

# EVALUATION OF THE ANALYTICAL PERFORMANCES OF THREE DIAGNOSTIC KITS BASED ON MULTIPLEX RT-PCR FOR THE DETECTION AND QUANTIFICATION OF HHV-6, HHV-7 E HHV-8 IN HUMAN SAMPLES USING THE SENTINAT® 200 AUTOMATED INSTRUMENT

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## Background

There are 8 Herpes viruses able to infect humans: they remain latent in the host even after the primary infection has resolved and can reactivate in physical conditions like immunodepression and transplantations. Related infections can be diagnosed by multiplex real-time PCR<sup>1</sup> assays. Aim of this study is to evaluate the analytical performances of STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 (Sentinel Diagnostics) for the detection and quantification respectively of *Human betaherpesvirus 6*, *Human betaherpesvirus 7* e *Human herpesvirus 8*<sup>2,3,4,5</sup> on different human matrices, using the fully automated system SENTINAT® 200 (Sentinel Diagnostics).

## Methods

Human plasma and blood samples were analyzed to evaluate the performances of STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 kits relatively to the analytical sensitivity (LoD, LoQ), linearity, precision and specificity on 22 pathogens, following the CLSI<sup>6</sup> (Clinical and Laboratory Standards Institute) guidelines. DNA from samples was extracted using SENTINAT® X48 Pathomag Extraction kit (Sentinel Diagnostics). The extraction, PCR set up and amplification reaction were performed with SENTINAT® 200, a fully automated, sample-to-result platform.



