Letter to the Editor

Interlaboratory Agreement in a Polymerase Chain Reaction-based Human Papillomavirus DNA Assay

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The validity of laboratory assays has a substantial impact on the results of biochemical/molecular epidemiological studies. As part of the quality-control procedure incorporated in studies nested within a larger screening cohort (1, 2), we tested HPV DNA in cervical swabs from a subset of study subjects from two earlier studies [a case-control study (2) and a study of HPV DNA in cervical swabs from a subset of study subjects from a nested within a larger screening cohort (1, 2)], we tested HPV DNA in cervical swabs from a subset of study subjects from two earlier studies [a case-control study (2) and a study of HPV persistent infection (1)] in two laboratories to assess interlaboratory variation.

Cervical cells were collected at screening by ViraPap kits, consisting of a Dacron swab for scraping cells and 1 ml Digene standard transport medium for DNA preservation (Digene Diagnostics, Silver Spring, MD). In an earlier case-control study nested within the screening cohort (2), cervical cells of study subjects were tested for the presence of HPV DNA of types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, and 68 and a mixture of clinical types for which type numbers had not been assigned at the time of testing, including PAPB8 (now known as HPV 66), PAP155, PAP238A (now known as HPV 73), PAP291, and W13B, at laboratory 1 by an L1 consensus primer PCR-based method (3, 4). The amplification reactions included the modified consensus primer pair MY09/MY11 (3). Amplification products were hybridized with a generic HPV probe mixture to determine positivity and with specific oligonucleotide probes to identify individual types. On the basis of the results from laboratory 1, the same aliquot from the same cervical swab of each of the 29 women whose HPV status was unusual was sent to laboratory 2 for retesting with the same procedure. The probes used in dot blot hybridization at laboratory 2 were similar to those employed at laboratory 1, with the exception that types 52 and 57 were not included at laboratory 2, and types 61, 62, 64, 66, 67, 69, and 70 were added to laboratory 2. For four of these women, swabs had been collected at two different times (once at the initial pap smear screening and once 2–5 months later at the time of confirmatory biopsy) for an earlier study of persistent HPV infection (1). These eight samples were treated as independent and tested at both laboratories. Therefore, a total of 33 cervical samples was tested independently at two laboratories. Staff from both laboratories were unaware of the pathological diagnoses and HPV status of these 29 subjects.

The 29 women included 1 control (a non-case subject from a case-control study) with high risk and multiple types of HPV infection (types 16, 39, 53, and 55; Ref. 2), 6 who had confirmed koilocyotic atypia (after two independent reviews) but were HPV negative (2), 4 who had high-grade cervical neoplasia but were HPV negative (2), and 4 cases with cervical neoplasia from an earlier study of persistent infection who had discordant HPV results (HPV positivity) from cervical samplings at two different times (1); we also included 14 subjects from the same cohort with biopsy-proven cervicitis (a random sample of all subjects with cervicitis in an earlier study).

HPV results for these 29 women (33 samples) from laboratories 1 and 2 are listed in Table 1. With respect to HPV positivity, of the 33 samples tested in both laboratories, only 3 had discordant results. Of these three, two tested negative at laboratory 1, but their DNA was not amplifiable in laboratory 2, and one tested low positive (<25 copy amplification level, and specific type was not determined) at laboratory 1 but negative at laboratory 2. Excluding the two nonamplifiable samples, the overall agreement for HPV positivity was 97%. In addition, for the nine samples that tested positive at both laboratories, the specific types of HPV were nearly the same. For the six samples with only a single type of HPV infection, the agreement on HPV positivity and specific type was 100%. For the three samples with more than one type of HPV infection, laboratory 1 reported more individual types (among these three samples, HPV types 51, 53, 55, 56, and ME180 were not reported by laboratory 2).

In summary, consistent with a previous anal cancer study reporting a high (around 90%) level of agreement of HPV detection and typing between two laboratories (5), our results, using cervical cells, suggest that interlaboratory variation in L1 consensus primer PCR can be quite small when well-validated testing methods are employed by quality laboratories. The slight variation may have been due to differences between laboratories in interpretation of weakly positive signals or to failure to amplify or detect a very small amount of HPV DNA. However, when comparing results across studies and populations, it is important to recognize that not all PCR-based HPV results are reliable.
Table 1  HPV DNA status measured by two laboratories

<table>
<thead>
<tr>
<th>Category of subjectsa, b</th>
<th>HPV DNA results on identical samples</th>
<th>Laboratory 1</th>
<th>Laboratory 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control with multiple-type infection (n = 1)</td>
<td>1 positive (types 16, 39, 53, 55)</td>
<td>1 positive (types 16, 39)</td>
<td></td>
</tr>
<tr>
<td>Cases with koilocytic atypia (n = 6)</td>
<td>6 negative</td>
<td>6 negative</td>
<td></td>
</tr>
<tr>
<td>Cases with high-grade cervical neoplasia (n = 4)</td>
<td>4 negative</td>
<td>4 negative</td>
<td></td>
</tr>
<tr>
<td>Cases with discordant HPV results from screening and confirmatory biopsy (n = 8)</td>
<td>3 negative</td>
<td>2 negative, 1 not amplifiable</td>
<td></td>
</tr>
<tr>
<td>Cases with biopsy-proven cervicitis (n = 14)</td>
<td>5 positivec</td>
<td>5 positivec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 negative</td>
<td>9 negative, 1 not amplifiable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 positivec</td>
<td>3 positivec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 low positivec</td>
<td>1 negative</td>
<td></td>
</tr>
</tbody>
</table>

a 29 women with 33 cervical samples.
b Cases from a previous persistent infection study (1); four women with eight cervical samples.
c Only two samples had different HPV types from these two laboratories. One tested positive for HPV 16, 39, 53, and 55 at laboratory 1 but tested positive for only HPV 16 and 39 at laboratory 2; the other tested positive for HPV 52 and ME180 at laboratory 1 but tested positive for only HPV 52 at laboratory 2.
d Only one sample had discrepant types from these two laboratories. This sample tested positive for types 16, 51, 56, and a mixture of clinical types at laboratory 1 but tested positive for only HPV 16 at laboratory 2.
e Less than 25 copy amplification level, so specific type was not determined.

DNA test systems are the same, because of variability in primer sets, amplification conditions, and detection and typing methods (6).

References
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