT-PCR Kit 1.0 as a second-line diagnostic assay for identification of the four known Dengue virus serotypes in known Dengue-positive samples.

The limit of detection was determined using probit analysis after testing replicates of limited dilutions of quantified *in vitro* transcribed RNA containing the RT-PCR target sequence. The limit of detection was calculated for each of the four different Dengue virus serotypes.

The ability of the RealStar® Dengue Type RT-PCR kit 1.0 assay to differentiate between the four different Dengue virus types was evaluated by testing the 2013 and 2016 QCMD Dengue panels.

Analytical Sensitivity

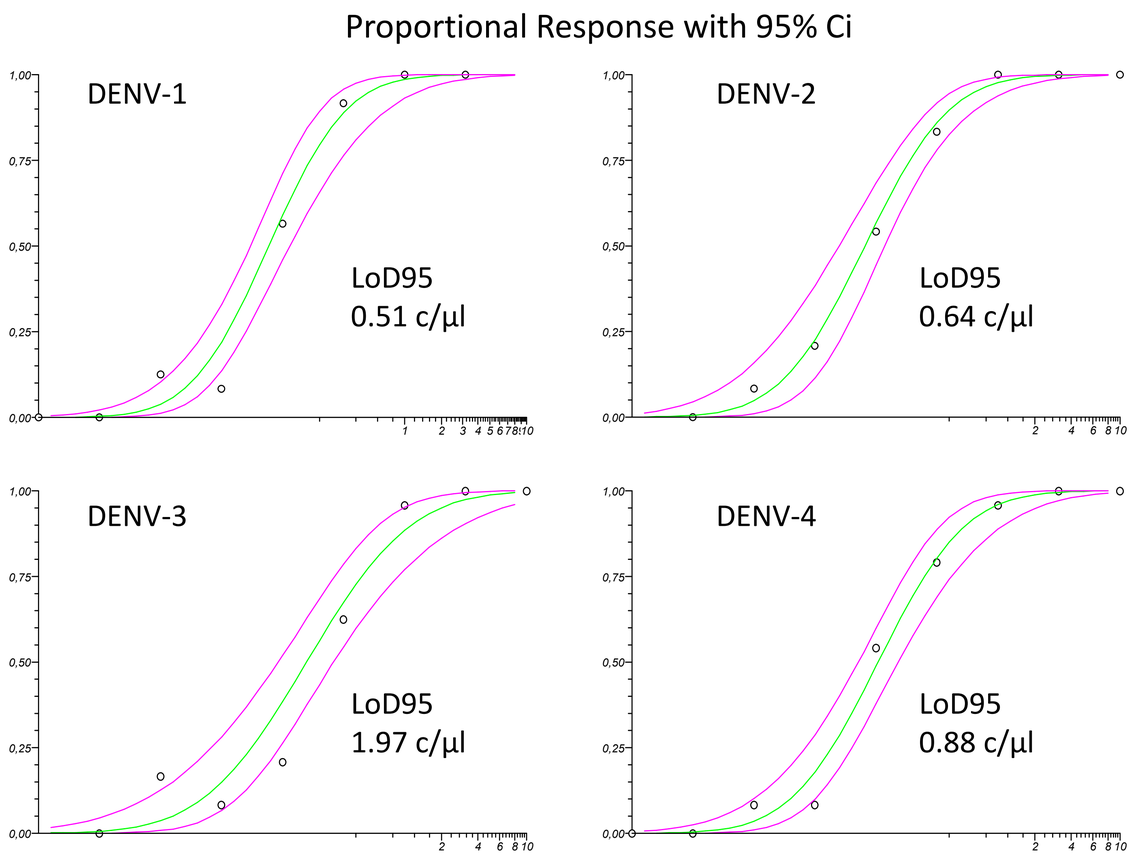

Figure 1

Probit analysis for the RealStar® Dengue Type RT-PCR Kit 1.0.

The analytical sensitivity (95% Limit of Detection, LoD95) was determined for each type by testing replicates of half-logarithmic dilutions of virus specific *in-vitro* transcribed RNA. The X-axis shows the concentration of RNA and the Y-axis the proportion of positive results. For DENV-1 the LoD95 is 0.51 c/µl (confidence interval (CI) 0.31 to 1.15); for DENV-2 0.64 c/µl (CI 0.37 to 1.47); for DENV-3 1.97 c/µl (CI 0.45 to 11.68); for DENV-4 0.88 c/µl (CI 0.51 to 2.03).

Diagnostic validation

Samples from the 2013 and 2016 QCMD Dengue panels were tested with the RealStar® Dengue RT-PCR Kit 2.0. Positive samples were subsequently typed with the RealStar® Dengue Type RT-PCR Kit 1.0. The observed results were 100% correct for both panels.

