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## Comparative analysis of three methods for HPV DNA detection in cervical samples.

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**Key words:** HPV detection; HC2; L1-Nested-PCR, E6/E7-PCR.

**Abstract.** High risk HPV infection is considered to play a central role in cervical carcinogenesis. HPV DNA testing has shown to be a very useful tool for screening and following cervical infections. The aim of this study was to compare three methods for HPV DNA detection, along with cytology and colposcopy analysis. Cervical samples were collected from 100 sexually active women in Mérida, western Venezuela. HPV infection was screened using Hybrid-Capture 2 (HC2), L1-Nested-PCR and E6/E7-PCR assays. 40% of the samples (40/100) were HPV positive by at least one of the DNA detection methods. HC2 detected HPV in 12% specimens. L1- and E6/E7-PCRs showed 50% sensitivity and 77% specificity. The agreement rate between HC2 and both PCR assays was 65%. Kappa value showed moderate concordance between HC2 and both PCR methods ( $\kappa=0.55$ ; CI 95%). Also moderate concordance was seen when L1- and E6/E7-PCRs were compared ( $\kappa=0.48$ ; CI 95%). There was a significant association between the Schiller test and E6/E7-PCR ( $p=0.006$ ) for HPV infection. An acceptable agreement between all three assays for HPV detection was observed. Nevertheless, different PCR formats need to be further analyzed in order to make the right choice of method for HPV testing.

## **Análisis comparativo de tres métodos para la detección del ADN de VPH en muestras de cuello uterino.**

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**Palabras clave:** detección de VPH; CH2; L1-Nested-PCR, E6/E7-PCR.

**Resumen.** La infección con VPH de alto riesgo es el principal factor etiológico asociado al desarrollo de carcinogénesis cervical y las pruebas de detección de ADN-VPH han mostrado ser una herramienta esencial para la pesquisa y seguimiento de estas infecciones. El objetivo del estudio ha sido comparar tres métodos para la detección del ADN viral, en combinación con los análisis colposcópico y citológico. Se obtuvieron muestras cervicales de 100 mujeres sexualmente activas, en Mérida, Venezuela. La detección de infecciones por VPH se realizó por Captura Híbrida 2 (CH2) y los ensayos de PCR "L1-Nested-PCR" y "E6/E7-PCR". 40% de las muestras (40/100) fueron positivas para VPH por al menos uno de los métodos aplicados. 12% de las muestras analizadas fueron positivas para VPH por CH2. Las dos PCR utilizadas mostraron un 50% de sensibilidad y 77% de especificidad. La coincidencia observada entre CH2 y las dos PCR fue del 65%. La determinación del valor Kappa mostró una concordancia moderada entre CH2 y ambos métodos de PCR ( $\kappa=0,55$ ; CI 95%). También existió concordancia moderada al comparar las PCR de las regiones L1 y E6/E7 de VPH ( $\kappa=0,48$ ; CI 95%). Hubo una asociación significativa entre el resultado del test de Schiller y la PCR E6/E7 ( $p=0,006$ ) para la infección por VPH. Se determinó una concordancia aceptable entre los tres métodos aplicados para la detección de VPH; sin embargo, las PCR deben ser analizadas en trabajos futuros con el fin de establecer las pruebas más adecuadas para la detección viral.

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### **INTRODUCTION**

Cervical carcinoma (CC) is a worldwide public health problem, also being one of the leading causes of cancer deaths in women in the developing world (1, 2). In Venezuela, CC constitutes a major cause of mortality in sexually active women (3-5). Currently, it is accepted that the infection with high-risk Human Papilloma Virus (HPV) genotypes is considered as a major risk factor related with progression of cervical lesions to intraepithelial neoplasia and/or cervical carcinoma and it is respon-

sible for at least 95% of CC (6, 7). It has been very well established that infection with specific high-risk HPV genotypes is necessary, but not sufficient, to cause virtually all CC, and to be most likely responsible for other anogenital neoplasms and oral squamous cell carcinomas (8).