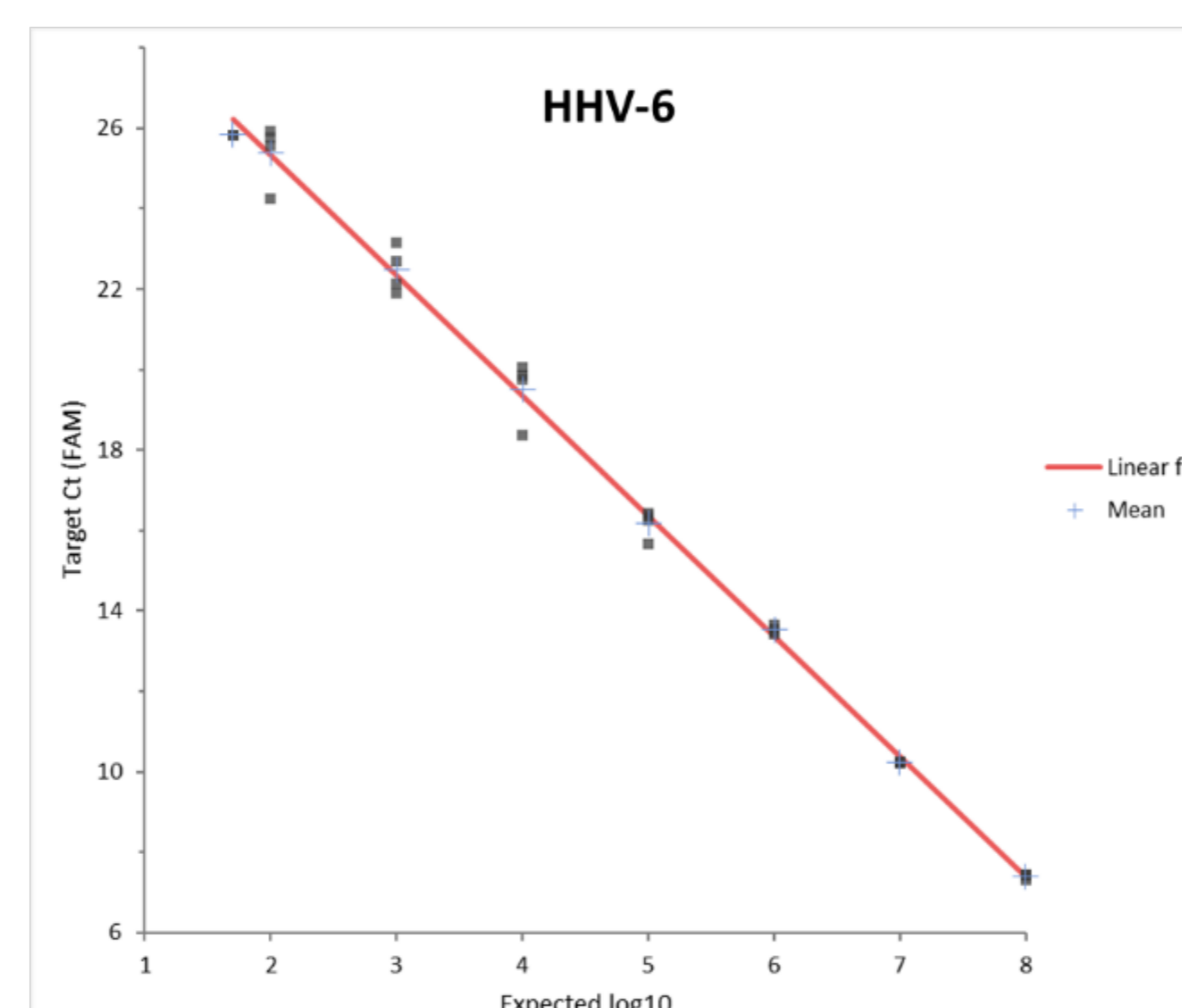
## Results

For STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 kits, the analytical sensitivity was evaluated by calculating the Limit of Detection (LoD) and the Limit of Quantitation (LoQ), using different concentrations of EDX virus particles (Exact Diagnostics, Bio-Rad Laboratories). Results showing a 95% probability of having a positive LoD and LoQ result were calculated on multiple sample replicates (**Table A**). The linearity of the STAT-NAT® SN 200 HHV-6 kit was investigated using an 8-level panel of the HHV-6 EDX virus particles.

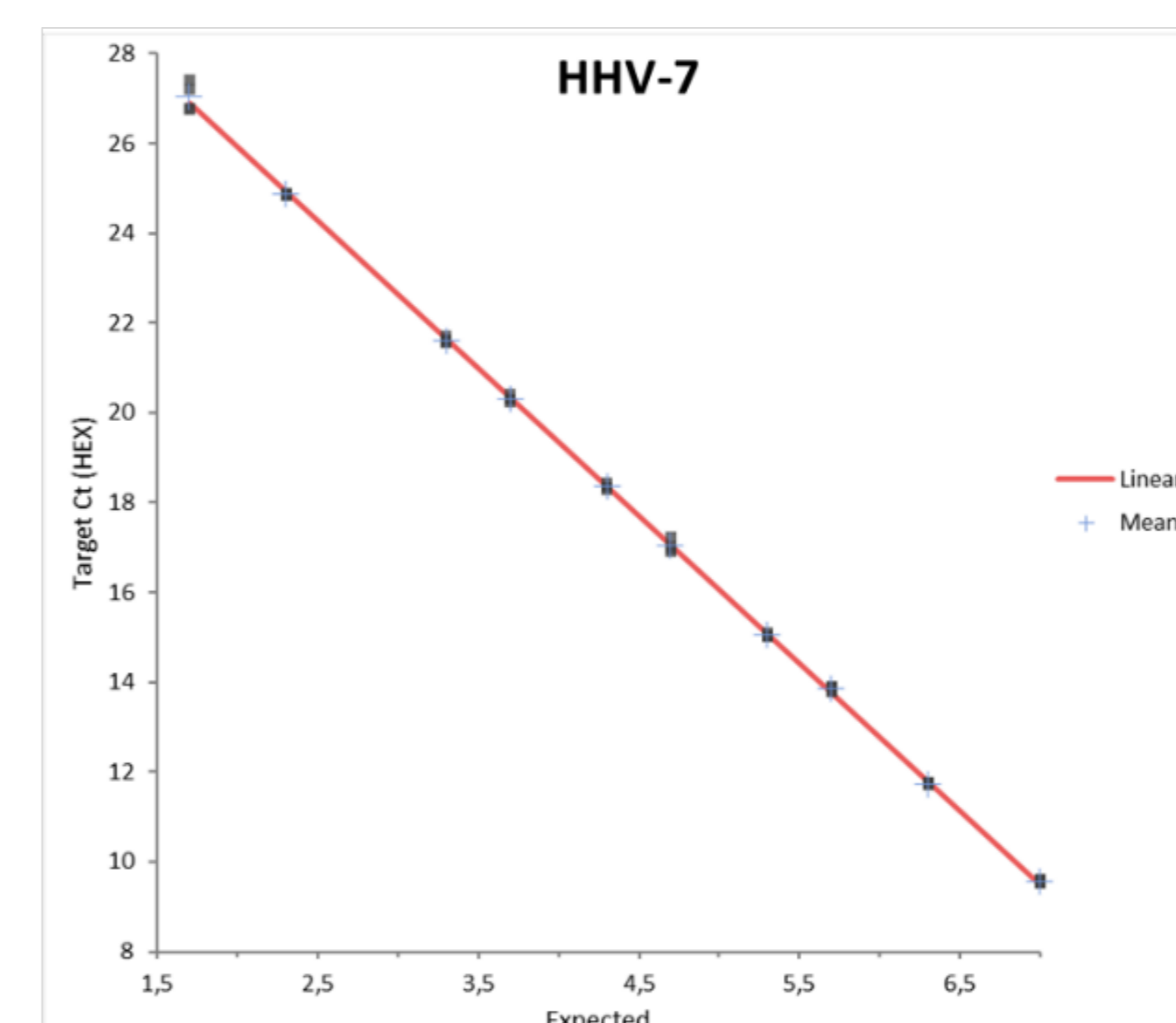
	HHV-6		HHV-7		HHV-8	
	LoD	LoQ	LoD	LoQ	LoD	LoQ
<b>Whole blood</b>	490 IU/mL	560 IU/mL	250 cps/mL	350 cps/mL	500 cps/mL	500 cps/mL
<b>Plasma</b>	380 IU/mL	380 IU/mL	202 cps/mL	300 cps/mL	200 cps/mL	300 cps/mL

