Supplemental Table 2. Primers, probes and qPCR conditions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Virus | Oligonucleotide name | Sequence (5’ – 3’) | GenBank accession numbers | Annealing positions | Amplicon size (bp) |
|  |  |  |  |  |  |
| HSV-1 | HSV-1 FWDLP1 | GTTGAGCTAGCCAGCGA | X14112.1 | 93560-93683 | 124 |
|  | HSV-1 REVLP1 | GTTAAGGACCTTGGTGAGC |  |  |  |
|  | HSV-1 probeLP1 | FAM-CGCGAACTGACGAGCTTTGTG-BHQ1 |  |  |  |
| HSV-2 | HSV-2 FWD-2-2 | CACACCACACGACAACAA | Z86099.2 | 46783-46872 | 90 |
|  | HSV-2 REVLP1 | TAGTTCAAACACGGAAGCC |  |  |  |
|  | HSV-2 probeLP1 | JOE-CGGCGATGACGGCAATAAA-BHQ1 |  |  |  |
| VZV | VZV FWDLP1 | GCGCAAGGCTATTAGAGC | KU529566.1 | 48283-48145 | 139 |
|  | VZV REVLP1 | ACATGGCAGAAATCCCTG |  |  |  |
|  | VZV probeLP1 | TxRd-CGCATACCCGGAAGTTCTTCAGAT-BHQ2 |  |  |  |
| HHV-6A | HHV6A FWD1-3 | CGGCCTCCAGAGTTGTAA | KP257584.1 | 133969-133894 | 76 |
|  | HHV6A REV 10 | TGTCCCTTCAACTACTGAATC |  |  |  |
|  | HHV6A LNA Probe A1 | FAM-C[+A]T[+G]TTGC[+T]A[+G]AAA[+G][+A]CT-BHQ1 |  |  |  |
|  | HHV6A LNA Probe A2 | FAM-AC[+A]T[+G]TTGC[+T]A[+C]AAA[+G][+A]CT-BHQ1 |  |  |  |
| HHV-6B | H6B FOTY1 | TTTGACAGGAGTTGCTGAG | AB021506.1 | 136176-136258 | 83 |
|  | H6B ROTY 1 | GGATTCAGGAAAAAGGTTCTAA |  |  |  |
|  | H6B PROBE MVP | JOE-AGGAAGCGTTTCGGTACACTTGGAG-BHQ1 |  |  |  |
| HHV-7 | HHV7 1. FWD | CTCGCAGATTGCTTGTTG | AF037218.1 | 88332-88490 | 159 |
|  | HHV7 1. REV | GCATACACCAACCCTACTGTAA |  |  |  |
|  | H7 MOP PROBE | TxRd-TTAGGCATCACGTTGGCATTG-BHQ2 |  |  |  |
| EBV | EBV FWD | CGGAAGCCCTCTGGACTTC | KF717093.1 | 153036-152947 | 90 |
|  | EBV REV | CCCTGTTTATCCGATGGAATG |  |  |  |
|  | EBV Probe | FAM-TGTACACGCACGAGAAATGCGCC-BHQ1 |  |  |  |
| CMV | H5 FWD211 | GTGYTCCGTGAATCGTTAC | AB329634.1 | 80396-80329 | 68 |
|  | H5 rev 211 | AGTCKACCTCGATATCACAAGTCG |  |  |  |
|  | H5 Probe 20 | TxRd-ACCCTGCTGCCGCCAGT-BHQ2 |  |  |  |
| HHV-8 | HHV8 fwd 3.1 | ATATACGGCGACACTGACTC | AP017458.1 | 13603-13761 | 159 |
|  | HHV8 REV 10 | GAGCAGAAGGCACTTGAAG |  |  |  |
|  | H8 Probe 300 | JOE-CGGAGGAGCTAGCGTCAATCA-BHQ1 |  |  |  |
| BuV | BuV Forward | ACAGTGTAGACAGTGGATTCAAACTT | JX02729 | 705-830 | 126 |
|  | BuV Reverse | GTTGTGGTTGGATTGTGGTTAGTTC | JX02729 |  |  |
|  | BuV NS1 probe | FAM-CGGAAGAGATTTTGACAGTGCYTAGCAA-BHQ1 | JX02729 |  |  |
| TuV | TuV Forward | CCAGAAAGCCGTATCACCAT | KJ495710 | 3085-3202 | 118 |
|  | TuV Reverse | AACCAAGTGTTTCTGATCTTATTGCT | KJ495710 |  |  |
|  | TuV VP2 probe | TxRd-ACACCAACAATCAACTGCCATACACACC-BHQ2 | KJ495710 |  |  |
| CuV | CuV Forward | TAACACATCCCAGAATYGTCACATA | KT868811 | 4245-4335 | 91 |
|  | CuV Reverse | TTCCATTGTCTTGGAGTGCG | KT868811 |  |  |
|  | CuV VP2 probe | JOE-AGTTKTCCTGACCACCAGAAGGTTCCA-BHQ1 | KT868811 |  |  |
| HBoV1 | HBoV1Forward | CCTATATAAGCTGCTGCACTTCCTG | NC\_007455 | 152-259 | 108 |
|  | HBoV1Reverse | AAGCCATAGTAGACTCACCACAAG | [NC\_007455](https://www.ncbi.nlm.nih.gov/nuccore/NC_007455) |  |  |
| HBoV2-4 | HBoV234Forward | GCACTTCCGCATYTCGTCAG | [FJ170279](https://www.ncbi.nlm.nih.gov/nuccore/FJ170279) | 50-230 |  |
|  | HBoV3Reverse | GTGGATTGAAAGCCATAATTTGA | ([EU918736](https://www.ncbi.nlm.nih.gov/nuccore/EU918736) |  | 102 |
|  | HBoV24Reverse | AGCAGAAAAGGCCATAGTGTCA | [FJ170279](https://www.ncbi.nlm.nih.gov/nuccore/FJ170279) |  | 101 |
|  | HBoV NS1 probe | FAM-CCAGAGATGTTCACTCGCCG-BHQ1 | [FJ170279](https://www.ncbi.nlm.nih.gov/nuccore/FJ170279) |  |  |
| B19 | B19 Forward | CCACTATGAAAACTGGGCAATA3\_ | KM065414 | 1469-1622 | 154 |
|  | B19 Reverse | G CTGCTTTCACTGAGTTCTTCA3 | KM065414 |  |  |
|  | B19 NS1 probe | FAM-AATGCAGATGCCCTCCACCCAG-BHQ1 | KM065414 |  |  |

