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Yellow fever set of molecular standards for assay verification

This set of four standards serves as yellow fever virus positive control material with defined concentration to test and verify molecular diagnostics for yellow fever virus.

Use

The four vials you receive contain cell culture supernatant of yellow fever virus (strain 17-D) infected Vero E6 cells. The samples were adjusted to a range of detection of yellow fever genome (high, medium and low viral load), aliquoted and, freeze-dried. The virus in each preparation was inactivated by heat treatment and additional γ-irradiation. No remaining infectivity could be proven.

Based on an elution volume of 60 µl in the RNA-extraction protocol, quantification by real-time RT-PCR assays using a molecular standard curve revealed the following results*

Assay	Ct value*/5 µL RNA	Copies*/5 µl RNA
Yellow fever RT-PCR (Domingo et al, JCM 2012; 50:12)	Calibrator-1: 26± 0.5 (CV 0.88 %) Calibrator-2: 29± 0.5 (CV 0.88 %) Calibrator-3: 32± 0.5 (CV 0.51 %) Calibrator-4: 36± 1 (CV 1.31 %)	Calibrator-1: 3.9E+04 Calibrator-2: 5E+03 Calibrator-3: 8.9E+02 Calibrator-4: 8E+01
RealStar®Yellow Fever Virus RT-PCR Kit	Calibrator-1: 24± 0.5 (CV 0.68 %) Calibrator-2: 28± 0.5 (CV 0.42 %) Calibrator-3: 31± 0.5 (CV 0.78 %) Calibrator-4: 34.5± 0.5 (CV 0.6 %)	

*Average of six measurements

To prepare the **calibrators** for verification of your RT-PCR assays, resuspend the lyophilized material in 100 µL of molecular biology grade water. Alternatively, you can resuspend the material in the sample volume specified in your extraction protocol. The samples have to be extracted at once without preparing aliquots of the starting material.
Aliquot them (12 microliters aliquots) and store at -80°C until use.
For verification of your YF RT-PCR assays analyze the four samples in duplicate in the same run.

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