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The diagnostic performance of classical molecular tests used for detecting human papillomavirus

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A B S T R A C T

Cervical samples were evaluated for human papillomavirus (HPV) presence using the hybrid capture-2 (HC2) assay and the polymerase chain reaction (PCR) with three different primer sets (GP5+/6+, MY09/11 and pU1M/2R). PCR results were compared to HC2 and results of all assays were compared to cytological and colposcopy findings. Post-test probability was assessed in individual assays and test combinations. HPV-DNA prevalence was 36.5% with HC2 and 55.2% with PCR. MY09/11 detected HPV-DNA in 38% of samples, GP5+/6+ in 19.1% and pU1M/2R in 16.4%. pU1M/2R and HC2 had the highest concordance (75.31%, $k = 0.39$ in the whole population; 74.1%, $k = 0.5$ in women with abnormal cytology). pU1M/2R had the best diagnostic performance, including optimal post-test probabilities and cervical abnormality detection (individually or in a panel of tests). Women positive for pU1M/2R may be at higher risk of disease progression; the assay performance when combined with a Pap smear in cervical cancer screening programs should be evaluated.

1. Introduction

Cervical cancer is the second oncological cause of death in women worldwide (Bosch and de Sanjose, 2002); as 85% of new cases occur in developing countries where access to screening programs is limited then this preventable disease constitutes a major public health issue (Franco et al., 2003). There are 36.4 cases/year/100,000 women age standardized incidence in Colombia associated with 18.2 cases/year/100,000 women age standardized mortality according to the latest report issued by the World Health Organization in association with the Institut Català d'Oncologia (WHO/ICO, Summary Report 2010).

The main risk factor associated with development of cervical cancer is persistent infection with a high risk human papillomavirus (HR-HPV) altering the physiological cell cycle control and promoting abnormal proliferation of affected cells (Schlecht et al., 2001). Even though HPV infection is a necessary cause of cervical cancer (Girianelli et al., 2009), many other risk factors are involved, such as the life-style of individuals and populations, sexual practices and intrinsic genetic characteristics; these have also been studied widely (Castellsague et al., 2002).

Most women (±90%) who become infected with HPV clear the infection spontaneously within a two-year period; however, a small number will develop cellular atypia but most will experience regression of the lesions requiring no medical intervention and only a small percentage will suffer persistent infection leading to malignant transformation (Moscicki et al., 2006). The Pap smear test is used widely for detecting such cervical abnormalities and, in spite of the high false negative rate and the fact that cellular changes are detected during late stages of infection, this method has proven to be effective in reducing the burden of cervical cancer-derived disease (Nanda et al., 2000). Many molecular biology techniques have become available for clinical and research purposes in response to the need for identifying infection during earlier stages and improving patient follow-up (Molijn et al., 2005).

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Hybrid capture-II (HC2; Digene, Gaithersburg, MD, USA) is a molecular technique which has been approved by the US Food and Drug Administration (FDA) for DNA-HPV identification; it uses RNA-labeled probes for targeting DNA sequences from 13 HR-HPV and 5 low-risk types (Vernick and Steigman, 2003). Although its use has been widespread in the medical field, it has several limitations including the inability to identify specific types and involves the possibility of crossed-reaction between two sets of probes (Poljak et al., 1999). By contrast, polymerase chain reaction (PCR) is a highly sensitive and specific technique which detects viral DNA even when sample substrate is scarce. The limitations associated with PCR-based HPV-DNA detection are related to primer selection and optimal protocol standardization (Iftner and Villa, 2003). Additionally, the PCR assay can detect residual HPV-DNA from recently resolved infections where cytology is normal; this lowers the sensitivity level for assessing which patients will develop cervical cancer.

The present study was aimed at comparing the performance of different molecular methods for detecting HPV-DNA in cervical samples: HC2 (reference test for HPV-DNA infection) and PCR. Three different primer sets were used for the latter; they were directed towards two viral regions whose integrity is differentially affected during viral integration into the host cell genome. All molecular tests were compared with cytological and colposcopy results for the same women as reference tests for assessing cervical conditions.

