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

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assisted laser desorption/ionization (MALDI)-TOF MS, with its higher resolution, is the capability of SELDI to enrich low-abundance proteins from complex matrices such as plasma, through, for example, the coupling of specific antibodies to the chip surfaces (4). Such an approach is an important methodologic aspect in "second phase" proteomics, which are characterized by repetitive investigation of the same protein to validate the protein phenotype in large population-based studies. This provides a basis for diagnostic progress in personalized medicine (5). For TTR, such an approach is relevant not only in the diagnosis of TTR-related amyloidosis but also in other diseases.

The true problem with on-chip immunopurification is not the resolution of the MS, which can be solved by use of specific available interfaces, but the efficient coupling of the antibody to the chip surface. When an on-chip immunoassay format is being used, it is important that the protein chip retains the antibody in an active state at high density. Results are greatly affected by functionality characteristics, such as the stability, affinity, and specificity of the antibody. On the basis of studies relating to microarrays, only 5%–20% of commercially available antibodies are suitable for one or the other microarray format (6).

References

1. Theberge R, Connors LH, Skinner M, Costello CE. Detection of transthyretin variants using immunoprecipitation and matrix-assisted laser desorption/ionization bioreactive probes: a clinical application of mass spectrometry. *J Am Soc Mass Spectrom* 2000;11:172–5.
2. Wang Z, Yip C, Ying Y, Wang J, Meng XY, Lomas L, et al. Mass spectrometric analysis of protein markers for ovarian cancer. *Clin Chem* 2004;50:1939–42.
3. Schweigert FJ, Wirth K, Raila J. Characterization of the microheterogeneity of transthyretin in plasma and urine using SELDI-TOF-MS immunoassay. *Proteome Sci* 2004;2:5.
4. Schweigert FJ. Characterization of protein microheterogeneity using mass spectrometry based immunoassays. *Brief Funct Genomic Proteomic* 2005;4:7–15.
5. Hanash S. Disease proteomics. *Nature* 2003;422:226–32.
6. MacBeath G. Protein microarrays and proteomics. *Nat Genet* 2002;32:526–32.

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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 Test™, has had the effect of improving the sensitivity of conventional cytologic screening with the potential for HPV testing of residual cellu-

lar 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 PreservCyt™ (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 PCO3/4 primer set, 200 μ M deoxynucleoside triphosphates, 10 \times 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 μ L. 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.

Sample cohort	Multiplex positive, n		Single PCR positive, n			
	MY09/11 ^a	CTP1/2 ^b	MY09/11	GP5+/6+ ^a	CTP1/2	Hsp60 ^b
GP ^c clinic (n = 100)	21	2	24	14	2	2
GUM clinic (n = 34)	10	34	10	10	34	34

^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 10⁸ to 10¹ 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 GGT GTT ACC GTT GCG A-3'; antisense, 5'-TAA TAA TCG TCT TTA ACA ACG 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 were 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

- World Health Organization. State of the art of new vaccines research and development: Initiative for Vaccine Research. Geneva: WHO, April 2003;21. http://www.who.int/vaccine_research/documents/en/stateofart_excler.pdf (accessed October 10, 2004).
- Mardh PA. Tubal factor infertility, with special regard to chlamydial salpingitis. *Curr Opin Infect Dis* 2004;17:49–52.
- Munoz N. Human papillomavirus and cancer: the epidemiological evidence. *J Clin Virol* 2000; 19:1–5.
- Renshaw AA, Young AN, Birdsong G, Styer P, Davey D, Mody D, et al. Comparison of performance of conventional and ThinPrep gynecologic preparations in the College of American Pathologists Gynecologic Cytology Program. *Arch Pathol Lab Med* 2004;128:17–22.
- Hoelund B. Implementation of liquid-based cytology in the screening programme against cervical cancer in the County of Funen, Denmark, and status for the first year. *Cytopathology* 2003;14:269–74.
- Cheung A, Szeto NE, Leung B, Khoo U, Ng A. Liquid-based cytology and conventional cervical smears: a comparison study in an Asian screening population. *Cancer* 2003;99:331–5.
- Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). *Arch Pathol Lab Med* 2003;127:946–9.
- FDA News. FDA approves expanded use of HPV test. Washington: FDA, March 31, 2003. <http://www.fda.gov/bbs/topics/news/2003/new00890.html> (accessed October 10, 2004).
- Manos MM, Ting Y, Wright D, Lewis A, Broker T, Wolinsky S. Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989;7:209–14.
- de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995;76:1057–62.
- Griffais R, Thibon M. Detection of *Chlamydia trachomatis* by the polymerase chain reaction. *Res Microbiol* 1989;140:139–40.
- Saiki RK, Scharf S, Faloona F, Mullis K, Horn G, Erlich H, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Biotechnology* 1992;24:476–80.
- Wood H, Reischl U, Peeling R. Rapid detection and quantification of *Chlamydia trachomatis* in clinical specimens by LightCycler PCR. In: Reischl U, Wittwer C, Cockerill F, eds. Rapid cycle real-time PCR—methods and applications. New York: Springer, 2002:115–32.

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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 γ heavy chains in serum. By contrast, we describe a case in which CZE failed to detect and characterize μ 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