1 **Supplemental Digital Content 1. Methodological Details of Molecular Viral Testing**

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3 ***Polymerase Chain Reaction (PCR)***

4 DNA samples were prepared from the paraffin embedded samples by de-paraffinization followed
5 by extraction of the DNA. The PCR assay was performed with primers HPV-L1 (150bp); 5'-
6 TTTGTTACTGTGGTAGATACTAC-3', 3'-CTTATACTAAATGTCAAATAAAAAG-5';
7 EBV-BamH1W (129bp); 5'- CCAGACAGCAGCCAATTGTC-3', 3'-
8 GGTAGAAGACCCCCTCTTAC-5'; KSHV-ORF-73 (293bp); 5'-
9 CCATCTCTTGCATTGCCAC-3', 5'-AACTACGGTTGGCGAAGTCA-3';HPV-6 (280bp); 5'-
10 TAGTGGGCCTATGGCTCGTC-3', 5' TCCATTAGCCTCCACGGGTG-3'; HPV-11(360bp) 5'-
11 GGAATACATGCGCCATGTGG-3', 5'-CGAGCAGACGTCCGTCCTCG-3'; HPV-16 (96bp)
12 5'-GGTCGGTGGACCGGTCGATG-3', 5'-GCAATGGTAGGTGTATCTCCA-3'; HPV-18
13 (115bp) 5'-CCTTGGACGTAAATTTTTGG-3', 5'-CACGCACACGCTTGGCAGGT-3'; HPV-31
14 (110bp) 5'-GGGATTGTTACAAAGCTACC-3', 5'-CGCTTAGTAGACGTCGTCGC-3'; HPV-
15 33 (114bp) 5'-CCACCACTGCTTCTTACCTC-3', 5'-ACCATTTTCATCAAATGGGA-3'; HPV-
16 45 (118bp) 5'-ACCATTTTCATCAAATGGGA-3', 5'TCCCTACGTCTGCGAAGTCTTTC-3';
17 JC (133bp) 5' GAAGAACCCAAAAACTATTTGTTGAAA 3', 5'
18 GCCTAACTGGAGACAATCTAGAATAATAGTC 3'.

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20 The PCR conditions were as follows: For HPV- 94°C for 5 minutes, 94°C for 30 seconds, 48°C
21 for 30 seconds, and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and
22 then incubation at 4°C. For EBV-94°C for 5 minutes, 94°C for 30 seconds, 47°C for 30 seconds,
23 and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and then incubation at

24 4°C. For KSHV- 94°C for 5 minutes, 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30
25 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and then incubation at 4°C. For HPV-6-
26 94°C for 5 minutes, 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds, for 40
27 cycles; elongation at 72°C for 5 minutes; and then incubation at 4°C. For HPV-11- 94°C for 5
28 minutes, 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds, for 40 cycles;
29 elongation at 72°C for 5 minutes; and then incubation at 4°C. For HPV-16- 94°C for 5 minutes,
30 94°C for 30 seconds, 46°C for 30 seconds, and 72°C for 30 seconds, for 40 cycles; elongation at
31 72°C for 5 minutes; and then incubation at 4°C. For HPV-18- 94°C for 5 minutes, 94°C for 30
32 seconds, 47°C for 30 seconds, and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5
33 minutes; and then incubation at 4°C. For HPV-31- 94°C for 5 minutes, 94°C for 30 seconds,
34 48°C for 30 seconds, and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5 minutes;
35 and then incubation at 4°C. For HPV-33- 94°C for 5 minutes, 94°C for 30 seconds, 48°C for 30
36 seconds, and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and then
37 incubation at 4°C. For HPV-45- 94°C for 5 minutes, 94°C for 30 seconds, 48°C for 30 seconds,
38 and 72°C for 30 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and then incubation at
39 4°C. For JC- 94°C for 5 minutes, 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30
40 seconds, for 40 cycles; elongation at 72°C for 5 minutes; and then incubation at 4°C. The PCR
41 products were run on 2.5% agarose gel at 100 V for 1 hour.
42

43 Precautions against PCR contamination was maintained by preparing PCR reagents before each
44 assay in a master mixture that was then aliquoted. The preparation of the master mixture, the
45 extraction of the DNA and the addition of the template to the PCR mixture, and the thermal
46 cycling were performed in three different areas. General precautions against contamination,

47 including systematic use of aerosol-barrier-protected pipette tips, frequent changes of gloves, and
48 constant decontamination of surfaces with sodium hypochlorite, were strictly adhered to. The
49 sample extracts, along with a positive control and a water specimen as an additional negative
50 control to rule out contamination of the reagents or by aerosols, were then subjected to PCR as
51 described above.
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53 ***Immunohistochemistry (IHC)***

54 Immunohistochemistry for the presence of HPV, EBV, and KSHV in tissues was performed on 5
55 µm thick paraffin- embedded sections to detect virus specific antigens. We used commercial
56 antibodies HPV 16-L1 and HPV 18-E6 (DAKO Inc., Carpentaria, CA), and monoclonal antibody
57 S12 for EBV-LMP1, and monoclonal antibody derived from KSHV encoded LANA. We first
58 deparaffinized the slides 3x in xylene. The slides were serially dehydrated for 1 minute each in
59 100% ethanol, 90%, 80%, 70%, and 60%, and water. The slides were washed 1x in 1X PBS.
60 Slides were treated with 3% PFA for 30 minutes, then washed with 1X PBS. This was followed
61 by treatment with pepsin at 37oC for 10 minutes (2.5ul pepsin in 2.5ml 0.01N HCL at 37oC).
62 Slides were washed in 1X PBS and then blocked with 3% BSA for 30 minutes at room
63 temperature. The slides were incubated with primary antibody in 1:50 dilution in 1X PBS, or
64 undiluted ascites fluid and incubated for 1hour at RT. Slides were washed in 1X PBS, covered
65 with LINK (yellow), (DAKO LSAB + System HRP Ref K0679) for 30 minutes, washed with
66 1X PBS and covered by streptavidin peroxidase (RED), (DAKO LSAB + System HRP Ref
67 K0679) for 30 minutes, washed again in 1X PBS and finally treated with chromogen solution (1
68 drop of DAB chromogen to 1ml DAB substrate buffer), (DAKO LSAB + System HRP Ref

69 K0679) and allowed to develop at room temperature for 1-5 minutes. The slides were washed in
70 1X PBS and mounted using 30% glycerol.