

Divergent GemyCircularvirus in HIV-Positive Blood, France

Technical Appendix

Plasma sample preparation and NGS analysis

A 4-ml aliquot of the plasma sample was clarified by centrifugation, filtered (0.22 µm), and viral particles were concentrated by adding sterile polyethylene glycol (MW 8,000, Invitrogen, Cergy Pontoise, France) to a 4% final concentration before centrifugation (10,000 × g for 1 h at 4°C). The pellet was suspended in 600 µl of sterile water, treated with 550 U of Benzonase (Sigma, Saint-Quentin Fallavier, France), 300 U of DNase and 1.2 mg of RNase (both from Roche Diagnostics, Meylan, France), and incubated at 37°C for 45 min. Particle-protected nucleic acids were recovered using the High pure viral nucleic acids kit (Roche) and subsequently used for the preparation of a NGS library according to manufacturer's instructions (Nextera XT DNA sample preparation kit; Illumina, Paris, France); the final analysis was performed on a MiSeq sequencer (Illumina).

GemyC1c DNA specific detection and controls

Nucleic acids were tested for GemyC1c DNA by using a nested-PCR assay targeting the CP gene with primers C1cS1 (5'-GTTCCGATTTCCACCGATTTCCA-3') / C1cR1 (5'-TGCAACTCAACAGTTTCTTGGAG-3') for PCR-1 (206 bp), and primers C1cS2 (5'-GATTTCCAACAACAGAATCCTGG-3') / C1cR2 (5'-CTTGGAGGCCTTTGACGAAACAC-3') for PCR-2 (175 bp). Amplification conditions for the two consecutive PCR assays were 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 1 min. Dilutions of a cloned partial sequence of GemyC1c permitted to estimate the sensitivity of the amplification assay to be <5 copies of target sequence by limiting-dilution assay. Negative (sterile water) and positive controls (cloned GemyC1c partial sequence) were used systematically. The quality of

the nucleic acids extraction procedure was also checked by a control PCR targeting members of the family *Anelloviridae*, a highly prevalent group of DNA viruses in humans (1).

GemyC1c sequence (2,109 nt, GenBank accession no KP987887)

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1 taatattacc tctggacctt ggacccggac ccaggccggg tataaatagg tcccttcccc
61 cgcggagatg ttttttttgg ttgaggggta tgtcgcattt tacgtcatgg cttacgcaca
121 ccgtcgcctt cggaggcgca ggttcgcccc ccgccggcca acattcgccc gtcgccgtgt
181 cccccgtcgc tcttatggtc gccgtcgtcg gtcacccggt cgttccgttc ggtccattcg
241 gaacatagcg gctcgcgaagt gtcgcgacaa catgcttggc gttccgattt ccaccgattt
301 ccaacaacag aatcctggcc ctctcaccat gtttggatg accagccatg gatatttggt
361 ttctcccact gtcgctcgcg tcggttctct tgatgggtca ttacctcgcg gtattcacgc
421 caattccgct gagcgttggg agaccgggtg tttcgtcaaa ggctccaag aaactggtga
481 gttgcagcct aataatggag caggctggct gtggagacgc gtcgtatttt cctgcattgg
541 cctattggag gaattcccta ccaatagtg ggctgcgcc gactccactc gtgggtatgg
601 tcgcccatt tggaacatgc gggatggtag cgctgctgcc acccccctt acaacgaaat
661 ggccaattat ctttctgagg gtactcgatt gcgtgattgg atcaatcctt ttactgcgaa
721 gttggatcgc agtattgtta cggtacattc ggacacgaca cgtaccattc agagtaacaa
781 cccgaacggt acctacaaga ttttcaagcg gtattacccc gtgaacaggg gtattgtcta
841 tgcggatgac gagtctggaa gtgggtcgaa gaatgacgcc cactacgctg ctggtacgac
901 caaaggggat tcgggcgatc tgtttgtttt ggacctctt aatgctatca atgatagttc
961 ggagaatact atgaatttcg gagttcacgc caggatattac tggcatgagg gctctggaga
1021 gtaaagcctc tcccctatat aaactataat acaatttttt tccaaccaat catggtcgac
1081 gtcctcgtc attgggtcat tgttggatag ccaaatgcat ggtttacccc atgcatggtg
1141 cttcttcttc ctgtacctgt cagtacagta gaactctgtc tgttgaccca accagctctt
1201 gtatgctggg aagaatttga aacctcctg gatgtcgtcg aatactgcat acgaacagtc
1261 tggatcctgt tcatactctt ccaaagagaa taatccacca aagtaggcat gggtgccaaa
1321 tgacctcgcc aacattgtct taccagtctt tgaaggacc cacagataca agctcattcc
1381 acgcacctct gtcactcaga ggtttagccca attaggggta gggttagggt tagggtagg
1441 gttaggggta gggttagggt tacccttagg gttagcggag tacttacc aa tctgattgcc
1501 ggatagatgt tccctaacc attcccgaat ctcaccat at tgtgtaagat cgaacgagta
1561 ttggctaggg tgacgataag gtgcccgatc cactctaaac ttccaatctg cgtatgcaga
1621 gaatgaagt aatgatttga cgagatggct gaaaagatgc gctgacgcaa gttcaaaaaa
1681 ctctctcga gttgatgcat ctctgagttg gtcccaccca gcattctgtc gttcagcatt
1741 agaaccctgg ctaggttctg gtctgcgtag gctcccgcca catatgtcgc catctttcgt
1801 tgcgtaatcc caactgccac ttggacttcg gattgtagaa atgatatttg gatggaaacc
1861 ctcaacatcg aatttgcgca cgtctcgagt tctgtacttt ctgcgaaaat cgacgaaagc
1921 atgtagatgc gtcccacat cgccgtgcgc ttctctgccg acgatacaat ctgctgagag
1981 agccgagaat aactccacga ctgctgatgg tcggagttgg ccgattgcg agtaggtgaa
2041 taatccatag cgggcttggg atcgaatga tgacataagc tttttcggaa gggctctggtc
2101 cagagtttt
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Location:

CP: 68..1024; Rep1/Rep2: complement 997...2076; Overprinted ORF: complement 1392..2048.

Reference

1. Biagini P, Touinssi M, Galicher V, de Micco P. KIs virus and blood donors, France. *Emerg Infect Dis.* 2012;18:1374–5. [PubMed http://dx.doi.org/10.3201/eid1808.120442](http://dx.doi.org/10.3201/eid1808.120442)