Validation of a Multiplex PCR Assay for the Simultaneous Detection of Human Papillomavirus and Chlamydia Trachomatis in Cervical PreservCyt Samples.

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Validation of a Multiplex PCR Assay for the Simultaneous Detection of Human Papillomavirus and Chlamydia trachomatis in Cervical PreservCyt Samples

To the Editor:

Chlamydia trachomatis is the most common sexually transmitted bacterium worldwide (1) and a leading cause of infertility in women (2). Human papillomaviruses (HPVs) are the most important single agent causing carcinoma of the uterine cervix (3). Combined molecular screening for C. trachomatis and HPV could be justified given their propensity to cause asymptomatic infections, particularly in high-risk groups. Features of HPV infection of cells of the uterine cervix are traditionally reported by the Pap smear method (4). The introduction of liquid-based cytology, such as the ThinPrep® Pap TestTM, has had the effect of improving the sensitivity of conventional cytologic screening with the potential for HPV testing of residual cellular material in borderline or difficult cases (5–7). The US Food and Drug Administration (FDA) has recently cleared a hybrid capture-based system (HCII; Digene) for screening women over 30 years of age as an adjunct to Pap testing (8). Researchers have developed consensus primers for the detection of HPV DNA by PCR (9, 10). We developed and evaluated a multiplex PCR for the simultaneous detection of HPV and C. trachomatis from PreservCytTM (ThinPrep) solution.

The multiplex PCR was performed on 100 cervical PreservCyt fluid specimens collected from women attending their general practitioners for routine cervical screening. We used the MY09/11 primers (9) for HPV and plasmid primers for C. trachomatis (11), which generated fragments of 450 and 201 bp, respectively. Primers for the human β-globin gene were included in the multiplex as an internal DNA amplification control, generating a 110-bp product (12) (see Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol51/issue7/). The PCR mixture contained 5 pmol of each of the forward and reverse primers of the MY09/11 and CTP1/2 primer sets and 10 pmol of each of the forward and reverse primers of the CO03/4 primer set, 200 μM deoxynucleoside triphosphates, 10× PCR buffer [containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl], 2 mM MgCl2, and 1 U of Platinum Taq DNA polymerase (Invitrogen Ltd.) in a final volume of 20 μL. The PCR was initiated by a 10-min denaturation and enzyme activation step at 95°C and was completed by a 10-min extension step at 72°C. The

Table 1. Comparison of multiplex and single PCR for the detection of HPV and C. trachomatis in PreservCyt cervical samples.

<table>
<thead>
<tr>
<th>Sample cohort</th>
<th>Multiplex positive, n</th>
<th>Single PCR positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY09/11</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>CTP1/2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>GP clinic (n = 100)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>GUM clinic (n = 34)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

a Primers for the detection of HPV.
b Primers for the detection of C. trachomatis.
c Women who attended general practitioner (GP) clinics for routine cervical screening.
temperature cycles were as follows: 40 cycles of 30 s at 95 °C, 1 min at 57 °C, and 1 min at 72 °C.

The detection limit was estimated on serial dilutions, from $10^8$ to $10^1$ copies/μL of cloned HPV MY09/11 and the C. trachomatis CTP PCR product in the pCR® 2.1-TOPO® (Invitrogen Ltd). To evaluate the sensitivity and specificity of the multiplex PCR for the detection of each organism, we performed single PCRs on all samples: one PCR that detects HPV by use of the GP5+/6+ primers (10), generating a 150-bp product; and one that detects C. trachomatis by use of primers specific to the hsp60 gene of C. trachomatis (sense, 5′-GAT GTG GTT ACC GTG GCG A-3′; antisense, 5′-TAA TCG TCT TTA ACA ACC T-3′), generating a truncated version (309 bp) of a previously described product (13). PCRs were also performed with the MY09/11 and CTP1/2 primer sets singly.

In the screened population, 21% (21 of 100) of samples were positive for HPV by the multiplex PCR (Table 1). The MY09/11 primers identified 3 other samples as HPV positive that were not detected by the multiplex assay (Table 1). Either the MY09/11 or the GP5+/6+ primers confirmed all 21 of the samples positive by the multiplex assay. Two samples were positive for C. trachomatis in the multiplex assay (Table 1). These were confirmed positive by the CTP1/2 and Hsp60 primers in single PCRs, and no additional positive samples were detected. A positive sample was defined as positive by either the multiplex or any of the single PCRs for that organism. The sensitivity and specificity of the multiplex with respect to single PCR for the detection of HPV in the opportunistically screened samples was 89% (95% confidence interval, 70%–97%) and 100% (94%–100%; Table 1). The multiplex assay was 100% specific and sensitive for the detection of C. trachomatis with respect to single PCR in the screened population (Table 1).

To estimate the diagnostic sensitivity of the multiplex assay for the detection of C. trachomatis, we performed multiplex PCR on 34 Preserv-Cyt fluid specimens from women attending a genitourinary (GUM) outpatient clinic who had tested positive for C. trachomatis by the LCx (Abbott Laboratories). The sensitivity of the multiplex assay for the detection of C. trachomatis in the GUM clinic population was 100% with respect to the commercial LCx assay (Table 1). Ten of the 34 samples were positive for HPV by the multiplex assay. These results were confirmed by single PCRs (Table 1). The multiplex assay could detect as few as 100 copies of the C. trachomatis plasmid and 100 copies of the HPV genome per microliter of extracted DNA.

This is the first study looking at the detection of both HPV and C. trachomatis from a single ThinPrep sample by use of multiplex PCR. This simple multiplex is rapid and could be used to screen cervical ThinPrep samples for both HPV and C. trachomatis, particularly in a high-risk population.

References


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Detection of Heavy Chain Disease by Capillary Zone Electrophoresis

To the Editor:
Luraschi et al. (1) recently published the first report describing the use of capillary zone electrophoresis (CZE) coupled with immunosubtraction to detect and characterize low concentrations of free κ heavy chains in serum. By contrast, we describe a case in which CZE failed to detect and characterize κ heavy chain disease.

A 90-year-old woman who complained of weight loss presented with progressive enlargement of the left parotid gland, splenomegaly, and palpable inguinal lymph node