PERFORMANCE OF THE ONCOE6 TEST FOR HIGH-GRADE CERVICAL DISEASE AND CANCER DETECTION AMONG HONDURAN WOMEN

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INTRODUCTION

Cervical cancer is a public health problem in Honduras. Cytology-based screening has failed in reducing cervical cancer rates in the country, and moving towards HPV primary screening is under evaluation. The ESTAMPA study aims to evaluate triage techniques for HPV positive women aged 30-64 years within an HPV-based screening programme.

One of these techniques is the OncoE6 test, developed by Arbor Vita Corporation, a qualitative test that detects levels of E6 oncoprotein expressed by HPV types 16 and 18. Using a lateral flow format, the "E6 Test" can detect elevated levels of the cancer causing E6 oncoprotein through a simple procedure with visual readout. The test can be performed upon minimal training without the need for complex equipment or a cold chain.[1] This strategy has been evaluated in feasibility studies documenting a high positive predictive value and high specificity for detecting CIN3.[2]

OBJECTIVE

The test is robust, simple, fast (2.5 hours) and not very costly making it very promising for low-resource settings. In this work, we evaluated the performance of the OncoE6 test using samples from women in the ESTAMPA study, supplemented with samples from a referral population.

METHODS

Study population

Cervical samples were collected from two different groups of women. Two health centers from a rural community of Honduras where screening is not accessible for all women recruited 155 females between 30 and 64 years of age participating in a pilot cross sectional population-based cervical cancer HPV-screening project (ESTAMPA screening group). An additional group of 41 women attending colposcopy service at San Felipe General Hospital in Tegucigalpa after high grade cytology (HSIL or glandular lesions), were also invited to participate (referral group). All women agreed to participate in the study and signed an informed consent.

Sample processing

Samples were tested blindly with Hybrid Capture II (HC2), the LiPA (Genotyping kit HPV GP, version 2, primers GP5+/GP6+) for genotyping and the OncoE6 test. HPV positive women in the ESTAMPA group and all the HSIL's underwent colposcopy and biopsies were collected from visualised lesions and interpreted by a local pathologist.

Laboratory assays

Hybrid Capture (HC2) testing

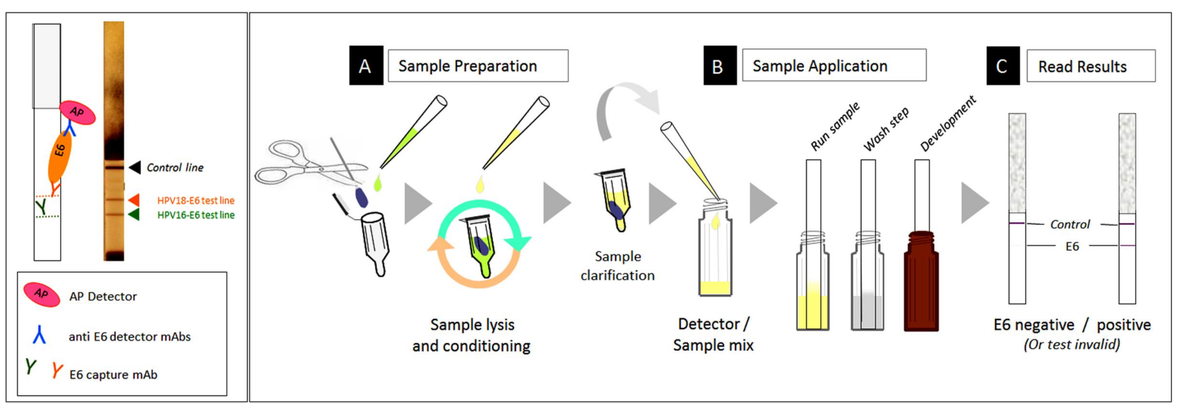
This is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. This test detects HR-HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The test was performed following the manufacturer´s procedures.

LiPA testing (Genotyping kit HPV GP, version 2)

Samples´ extracted DNA were amplified using GP5+/6+-PCR. Amplimers were used in a reverse hybridization assay (Diassay, the Netherlands) for the qualitative identification of HPV types classified as oncogenic risk 16, 18 , 26, 31, 33, 35, 39 , 45, 51 , 52, 53 , 56, 58 , 59, 66 , 68, 73 and 82, as well as the detection of the most common low risk HPV types 6, 11 , 30, 67 and 70.

ONCO*E6*™ CERVICAL TEST

Detection of E6 protein by high-affinity proprietary antibodies; capture mAb / HPV E6 / detector mAb sandwich; very high analytical sensitivity (pg to fg detection of E6 / <1000 cells); detection of HPV 16/18 E6 on two distinct test lines.