Given that HPV itself cannot be routinely grown in culture and that its detection relies mostly on a variety of molecular biology techniques; methods for detection HPV DNA sequences (including the possibility of HPV typing) have been introduced to improve diagnosis. However, it has been as-

sumed that DNA testing might eventually complement cytology for routine gynecological screening (9).

DNA testing allows identification of HPV especially in cervical intraepithelial neoplasia (CIN) and CC. Pap-smears with evidences of dysplasia and/or cancer, such as atypical squamous cells of undetermined significance (ASCUS), low-grade and high-grade atypical squamous cells, should undergo molecular HPV identification (10, 11).

The former molecular methods for HPV DNA detection were hybridization-based techniques such as Southern-blot, Dot-blot, and Slot-blot (12). More recently, two of the most widely used assays for detection of genital HPV types are PCR, using consensus primers, and the Hybrid-Capture 2 assay (Digene® Corporation, Gaithersburg, MD, USA). Assays based on hybridization after PCR, such as *Immo-Lipa* (Innogenetics, Gent, Holland) and *Linear Array* (Roche Diagnostics, Branchburg, NJ), or methods that specifically target and amplify each genotype are available to identify individual HPV types. Other PCR-based amplification techniques such as real-time PCR are currently available for detection and to estimate viral load as well (13-16).

The aim of this investigation was to compare two independent PCR assays. The first one, an adapted Nested-PCR assay targeting HPV L1 region (17), using consensus primers MY09/11 and GP5+/6+ (18, 19). Nested-PCR format allows an increase (10-15%) of sensitivity compared with one-step PCR format (20). Since it is well known that part of the L1 region may be lost in some cases during integration of viral genome, whereas the E6/E7 region is always retained and expressed (21, 22), another PCR assay was developed to amplify the early genes E6 and E7. Both amplification methods were tested in comparison and simultaneously with the FDA approved HC2 assay.

## PATIENTS AND METHODS

### Study population

A total of 100 cervical specimens were collected from a random sample of 100 patients out of a population of 395 sexually active women aged from 15 to 69 years old, attending the department of Obstetrics & Gynecology (Gyn/OB) at Instituto Autónomo Hospital Universitario Los Andes (IAHULA) in Mérida State, western Venezuela. Individuals and parents/guardians consent were obtained from each participant following the Helsinki and WHO ethics principles in human research (23). This study was also conducted in compliance with the local institutional ethical board.

Practitioners were instructed to obtain the sample from the transformation zone of the cervix using the DNA collection device (Digene®). Samples were stored at 4°C and transported to the Laboratorio de Microbiología y Salud Pública at the Departamento de Microbiología y Parasitología Clínicas, Universidad de Los Andes within the same day of sample collection. Simultaneously, clinical examination, Papanicolaou smear (Pap-smear) sampling and colposcopy were performed during the same procedure. Colposcopy evaluation was used as a routine diagnostic tool for cervical atypia, in accordance with the guidelines proposed in the Venezuelan Consensus Meeting in Human Papilloma Virus Infections, 2008 (24).

### Colposcopy

All study participants underwent a colposcopic examination of the cervix, vagina, and vulva by the Gyn/OB department specialists. Lesions in the transformation zone (TZ) were assessed by applying 5% acetic acid and iodine solution under  $\times 8$  to  $\times 12$  magnifications. The international nomenclature established by the International Federation for Cervical Pathology and

Colposcopy (IFCPC) was used to classify the colposcopic patterns (25).

### Cytology

Pap-smear analysis of the cervical cells was carried out by a trained cytopathologist (Departamento de Anatomía Patológica, Universidad de Los Andes), who adopted the conventional Bethesda terminology/classification (10).

Smears showing squamous and glandular abnormalities were not separated in our main analysis of low-grade intraepithelial lesions (LSIL) or high-grade intraepithelial lesions (HSIL) for simplicity of data presentation.

