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P1703

SENTINEL DIAGNOSTICS

EVALUATION OF THE ANALYTICAL PERFORMANCES OF TWO DIAGNOSTIC KITS BASED ON MULTIPLEX RT-PCR FOR THE DETECTION AND QUANTIFICATION OF BKV AND JCV IN HUMAN SAMPLES USING THE SENTiNAT® 200 AUTOMATED INSTRUMENT

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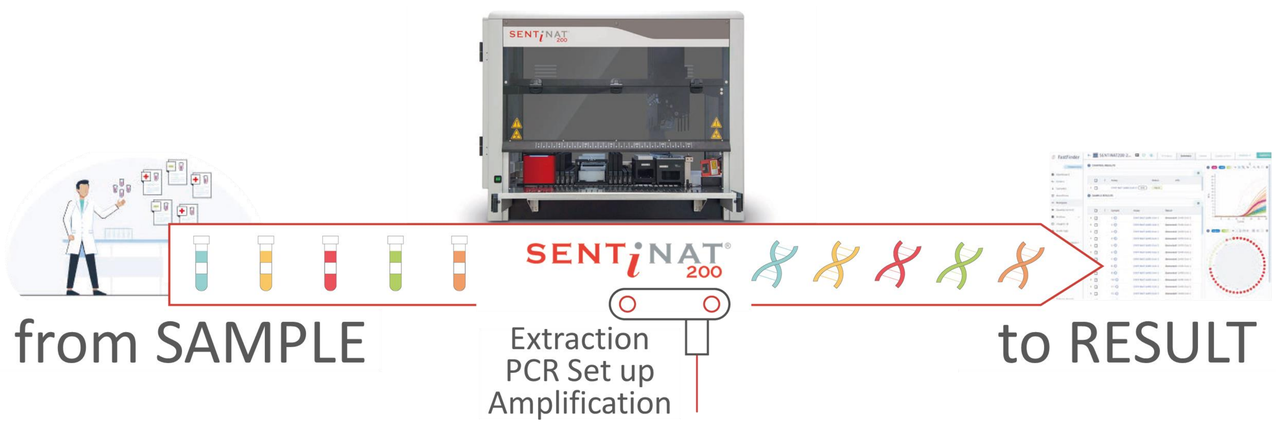
Background

Polyomaviruses are ubiquitous, species-specific, members of the *Polyomaviridae* family. *Human Polyomavirus* 1 (BKV) and *Human Polyomavirus* 2 (JCV) are the most commonly known viruses that can cause mild pathologies in childhood, and more serious disease states if reactivated or acquired by immunocompromised patients[1,2,3,4]. While BKV is mainly associated with nephropathies, JCV can cause progressive multifocal leukoencephalopathy. Both viruses are diagnosed by multiplex Real-Time PCR assays. The aim of this work was to evaluate the analytical performances of STAT-NAT® SN200 BKV and STAT-NAT® SN200 JCV (Sentinel Diagnostics) kits for the detection and quantification, respectively, of BKV and JCV on different human matrices, using the fully automated system SENTiNAT® 200 (Sentinel Diagnostics).

Methods

Human plasma and urine samples were analyzed to evaluate the performances of STAT-NAT® SN200 BKV kit and human plasma and blood samples were analyzed to evaluate the performances of STAT-NAT® SN200 JVC kit to determine the analytical sensitivity (LoD, LoQ), linearity, precision and specificity on 22 pathogens, following the CLSI[5] (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 the STAT-NAT® SN200 BKV and STAT-NAT® SN200 JCV kits, the analytical sensitivity was evaluated by calculating the Limit of Detection (LoD) and the Limit of Quantitation (LoQ), using different concentrations of EDX (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**).