Table 1: Dengue QCMD 2013 Results

Sample ID	RealStar® Dengue RT-PCR Kit 2.0 [Ct-value]	RealStar® Dengue Type RT-PCR Kit 1.0 [Ct-value / serotype]	QCMD Sample Content
DENVRNA13-01	26.81	21.55 / DENV-1	Dengue virus Type 1
DENVRNA13-02	29.16	27.32 / DENV-1	Dengue virus Type 1
DENVRNA13-03	29.45	24.74 / DENV-1	Dengue virus Type 1
DENVRNA13-04	34.65	28.33 / DENV-1	Dengue virus Type 1
DENVRNA13-05	34.78	31.21 / DENV-1	Dengue virus Type 1
DENVRNA13-07	35.17	36.29 / DENV-2	Dengue virus Type 2
DENVRNA13-08	32.11	26.60 / DENV-3	Dengue virus Type 3
DENVRNA13-09	35.07	30.11 / DENV-3	Dengue virus Type 3
DENVRNA13-10	33.92	25.86 / DENV-4	Dengue virus Type 4
DENVRNA13-11	negative	Not tested	Non Dengue virus flaviviruses
DENVRNA13-12	negative	Not tested	DENV Negative VTM

Table 2: Dengue QCMD 2016 Results

Sample ID	RealStar® Dengue RT-PCR Kit 2.0 [Ct-value]	RealStar® Dengue Type RT-PCR Kit 1.0 [Ct-value / serotype]	QCMD Sample Content
DENVRNA16-01	27.83	21.66 / DENV-1	Dengue virus Type 1(core)
DENVRNA16-02	36.35	31.27 / DENV-1	Dengue virus Type 1(educational)
DENVRNA16-03	35.33	33.74 / DENV-2	Dengue virus Type 2 (educational)
DENVRNA16-04	32.62	31.19 / DENV-2	Dengue virus Type 2 (core)
DENVRNA16-05	31.4	25.42 / DENV-3	Dengue virus Type 3 (core)
DENVRNA16-06	negative	Not tested	Dengue virus negative (core)
DENVRNA16-07	30.05	26.03 / DENV-4	Dengue virus Type 4 (core)
DENVRNA16-08	30.85	24.76 / DENV-1	Dengue virus Type 1 (core)
DENVRNA16-09	34.17	28.05 / DENV-1	Dengue virus Type 1 (core)
DENVRNA16-10	negative	Not tested	Non dengue virus flaviviruses (core)

Figure 2: Amplification curves of QCMD panel samples with the different Dengue virus Type assays contained in the RealStar® Dengue Type RT-PCR Kit 1.0. Samples were run on a CFX96™ Real-Time System (Bio-Rad). Fluorescence signals of DENV-1 and DENV-3 assays are measured in the FAM channel and the signals of the DENV-2 and DENV-4 assays in the Cy5 channel.

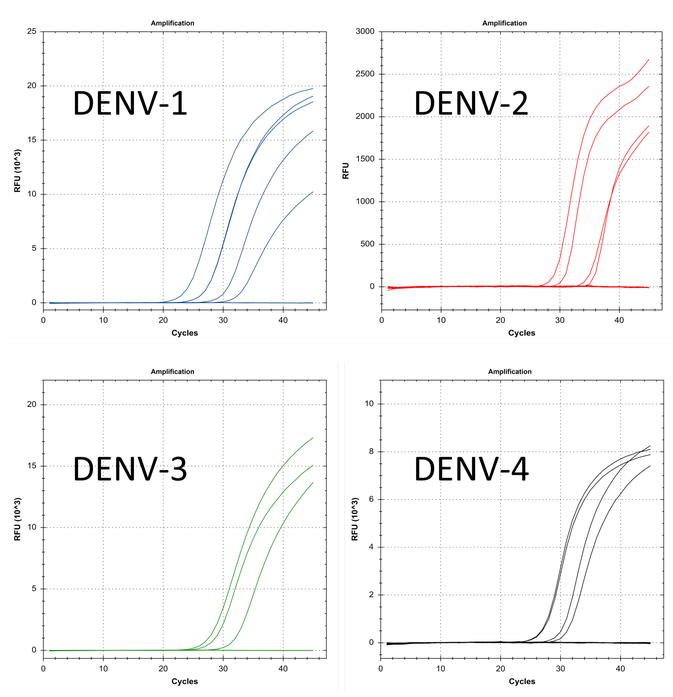

Table 3: Reactivity and cross-reactivity of the Dengue assays 1-4 contained in the RealStar® Dengue Type Kit.

	RealStar® Dengue Type RT-PCR 1.0 assay			
Virus RNA	DENV-1	DENV-2	DENV-3	DENV-4
Dengue virus 1	+	–	(+)*	–
Dengue virus 2	–	+	–	–
Dengue virus 3	–	–	+	–
Dengue virus 4	–	–	–	+

* weak cross-reactivity of the Dengue virus 3 assay with high RNA titers of Dengue virus serotype 1

Conclusion

The RealStar® Dengue Type RT-PCR Kit 1.0 enables the user to distinguish successfully between the four different Dengue virus serotypes in Dengue virus positive samples.

References

[1] WHO Fact Sheets. 2016. *Dengue and severe Dengue.* [ONLINE] Available at: http://www.who.int/mediacentre/factsheets/fs117/en/. [Accessed 10 October 2016].

[2] Rico-Hesse, R. (2009). Dengue virus markers of virulence and pathogenicity. *Future Virology,* 4(6), 581-589.

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