### Specimen processing and HC2 testing

Cells in liquid transport medium were pelleted by centrifugation. The pellet was used for DNA isolation in silicon-based column technology using a *QIAamp DNA Mini Kit* (QIAGEN®, Hilden, Germany). Purified DNA was eluted in 100  $\mu$ L of buffer in accordance with the manufacturer's recommendations. DNA-quantification was performed in each sample at 260 nm wavelength (UV1101/1101T, Biotech, Cambridge, UK) which was stored at -20°C until processing. A 75 ng aliquot of the DNA was used for HC2 (Digene®) using both HPV high- and low-risk probe cocktails separately with the help of the Rapid Capture System in accordance with the manufacturer's protocol. The samples were sorted into the following groups based on the HC2 result: group 1 consisted of HC2-positive samples with a high-oncogenic risk; group 2 consisted of HC2-positive samples with a low-oncogenic risk; group 3 consisted of HC2-positive samples for both groups, and group 4 consisted of HC2-negative samples.

### PCR amplification of late (L1) and early (E6/E7) genome regions

DNA extraction processes and all pre- and post-PCR procedures were carried out in separate rooms and cabinets. Buffer and blank controls were always included for the extraction protocol to obtain sufficient numbers of negative controls in order to monitor contamination. To perform a head-to-head comparison of the two assay systems, efforts were made to standardize the quantity of the starting material, therefore a 100 ng of total DNA was used for each PCR assay in a final volume of 25  $\mu$ L. The *HotStar Taq DNA Polymerase Master Mix* (QIAGEN®) was used for all PCR tests. The consensus sequences MY09/11 (18) and GP 5+/6+ (19) were used to amplify L1-region using a Nested-PCR assay. Consensus primers MY09/11 were used for the first PCR, in a mix containing *HotStar Taq DNA Polymerase Master Mix* (QIAGEN®), and 10 pmol of primers PC04/GH20 for the simultaneous amplification of a 248bp product of the human  $\beta$ -globin housekeeping gene (26). A commercially available positive control was used in each PCR assay (HPV-C001, Maxim Biotech, USA). Amplifications were performed with an initial denaturation step of 15min at 95°C, followed by 40 cycles (1 min of denaturation at 94°C, annealing at 55°C/1 min and an extension step at 72°C/1 min) and a final extension of 72°C/10 min. In the second-PCR, using primers GP5+/6+, 1 microliter of the first PCR product was used as template. This reaction was performed in 35 cycles (94°C/1 min, 40°C/2 min and 72°C/1.5 min) with a final extension of 72°C/10 min [adapted from Sotlar *et al.* (17)].

Early genome regions (E6 and E7) were amplified using the consensus sequences GP-E6 3F/5B/6B (17), and 10

pmol of primers PC04/GH20, with an initial denaturation step of 15 min at 95°C, followed by 40 cycles (1 min of denaturation at 94°C, annealing at 55°C/1 min and a extension step at 72°C/1 min) with a final extension step of 72°C/10 min. A commercially available positive control was used in each PCR assay (HPV-4011, Maxim Biotech, USA). All PCRs were performed on an ABI 2400 instrument (Applied Biosystems) and amplification products were visualized on 2% agarose gels containing 10  $\mu$ L of ethidium bromide/100 mL agarose, under UV light (UV transilluminator, Vilber Lourmat, France).

All these assays were performed under stringent conditions, which included separate laboratories for reagent preparation, sample DNA isolation, and thermal cycling.

