Clinical Chemistry

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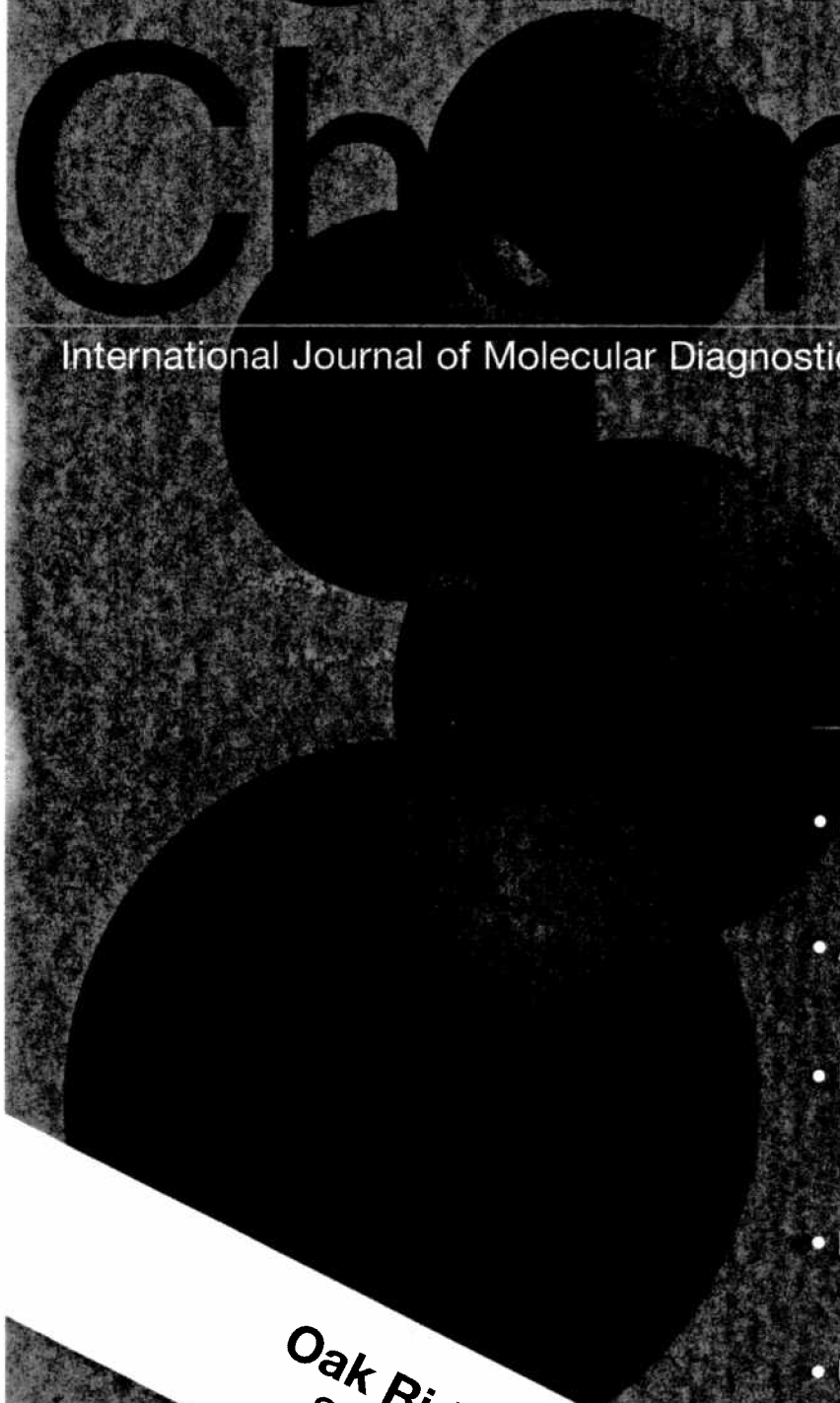

- Rapid Detection and Identification of Bacteria
  See Pages 1985 and 1997
- Adiponectin Gene Variations and Cardiovascular Risk
  See Page 2021
- Health Outcomes Associated with Current and Previous Definitions of Acute Myocardial Infarction
  See Page 2028
- Lipoprotein Subclasses and Coronary Artery Disease
  See Page 2049
- Urinary Metanephrines to Screen for Pheochromocytoma
  See Page 2060

Oak Ridge Conference
See Pages 2129–2175

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A Magnetic Immunochromatographic Strip Test for Detection of Human Papillomavirus 16 E6, *Roger B. Peck,[1*] Johannes Schweizer,[2] Bernhard H. Weigl,[1] Chamorro Somoza,[2] Jon Silver,[2] John W. Sellors,[1] and Peter S. Lu[2]* ([1] PATH, Seattle, Washington 98107; [2] Arbor Vita Corp., Sunnyvale, California 94085; * address correspondence to this author at: PATH, 1455 NW Leary Way, Seattle, WA 98107; fax 206-285-6619, e-mail rpeck@path.org)

Cervical cancer kills 230 000 women annually. Low-resource regions of the world are disproportionately burdened with 80% of the cases. Efficient screening methods are the key to decreasing the death toll from this disease. High-risk human papillomavirus (HPV) types have been identified as the etiological agent for >99% of cervical cancers. Infection with HPV is ubiquitous and is often resolved by the host. High-risk HPV infections leading to cervical cancer require the production of both HPV E6 and E7 oncoproteins *(1–5)*. Thus, an assay capable of detecting high-risk HPV E6 from cervical swab samples may have a high positive predictive value and may help to accurately identify women at increased risk of progression to cancer.

