A New HPV-DNA Test for Developing Countries

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Paul S. Eder,[1] Irina Nazarenko,[1] Roger Peck,[2]
Bernhard Weigl,[2] John Sellors,[2] and Attila Lorincz[1]

[1]Digene Corporation, Gaithersburg, USA [2]PATH, Seattle, USA

Digene Corporation's Program for New HPV-DNA Test

- Partnership with PATH, headquartered in Seattle, seeking:

  To develop and commercialize a rapid batch diagnostic HPV test designed for use in developing countries, based on a modification of the Hybrid Capture® technology.

PATH
A catalyst for global health

Motivation

- **Cervical cancer is the most common cancer among women in developing countries.**
  - More than 230,000 deaths annually.
  - Low-resource regions comprise 80% of all new cases.
- **Cervical cancer is preventable with early detection and treatment. What is needed:**
  - Validated screening test.
  - Adequate coverage of the population at risk.
  - Increased accessibility to effective treatment.

Rationale

- **Overwhelming causal association between infection with some types of HPV and cervical cancer.**
- **IARC Cervix Cancer Working Group Evaluation, (Apr 2004) concludes that there is "*sufficient evidence*" that HPV testing, as the primary mode of screening can reduce cervical cancer incidence and mortality.**
- **Highly sensitive and reproducible tests to detect HPV DNA are routinely available.**

Challenges

- **Produce a robust assay in a field setting.**
- **Deliver high specificity and sensitivity.**
- **Improve cost-effectiveness.**
- **Simplify the protocol.**
- **Shorten testing time.**
- **Adapt to allow high-throughput testing.**
- **Increase access to underserved women (portability).**
- **Adopt the use of self-collected samples.**

Details

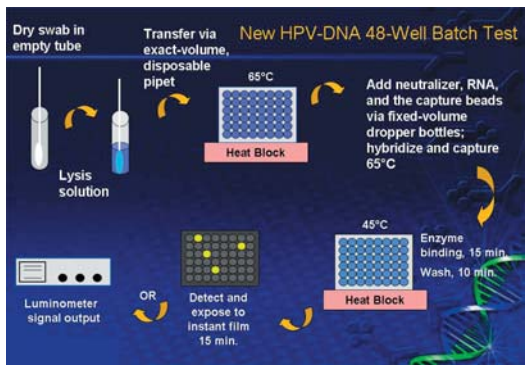
- **Reduce assay time to 2 hours.**
  - Results and treatment in same clinic visit.
- **Improve target capture with bead-based matrix and alternative antibody.**
  - Greater speed and reproducibility; lower costs.
- **Simplify detection and signal output.**
  - Cheaper, simpler, faster, equally sensitive readout.
- **Stabilize labile kit components for extended periods.**
  - Geographical climates and cold-chain uncertainty will limit solution stability.

Current Steps in the hc2 Format

- **Denaturation of target for 45 minutes at 65°C.**
- **Hybridization of RNA probe for 60 minutes at 65°C.**
- **Capture with anti-hybrid antibody (Ab) on plate for 60 minutes at room temperature (RT).**
- **Detection with Ab-enzyme for 30 minutes at RT.**
- **Wash 15 minutes.**
- **Detection substrate for 15 minutes at RT.**
- **Signal output: luminometer 15 minutes.**

Total assay time: ~4 hours

New HPV-DNA 48-Well Batch Test



Improved Assay Times: hc2 *versus* New HPV-DNA Test

Step	hc2	New
Denature specimen	45 min.	45 min.
Hybridize with RNA probe	60 min.	--
Capture on microplate	60 min.	60 min.
React with Ab-conjugate	30 min.	15 min.
Wash	10 min.	10 min.
Incubate with chemiluminescent substrate	15 min.	15 min.
Signal output	5 min.	--
Total elapsed time	~4 hours	~2.5 hours

Analytical Sensitivity of New HPV-DNA Test

Version	Trials	Replicates	1 pg/mL S/N	95% CI	Range
Two-plate	14	8	8.9	1.1	6.8-11.8
One-plate	29	8	9.4	1.9	6.0-17.0

1 pg/mL = 5000 copies HPV 16 plasmid DNA

Acceptable CV must be below 20%

Background Signals from STM Negative Clinical Pools

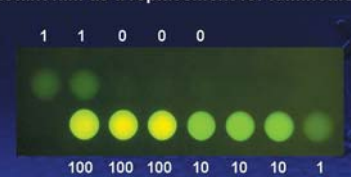
Target Concentration (pg/mL)	RLU/CO	%CV (n=8)
0	1	16
1.0	1.0	18
Negative Clinical Pool	0.2	6

Cutoff (CO) is defined as the RLU signal of a 1-pg/mL target sample

Clinical specimen is defined as HPV-positive if RLU/CO≥1

Simplified Detection: Visual Imaging

Instant film as a replacement for luminometer



Triplicate HPV-16 target conc. (pg/mL)

Stabilization of Kit Components Under High Temperatures

- **Labile kit reagents air-dried in a disaccharide formulation to a glassy state.**
- **Storage at elevated temperatures for up to 3 months.**
- **Reconstituted activity in hc2 assay: 80-100% of control.**

Some Ongoing Issues

- **Further optimization: assay time and collection medium.**
- **Further reduction in plastics and consumables.**
- **How many and which types to include in probe mix?**
  - Typing study to generate prevalence data (Digene).
  - Cost-effectiveness modeling (PATH).
  - Systematic review of the literature (PATH).
- **What kinds of specimens should be employed?**
  - Provider-collected cervical? Self-collected? Brush? Swab?
- **Local manufacture of some assay components?**

Progress to Date

- **Single-plate, bead-based assay performed in ~2.5 hours.**
- **Analytical sensitivity below 5000 copies.**
- **Incorporated an improved Ab as capture protein.**
- **Neither electricity nor running water required.**
- **Most of the plastics and consumables eliminated.**
- **Heat-stabilized labile kit reagents for >90 days at 40°C.**
- **Developing a cheaper, simpler, equally sensitive visual signal output.**

DIGENE