2. Materials and methods

2.1. Patient characteristics

A total of 400 women were considered for this study; their voluntary attendance at four Colombian medical centers involved taking a routine cytology test between August 2007 and December 2008. Three of these centers were located in the capital city (Bogota): (i) "Fontibón" ($n = 47$), (ii) "Bosa" ($n = 115$), (iii) "Engativa" ($n = 105$); the fourth center was located in a popular tourist destination near Bogotá, (iv) "Girardot" ($n = 133$).

2.2. Ethical approval

This study was approved by the pertinent ethics committees at Fundación Instituto de Inmunología de Colombia, Hospital Fontibón, Hospital Pablo VI in Bosa, the Hospital de Engativá and the Nuevo Hospital San Rafael in Girardot. Prior to sample testing, all the women received information about the purpose of the study, as well as the risks and benefits associated with it. They then signed a written informed consent form and completed a questionnaire so that sociodemographic data and the risk factors of interest could be obtained. After the samples had been processed, the official results were sent to all the institutions participating in this study and included in the patients' medical records, thereby providing valuable information for the primary care doctor.

2.3. Collection of cervical cells

The Pap smear samples were collected and processed following official Colombian health service guidelines. Women whose samples showed any degree of abnormality were given a colposcopy examination, following the recommendations and procedures laid down in the mandatory Colombian unified health plan (Fig. 1). The Bethesda system was used for reporting the cytological and colposcopy findings (Solomon et al., 2002), and all cases were reviewed by an experienced gynecological pathologist.

2.4. Human papillomavirus DNA detection

Simultaneously, cervical samples were taken for both HPV-PCR and HC2; 95% ethanol (Lema et al., 2001) and Specimen Transport Medium (STM; Digene, Gaithersburg, MD, USA) were used as means of preservation and transport, respectively. An independent entity (Laboratorio-SIPLAS, Sociedad Interdisciplinaria para la Salud) did the HC2 tests based on commercially validated protocols for detecting high-risk HPV types. Test results from all study sites were masked from researchers until data analysis was completed.

DNA was extracted from epithelial cells found in specimens for PCR using a QuickExtract DNA extraction solution commercial kit (Epicentre, Madison, WI), following the manufacturer's instructions. PCR for human β-globin was carried out on all the samples processed using the GH20 and PC04 primers which amplified a 262 base pair segment; this PCR was used to verify the quality of the DNA (Saiki et al., 1985).

The samples showing human β-globin gene amplification were analyzed using three primer sets (GP5+/6+, MY09/11 and pU1M/2R). PCR amplifications were carried out simultaneously and independently in the standardized conditions reported by previous studies (de Roda Husman et al., 1995; Fujinaga et al., 1991; Gravitt et al., 2000), including measures for preventing contamination. Samples which had amplification products using any of the three primer sets were considered to have had positive PCR results for HPV infection. Positive controls were included in all assays; plasmids containing the HPV-16 L1 gene extracted from transfected Sf21 cells were used in the assays carried out with GP5+/6+ and MY09/11, while known positive samples were used in assays carried out with pU1M/2R. DNase- and RNase-free water were used as PCR negative control in all amplifications.

2.5. Statistical analysis

Descriptive statistics were used for clinical data to summarize the characteristics of the baseline population, including the main clinical and demographic variables. A concordance test (Kappa) estimated the agreement between the results for methods tested in the population as a whole and in different categories, according to the presence of cervical abnormality. Kappa values were classified as follows: 0–0.2 = poor, 0.21–0.4 = slight, 0.41–0.6 = fair, 0.61–0.8 = moderate, 0.81–0.99 = substantial and 1.0 = perfect agreement (Remmerbach et al., 2004).