### Statistical analysis

Patient information was collected in a particular formulary designed ad hoc. Data base and statistical analyses were performed using the EPI Info 2008, version 3.5. A descriptive analysis of the variables was firstly carried out; the percentages were calculated for qualitative variables, means, and standard deviation for numerical variables. The agreement rate, squared-Chi, Fischer test P value (a measure of the imbalance in the distribution of discordant pairs), and Kappa value (27), were calculated. Depending upon concordance force, a qualitative scale was used, <0-20: Poor; 0.21-0.40: Weak; 0.41-0.60: Moderate; 0.61-0.80: Good; 0.81-1.00: Very Good (28). Calculation was performed in a 95% of confidence interval (CI). The sensitivity of PCR assays relative to the HC2 method was the proportion of PCR-positive samples among those in which HC2 were positive, and the specificity of the PCR relative to that of the HC2 method was the proportion of PCR-negative samples, among those in which were HC2-negative samples. The pre-

dictive value of a positive HPV PCR result was the proportion of HC2-positive samples, among those in which samples were PCR-positive, and the predictive value of a negative HPV PCR result was the proportion of the HC2-negative samples, with PCR-negative samples. Similar analyses were performed post hoc by grouping all data regardless the HC2 status group and comparing HPV- late region-PCR versus HPV-early region-PCR assays.

## RESULTS

Cross-tabulation between the HPV PCR assay and the HC2 assay was performed using a total of 100 patients. The common practice of HC2 screening (29) classified HC2 positive/negative samples as high- and low-risk positive assays. Analysis of HC2 assay results along with in-house PCRs for L1 and E6/E7-regions are showed in Table I and summarized in Table II. HC2 allowed detection of HPV in 12% of the cases, while L1-PCR and E6/E7-PCR assays amplified HPV DNA in the 26%. Taking into account overall data, combined analysis (at least one of the assays) showed HPV-positive results in 40% of the samples, while 60% were negative for HPV DNA.

The agreement between both PCR assays (L1 versus E6/E7 regions) was high as shown in Fig. 1. Amplification occurred in 16% using both genomic regions, while 10% of samples amplification occurred only with L1-region and 10% using E6/E7 assays. Sixty-one percent were HPV-negative for these molecular methods.

The agreement rate between HC2 versus both PCR assays (L1 versus E6/E7 regions) was 65% (Fig. 2). Kappa co-efficiency showed moderate concordance ( $\kappa=0.55$ ; CI 95%) between HC2 and L1-PCR, likewise between HC2 and E6/E7-PCR showing a moderate agreement ( $\kappa=0.55$ ; CI 95%). As also shown in Fig. 2, Moderate concordance

**TABLE I**  
COMPARATIVE ANALYSIS OF HC2 ASSAY AND IN-HOUSE PCRS FOR L1 AND E6/E7-REGIONS FROM CERVICAL SAMPLES OF WOMEN ATTENDING THE GYN/OB OUTPATIENT CLINIC (IAHULA). MÉRIDA STATE, WESTERN VENEZUELA. 2008

Patient #	HC2	PCR Amplifications		Patient #	HC2	PCR Amplifications	
		L1 Region	E6/E7 Region			L1 Region	E6/E7 Region
001	+	+	+	022	-	-	+
002	+	-	-	023	+	+	-
003	+	+	+	024	-	+	+
004	+	+	+	025	-	-	+
005	+	-	+	026	-	+	-
006	+	+	-	027	+	-	-
007	-	-	+	028	-	+	-
008	+	-	+	029	-	+	-
009	-	+	-	030	+	-	-
010	-	+	+	031	-	-	+
011	-	+	+	032	-	+	-
012	-	+	+	033	+	-	-
013	-	+	+	034	-	+	-
014	-	+	+	035	-	+	+
015	-	-	+	036	-	+	+
016	-	-	+	037	-	+	-
017	-	-	+	038	-	+	+
018	-	-	+	039	-	+	+
019	-	+	+	040	+	+	+
020	-	+	+	Positives (Total)	12	26	26
021	-	+	-				

**TABLE II**  
SUMMARY OF HC2 ASSAY AND IN-HOUSE PCRS FOR L1 AND E6/E7-REGIONS COMPARISON FROM CERVICAL SAMPLES OF WOMEN ATTENDING THE GYN/OB OUTPATIENT CLINIC (IAHULA). MÉRIDA STATE, WESTERN VENEZUELA. 2008