Statistical analysis

Estimates of sensitivity and specificity with corresponding 95% confidence intervals (95CI) for detection of cervical intraepithelial neoplasia grade 2 or worse lesions (CIN2+) of the OncoE6 test were obtained.

RESULTS

Ninety five women tested positive for HPV: 93 in both HC2 and LiPA and two only in LiPA. Twenty-one women tested positive for HPV16 and nine for HPV18 oncoproteins using the OncoE6 test. A total of seven CIN2's, 6 CIN3's and 31 cancers were diagnosed. Two CIN2's and one cancer were detected in women in the screening group; all other cases were diagnosed in the referral group. The OncoE6 test was positive in all CIN2+ associated to HPV16/18 (three CIN2, four CIN3 and 19 cancers), in one cancer that tested negative in all other tests, and in three women with no high-grade disease. The sensitivity of the OncoE6 was 61.4% (95CI:45.5%-75.6%) for detection of CIN2+ and 100% (94.3-100) for CIN2+ associated to HPV 16 or HPV 18; the specificity for less than CIN2 was 98% (95CI:94.3%-99.6%).

Table 1. No. of women with CIN2+ and without disease by HPV type (16/18, OTHER and NEG) and E6 result

	CIN2+ on Histology			No disease (<CIN2, Colpo neg)			
	HPV 16/18	HPV OTHER	HPV NEG	HPV 16/18	HPV OTHER	HPV NEG	Total
E6 positive	26*	0	1**	3***	0	0	30
	100%	0%	100%	23.1%	0%	0%	15.3%
E6 negative	0	17	0	10	39	100	166
	0%	100%	0%	76.9%	100%	100%	84.7%
Total	26	17	1	13	39	100	196
	100%	100%	100%	100%	100%	100%	100%

* One HC2 neg, HPV18, E6 18 with cancer and one HC2 neg, HPV67 with cancer. ** One HC2 neg, GP5/GP6 neg, E6 16 with cancer. *** One HC2+, HPV 18, HPV 66, E6 18 with CIN1; one HC2+, HPV 16, E6 16 & negative colposcopy; one HC2+, HPV 18, E6 18 & colposcopy minor (no biopsy result).

Table 2. No. of women with CIN2+ and without disease by HPV type (16/18 and OTHER/NEG) and E6 result

	CIN2+ on Histology		No disease (<CIN2, colp neg)		
	HPV 16/18	HPV OTHER/ NEG	HPV 16/18	HPV OTHER/ NEG	Total
E6 positive	26	1*	3**	0	30
	100%	5.6%	23.1%	0%	15.3%
E6 negative	0	17	10	139	166
	0%	94.4%	76.9%	100%	84.7%
Total	26	18	13	139	196
	100%	100%	100%	100%	100%

* Case detected only by the OncoE6 test (type 16). ** One HPV 18, HPV66, E6 18 with CIN1; one HPV16, E6 16 & negative colposcopy; one HPV18, E6 18 & colposcopy minor (no biopsy result).

Table 3. No. of women with CIN2+ and without disease by HPV type and OncoE6 result

	CIN2+ on histology			No disease	
	HPV 16/18	HPV OTHER/ NEG	ALL	(<CIN2, colp neg)	Total
E6 positive	26	1*	27	3**	30
	100%	0.06%	61.4%	2.0%	15.3%
E6 negative	0	17	17	149	166
	0%	94.4%	38.6%	98.0%	84.7%
Total	26	18	44	152	196
	100%	100%	100%	100%	100%

* Case detected only by the OncoE6 test (type 16). ** One HPV 18, HPV66, E6 18 with CIN1; one HPV16, E6 16 & negative colposcopy; one HPV18, E6 18 & colposcopy minor (no biopsy result).

Table 4. E6 performance for detection of CIN2+

For detection of CIN2+	Estimate	(95%CI)
All CIN2+ (n=44)		
Sensitivity	61.4%	(45.5%-75.6%)
Specificity	98.0%	(94.3%-99.6%)
CIN2+ associated to HPV16/18 (n=26) [1]		
Sensitivity	100.0%	(86.8%-100.0%)
Specificity	98.0%	(94.3%-99.6%)

- No disease: histology<CIN2 or negative colposcopy
- E6 test positive if positive for HPV16 or HPV 18

[1] Based on GP5/GP6. CIN2+ not associated to HPV16/18 excluded

CONCLUSIONS

Since the transformation of epithelial cells requires an elevated expression of the E6 and E7 proteins, their detection represent an attractive viral marker of risk. In this work, the OncoE6 test showed high concordance with the LiPA genotyping, detected 61% of all precancer and cancers, and 100% of cases associated to HPV16/18. In addition, it showed very high specificity highlighting its potential to be used to triage HPV positive women and possibly allowing for more effective clinical follow-up.

BIBLIOGRAPHY

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Disclosure
The authors certify that they have no potential conflicts of interest to report.