Supplemental Online Content

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eMethods.

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

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HPV DNA detection

DNA was extracted from biopsy specimens and/or smears by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and stored at -20°C until further processing.

The detection and genotyping of HPV DNA were performed as described elsewhere [1]. Briefly, the presence and purity of the human DNA were confirmed by PCR with primers MS3/MS10bio specific for the beta-globin gene. HPV DNA detection and typing were done with a mixture of broad spectrum BSGP5+ / 5′-end biotin labelled BSGP6+ primers which amplify the 150 bp fragment of the L1 gene [2,3]. After amplification, an aliquot of 10 µl from each reaction product was electrophoretically separated on 3% NuSieve 3:1 agarose gel. Thereafter reverse line blot hybridization of biotin labelled PCR amplicons was performed with HPV-type specific probes covalently bound on membrane. This method enables the detection of at least 37 different HPV types and determines positivity for multiple HPV genotypes in a single assay [2–4].

Negative results of HPV detection and genotyping were further confirmed in PCR reactions with degenerate primers MY09/MY11 and HMB01 followed by agarose gel electrophoresis as specified before [1]. The remaining PCR amplicon aliquots of positive samples were purified using the MinElute™ Gel Extraction Kit (Qiagen, Hilden, Germany) and directly sequenced using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. The sequence analysis was performed on the ABI PRISM 3500 gene analyser (Applied Biosystems, Foster City, CA). The nucleotide sequences were analysed with the GenBank database using the BLAST server at the National Centre for Biotechnology Information (NCBI, Bethesda, USA).

Serological assays

The presence of antibodies to HPV was tested using an in-house L1 virus-like particles (VLPs) based enzyme-linked immunosorbent assay (ELISA) as described previously [5]. HPV6, 11, 16, 18, 31, and 33 VLPs were prepared by means of recombinant baculoviruses/insect cells expression system and purified on CsCl gradients. The final product was subjected to SDS PAGE, Western blotting, and ELISA with specific monoclonal antibodies. Briefly, to detect specific antibodies, wells of microtiter plates were coated with respective purified VLPs in phosphate-buffered saline (PBS), unbound antigen was removed, nonspecific binding sites were blocked, and wells were incubated in duplicate with human sera diluted 1:25. Following incubation, antibodies bound were detected with donkey horseradish peroxidase linked anti–human IgG (H and L chain), and the reaction was visualized by o–phenylenediamine. The colour reaction was stopped, and optical densities were read at 492 and 630 nm with an Infinite 200 microplate reader (TECAN, Austria). Background reactivity was determined in wells without antigen. Their absorbances were subtracted from the corresponding values obtained in the presence of the antigen. Control sera known to be positive and negative were tested on each plate. The cut-off (CO) level for each particular plate/antigen was represented by a mean absorbance plus two standard deviations (SD) after eliminating the outliers. All ELISA results were represented as a ratio between the absorbance obtained with the tested sample and appropriate CO value (OD index), which expresses the strength of the antibody response. Samples with an OD index of ≤ 1 were considered non-reactive. To confirm the results, all samples within 10% above the CO value as well as about one quarter of all serum samples were retested. To determine the level of antibody response in immunized subjects, the sera were three-fold diluted beginning at 1:50 to 1:12 150. The titre was the reciprocal of the last positive dilution.

References:

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