Tests	HC2 Positive	HC2 Negative	Kappa value (95% CI)
L1 PCR Positive	6	20	
L1 PCR Negative	6	8	0.55
<b>TOTAL</b>	<b>12</b>	<b>28</b>	
E6/E7 PCR Positive	6	20	
E6/E7 PCR Negative	6	8	0.55
<b>TOTAL</b>	<b>12</b>	<b>28</b>	

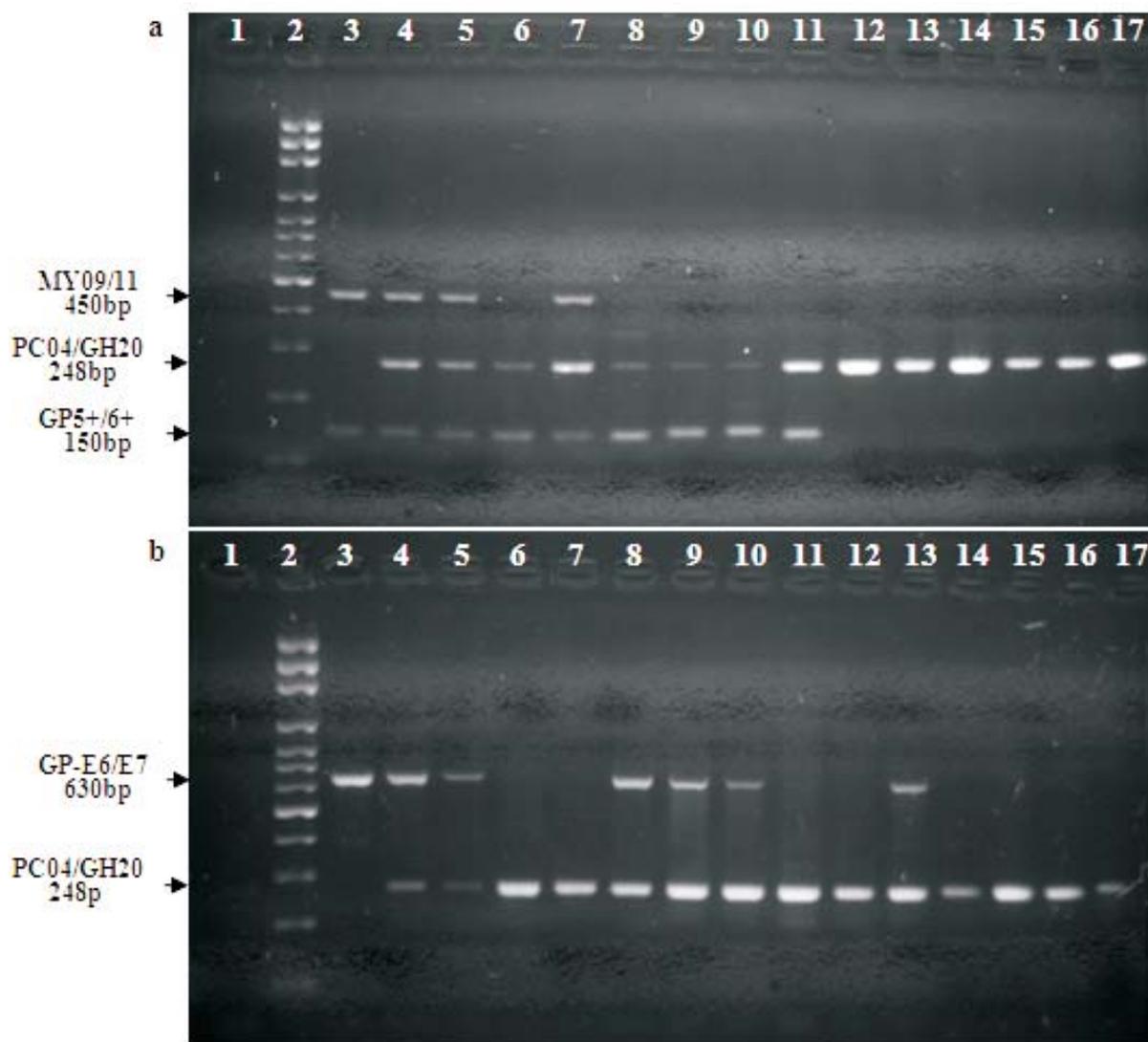


Fig. 1. Agreement between L1-Nested-PCR and E6/E7-PCR assays. DNA from cervical samples was isolated and amplified by PCR (see methods), amplification products were visualized on 2% agarose gels containing 10  $\mu$ L of ethidium bromide/100 mL. PCR with primers PC04/GH20 was performed for the simultaneous amplification of a 248-bp product of the human beta-globin housekeeping gene. a) L1-Nested-PCR assay. b) E6/E7-PCR assay. (1) Negative control; (2) Molecular weight ladder; (3a) Positive control HPV-C001 MB, amplicon of 450bp, consensus sequences MY09/11 and 150bp, consensus sequences GP5+/6+; (3b) Positive control HPV-C001 MB, amplicon of 630bp, consensus sequences GP-E6/E7; (4-5; 8-10) HPV DNA positive samples for both L1 and E6/E7 regions; (6-7; 11) HPV DNA positive samples for L1-region only; (13) HPV DNA positive samples for E6/E7-region only; (12; 14-17) HPV DNA negative samples for both L1 and E6/E7 regions. This in agarose gel electrophoresis shows a duplicate assay.

