Quantiplus® JC Virus Quantitation Kit (Real-Time Quantitative PCR Kit)

QT-JCV-25 : 25 rxns
QT-JCV-50 : 50 rxns
QT-JCV-100 : 100 rxns

RUO

PI/QTJCV-01

Intended Use

Quantiplus® JC Virus Quantitation Kit is a Real-Time PCR based in vitro diagnostic assay for detection/quantitation of John Cunningham Virus in human plasma, urine, and CSF. The test is used to monitor active viral infection. The kit consists of amplification mix along with Primers and probes (PPM) for JCV, Internal Control, Standards (JCQS1-JCQS4), and Internal Control (Huwel IC-B mix). The Huwel IC-B mix is a second amplification system used to identify possible PCR inhibition without affecting the analytical sensitivity of the assay.

Background Information

JCV is the etiologic agent of progressive multifocal leuko-encephalopathy (PML) which is mainly seen in HIV patients, organ transplant patients and other immune-deficient syndromes. In addition to PML, JCV also causes nephropathy in the renal transplant setting, although with considerably less frequency than BKV. JCV should always be considered in an immune-compromised patient with progressively deteriorating central nervous system (CNS) function.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-JCV-25)	50 rxns (QT-JCV-50)	100 rxns (QT-JCV-100)
Amber	Huwel JCV Ready Mix	JCV, internal control probes, primers with Amplification Mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Natural	Huwel IC-B Mix	Internal Control	1 x 300 µL	1 x 300 µL	2 x 300 µL
Pink	Huwel JCQS1	2 X 10^4 copies/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel JCQS2	2 X 10^3 copies /µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel JCQS3	2 X 10^2 copies /µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel JCQS4	2 X 10^1 copies /µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	Huwel PW	Purified water	1 x 500 µL	1x 500 µL	2 x 500 µL

Note: Please pay attention to the cap color coding and the tube contents.
Huwel PW (Molecular biology grade purified water)

Storage and Transportation Conditions

The kit should be transported at temperatures below −20 °C. The kit is stable until the expiry date mentioned on the package, if the storage temperature is within −20 ±5 °C. More than 4X freezing and thawing cycles reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

Target Sequence	VP1 gene
Specificity	100%
Sensitivity	0.04 copies/µL or 10 copies/mL
Linear range	0.4 copies/µL to 2 x 10^8 copies/µL (100 copies/mL to 5 x 10^{10}copies/mL)
Validated Specimen	Plasma (K2EDTA-Blood), CSF, urine
Reporting Units	1 copy/µL = 5.4 IU/µL

Assay Procedure

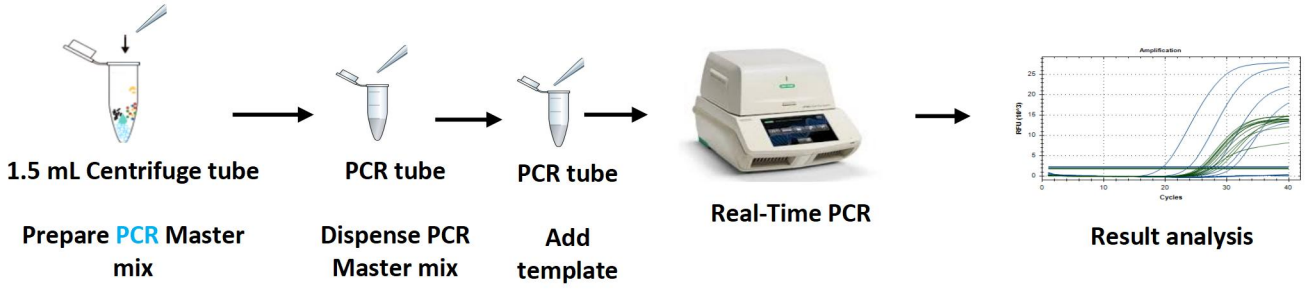
DNA Extraction

Quantiplus® JC Virus Quantitation Kit (Real-Time Quantitative PCR Kit) has been validated using the following Viral DNA extraction kits: Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	QIAamp® DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other Viral DNA extraction Kits.
IC-B mix can be added at the extraction step or while setting up the PCR
The sample volume for CSF is 100-500 µL based on the availability of clinical specimen and the final extraction volume is 100 µL..

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26µL)
Huwel JCV Ready Mix	15.0
Huwel IC-B Mix	1.0
Extracted DNA/ Huwel JCQS1- Huwel JCQS4 / Huwel PW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	15 min.
2 (PCR cycling)	40	95	15 sec.
		60*	1 min.
***Plate read/Data acquisition in FAM and Yakima Yellow/ VIC/HEX channels**			

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below and also the slope of the standard curve (standards in FAM channel) is between –3.1 to –3.6, (at least three standards should be included,) and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of unknown samples as mentioned in Table 2

Table 1:

Control	FAM (JCV)	Yakima Yellow/VIC/HEX (Internal Control)
If Internal Control (IC-B Mix) is added during extraction		
Standards (JCQS1 to JCQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Standards (JCQS1 to JCQS4)	√	√
Negative Control (NC)	-	√

Table 2:

S.No	FAM (JCV)	Yakima Yellow/VIC/HEX (IC)	Fluorophore → / Interpretation ↓	Conclusion
1	√	√	JCV DNA detected within quantitation range	Proceed for further Analysis
2	√	-		
3	-	√	JCV DNA below quantitation limit	
4	-	-	Possible inhibition of PCR	Dilute the DNA sample (1:100) and repeat the Assay

Note: All the Target channels (FAM and Yakima Yellow/ VIC/HEX) to be analyzed individually.

To convert the results of copies/µL obtained using the standard curve to copies/mL of the sample use the formula mentioned below.

$$\text{Result (copies/mL)} = \frac{\text{Result (copies/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

*** For calculating the result of diluted sample (1:100); multiply the observed copies/mL value by dilution factor, 100**

$$\text{Result of 1:100 diluted sample (copies/mL)} = \text{Dilution Factor x (100)} \frac{\text{Result (copies/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Reporting comments

Results in copies/mL	Comments
Target not detected	JCV DNA not detected in the given sample
<100	JCV DNA detected but below the lower limit of the linear range of the assay. The reproducibility of the positive result is not assured
100 to $5X10^{10}$	JCV DNA detected within the linear range of the assay
≥5 $X10^{10}$	JCV DNA detected but above linear range of the assay, dilute the sample and repeat the assay for accurate result.

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96

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