**Table A. LoD and LoQ in different matrices (whole blood, plasma).** The tested dilution range of HHV-6 viral particles is from 1x10<sup>2</sup> IU/mL to 1x10<sup>3</sup> IU/mL, while for HHV-7 and HHV-8 it's from 1x10<sup>2</sup> cps/mL to 1x10<sup>3</sup> cps/mL.

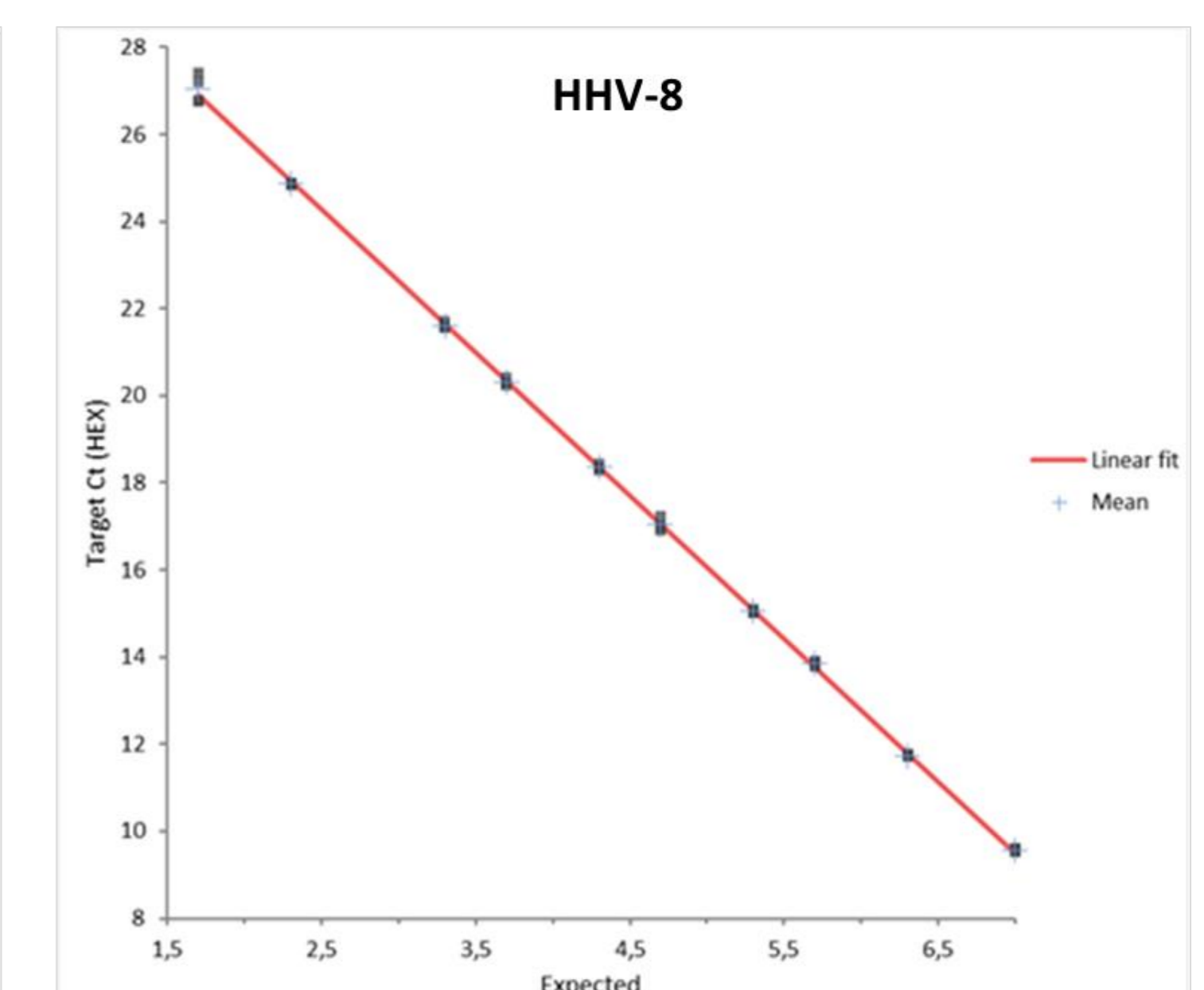
The HHV-6 assay presents a linear trend from 5x10<sup>1</sup> to 1x10<sup>8</sup> IU/mL (**Fig. 1**). Linearity of the STAT-NAT® SN200 HHV-7 kit was investigated using an 8-level panel of EDX HHV-7. The STAT-NAT® SN200 HHV-7 assay shows a linear trend from 1x10<sup>2</sup> to 1x10<sup>7</sup> cps/mL (**Fig.2**). For the STAT-NAT® SN200 HHV-8 kit, linearity was investigated using a 9-level panel of EDX HHV-8. The STAT-NAT® SN200 HHV-8 assay presents a linear trend from 2x10<sup>2</sup> to 1x10<sup>7</sup> cps/mL (**Fig.3**). The precision of the kits was performed by evaluating the degree of agreement between the quantities obtained on the same analyte in multiple replicates. The %CV of reproducibility and repeatability is <10% for HHV-6, HHV-7 and HHV-8. Analytical specificity is 100% and has been demonstrated using a panel of 22 different pathogens for each assay. No cross-reactivity was observed with any of the organisms tested (**Table B**). The various STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 assays were also evaluated for the presence of exogenous and endogenous interfering substances in selected human samples (**Table C**).



