March 14, 2009

To: The Editor

BMC Clinical Pathology

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

Dear Editor:

Thank you and the reviewer for the very constructive guidelines to revise the above-referenced manuscript. The authors have made the revisions accordingly and submit hereby the revised manuscript for your consideration in response to the reviewer’s comments point-by-point as follows.

Major points of revision:

#1: On page 16, one paragraph in red color is added to address the limitation of this approach in detection of HPV DNA in clinical samples.

“Our data have demonstrated that the sensitivity of the MY09/MY11 amplification of HPV DNA is relatively low. More than half of the positive cases are only detected in the nested PCR using GP6/MY11 primers after routine second amplification. In this protocol, only when a case was found to be positive for a 450 bp MY09/MY11 primary PCR amplification with a concomitant negative 190-200 bp nested GP6/MY11 PCR result, a supplementary long nested PCR using the GP5/MY09 primers was performed to amplify the 380-395 bp segment of DNA downstream of the GP5 primer-binding site. Since the latter long nested PCR is not routinely performed on all samples, this procedure may miss some MY09/MY11 PCR negative cases which can be demonstrated in a GP5/MY09 nested PCR setting only.”

#2. One paragraph is added in red color on page 18 to address the possibility of selective amplification of a particular HPV genotype to the exclusion of others in
mixed HPV infections, which might have caused a low percentage of mixed HPV infections observed in this series.

“However, since consensus PCR primers do not amplify DNA from different HPV genotypes with the same degree of efficiency, it is possible that one single HPV DNA template is preferentially amplified during its exponential replication under the nest PCR setting to the exclusion of other concomitant HPV in mixed HPV infections. This preferential DNA amplification might have accounted for the low 8% multiple HPV infections observed in this series.”

Minor essential revisions:

1) On page 17, one paragraph in red color is added to address the potential difference in HPV genotype prevalence between women of two age groups in this suburban US population.

“The cohort of 3222 patients used in this study consists of two age groups and includes 2633 cases previously analyzed. According to the data reported in the latter study [30], the prevalence rates of the first 5 most common “high-risk” HPV genotypes are HPV-16 (22.9% in women below 30; 14.8% in older women), HPV-52 (6.4% in women below 30; 10.0% in older women), HPV-18 (6.4% in women below 30; 7.1% in older women); HPV-31 (2.7% in women below 30; 4.1% in older women; and HPV-56 (5.5% in women below 30; 2.7% in older women). Based on our experience, HPV-16 seems to be more prevalent in women below 30, and HPV-52 is more frequently detected in women age 30 or older in this suburban US female population who are under the care of board-certified gynecologists in private practice.”

2) The 4 figures have been reduced to 2, as recommended.

A few typographic errors in the original manuscript have also corrected in red color.

Thank you for your consideration of the revision and please contact the undersigned if further revisions are needed.

Sincerely,

Sin Hang Lee, MD
On behalf of all co-authors