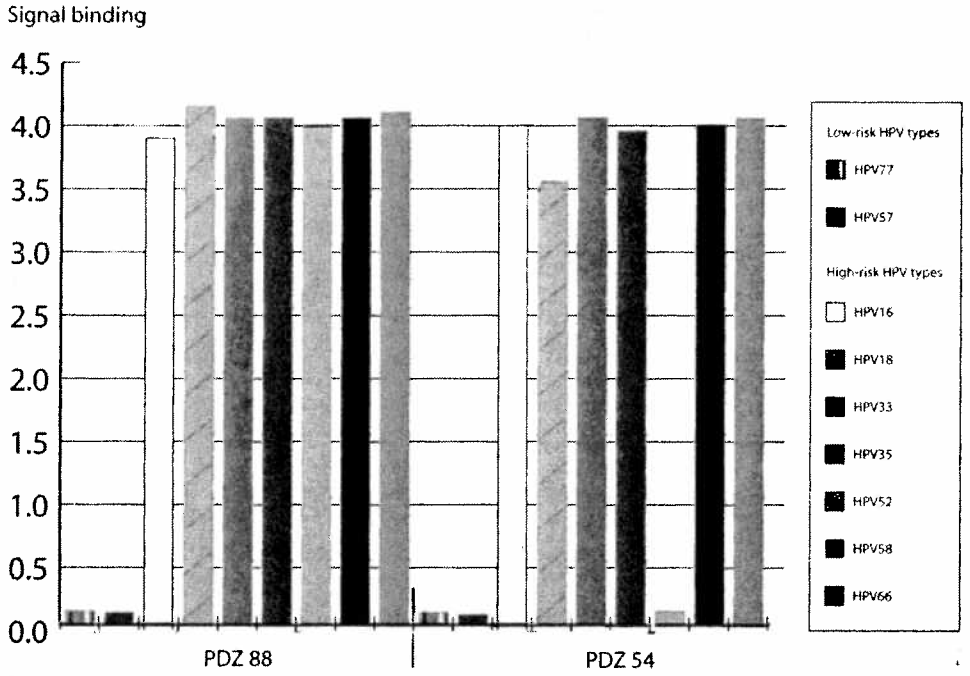
Internal studies have shown that cervical cancer cell lines produce ~1 ng of E6 per 1 000 000 cells (data not shown). Work is continuing to quantify E6 amounts in cervical cell samples from clients with cancer, high-grade lesions (cervical intraepithelial neoplasia 2/3), and low-grade lesions (cervical intraepithelial neoplasia 1). Protein determinations performed on provider-collected cervical swabs indicate that the swabs collect 500 000 to 2 000 000 cells.

PDZs (named for the first 3 described domain-containing proteins—postsynaptic density 95, *Drosophila* large disc, and zona occludens) are a conserved class of protein domains that engage in protein-protein interactions by binding PDZ ligands (PLs). PLs usually consist of short C-terminal sequences following the general motif: X-S/T-X-V/L.

Cellular proteins exhibiting PDZ domains fulfill widespread biological functions, including cell-to-cell contact, intercellular signaling, and cell polarity. Numerous viruses encode proteins that have PLs, thus allowing the viral protein to interact with cellular PDZ domain-containing proteins. E6 proteins of only high-risk HPV types interact with PDZs *(6–9)*. Arbor Vita Corporation and PATH, a nonprofit, international health agency, have collaborated on the development of an assay based on the specific interaction of high-risk HPV E6 with PDZ protein and detection via an anti-HPV E6 monoclonal antibody. A sandwich ELISA has been developed and is now being adapted to an immunochromatographic strip platform for use in low-resource settings.

