NEW! Molecular diagnostic tests to help transplantation and immunosuppressed patients

genesig® Real-Time PCR BKV Kit (CE), and genesig® Real-Time PCR EBV Kit (CE)

The new molecular kits have been developed to provide quantitative detection of viral DNA extracted from blood plasma and urine (BKV kit), or blood plasma and whole blood (EBV kit) from immunocompromised patients. The kits have been designed to run using the Roche LightCycler® 480 II PCR instrument. Quantification Standards in the kits are calibrated against the First World Health Organization International Standards for Virus Nucleic Acid Amplification Techniques (NIBSC code 14/212), and demonstrate the kits have strong levels of sensitivity and specificity performance as well as reproducibility.

> **The genesig Real-Time PCR BKV Kit (CE), and genesig Real-Time PCR EBV Kit (CE) allow monitoring of viral load alongside the patients medication regimes to help increase the likelihood of successful transplantation.**

Description of genesig Real-Time PCR BKV Kit (CE)

The genesig Real-Time PCR BKV Kit (CE) is intended to be used on the Roche LightCycler® 480 II PCR platform to achieve quantitative detection of BKV DNA extracted from blood plasma and urine from immunocompromised patients in association with the designated extraction instrument and kits:

- Automated extraction - Roche MagNA Pure 24 and Roche MagNA Pure 24 Total NA Isolation Kit (for plasma and urine samples)
- Manual extraction - QIAGEN QIAamp® DNA Mini Kit (for plasma samples), or QIAGEN QIAamp® Viral RNA Mini Kit (for urine samples)

Description of genesig Real-Time PCR EBV Kit (CE)

The genesig Real-Time PCR EBV Kit (CE) is intended to be used on the Roche LightCycler® 480 II PCR platform to achieve quantitative detection of EBV DNA extracted from whole blood and blood plasma from immunocompromised patients in association with the designated extraction instrument and kits:

- Automated extraction - Roche MagNA Pure 24 and Roche MagNA Pure 24 Total NA Isolation Kit
- Manual extraction - QIAGEN QIAamp® DNA Mini Kit

Kit controls and standards

The kits include an Internal Control to identify possible PCR inhibition, to measure extraction purity and to confirm the integrity of PCR run. Quantification Standards (QS) are included in the kits which are calibrated against the First World Health Organization International Standards for Virus Nucleic Acid Amplification Techniques (NIBSC code 14/212). The Quantitation Standards demonstrate that the kits have strong levels of sensitivity and specificity performance, as well as allowing harmonisation of results across labs. The Quantification Standards can be used individually as positive controls, or together to generate a standard curve which can be used to determine the concentration of specific viral DNA in a sample.

Background to BKV and EBV

BK virus (BKV) is a polyomavirus and Epstein-Barr Virus (EBV) is a gamma herpesvirus, both viruses can cause disease in humans. BKV primary infection usually occurs in childhood leading to symptoms similar to a common cold followed by lifelong latent infection. BKV causes disease during transplantation, including BKV-associated nephropathy (BKVN) in kidney transplantation which is an increasing problem and leads to renal injury and graft loss. BKV also leads to hemorrhagic cystitis in hematopoietic cell transplantation (HCT). EBV primary infection usually occurs in childhood leading to asymptomatic viremia followed by lifelong latent infection, while later infection can lead to mononucleosis. EBV has oncogenic potential and is associated with a range of cancers, including Post-Transplant Lymphoproliferative disorder (PTLD) in post-transplant recipients.

> **To prevent the post-transplantation diseases caused by both viruses there is increasing monitoring for BKV and EBV DNAemia after transplantation and in patients undergoing immunosuppression.**

Performance data

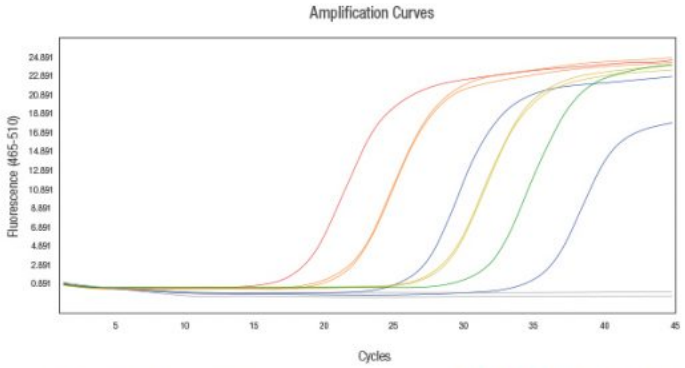
The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed across 3 clinical specimens over 3 days producing at least 30 replicates for each sample type and extraction system combination. **genesig Real-Time PCR BKV Kit (CE) analytical sensitivity:**