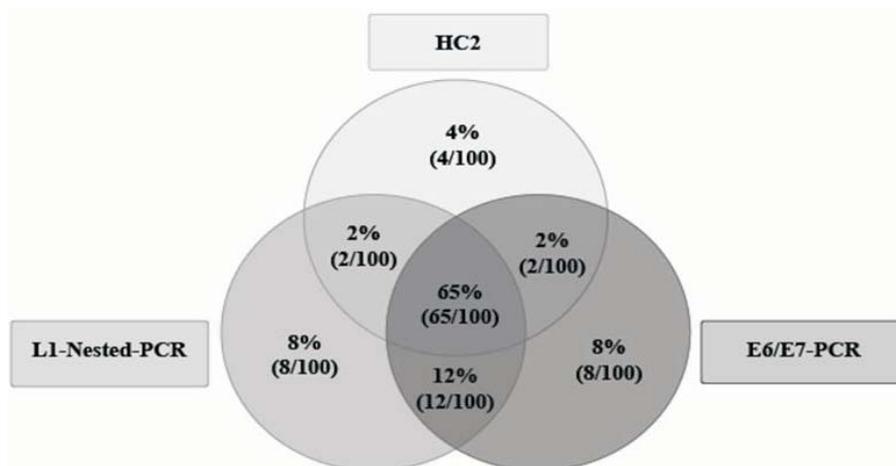


Fig. 2. Relative agreement rates between HC2 and in-house PCR assays: L1-Nested-PCR versus E6/E7-PCR, according to the results reported in patients evaluated for the detection of HPV DNA. Cross-section between the three circles represents identical results, both positive and negative.

was also detected when L1-PCR and E6/E7-PCR were compared ( $\kappa=0.48$ ; CI 95%).

Table III shows the varying degrees of dysplasia and the histological findings of patients' cervical colposcopy versus findings of molecular assays for HPV detection. There was a significant association ( $p=0.006$ ; 2-tail-square-Chi-Mantel-Haenszel Test) between the Schiller test and HPV E6/E7-PCR detection. Otherwise, Pap-smear examination of cervical samples showed that 9% of the samples had cytological changes suggesting HPV infection (Table IV) with changes described as LSIL, from which 7 out of 8 were HPV-positive by at least one of the molecular assays. Correlation between results of the cytological diagnosis and HPV diagnosis by both L1- and E6/E7-PCR tests, showed that 6 out of 8 of the HPV positive samples had cytological changes suggesting HPV infection.