For Luminex

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| BKPyV | BKPyV Forward/Reverse | ACAGAGGTTATTGGAATAACTAG/ACTCCCCTGCATTTCCAAGGG | DQ305492 | 1952-2094 | 143 |
|  | Probe | CTTAACCTTCATGCATTGG |  |  |  |
| JCPyV | JCPyV Forward/Reverse | AATGAGGATCTAACCTGTGGAA/CTGCACCATTGTCATGAGTTGCTTG | J02226 | 1742-1868 | 127 |
|  | Probe | ATGAATGTGCACTCTAATGG |  |  |  |
| KIPyV | KIPyV Forward/Reverse | TTGGATGAAAATGGCATTGG/TAACCCCTTCTTTGTCTAAAATGTAGCC | EF127906 | 2263-2404 | 142 |
|  | Probe | CTTGGAACAGCTAATAGTAGGGTC |  |  |  |
| WUPyV | WUPyV Forward/Reverse | TTGGATGAAATGGCATTGG/TAACCCTTCTTTGTCTAAAATGTAGCC | EF444554 | 2411-2552 | 142 |
|  | Probe | GAGTACATACAGGGCTTTCCAG |  |  |  |
| MCPyV | MCPyV Forward/Reverse | TTCCATCTTTATCTAATTTTGCTT/GGCCTAGTTTTAGATTACCAGAC | EU375803 | 3757-3900 | 144 |
|  | Probe | AGTAATAGGCCCACCATTTGT |  |  |  |
| HPyV6 | HPyV6 Forward/Reverse | TTGCTTCTGGATCCAATACTGC/GGCCTCAGGAATTTCAGGCAA | HM011558 | 1426-1556 | 131 |
|  | Probe | TGGATGCTGGTTCATCTCTG |  |  |  |
| HPyV7 | HPyV7 Forward/Reverse | AAGCAGCTACAACTGGGAACTT/GGCCTCAGGAATTTCAGGCAA | HM011566 | 1450-1574 | 125 |
|  | Probe | GCCTACCTTATCCTATGAGTG |  |  |  |
| TSPyV | TSPyV Forward/Reverse | AGAATGTATGATGACAAAGGTAT/TCTGTAGTTTCCAGTTAGAAAC | GU989205 | 1722-1832 | 111 |
|  | Probe | TGAGGGAATGAATTTCCATATGTT |  |  |  |
| HPyV9 | HPyV9 Forward/Reverse | ATCTATGGCTCATCCTCAGG/GTAGAGCTAGCAACTAGGCCT | KC831440 | 1862-1968 | 107 |
|  | Probe | AGTGCAGGGTACCACTCTC |  |  |  |
| HPyV10 | HPyV10 Forward/Reverse | GTCCAGTTCCTACTAAAGTTCCT/TACATCATTGCCCATCCTTGGTT | JQ89892 | 1501-1628 | 106 |
|  | Probe | GCCGGACACCACAATGACA |  |  |  |
| STPyV | STPyV Forward/Reverse | TGAATATGATCCGTGCCAA/ACTGCATCAGGGCCTACTTG | JX463184 | 1318-1446 | 129 |
|  | Probe | CCTCCTCCAACATGTGTTCC |  |  |  |
| HPyV12 | HPyV12 Forward/Reverse | GTAATGGCACCCAAGAGGAA/GGGGATTTAGAAAGGCCTCA | JX308829 | 1402-1558 | 157 |
|  | Probe | CCCAGCAGTGTCCCTAAATT |  |  |  |
| NJPyV | NJPyV Forward/Reverse | TGTGTGCCAAAGAAGTGTCCT/TCTGTCACCTGTTGGAGCATT | KF954417 | 1113-1271 | 159 |
|  | Probe | CTGATGCTACTACTGAAATTGAA |  |  |  |

