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PCR KIT FOR SIMULTANEOUS QUANTITATIVE DETECTION OF BKV, JCV AND ADV A-F IN IMMUNOCOMPROMISED CHILDREN

DIAGNOSTICS AND BIOMARKERS

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Background: Polyomavirus and adenovirus infection (PVI, AdVI) are one of known causes of posttransplant complications after kidney and hematopoietic stem cells (HST) transplantation under conditions of immunosuppressive therapy. The abstract presents the results of development of the PCR-kit that provides simultaneous detection of BKV, JCV and AdV A-F.
Methods: We used hot-start DNA-polymerase, 10x buffer, $MgCl_2$, dNTP mix (Syntol). For the development of control samples were used pET20b, pJET vectors and MS2 bacteriophage genomic RNA template. The PCR fragments were cloned using BamHI, HindIII restrictases and T4 DNA ligase (Thermo Scientific). This kit was tested on 181 samples from 69 kidney-recipients, 367 samples from 32 HSC received children.
Results: The developed kit is using for real-time hybridization-fluorescence detection of amplified products. It includes buffer, BKV+JCV and AdV+internal PCR control primers and probes mixes, 2 pairs of calibrators (10^5 and 10^7 copies/ml), internal PCR control, negative extraction control and DNA-polymerase. The target for BKV and JCV DNA detection was 228 bp of large T-antigene gene, for AdV DNA – 94 bp of hexon gene. Our tests showed that the kit has 10^3 copies/ml sensitivity for the identification of BKV, JCV and AdV DNA. Specifity test was carried out with clinical samples positive for CMV, EBV, HHV-6, HHV-7, PV B19 DNA, astrovirus, norovirus, enterovirus RNA. No one positive result was obtained. In the group of kidney-recipients viruses were detected in 36%, among these, 78.7% was present JCV, 19% - BKV, 2.3% –AdV. Among HSC-recipients BKV detection frequency was 53.1%, AdV – 50%.
Conclusions/Learning Points: The developed kit for simultaneous quantitative detection of BKV, JCV and AdV A-F showed good results in fast diagnosis and prediction of viral complications development in pediatric recipients of kidney and hematopoietic stem cells.