## DISCUSSION

Two main stages could be considered in HPV infection epidemiology, in one hand, type distribution of cervical HPV infection

in women with normal cytology and, in the other hand, in women with abnormal cytology. Recently, a meta-analysis of relevant studies concerning the worldwide prevalence and genotype distribution of cervical HPV DNA in women with normal and abnormal cytology has been published, concluding that HPV testing in women was associated with a significant reduction of progression of cervical lesions to advanced CC and deaths from CC (30). In the present manuscript, analyses of HPV detection by HC2 versus two in-house PCR methods were performed in 100 cervical samples from sexually active women examined in a local university hospital. The overall detection was estimated to be 40%, in contrast with World Health Organization estimation that described a 10% of prevalence in patients with normal cytology, and 87.2% in cervical cancer cases (31), although the main objective of this study was not to calculate any prevalence.

In Venezuela, there are two studies performed in Mérida State revealing a prevalence of HPV infection among the general population that range between 12.54% and 51.9%, using HC2 and PCR, respectively

**TABLE III**  
CORRELATION BETWEEN RESULTS OF COLPOSCOPY AND HPV DNA DETECTION

HPV detection by molecular biology methods <sup>∞</sup>		Colposcopic changes suggestive of HPV infection				Total
		Schiller Test Positive		Schiller Test Negative		
		n	%	n	%	
Positive*		17	17.0	23	23.0	40
Negative		15	15.0	45	45.0	60
Total		32	32.0	68	68.0	100
HC2	Positive	4	4.0	8	8.0	12
	Negative	28	28.0	60	60.0	88
L1-Nested-PCR	Positive	12	12.0	14	14.0	26
	Negative	20	20.0	54	54.0	74
E6/E7-PCR	Positive	14	14.0	12	12.0	26
	Negative	18	18.0	56	56.0	74

<sup>∞</sup>HPV detection by molecular biology: colposcopic changes suggestive of HPV infection associate to Hybrid-Capture 2 (HC2),  $p=0.916$ ; colposcopic changes associate to Nested-PCR-L1 (MY 09/11 and GP 5+/6+),  $p=0.07$ ; and PCR-E6/E7 (GP E6/E7),  $p=0.006$ . \* "Positive" means being positive for any of the three tests.

**TABLE IV**  
CORRELATION BETWEEN CYTOLOGY AND HPV TESTING

HPV detection by molecular methods <sup>∞</sup>		Cytology findings							
		Cytology changes not suggestive of HPV		Cytology changes suggestive of HPV				Unsatisfactory smears	
				ASCUS-H*		LGIL <sup>‡</sup>			
		n	%	n	%	n	%	n	%
Positive*		31	31.0	1	1.0	7	7.0	1	1.0
Negative		59	59.0	-	-	1	1.0	-	-
Total		90	90.0	1	1.0	8	8.0	1	100.0
HC2	Positive	8	8.0	1	1.0	2	2.0	1	1.0
	Negative	82	82.0	-	-	6	6.0	-	-
L1-Nested-PCR	Positive	19	19.0	1	1.0	6	6.0	-	-
	Negative	71	71.0	-	-	2	2.0	1	1.0
E6/E7-PCR	Positive	18	18.0	1	1.0	6	6.0	1	1.0
	Negative	72	72.0	-	-	2	2.0	-	-

<sup>∞</sup>HPV detection by molecular biology: Cytology changes suggestive of HPV infection associated to Hybrid-Capture 2 (HC2),  $p=0.027$ ; changes associated to L1-Nested-PCR (MY 09/11 & GP 5+/6+),  $p=0.0002$  and E6/E7-PCR (GP E6/E7),  $p=0.0001$ . \*ASCUS-H: Atypical Squamous Cells High not excluding High-grade Intraepithelial Lesion (HSIL). <sup>‡</sup>LGIL: Low-grade Intraepithelial Lesion. \* "Positive" means being positive for any of the three tests.