Independent multivariate logistic regression models were run, including the results for all five methods assessed (i. HC2, ii. PCR, iii. GP5+/6+, iv. MY09/11, v. pU1M/2R) as dependent variables, and cytological or colposcopy findings as independent variables. Odds ratios (OR) and 95% confidence intervals (95% CI) were adjusted to a group of covariables (age, ethnicity, marital status, smoking, age of first intercourse, number of sexual partners, pregnancies, contraceptive methods used and history of sexually transmitted diseases (STDs)), all of them are variables that have been associated with an increased risk of infection, however, correlation does not prove causation. A <0.05 P value was considered significant. The Hosmer–Lemeshow goodness-of-fit test was used for assessing the suitability of the logistic model.

All PCR test results were compared to HC2 to evaluate each assay's performance as a tool for detecting HPV infection; the five molecular assay results were then compared to those obtained by cytology or colposcopy. Sensitivity and specificity data were adjusted as these tests are considered to be an imperfect gold standard, given the sensitivity and specificity values reported in previous studies for each of them (Cox et al., 1995; Kumar et al., 2007; Wu et al., 2005). This adjustment was based on an algorithm assuming conditional independence (Zhou et al., 2008). Predictive values and likelihood ratios were calculated for each comparison.

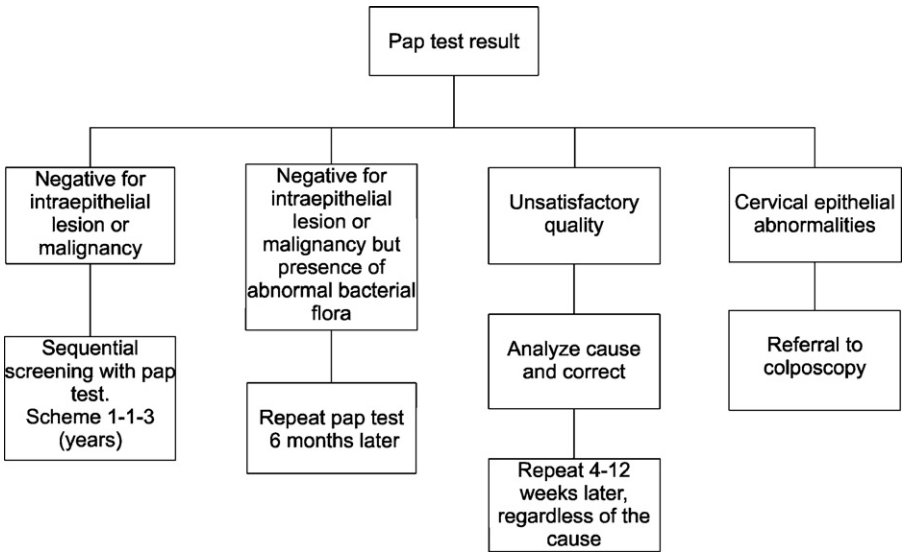

Fig. 1. Screening strategies for cervical cancer detection and control in Colombia. Routine screening according to the guidelines of the cervical cancer detection and control program of the general health security system in Colombia (Posso et al., 2005).

The likelihood ratios for a positive test result (LR+) or a negative test result (LR−) were used to calculate post-test probability, a predictive analysis being made for diagnostic probability using a specific method or combinations of methods. The pre-test probability of HPV infection was compared to the estimated later probability of disease using the information provided by the diagnostic test. The difference between previous probability and later probability was used as a way to analyze the efficiency of the specific diagnostic method or combinations of methods used in parallel. A Diagnostic Test Calculator was used for making the LR calculations (Schwartz, 2002–2007) and STATA 10 software was used for the rest of the analyses.

3. Results

Three of the samples were excluded from statistical analysis due to the incompleteness of the required data. The mean age of the population analyzed ($n = 397$) was 34.7 ± 10.4 years [range: 16–63]. Table 1 shows the distribution of other socio-demographic variables and the risk factors included in this study.

HPV infection prevalence was 55.2% when screening with PCR and 36.5% when using HC2. When discriminating PCR results according to the primer set used, positive readings were found in 19.1% of the samples when using GP5+/6+, 38.0% with MY09/11 and 16.4% with pU1M/2R.

Abnormal cytology findings were detected in 36.0% ($n = 143$) of total samples processed, of which HPV-DNA was detected in 53.8% ($n = 77$) by HC2 and 62.2% ($n = 89$) by PCR; among PCR positive samples, 37.1% ($n = 33$) of them were detected with GP5+/6+, 68.5% ($n = 61$) with MY09/11 and 47.2% ($n = 42$) with pU1M/2R. Of the total number of lesions confirmed by colposcopy ($n = 99$, 24.9%), HC2 detected 56.6% ($n = 56$) and PCR 63.6% ($n = 63$), whereas GP5+/6+ detected HPV-DNA in 30.1% ($n = 19$) of the samples, MY09/11 in 80.9% ($n = 51$) and pU1M/2R in 47.6% ($n = 30$).

A total of 42 (10.6%) samples proved positive in HC2 and negative in PCR, most of them having relative luminescence units (RLU) values ranging from 1.1 to 5 (95.2% ($n = 40$)). Within this subpopulation 17 (40.5%) women also had abnormal cytology, 11 (64.7%) of whom were diagnosed as having some degree of cervical lesion in colposcopy evaluation. Another subgroup

Table 1
Sociodemographic characteristics of the population studied.

Variable (n) category	Individuals per category n (%)[a]
Age (396 women)	
≤20	30 (7.6)
21–30	129 (32.6)
31–40	117 (29.5)
≥41	120 (30.3)
Ethnic background (374)	
White	73 (19.5)
Mestizo	296 (79.2)
African-American	5 (1.3)
Marital status (390)	
Single	90 (23.1)
Married	263 (67.4)
Divorced/widowed	37 (9.5)
Smoker (394)	
No	303 (76.9)
Yes	91(23.1)
Age of first intercourse (396)	
≤16	146 (36.9)
17–19	160 (40.4)
≥20	90 (22.7)
Sexual partners ($n = 390$)	
1	133 (34.1)
2–3	196 (50.3)
≥3	61 (15.6)
Pregnancies (394)	
None	33 (8.4)
1–2	177 (44.9)
≥3	184 (46.7)
Contraceptive methods (380)	
No method used	120 (31.6)
Hormonal	72 (18.9)
Other	188 (49.5)
STD (380)	
Yes	62 (16.3)
No	318 (83.7)

[a] Categories had less than 397, given that data was missing from the surveys. STDs: history of sexually transmitted disease.

Table 2
Degree of cervical abnormality according to cytology and colposcopy results.

Diagnostic test	Total, *n* (%)[a]	ASCUS, *n* (%)[b]	LSIL, *n* (%)[b]	HSIL, *n* (%)[b]
Cytology	368 (92.7)	90 (24.5)	46 (12.5)	7 (1.9)
Colposcopy	196 (49.4)	–	95 (48.5)	4 (2.0)

[a] Percentage of individuals having a result recorded for each test in the total population ($n = 397$).
[b] Rate of subjects having positive test from the total of individuals with the result for each particular technique. – Abnormality not detectable by colposcopy, ASCUS: atypical squamous cells of undetermined significance, HSIL: high-grade squamous intraepithelial lesions, LSIL: low-grade squamous intraepithelial lesions.

consisted of 116 individuals testing positive in PCR and negative in HC2 (29.2%), 25.0% ($n = 29$) of whom had abnormal cytology and more than half of them (62.1%, $n = 18$) had abnormal findings by colposcopy.

Table 2 shows cervical cytology and colposcopy results based on the degree of abnormality. A group of 37 women (9.3%) had negative results for all molecular tests, in spite of having abnormal findings in cytology; 67.6% ($n = 25$) of these women had readings consistent with atypical squamous cells of undetermined significance, 29.7% ($n = 11$) with low-grade squamous intraepithelial lesion and 2.7% ($n = 1$) with high-grade squamous intraepithelial lesion. All of them underwent colposcopy and lesions were identified in 67.5% ($n = 25$) of the cases, including a patient reported as having high-grade squamous intraepithelial lesion.

Overall agreement between HC2 and PCR was estimated to be 60.2% ($k = 0.2$) and the tests having the highest agreement were PCR using pU1M/2R and the HC2 assay (75.3%, k: 0.4). The correlation between the rest of the tests used was poor (see Supplementary file 1). An even higher agreement between HC2 and PCR was observed when cytology and colposcopy results were categorized as being normal/abnormal, having 67.8% (k: 0.3) and 70.7% (k: 0.4) agreement rates, respectively. The pU1M/2R primer set had the highest correlation with HC2 results among the individual PCR assays (74.1%, k: 0.5 for abnormal cytology and 71.7%, k: 0.5 for colposcopy-proven lesion).

Table 3 shows the ORs (adjusted for risk factors, as described previously) showing the association between tests and lesion severity in cytology (A) and colposcopy (B). An additional statistical analysis was run to estimate the ORs, cytology being recorded as either normal or abnormal. These results showed that the association between the pU1M/2R primer set (5.2-CI: 2.4–10.9) and the HC2 assay (2.9-CI: 1.7–5.0) was still significant, whereas colposcopy was only significantly associated with HC2 (2.5-CI: 1.2–5.8) (data not shown).

Table 4 shows the features for each assay regarding its promise as a diagnostic test (sensitivity, specificity, predictive values and likelihood ratios) based on the prevalence obtained from results yielded by techniques considered as standard (HC2 for HPV infection and cytology or colposcopy for cervical abnormalities). Table 5 shows the post-test probabilities for single tests and combinations of tests using the prevalence reported by reference tests found in the pertinent literature.

4. Discussion

Prior studies conducted all around the globe have reported HPV-DNA prevalence determined by PCR, ranging from 5% in certain European regions (de Sanjose et al., 2007) to 50% in some Latin-American countries (Tabora et al., 2009); such significant variation could be related to intrinsic population characteristics and the screening methods used (Bosch and de Sanjose, 2003). Previous research conducted in Colombia using a single primer set (GP5+/6+) to estimate HPV-DNA prevalence in patients having normal cytology has reported positive findings in 14.8% of its sample (Molano et al., 2002). 49.2% prevalence was detected later in cytology samples during 2009 using two different primers sets (GP5+/6+ and MY09/11) (Soto-De Leon et al., 2009), similar to that being reported now using three primers sets (55.2%).

Such relatively higher HPV prevalence could have been the result of the simultaneous use of primers targeting two viral genome regions: (i) GP5+/6+ (Remmerbach et al., 2004) and MY09/11(Qu et al., 1997) both directed towards the L1 late protein promoter and (ii) pU1M/2R, directed at the E6–E7 genes (Fujinaga et al., 1991). This approach was based on published data suggesting that the viral genome undergoes changes during infection thereby allowing it to become integrated into the genome host cell, resulting in total or partial loss of DNA segments. The only regions consistently found to remain intact through the whole microorganism life-cycle are the long control region and E6–E7 oncogenes (Rauber et al., 2008), supporting their use as targets for molecular screening techniques, even in patients having active infection where integration may have occurred already.

The percentage of HPV-DNA detection using HC2 (36.5%) was higher than that usually reported for European countries where prevalence is close to 10% (Giorgi Rossi et al., 2010) and was lower than that for Brazil where HPV-DNA has been detected in 44.9% of samples (Carestiato et al., 2006). Data obtained in the present study using the above test as reference was still high, being consistent with data from other Latin-American countries where population demographics and screening characteristics are similar. Furthermore, the observations indicated that PCR was able to detect viral DNA in more patients compared to HC2, especially in women having abnormal cytology (62.2% and 53.8%, respectively). The above arguments support the previously suggested notion that the

Table 3
Multivariate logistic regression analysis showing the association between test result and cervical lesion severity.

A.	Cytology ($n = 369$), OR (95% CI)				
	GP5+/6+	MY09/11	pU1M/2R	PCR	HC2
ASCUS	1.6 (0.8–3.1)	1.19 (0.7–2.1)	5.09* (2.2–11.8)	1.45 (0.8–2.6)	2.57* (1.4–4.8)
LSIL	0.42 (0.1–1.2)	1.75 (0.8–3.7)	4.65* (1.7–12.5)	1.45 (0.7–3.1)	2.94* (1.3–6.5)
HSIL	–	–	48.61* (6.2–381.1)	1.04 (0.2–5.8)	30.12* (2.8–324.5)
B.	Colposcopy ($n = 196$), OR (95% CI)				
	GP5+/6+	MY09/11	pU1M/2R	PCR	HC2
LSIL	0.4 (0.2–0.9)	0.9 (0.4–1.8)	1.7 (0.7–4.0)	0.3 (0.1–0.7)	2.5* (1.6–5.2)
HSIL	0.6 (0.5–6.8)	0.3 (0.0–4.3)	8.0* (0.7–89.7)	0.2 (0.0–1.9)	4.4 (0.7–54.2)

Odd ratios (OR) adjusted for the variables included in Table 1. A. Lesion reported in cytology. B. Lesion described in colposcopy. – The estimator could not be calculated because one of the fields contained no data during the dispersion analysis. ASCUS: Atypical squamous cells of undetermined significance, CI: confidence interval, HSIL: high-grade squamous intraepithelial lesions, LSIL: low-grade squamous intraepithelial lesions. For detailed information on the raw data and OR calculations, see Supplementary file 2.
* Values having $P < 0.05$.

Table 4
Characteristics for each molecular test as a diagnostic tool compared to references test results.

Test	Reference test	Sensitivity % [95% CI]	Specificity % [95% CI]	PPV [95% CI]	NPV [95% CI]	LR (+) [95% CI]	LR (−) [95% CI]
GP5+/6+	HC2	19.2 [11.8–28.1]	80.9 [71.9–88.2]	53.9 [42.1–65.5]	67.6 [62.2–72.7]	2.0 [1.4–3.0]	0.8 [0.7–0.9]
	Cytology	20.1 [12.7–28.2]	79.9 [70.8–87.3]	44.6 [33.0–56.6]	62.6 [56.8–68.1]	1.3 [0.8–1.9]	0.9 [0.8–1.0]
	Colposcopy	26.1[17.7–35.7]	73.8 [64.3–82.3]	37.3 [24.1–51.9]	44.8 [36.6–53.3]	0.6 [0.4–0.9]	1.2 [1.0–1.4]
MY09/11	HC2	38.1 [28.5–51.9]	62.0 [51.8–71.5]	41.7 [33.8–50.0]	66.7 [60.4–72.5]	1.2 [1.0–1.6]	0.9 [0.7–1.0]
	Cytology	39.4 [29.4–47.3]	60.6 [50.7–70.6]	42.1 [33.9–50.5]	63.2 [56.5–69.6]	1.1 [0.9–1.5]	0.9 [0.8–1.1]
	Colposcopy	52.3 [41.8–62.1]	47.7 [37.9–58.2]	49.5 [39.5–59.5]	48.4 [37.9–59.0]	1.0 [0.7–1.2]	1.0 [0.8–1.4]
pU1M/2R	HC2	16.5 [9.43–24.7]	83.7 [75.3–90.6]	86.2 [75.3–93.5]	73.2 [68.1–77.9]	11.0 [5.5–21.0]	0.6 [0.6–0.7]
	Cytology	16.1 [13.8–18.4]	84.0 [81.6–86.2]	71.2 [57.9–82.2]	67.3 [61.8–72.5]	3.9 [2.3–6.6]	0.8 [0.7–0.8]
	Colposcopy	22.6 [14.3–31.4]	77.5 [68.6–85.7]	66.7 [51.0–80.0]	54.3 [46.0–62.4]	2.0 [1.1–3.4]	0.8 [0.7–1.0]
PCR	HC2	55.3 [44.7–65.0]	44.9 [35.0–55.3]	47.0 [40.3–53.9]	76.4 [69.5–82.4]	1.5 [1.3–1.8]	0.5 [0.4–0.7]
	Cytology	56.0 [45.7–65.9]	44.0 [34.1–54.3]	43.2 [36.3–50.3]	66.7 [58.8–73.9]	1.2 [1.0–1.4]	0.8 [0.6–1.0]
	Colposcopy	72.3 [62.1–80.5]	27.6 [14.5–37.9]	44.4 [36.0–52.9]	33.3 [21.1–47.5]	0.8 [0.6–0.9]	2.0 [1.2–3.2]
HC2	Cytology	36.8 [25.6–47.2]	63.4 [52.8–77.4]	57.0 [48.2–65.5]	71.7 [65.4–77.4]	2.1 [1.6–2.7]	0.6 [0.5–0.7]
	Colposcopy	43.7 [33.1–53.3]	56.5 [45.7–65.9]	65.9 [54.8–75.8]	61.3 [51.5–70.4]	1.9 [1.3–2.7]	0.6 [0.5–0.8]

The molecular test results with the prevalence yielded by reference tests, HC2 for HPV infection and cytology-colposcopy for cervical abnormalities were compared for doing these calculations. CI: Confidence interval, HC2: Hybrid capture-II assay, LR: likelihood ratio, NPV: negative predictive value, PPV: positive predictive value. All calculations regarding the LR are included in the Supplementary file 3 (HC2/cytology/colposcopy).

difference in prevalence data yielded by these techniques could have been due to HC2 failing to detect viral DNA in the specific subset of infected patients having low amounts of HPV-DNA (Jastania et al., 2006).

Regarding cytology reports, it was observed that the prevalence of low- and high-grade squamous intraepithelial lesions was higher than that reported in previous studies from around the world; however, other studies carried out in Colombia have found a high prevalence of cervical abnormality (Uribe et al., 2006). This could have been related to low cervical cancer prevention and control program coverage regarding the Colombian population, meaning that activities aimed at identifying women at greater risk of developing this disease should become intensified. The fact that a group of women had negative molecular results and cervical abnormalities emphasizes the important role of cervical cytology as an effective screening strategy in reducing the burden of cervical cancer. In spite of this, the data has also revealed limitations in detecting HPV-infected women when using just Pap smears, thereby indicating that adding molecular techniques to screening regimes would be of great use.

Agreement was low between the techniques used here, similar to that reported in other studies (Castle et al., 2003); this may have been related to the fact that they target different viral genome elements. The highest concordance was found between HC2 and pU1M/2R which target early genome regions, while the other primer sets were aimed at annealing within the L1 protein promoter. Agreement between tests increased when they were tested in the population suffering cervical abnormalities. This could be explained by spectrum bias or have been related to the hypothesis proposed by previous field research stating that low agreement regarding the results for women having normal smears may have been due to fewer viral copies being present during early disease stages (Munoz et al., 2006), thereby decreasing the ability of HC2 to detect DNA since it is a technique based on signal amplification as opposed to PCR assays which amplify target-specific DNA sequences.

A significant association between molecular tests and cervical abnormality was found, showing that pU1M/2R and HC2 had high odds of detecting women with low-grade squamous intraepithelial lesion compared to the normal group; the likelihood of detecting women with high-grade squamous intraepithelial lesion increased by up to 5 times (consistent with colposcopy and cytology results). This could have been related to the fact that they both recognized early viral genes (E6–E7) considered to play a critical role in malignant transformation secondary to HPV infection (Lorincz, 1996).

Analyzing post-test probabilities for combinations of techniques showed that pU1M/2R had the best diagnostic features and performance as it had a very strong association with a confirmatory HPV infection result and cervical abnormality, yielding results superior to those from HC2 and PCR. It is thus suggested that the molecular identification of HPV infection targeting genome regions remaining unchanged during infection is a valid approach; also, this argument can be supported by the findings related to women evaluated by colposcopy where the highest post-test probabilities included negative readings of molecular tests directed towards late viral regions, which presumably become lost during advanced stages. It should be taken into account here that the good analytical sensitivity reported for GP5+/6+ and MY09/11 in other publications

Table 5
Post-test probabilities for test combinations.

Reference test	Pre-test percentage	Post-test percentage for single test (test results)	Post-test percentage for test combinations (test results)		
			Two	Three	Four
HC2	36.5	86.4: pU(+) 23.7: PCR(−)	92.8: pU(+) and GP(+) 23.4: pU(−) and GP(−)	94.1: pU(+), GP(+) and MY(+) 21.0: pU(−), GP(−) and MY(−)	N/A N/A
Cytology	36.0	71.2: pU(+) 28.3: HC(−)	83.8: pU(+) and HC(+) 23.0: pU(−) and HC(−)	86.8: pU(+), GP(+) and HC(+) 21.6: pU(−), HC(−) and MY(−)	88.2: pU(+), GP(+), HC(+) and MY(+) 20.6: pU(−), MY(−), HC(−) and GP(−)
Colposcopy	24.9	66.7: pU(+) 38.7: HC(−)	79.1: pU(+) and HC(+) 34.2: pU(−) and HC(−)	79.9: pU(+), HC(+) and MY(−) 27.8: HC(−), GP(−) and MY(+)	82.5: pU(+), HC(+), GP(−) and MY(−) 22.1: pU(−), HC(−), MY(+) and GP(+)

The table features the test combinations showing the highest positive predictive value, and the results with the lowest negative predictive value. Calculations were made using reference tests as data sources, HC2 for HPV-DNA and cytology-colposcopy for cervical abnormality. N/A, not applicable considering that HC2 is regarded as the reference test for HPV-DNA infection. All calculations can be seen in Supplementary file 3 (Combined, pag. 1 and 2).

should not be mistaken as good clinical performance (Kinney et al., 2010).

Even though the pU1M/2R primer set had greater precision in detecting infection in women having any degree of cervical abnormality, the percentage of positive samples was lower than that found with primer sets directed against late viral regions. Although results obtained with late primer sets would not be as clinically relevant as those yielded by pU1M/2R, it is worth stressing that they might contribute towards gathering knowledge regarding epidemiology and viral biology during infection stages where viral genome integration has not happened. The detection spectra in different viral types were variable for each primer set, which is why it would be advisable to use primer combinations in HPV identification studies.

In conclusion, test performance evaluation showed that the PCR using the pU1M/2R primer set is an optimal screening tool for HPV-DNA detection and its results indicate that it is a more reliable indicator of malignant transformation, when compared to the more frequently used GP5+/6+ and MY09/11 sets; however, these sets have been shown to be useful for monitoring women having persistent HPV infections, remaining as valuable tools for the early prevention of cervical carcinogenesis. This finding has led us to wonder whether including pU1M/2R in cervical cancer screening programs, along with the Pap smear, might provide doctors with information for the early recognition of women at a higher risk of developing cancer. This would also take into consideration that the costs of applying an additional PCR test could be less expensive compared to treatment costs after the disease has developed. Nonetheless, additional longitudinal and diagnostic cost-effectiveness studies are required to assess the diagnostic accuracy of such test during the different stages of the clinical course of HPV infection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jviromet.2012.05.023.

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