Reviewer's report

Title: Validation of human papillomavirus genotyping by signature DNA sequence analysis

Version: 3 Date: 24 February 2009

Reviewer: Karin Milde-Langosch

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Most commercial HPV detection systems which rely on probe hybridization are limited in their specificity in HPV genotyping. Therefore, a sophisticated PCR system followed by direct sequencing and BLAST identification of HPV type might be a very sensitive and specific alternative. In the present study, Lee et al. carefully described the application of this method in 3222 cytological samples, 352 of which were positive for one or more out of 35 HPV genotypes. Obviously, the authors decided to present the data obtained in this study in two publications: one merely presenting the technical approach and one presenting and discussing the HPV results in the analyzed population (ref. 29 in references). In this manuscript, they describe the varying amplification efficiencies for different HPV types and the need to carefully select the sequence used for BLAST analysis.

Yet, the manuscript would be improved if the authors clearly state the limitations of this method and discuss the results:

1. In 9 cases, MY09/MY11 amplification was positive, but the following nested GP5+/GP6+ PCR was negative. In many of these cases, typing could be performed by the use of alternative primer pairs. Since the sensitivity of the MY09/MY11 amplification is relatively low and more than half of the positive cases was only detected in the second round of amplification, it can be assumed that a significant number of HPV-positive cases (MY09/MY11-negative, GP5+/GP6+-negative, but probably GP5/MY09- or GP6/MY11-positive) was missed by this approach.

2. A certain number of double or multiple infections might be missed due to different PCR efficiency, i.e. if a sample contains a high HPV 11 copy number and a low HPV 56 copy number, the HPV 56 amplicon might be masked by the HPV 11 amplification product after 60 PCR cycles. Thus, the true number of multiple infections is probably higher than the 8% found in this study.

Minor essential revisions

1. Since the cohort analyzed in this study consisted of two types of patients (women > 30 y and women < 30y with ASCUS), it would be an interesting additional information how these two sub-cohorts differed in HPV type distribution (this issue was obviously addressed in a separate publication which was submitted elsewhere).

2. The 4 figures are partly redundant. Since everybody who is familiar with DNA
sequencing knows about the problems of interpretation illustrated in Fig. 3 and 4, the figures should be partially omitted or all figures concentrated in 1 composite panel.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'