**Supplementary Material**

**HPV DNA Detection** [1, 2]

*Swab specimens*

Each of the endo-ecto cervical swabs and labial/vulvar/perineal/perianal swabs was placed in 1 ml of Digene specimen transport medium (STM). Each specimen was divided into three pristine aliquots and frozen. DNA was extracted from 200 l of each of the swab specimens using the Qiagen QIAamp 96 DNA blood kit in a 96-well format as follows. The purified DNA was eluted in 200 l of Qiagen AE buffer. HPV-negative MRC5 cells (500,000 cells/well) and HPV-positive SiHa cells (2,500 cells/well) were included in the processing of each plate to serve as positive controls for DNA isolation and HPV detection. Negative control wells included in plate processing contain 200 l of STM.

*Cervical lesions (biopsy or surgical excisional specimen)*

The specimens were formalin fixed, paraffin embedded, and cut into 4-m thin sections. Thin sections designated for PCR testing were placed into individual sterile tubes and shipped to Merck for DNA extraction and multiplex HPV PCR testing. DNA was extracted from 200 l of each specimen using the Qiagen Spin blood kit in a 96-well format. For thin sections, an overnight digestion of the paraffin-embedded tissues using Qiagen proteinase K in ATL buffer in a background of 0.1 g/ml Escherichia coli DNA at 56°C was performed in lieu of the proteinase digestion step in the Spin blood kit. DNA isolation from digested thin-section specimens then proceeded according to the manufacturer’s protocol. HPV-negative MRC5 cells (500,000 cells/ well) and HPV-positive SiHa cells (2,500 cells/well) were included in the processing of each plate to serve as positive controls for DNA isolation and HPV detection. Negative-control wells included in plate processing contained 200 l of overnight digest buffer for biopsy thin-section batches. All isolated DNAs were eluted into 200 l of AE buffer.

*HPV Type-specific Multiplex PCR assays*

Real-time, HPV type-specific, multiplex HPV PCR assays were used. Samples were tested in human -globin and individual type-specific and gene-specific real-time multiplex HPV PCR assays for the L1, E6, and E7 open reading frames (ORFs) of HPV6, HPV11, HPV16, HPV18, HPV31, HPV45, HPV52, and HPV58 and the E6 and E7 ORFs of HPV33, HPV35, HPV39, HPV51, HPV56, and HPV59 [3-5]. The assays were performed in a 96-well format with 25-l/well reaction volumes. Each well contained 4 l of sample DNA (regardless of DNA concentration), QuantiTect PCR master mix (Qiagen, Inc.), uracil-DNA glycosylase (0.5 U/reaction; Invitrogen), HPV type- and ORF-specific fluorescent oligonucleotide probes, and the HPV type- and ORF-specific primers. A sample was considered PCR positive for a specific HPV type if a minimum of 2 ORFs were PCR positive, and was considered PCR negative for a specific HPV type if all ORFs were PCR negative. A negative HPV result was considered valid only if a portion of the human -globin ORF was amplified in a separate reaction to verify that adequate DNA was present in the specimen. Samples that resulted in a single-ORF-positive result were retested from a fresh aliquot of the specimen for verification. The analytic sensitivity for the 14 PCR assays is similar. In the PCR assay validations, the lower limits of detection for all 14 HPV assays were below 20 copies/test [2].

Reference List

1. Roberts CC, Swoyer R, Bryan JT, Taddeo FJ. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with the linear array HPV genotyping PCR assay and influence of DNA extraction method on HPV detection. J Clin Microbiol 2011;49:1899-1906.

2. Else EA, Swoyer R, Zhang Y et al. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with INNO-LiPA HPV genotyping extra assay. J Clin Microbiol 2011;49:1907-1912.

3. Jansen KU, Taddeo FJ, Li W, DiCello AC. Fluorescent multiplex HPV PCR assays using multiple fluorophores. WO patent 2003/019143 A2. 2003.

4. Taddeo FJ, Skulsky.D.M., Wang XM, Jansen KU. Fluorescent multiplex HPV PCR assay. WO patent 2006/116303 A2. 2006.

5. Taddeo FJ, Skulsky DM, Wang XM, Jansen KU. Real-time HPV PCR assays. WO patent 2006/116276 A2. 2006.