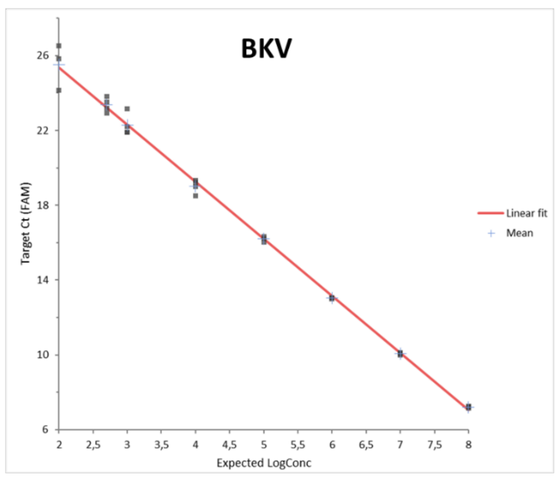
Linearity of the STAT-NAT® SN200 BKV kit was investigated using an 8-level panel of EDX BKV virus particles (Exact Diagnostics). The BKV assay presents a linear trend from a concentration of $1x10^2$ to $1x10^8$ IU/mL (**Figure 1**). As for BKV, also the linearity of the STAT-NAT® SN200 JCV kit was investigated using an 8-level panel of EDX JCV (Exact Diagnostics) and the assay shows a linear trend from $1x10^2$ to $1x10^8$ IU/mL (**Figure 2**).

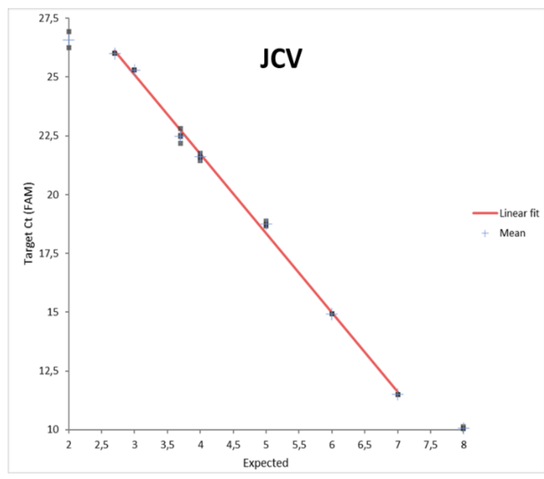
Precision of the kits has been calculated by evaluating the degree of agreement between the quantities obtained on the same analyte in multiple replicates. The %CV of reproducibility and repeatability is <10% for both the STAT-NAT® SN200 BKV assay and the STAT-NAT® SN200 JCV assay.

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**). STAT-NAT® SN200 BKV and STAT-NAT® SN200 JCV assays were also evaluated for the presence of exogenous and endogenous interfering substances in selected human samples (**Table C**).

	BKV		JCV	
	LoD	**LoQ**	**LoD**	**LoQ**
Whole blood	-	-	500 IU/mL	750 IU/mL
Plasma	190 IU/mL	200 IU/mL	500 IU/mL	600 IU/mL
Urines	200 IU/mL	250 IU/mL	-	-

Table A. LoD and LoQ in different matrices (whole blood, plasma and urine). The tested dilution range of BKV viral particles is from $1.5x10^2$ IU/mL to $1x10^3$ IU/mL, while for JCV it is from $1x10^2$ IU/mL to $9x10^2$ IU/mL.





Figures 1 and 2. Linearity plots of BKV and JCV in plasma matrix. Linearity of STAT-NAT® SN200 BKV and STAT-NAT® SN200 JCV kits was investigated in the range from $1x10^2$ to $1x10^8$ IU/mL.

Pathogens	BKV Cross-reactivity (Yes/No)	JCV Cross-reactivity (Yes/No)
Enterovirus	NO	NO
Adenovirus	NO	NO
Streptococcus pneumoniae	NO	NO
Herpes Simplex Virus 1	NO	NO
Herpes Simplex Virus 2	NO	NO
Varicella-Zoster virus	NO	NO
Epstein-Barr virus	NO	NO
Human herpes virus 8	NO	NO
Human immunodeficiency virus 1	NO	NO
Human immunodeficiency virus 2	NO	NO
Cytomegalovirus	NO	NO
Staphylococcus aureus	NO	NO
Streptococcus pyogenes	NO	NO
Staphylococcus epidermidis	NO	NO
BK polyomavirus	-	NO
Hepatitis B virus	NO	NO
Enterococcus faecalis	NO	NO
Klebsiella pneumoniae	NO	NO
Human betaherpesvirus 7	NO	NO
JC polyomavirus	NO	-
Parvovirus B19	NO	NO
Toxoplasma gondii	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 BKV and STAT-NAT® SN200 JCV 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
Glucose	1000 mg/dL

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, used in combination with the SENTiNAT® 200 instrument, offer a complete, automated, sample-to-result solution for the simultaneous detection and quantification of BKV and JCV in different human matrices, providing sensitive, precise and reproducible results.

REFERENCES

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