**Figure 1. Linearity plot of HHV-6 in plasma matrix.** Linearity of the STAT-NAT® SN200 HHV-6 kit has been investigated in the range from 5x10<sup>1</sup> to 1x10<sup>8</sup> IU/mL.



**Figure 2. Linearity plot of HHV-7 in plasma matrix.** Linearity of the STAT-NAT® SN200 HHV-7 kit was tested in the range of 1x10<sup>2</sup> to 1x10<sup>7</sup> cps/mL.



**Figure 3. Linearity plot of HHV-8 in plasma matrix.** Linearity of the STAT-NAT® SN200 HHV-8 kit was evaluated in the range from 2x10<sup>2</sup> to 1x10<sup>7</sup> cps/mL.

Pathogens	HHV-6 Cross-reactivity (Yes/No)	HHV-7 Cross-reactivity (Yes/No)	HHV-8 Cross-reactivity (Yes/No)
Enterovirus	NO	NO	NO
Adenovirus	NO	NO	NO
Streptococcus pneumoniae	NO	NO	NO
Herpes Simplex Virus 1	NO	NO	NO
Herpes Simplex Virus 2	NO	NO	NO
Varicella-Zoster virus	NO	NO	NO
Epstein-Barr virus	NO	NO	NO
Human herpes virus 8	NO	NO	-
Human immunodeficiency virus 1	NO	NO	NO
Human immunodeficiency virus 2	NO	NO	NO
Cytomegalovirus	NO	NO	NO
Staphylococcus aureus	NO	NO	NO
Streptococcus pyogenes	NO	NO	NO
Staphylococcus epidermidis	NO	NO	NO
BK polyomavirus	NO	NO	NO
Hepatitis B virus	NO	NO	NO
Enterococcus faecalis	NO	NO	NO
Klebsiella pneumoniae	NO	NO	NO
Human betaherpesvirus 7	NO	-	NO
JC polyomavirus	NO	NO	NO
Parvovirus B19	NO	NO	NO
Toxoplasma gondii	NO	NO	NO
Human herpes virus 6	-	NO	NO

**Table B. Evaluation of in vitro cross-reactivity.** A panel of 22 pathogens was used for STAT-NAT® SN200 HHV-6, STAT-NAT® SN200 HHV-7 and STAT-NAT® SN200 HHV-8 kits.

Interfering substances	Tested concentrations
Valganciclovir	10 µg/mL
Prednisone	22,2 µg/mL
Cidofovir	20 µg/mL
Cefotaxime	214 µg/mL
Mycophenolate mofetil	40 µg/mL
Vancomycin	50 µg/mL
Tacrolimus	100 ng/mL
Famotidine	200 µg/mL
Valacyclovir	100 µg/mL
Leflunomide	100 µg/mL
Triglycerides	500 mg/dL
Conjugated bilirubin	0,25 g/L
Unconjugated bilirubin	0,25 g/L
Albumin	58,7 g/L
Hemoglobin	0,25 g/L
Human genome	2 mg/L

**Table C. Exogenous and endogenous interferents tested.** The results obtained show an irrelevant interfering effect of the endogenous or exogenous molecules on the analytical sensitivity of the kits.

## Conclusions

The evaluated STAT-NAT® kits, combined with the SENTINAT® 200 instrument, offer a complete, automated, sample-to-result solution for the simultaneous detection and quantification of HHV-6, HHV-7 and HHV-8 in different human matrices, providing sensitive, precise and reproducible results.

## REFERENCES

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