- Plasma and urine samples extracted with the MagNA Pure 24 Total NA Isolation Kit was found to be 70 IU/ml
- Plasma samples extracted with the QIAamp DNA Mini Kit was found to be 80.63 IU/ml
- Urine samples extracted with the QIAamp Viral RNA Mini Kit was found to be 161.25 IU/ml

genesig Real-Time PCR EBV Kit (CE) analytical sensitivity:

- Plasma samples extracted with the MagNA Pure 24 Total NA Isolation Kit was found to be 337.5 IU/ml
- Blood samples extracted with the MagNA Pure 24 Total NA Isolation Kit was found to be 168.8 IU/ml
- Plasma samples extracted with the QIAamp DNA Mini Kit was found to be 213.13 IU/ml
- Blood samples extracted with the QIAamp DNA Mini Kit was found to be 426.25 IU/ml

genesig® Real-Time PCR BKV Kit (CE) allows for reliable quantitation of BKV viral load



Blue = BKV positive samples, Grey = BKV negative samples, Red = QS1, Orange = QS2, Yellow = QS3, Green = QS4

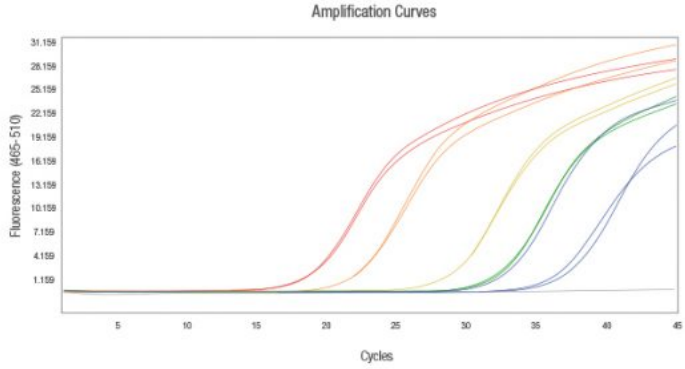
Detection of real clinical BKV plasma samples using the genesig Real-Time PCR BKV Kit (CE) on the Roche LightCycler® 480 II. DNA from plasma samples was extracted using the QIAamp DNA Mini Kit.

The Quantification Standards (QS) in the kit allow the viral load of each BKV sample to be calculated in International Units or copy number.

The kit demonstrates strong amplification even in BKV samples with a low viral load, amplifying after 30 cycles. The analytical sensitivity of this kit is 80.63 IU/mL in plasma samples.

The kit also demonstrates excellent accuracy when tested with negative BKV samples. No BKV was detected in the negative samples in this data.

genesig® Real-Time PCR EBV Kit (CE) allows reliable quantitation of EBV viral load



Blue = EBV positive samples, Grey = EBV negative samples, Red = QS1, Orange = QS2, Yellow = QS3, Green = QS4

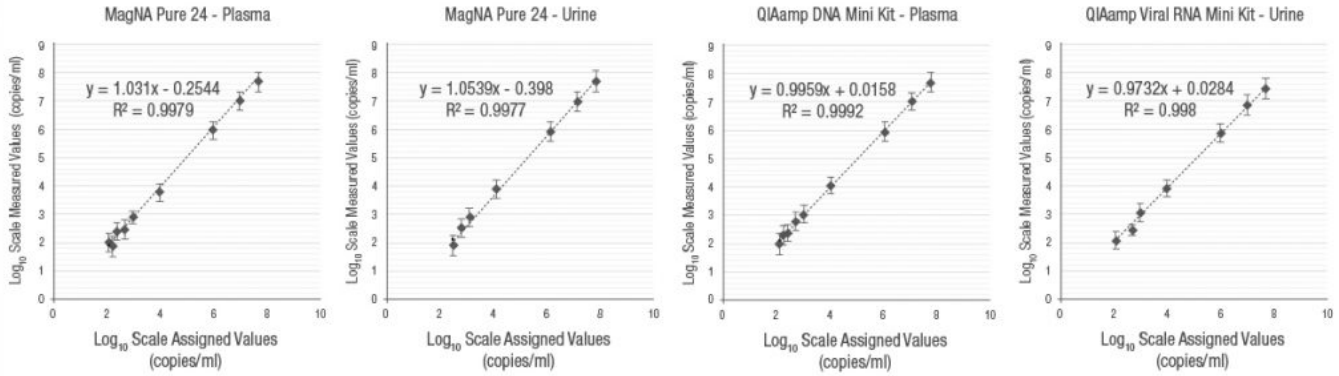
Detection of real clinical EBV whole blood samples using the genesig Real-Time PCR EBV Kit (CE) on the Roche LightCycler® 480 II. DNA from whole blood samples was extracted using the QIAamp DNA Mini Kit.