All qPCRs were done in a volume varying between 20 to 25 μl depending on the virus assays used [1-5] and were performed with AriaMx Realtime PCR System (Agilent Technologies, Santa Clara, CA). The reactions consisted of Maxima probe qPCR Master Mix (Thermo Scientific, Walthan, MA) with or without ROX as passive reference dye or 2 x Taqpath Proamp Multiplex Master Mix (Fischer Thermo Scientific, Walthan, MA). Besides the mastermix and each primer pairs and probe, the reactions consisted of 2.5-5 µl template, and molecular biology-grade H2O to a final volume of 20-25 µL. After initial denaturation and enzyme activation at 95° C for 10 min, the amplification consisted of 40-45 cycles of 90-95°C and 1 min at 60-62°C, depending on each assay. Each run included plasmid and no-template controls. Strict laboratory procedures were followed to prevent PCR contamination, including separate spaces for handling of samples, Master Mix ingredients, and plasmid templates. The multiplex PCR assay with Luminex-based detection system for polyomaviruses was performed with 2720 Thermal Cycler (Applied Biosystems, Waltham, MA) [6, 7] . The reaction consisted of 2 x Qiagen multiplex PCR Master Mix, forward and reverse primer and 5 µl template and molecular biology-grade H2O to a final volume of 25 µl. The microbeads were obtained from Luminex Corp (Hertogenbosch, The Netherlands), and coupling was done according to manufacturer´s instructions.

References:

1. Toppinen M, Norja P, Aaltonen LM, Wessberg S, Hedman L, Söderlund-Venermo M, Hedman K. A new quantitative PCR for human parvovirus B19 genotypes. J Virol Methods. 2015; doi:S0166-0934(15)00082-8.

2. Pyöriä L, Jokinen M, Toppinen M, Salminen H, Vuorinen T, Hukkanen V, Schmotz C, Elbasani E, Ojala PM, Hedman K, Välimaa H, Perdomo MF. HERQ-9 Is a New Multiplex PCR for Differentiation and Quantification of All Nine Human Herpesviruses. mSphere. 2020; doi:10.1128/mSphere.00265-20.

3. Kantola K, Sadeghi M, Antikainen J, Kirveskari J, Delwart E, Hedman K, Söderlund-Venermo M. Real-time quantitative PCR detection of four human bocaviruses. J Clin Microbiol. 2010; doi:10.1128/JCM.00686-10.

4. Väisänen E, Fu Y, Koskenmies S, Fyhrquist N, Wang Y, Keinonen A, Mäkisalo H, Väkevä L, Pitkänen S, Ranki A, Hedman K, Söderlund-Venermo M. Cutavirus DNA in Malignant and Nonmalignant Skin of Cutaneous T-Cell Lymphoma and Organ Transplant Patients but Not of Healthy Adults. Clin Infect Dis. 2019; doi:10.1093/cid/ciy806.

5. Väisänen E, Lahtinen A, Eis-Hübinger AM, Lappalainen M, Hedman K, Söderlund-Venermo M. A two-step real-time PCR assay for quantitation and genotyping of human parvovirus 4. J Virol Methods. 2014; doi:S0166-0934(13)00415-1.

6. Wang Y, Strassl R, Helanterä I, Aberle SW, Bond G, Hedman K, Weseslindtner L. Multiplex analysis of Human Polyomavirus diversity in kidney transplant recipients with BK virus replication. J Clin Virol. 2019; doi:S1386-6532(19)30195-7.

7. Sadeghi M, Wang Y, Ramqvist T, Aaltonen LM, Pyöriä L, Toppinen M, Söderlund-Venermo M, Hedman K. Multiplex detection in tonsillar tissue of all known human polyomaviruses. BMC Infect Dis. 2017; doi:10.1186/s12879-017-2479-5.