The nearly complete set of PDZ domains present in humans were generated as prokaryotic recombinant fusion proteins and screened for binding to high-risk HPV E6 with an ELISA approach. Fig. 1A shows a subset of the data generated. PDZ 88 demonstrated high affinity to high-risk HPV E6 but not to low-risk HPV E6. E6 PL

A



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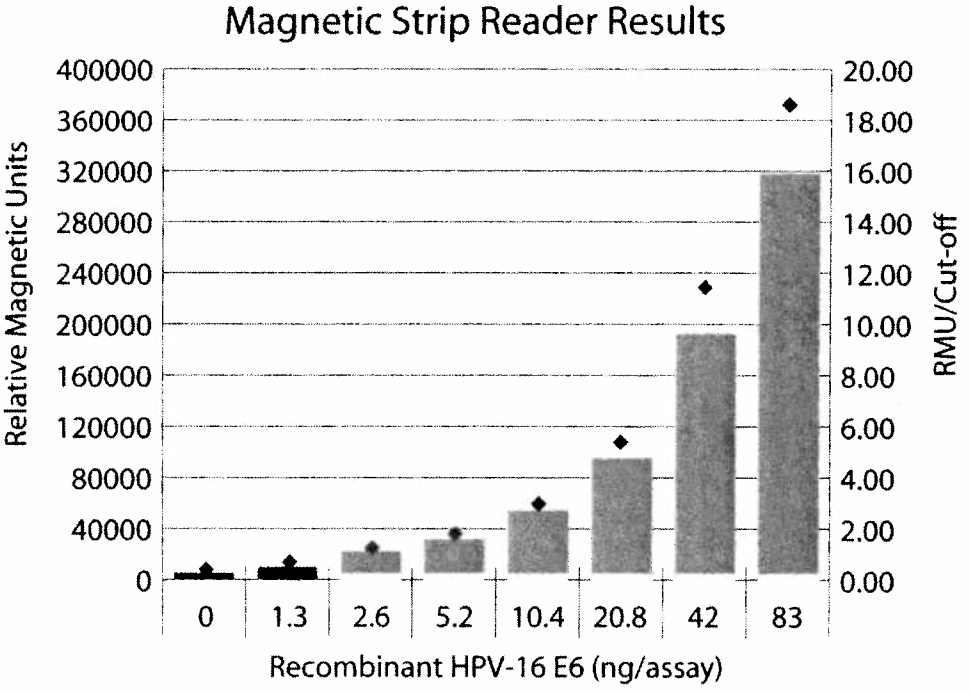

Raw RMU	5570.5	10030	16859	26141	48758	89865	186931	312240
RMU/Cut-off	0.33	0.60	1.01	1.56	2.92	5.38	11.19	18.68

Fig. 1. Results from a subset of E6-PDZ binding data and immunochromatographic strip results using PDZ 88.

(*A*), selection of the PDZ oncogenic E6 detector. (*B*), magnetic strip reader results.

sequences representing all known high-risk HPV E6 were tested, and PDZ 88 interacted with all of them with greatest binding strength. Therefore, PDZ 88 was selected as the capture reagent. The recognition of PDZ 88 to only high-risk HPV E6 (e.g., HPV types 16, 18, 33, 35, 52, 58, and 66) but not low-risk HPV E6 (e.g., HPV types 77 and 57) provides a high degree of selectivity for the assay.

Antibodies to HPV E6 from high-risk HPV types were generated. The antibodies were screened for affinity and cross-reactivity with other HPV E6. The antibody with the strongest reactivity to HPV-16 E6 was selected as the detector.

The magnetic immunochromatographic strip was selected for evaluation as the assay platform on the basis of ease of use and acceptability in low-resource settings, inherent sensitivity of the technology, and the ability to quantify results. MagnaBioSciences has developed a magnetic immunochromatographic test platform including an assay cassette and reader that is suitable for developing quantitative assays for a variety of analytes. The assay described here was developed on this platform. Additional studies for the method have shown that strip-to-strip reproducibility yields a CV of <10%, and reader reproducibility yields a CV of 1.5% (data not shown).

The PDZ capture reagent was immobilized on a nitrocellulose membrane. Assays were assembled with a laminate backing, membrane with immobilized PDZ, hydrophilic sample pad, absorbent pad, and membrane cover tape. Test strips were cut to a width of 7.5 mm. The soluble-phase detection antibody was covalently conjugated to superparamagnetic particles.

Doubling dilutions of recombinant HPV-16 E6 from 332 to 5.2 μg/L were prepared in the proprietary assay running buffer containing Tris, surfactants, and blocking agents. The detection reagent was mixed with the sample, and 250 μL was applied to the strip. On migration of the sample, the strips were inserted into the magnetic strip reader. The strip reader generated data curves. Peak height was selected, and data were reported as relative magnetic units (RMUs).

The assay cutoff was conservatively set at 3 times the mean RMU of the negative controls. The RMU value for each sample was divided by the cutoff. Samples >1 were considered positive.

Lower amounts of the target protein led to lower RMU values (Fig. 1B). Strips titrated with recombinant HPV-16 E6 and read on the magnetic strip reader exhibited a linear response.

An initial prototype of the magnetic immunochromatographic strip assay is capable of detecting recombinant HPV-16 E6 down to 2.6 ng/assay (~2 600 000 cells). An early version of the assay in an ELISA format detected oncogenic E6 from cervical cancer cell lines down to 30 pg/assay (~30 000 cells). Future steps include further optimization to decrease detection limits and evaluation of cell cultures and cervical cell swabs from clients. Once utility with HPV16 is demonstrated, additional high-risk HPV types will be added to the assay.

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