(3,32). Other reports from Cojedes State (central Venezuela) reported a prevalence of 27% (33). In other Venezuelan regions such as Caracas and Sucre, HPV infection have been detected in samples from cervical lesions with prevalence varying from 43% to 98% using different molecular methods (34-37).

Hybrid-Capture 2 assay has been the recommended method by the FDA Committee as a gold standard for diagnosis of HPV in clinical samples. It has been proposed that primary screening with HPV DNA testing should be restricted to women of 30 years of age or older with previous abnormal cytology report (29, 38). HC2 assay was used in this study without discrimination of any age range or cytology status, which could be a limitation of our data; however, transient HPV infections are more common among younger women than older women. Therefore, molecular detection of HPV can show variations depending upon the methods of screening or gene targeting (11, 39), and sample collection (40).

The comparisons described herein examine the results of two PCR methods designed for screening of HPV showing a comparable agreement between sensitivity and specificity of both L1 and E6/E7 genes amplification. To ensure the accuracy and consensus of PCR tests, stringent criteria governing the acceptance of a PCR result were established, with controls being within specifications. Similar results have been reported for detection of early genes by others, particularly for HPV16, 18, 33, 43, and 49 genotypes, where as low as  $10^2$  to  $10^4$  viral DNA copies can be detected (41). Likewise, L1-PCR assay established by Sotlar *et al.* (17), could detect as low as  $10^2$  viral DNA copies, for genotypes 31, 56 and 58.

In this study, results showed that the sensitivity of the L1-region and the E6/E7-region PCR assays were overall the

same compared to HC2; the agreement between the three methods for each patient taken individually was 65% (Fig. 2), with a Moderate concordance rate between HC2 and both PCR assays (L1 versus E6/E7 regions). Depending upon the DNA assay, variability in the agreement between HC2 and PCR technology has been described (42, 43); for this reason, it has been proposed that the performance of some comparative studies using different methods for HPV DNA detection would be advisable in a high population of patients including the most reliable and homogeneous conditions. Nevertheless, standardization and use of the MY/GP PCR system could aid physicians in providing more reliable HPV screening methods. The relationship between oncogenic HPV and cancer indicates the relevance of being able to quickly detect specific HPV types when it is suspected that a lesion may harbor HPV. This would aid in the assessment of lesions and impact on the decision whether to treat it before its progression to a more severe form of the disease.

Finally, when results were compared for colposcopy and conventional cytology for HPV diagnosis based upon HPV DNA detection, it was found that the second one has a better predictive value for HPV diagnosis than colposcopy. It is recognized that HPV testing has not or little value in the management of women found to have LSIL on cytology evaluation; yet results obtained in a randomized screening study showed that detection for persistent HPV type-specific infection by molecular biology methods in combination with cytological diagnosis, increased the sensitivity for detecting CIN II and CIN III compared with cytology screening studies alone (44). Nevertheless, the American Society for Colposcopy and Cervical Pathology (ASCCP) recommends that these women should undergo colposcopy instead of HPV

testing (45). Therefore, colposcopy is a well-recognized tool for detection of cervical lesions and its application can reduce mortality due to cervical cancer in geographical areas where molecular methods are not available (46).

In conclusion, the agreement rate between HC2 versus both PCR assays was moderate, as well as the concordance between L1-PCR and E6/E7-PCR for HPV DNA detection. Molecular biology methods need to be further analyzed in order to make the right decision when selecting the proper HPV method for screening along with cytology or colposcopy procedures. A nested PCR approach may be required to confirm negativity or to detect low levels of HPV. Standardization of highly sensitive PCR methods for the detection of high-risk HPV types depends on variables such as a particular gene region and primer sequences design need to be homogenous between laboratories for robust routine mass screening and for evaluating the value of PCR-based HPV testing in cervical cancer screening programs feasible in the near future.

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