The Quantification Standards (QS) in the kit allow the viral load of each EBV sample to be calculated in International Units or copy number.

The kit demonstrates strong amplification even in EBV whole blood samples with low viral load, amplifying after 30 cycles. The analytical sensitivity of this kit is 426.25 IU/mL in whole blood samples.

The kit also demonstrates excellent accuracy when tested with negative EBV samples. No EBV was detected in the negative samples in this data.

genesig® Real-Time PCR BKV Kit (CE) linear range plots

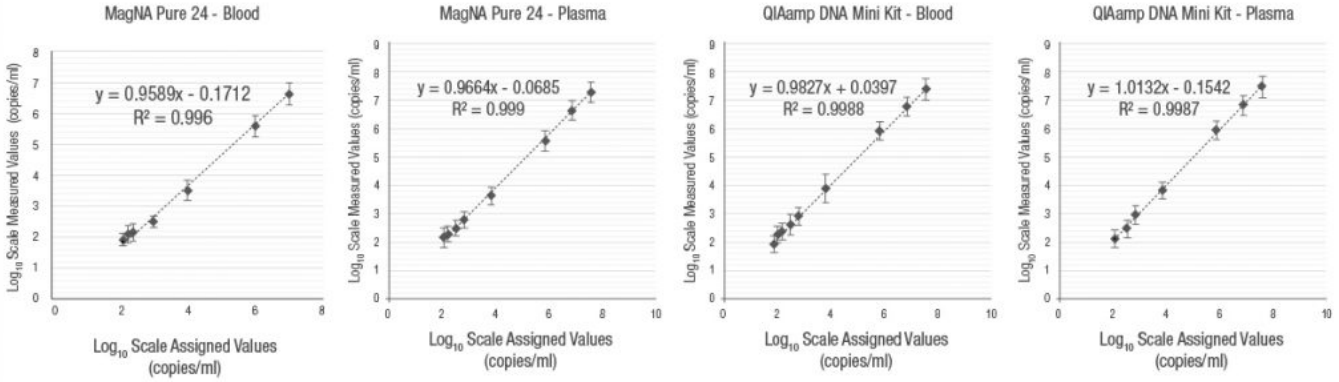


The linear range of the genesig Real-Time PCR BKV Kit (CE) was determined with samples contrived with Primerdesign positive control template, to represent a dilution series ranging from 1.25 x 10^2 to 5.00 x 10^7 copies/ml. This testing was conducted on the Roche LightCycler® 480 II for both plasma and urine sample types, with separate analyses conducted for each of the recommended extraction systems and kits: Automated extraction - Roche MagNA Pure 24; Manual extraction - QIAGEN QIAamp® DNA Mini Kit (for plasma samples), or QIAGEN QIAamp® Viral RNA Mini Kit (for urine samples).

The qPCR results were plotted on a log scale scatter plot, with the expected and measured analyte values plotted on the x and y axes respectively. Error bars show the log-transformed relative error.

The presentation of a linear trend line with an R2 value > 0.98 was deemed reflective of an assay performing within its linear range.

genesig® Real-Time PCR EBV Kit (CE) linear range plots



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PRODUCT DESCRIPTION **KIT SIZES** **CATALOGUE NO.** genesig Real-Time PCR BKV Kit (CE) 100 reactions Z-Path-BKV-CE genesig Real-Time PCR EBV Kit (CE) 100 reactions Z-Path-EBV-CE Kit includes: oasig® Lyophilised qPCR Master Mix, Primer & Probe Mix, 4 Quantitation Standards, Internal Extraction Control, No Template Control, Resuspension Buffers *The genesig Real-Time PCR BKV Kit (CE), and genesig Real-Time PCR EBV Kit (CE), are CE-IVD marked and intended for in vitro diagnostic use in Europe.*