SUPPLEMENTAL DIGITAL CONTENT

This appendix has been provided by the authors to give readers additional information about their work.

**Supplement to:** Angela Chiereghin, Patrizia Barozzi, Evangelia Petrisli et al. Multicenter prospective study for laboratory diagnosis of HHV8 infection in solid organ donors and transplant recipients and evaluation of the clinical impact after transplantation.

**MATERIALS AND METHODS**

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**HHV8 SEROLOGICAL TESTS**

Four internationally distributed indirect immunofluorescence (IFA) and 2 enzyme-linked immunosorbent (ELISA) tests made by 3 different companies ie, Advanced Biotechnologies Incorporated (ABI, Columbia-USA), Biotrin-Diasorin (Dublin, Ireland) and Scimedx Corporation (Denville, New Jersey-USA) were utilized. IFA and ELISA tests made by ABI are for research use only, IFA and ELISA tests made by Biotrin-Diasorin and IFA by Scimedx Corporation have In Vitro Diagnostic-European Conformity marking.

**ABI IFA test:** the HHV8 IgG antibody IFA Kit uses a mixture of HHV8 infected/induced KS-1 cell line derived from a body-cavity-based lymphoma (No longer commercially available).

**Biotrin-Diasorin IFA test:** The Human Herpes Virus 8 IgG IFA Kit is based on human lymphocytes that express lytic viral antigens (commercially available also in Canada).
**Scimedx Corporation IFA tests:** The Human Herpesvirus 8 Lytic and Latent IFA Kits use human lymphocytes expressing lytic and latent HHV8 antigens, respectively.

**ABI ELISA test:** the HHV8 IgG antibody ELISA Kit is made from a whole virus extracted derived from sucrose gradient purified HHV8 virions isolated from the KS-1 cell line derived from a body-cavity-based lymphoma.

**Biotrin-Diasorin EIA test:** the Human Herpes Virus 8 IgG ELISA Kit uses lytic peptide epitopes derived from different viral proteins (No longer commercially available).

All tests were performed following manufacturer’s instruction.

**VALIDATION OF THE HHV8 SEROLOGICAL TESTS**

All results obtained by each IFA assay were evaluated by 2 different investigators. The samples were considered HHV8-positive or HHV8-negative when there was consistency between the 2 evaluations otherwise the samples were considered HHV8-border-line (BL). All twenty samples collected from the healthy adults resulted negative in each serologic test, but not all tests detected anti-HHV8 serum antibodies in the 20 KS cases. Based on this variable performance of the different serological HHV8 tests observed (data not shown), patients were considered HHV8-seropositive when samples resulted positive in at least 2 HHV8-specific assays (IFA and/or ELISA). Patients were HHV8-seronegative when samples resulted negative in all tests. Patients were considered HHV8-not defined when samples resulted positive and/or BL in 1 serologic test.

**HHV8 MOLECULAR TESTS**

**Quantitative real-time PCR assay (HHV8 Q-PCR Alert Kit, ELITech Group, Italy):** Blood samples were collected in ethylenediaminetetraacetic acid-anticoagulated tubes. DNA was extracted from 200 μL of plasma and 100 μL of WB using the NucliSens easyMAG System (bioMerieux, Marcy l’Etoile, France) per the manufacturer’s instructions and eluted in 50 μL and 25 μL, respectively. An aliquot of 5 μL of these extracted DNA samples was used for the quantitative real-time PCR on the ABI
Prism 7300 real-time PCR System (PE Applied Biosystem, Foster City, Calif, United States). The PCR assay targets gene KS330 codifying the HHV8 capsid protein. The analytical sensitivity of the assay is 10 copies of target DNA per amplification reaction. The lower limit of quantification of the assay is 500 copies/mL plasma and WB.

**HHV8 in-house nested PCR assay:** DNA was extracted from 500 µL of plasma using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) per the manufacturer’s instructions and eluted in 40 µL. For nested PCR, 10 µL of these extracted DNA samples was used. The 1st round PCR used primers from ORF26, which generated a 233-bp PCR product. The primer sequences were: sense 5′AGCCGAAAGGATTCCACCAT3′, and antisense 5′TCCGTGTTGTCTACGTCCAG3′. The 2nd round PCR used inners primers, which generated a 172-bp PCR product. An aliquot of 5 µL of products from the 1st round PCR was reamplified in the 2nd round PCR. The primer sequences were: sense 5′GTGCTCGAATCCAACGGATT3′ and antisense 5′ATGACACATTGGTGTATAT3′. The PCR program for both the 1st and 2nd round was 95°C for 10 minutes, followed by 44 cycles of 95°C for 30 seconds, 58°C for 1 minute, 72°C for 1 minute and 30 seconds, followed by a 7-minute extension at 72°C. The analytical sensitivity of the assay is 5 copies of target DNA per amplification reaction.

**Statistical analysis**

In the overall study population, HHV8-seroprevalence was compared to gender and age classes using Fisher’s exact test and to grouped place of birth using chi-square test.

The agreement of each test with our hypothesized reference standard was obtained evaluating Cohen’s kappa on the overall study population of 766 solid organ donors and recipients. Agreement was considered almost perfect when kappa ranged 0.81-1.00 and substantial when kappa ranged 0.61-0.80. Two separate agreement assessments were carried out, with border-line test results assigned to positive and negative test results, alternatively. This was done to estimate the potential loss of accuracy that could occur when assigning a diagnosis to borderline cases.
The relation between the administration of antiviral agent valganciclovir (VGCV) as cytomegalovirus (CMV) prophylaxis and posttransplant HHV8 infection was also assessed by Fisher’s exact test.
REFERENCES:

