Classical Molecular Tests Using Urine Samples as a Potential Screening Tool for Human Papillomavirus Detection in Human Immunodeficiency Virus-Infected Women

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Human papillomavirus (HPV) is the main risk factor associated with the development of cervical cancer (CC); however, there are other factors, such as immunosuppression caused by the human immunodeficiency virus (HIV), that favor progression of the illness. This study was thus aimed at evaluating the functionality of classical PCR-based molecular tests for the generic identification of HPV DNA (GP5+/GP6+, MY09/MY11, and pU1M/2R primers, individually or in combination) using cervical and urine samples from 194 HIV-positive women. Infected samples were tested with type-specific primers for six high-risk types (HPV-16, -18, -31, -33, -45, and -58) and two low-risk types (HPV-6 and -11). HPV infection prevalence rates were 70.1% for the cervical samples and 63.9% for the urine samples. HPV-16 was the most prevalent viral type in the cervical and urine samples, with higher rates of multiple infections than single infections detected in such samples. HPV DNA detection by PCR (mainly with the pU1M/2R primer set) in urine samples was positively associated with abnormal cytological findings (atypical squamous cells of undetermined significance/squamous intraepithelial lesions [ASCUS/SIL]). It was determined that the operative characteristics for detection of cytological abnormalities were similar for cervical and urine samples. This suggested using PCR for the detection of HPV DNA in urine samples as a potential screening strategy for CC prevention in future prevention and control programs along with currently implemented strategies for reducing the impact of the disease, i.e., urine samples are economical, are easy to collect, have wide acceptability among women, and have operative characteristics similar to those of cervical samples.

Cervical cancer (CC) has a great impact on the global female population, having age-standardized incidence and mortality rates of 15.2 and 7.8 cases per 100,000 people, respectively (see http://globocan.iarc.fr). Most new cases occur in developing countries, where screening programs based primarily on the Papanicolaou (Pap) test are limited by several factors, such as sampling and detection errors (1). In addition to reduced coverage, the test's low acceptability among women yields reduced effectiveness in CC prevention programs (2).

CC patients have usually suffered persistent infections by high-risk human papillomavirus (HPV) (3); however, most immunocompetent women (>90%) manage to eliminate such viral infections (4) and spontaneous regression occurs in most cases (around 60% with low-grade lesions) (5), with this being linked to the efficiency of a particular female subject's immune system (6). In contrast, spontaneous regression rates are considerably reduced for immunocompromised women in occasional cases involving HIV coinfection (7), leading to a decline in the number of CD4$^+$ T lymphocytes (8). The probability of elimination of infection and of lesion regression is low in such populations with high HPV infection prevalence rates (5).

Strategies aimed at reducing the impact of CC have been implemented for detection of HPV infections before tissue alterations become evident. These strategies, based on detection of DNA in cervical samples, have led to infected women being identified and have facilitated their monitoring (9); however, the strategies are limited by the discomfort involved in sample collection during gynecological examinations and the requirement for specialized personnel for sample collection. The potential of such screening strategies has thus been restricted (10).

Proposed self-sampling from urine constitutes a foundation for alternative collection methods that are practical, are economical, and have high acceptability among women (2); even though this approach usually yields lower cell counts than direct exfoliation sampling methods, it has been reported to be a convenient screening method, as the viral load in these specimens is associated with the presence of cervical lesions (11). In addition, using urine samples could contribute to strengthening screening programs, as this approach should decrease lesions caused in the cervical epithelial tissue (as with the conventional smear test) and would not stimulate the natural progression of the illness (i.e., tissue damage during smear testing facilitates viral entry) (12). HPV DNA detection in urine samples can be used for screening of at-risk populations, such as women with compromised immunity (3). This study was thus aimed at evaluating the HPV infection-detecting

capacity of classical molecular tests (GP5+/GP6+, MY09/MY11, and pU1M/2R primers, used singly or together) with HIV-infected women's cervical cell samples and self-collected urine samples, comparing test functionality for identifying viral DNA with cytological findings.

MATERIALS AND METHODS

Clinical samples. This study included HIV-positive female subjects with a diagnosis confirmed by Western blotting who were voluntarily attending the Asistencia Científica de Alta Complejidad S.A.S. CC screening program in Bogotá (Colombia). Each woman was informed of the purpose of the study, signed an informed consent form, and completed a specifically prepared survey that was used to collect information pertaining to sociodemographic characteristics and related risk factors.

Initial urine midstream sampling involved self-sampling and storage at 4°C until the samples were processed (within 72 h after collection). Samples were then taken for conventional cervical Pap testing and for molecular identification of HPV DNA; the latter samples were then stored in 95% ethanol as the preservation and transport medium (13). The Pap tests were performed and interpreted at the Instituto de Diagnóstico Médico (IDIME); the findings were reported using the Bethesda classification system (14). This study was approved and supervised by the ethics committee of the participating institutions, i.e., the Asistencia Científica de Alta Complejidad and the Fundación Instituto de Inmunología de Colombia.

Sample processing. The total volume (around 40 ml) of the urine samples was centrifuged at 2,300 × g for 20 min at 4°C. Cervical sample cells were taken from the cytobrush, and 50% of the samples were centrifuged at 15,000 × g for 10 min at 4°C (the other 50% was stored for inclusion in the cell bank). The cell pellet obtained from each sample source was subjected to total DNA extraction using a commercial DNA extraction kit (QuickExtract; Epicentre, Madison, WI), following the manufacturer's instructions. Subsequently, DNA sample integrity was verified by independent PCR amplification of the human β-globin gene, using GH20/PC04 and PC03/PC04 primer sets (15).

HPV detection and type-specific distribution. The positive samples for amplification of the human β-globin gene were subjected to three simultaneous and independent PCR amplifications. Three sets of primers previously reported in the literature and evaluated by our group were used to target two regions of the viral genome for the generic identification of HPV infection. The first targeted region corresponded to the genes encoding the late viral protein L1. Two primer sets were used for this, i.e., GP5+/GP6+, which is characterized by detecting infections with reduced viral loads (16), and MY09/MY11, which identifies women infected with more than one type of HPV (multiple infections) (16). The second targeted region corresponded to the region encoding oncoproteins E6 and E7, which was targeted using the pU1M/2R primer set; this set has shown great precision for detecting infections in women with abnormal cytological findings in previous studies (17).

The samples that were positive with any of the three generic primer sets were amplified with type-specific primers for six high-risk types (HPV-16, -18, -31, -33, -45, and -58) that are linked to >85% of CC cases (18) and two low-risk types (HPV-6 and -11) that are reported to be more globally prevalent (19). Independently evaluated samples showing an amplification product for each type-specific reaction were considered positive for each viral type. The PCR amplification conditions and controls used have been reported previously (17, 20). All PCR products were assessed by visualization in 2% agarose gels.

Statistical analysis. Means and standard deviations (SDs) were used to describe continuous variables; categorical variables were expressed in terms of frequencies and percentages. The frequencies of events of interest were reported together with their corresponding 95% confidence intervals (CIs), calculated using the bootstrap method.

Agreement between the results for detecting HPV infection in the two sample sources was evaluated with kappa coefficients and corresponding 95% confidence intervals. Agreement in the detection of each viral type in the cervical samples and urine samples was calculated according to the results of each HPV DNA detection test. The strength of association between each test result and the variables of interest, such as sociodemographic characteristics, coinfection, and cytological findings, was measured using crude odds ratios (ORs) (with corresponding 95% CIs).

The operative characteristics of the cervical and urine sample HPV tests were evaluated to determine their sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs), taking cytological results as an imperfect gold standard; this adjustment was based on an algorithm assuming conditional independence (21) and took previously reported sensitivity (87%) and specificity (66%) values into account regarding cervical cytology in a population of HIV-infected women (22). Stata 10 software was used for the statistical analysis.

RESULTS

Fifty-one of the 245 HIV-positive women enrolled were excluded; 18 of the samples collected did not match (the sample from one of the sources was missing), 23 of the specimens (cervical or urine specimens) tested negative for amplification of the human β-globin gene, and 10 of the samples were missing or reported unsatisfactory in Pap test results. A total of 194 paired samples were included for statistical analysis (mean age, 38.0 years; SD, 10.8 years). In terms of sociodemographic characteristics, most women (45.4% [$n = 88$] [95% CI, 38.2 to 52.6%]) were <34 years of age, primarily being white (68.4% [$n = 130$] [95% CI, 61.3 to 75.0%]) or of mestizo ethnicity (27.9% [$n = 53$] [95% CI, 21.6 to 34.8%]). Variables related to risk factors showed that 60.8% ($n = 115$) (95% CI, 53.5 to 63.8%) of the women had started sexual activity before the age of 18 years, 36.7% ($n = 69$) (95% CI, 29.8 to 44.0%) stated that they had had four or more sexual partners, and the most widely used contraceptive method was the barrier method, being used by 32.9% ($n = 52$) (95% CI, 25.7 to 40.8%) of the women.

The rates of HPV infection prevalence (defined as samples having a positive result with any of the three primer sets evaluated, i.e., GP5+/GP6+, MY09/MY11, and/or pU1M2R) were 70.1% ($n = 136$) (95% CI, 63.4 to 76.8%) in cervical samples and 63.9% ($n = 124$) (95% CI, 56.9 to 70.9%) in urine samples, with greater positivity frequency being found in the cervical samples than in the urine samples; this difference was statistically significant (χ^2 [1 degree of freedom] = 6.9486; $P = 0.008$). Table 1 describes HPV infection frequencies for both sample sources according to sociodemographic and risk factor categorization.

Single and multiple (simultaneous infection by more than one type of HPV) infection frequencies were defined. The overall single infection prevalence rates were 19.6% ($n = 38$) (95% CI, 14.2 to 25.9%) for cervical samples and 25.8% ($n = 50$) (95% CI, 19.8 to 32.6%) for urine samples. These rates were lower than multiple infection prevalence rates, i.e., 54.6% ($n = 106$) (95% CI, 47.4 to 61.8%) in cervical samples and 40.2% ($n = 78$) (95% CI, 33.2 to 47.5%) in urine samples. The rates of multiple infections for cervical and urine samples were not statistically significantly different (χ^2 [1] = 0.0126; $P = 0.911$).

The distribution of PCR results for generic identification of HPV DNA and type-specific identification considering the presence of any of the three infection levels (none, single, or multiple) is detailed in Table 2. The percentages of HPV DNA detection were similar for all primer sets. It was found that HPV-16 was the most prevalent viral type in both cervical and urine samples. However, the distributions of HPV of the remaining types were differ-

TABLE 1 Sociodemographic characteristics and HPV positivity regarding the population being studied according to cervical and urine sample results

| Characteristic[a] | Total no. (%) | HPV infection in: | | | |
| | | Cervical samples | | Urine samples | |
		n (%)	P	n (%)	P
Age (n = 194)					
20–34 yr	88 (45.4)	65 (73.9)	0.053	54 (61.4)	0.038
35–49 yr	70 (36.1)	42 (60.0)		52 (74.3)	
≥50 yr	36 (18.5)	29 (80.6)		18 (50.0)	
Ethnicity (n = 190)					
White	130 (68.4)	91 (70.0)	0.990	80 (61.5)	0.444
Indigenous	3 (1.6)	2 (66.7)		2 (66.7)	
Mestizo	53 (27.9)	38 (71.7)		35 (66.0)	
Black	4 (2.1)	3 (75.0)		4 (100.0)	
Age at first intercourse (n = 190)					
<18 yr	115 (60.9)	83 (72.2)	0.631	71 (61.7)	0.533
≥18 yr	74 (39.1)	51 (68.9)		49 (66.2)	
Lifetime no. of sexual partners (n = 188)					
1	30 (16.0)	21 (70.0)	0.997	20 (66.7)	0.914
2	46 (24.4)	33 (71.7)		28 (60.9)	
3	43 (22.9)	30 (69.8)		26 (60.5)	
≥4	69 (36.7)	49 (71.0)		45 (65.2)	
Contraceptive method (n = 158)					
None	32 (20.3)	20 (62.5)	0.029	19 (59.4)	0.252
Hormonal	5 (3.1)	3 (60.0)		2 (40.0)	
Barrier	68 (43.0)	54 (79.4)		43 (63.2)	
Barrier plus hormonal	3 (1.9)	0 (0.0)		3 (100.0)	
Barrier plus other	18 (11.4)	10 (55.6)		10 (55.6)	
Surgery	32 (20.3)	22 (68.7)		25 (78.1)	
Cigarette smoking (n = 187)					
No	159 (85.0)	112 (70.5)	0.379	103 (64.8)	0.960
Yes	28 (15.0)	22 (78.6)		18 (64.3)	
Pregnancies (n = 192)					
None	9 (4.8)	6 (66.7)	0.935	7 (77.8)	0.087
1	45 (24.2)	32 (71.1)		27 (60.0)	
2	47 (25.3)	31 (66.0)		36 (76.6)	
3	46 (24.7)	34 (73.9)		23 (50.0)	
≥4	39 (21.0)	28 (71.8)		25 (64.1)	

[a] Categories involved less than 194 women, given that data were missing from the surveys. HPV infection was considered positive when the PCR results using any of the three primer sets (GP5+/GP6+, MY09/11, and/or pU1M2R) were positive. P values correspond to sampling distribution probabilities of chi-square distributions.

ent between sample sources. It was also noted that all HPV types tested in the population were found more frequently in multiple infections for both sample sources.

The results showed associations between HPV DNA detection for both cervical and urine samples and the presence of multiple infections. This was determined with OR values for association with positive GP5+/GP6+ results of 8.1 (95% CI, 3.8 to 17.2) for cervical samples and 3.7 (95% CI, 1.9 to 7.0) for urine samples and values for association with positive MY09/MY11 results of 7.0 (95% CI, 3.4 to 14.4) for cervical samples and 5.0 (95% CI, 2.5 to 9.8) for urine samples. The strongest association was found with pU1M/2R, for which the adjusted OR values were 12.5 (95% CI, 5.2 to 30.1) for cervical samples and 6.7 (95% CI, 3.2 to 14.0) for urine samples. All associations were statistically significant (P = 0.000).

The overall agreement between the molecular detection of HPV infection in cervical and urine samples was 63.9% (κ = 0.18 [95% CI, 0.05 to 0.33]). Table 3 shows the agreement obtained for the six HPV types evaluated in both types of samples according to their detection by the three HPV DNA detection tests (GP5+/GP6+, MY09/MY11, and/or pU1M2R). The frequency of cytological abnormalities in the analyzed population was 28.9% (n = 56) (95% CI, 22.6 to 35.8%); 35.7% (n = 20) (95% CI, 23.4 to 49.6%) of those subjects were classified as having atypical squamous cells of undetermined significance (ASCUS), 57.2% (n = 32) (95% CI, 43.2 to 70.3%) had low-grade squamous intraepithelial lesions (LSILs), and 7.1% (n = 4) (95% CI, 1.9 to 17.3%) had high-grade squamous intraepithelial lesions (HSILs).

Table 4 describes the molecular test results for each sample source according to the Pap test report. An association was found between HPV infection in urine samples and LSIL findings, with HPV infection being 4.0-fold (95% CI, 1.4- to 11.4-fold) more frequent in women with this type of abnormality. No association was found for the cervical samples. The only association found in evaluations of the results for each primer set (conducted independently) in urine samples was between positive pU1M/2R results and LSIL findings (OR, 2.8 [95% CI, 1.3 to 6.4]).

The operative characteristics were adjusted by bearing in mind the presence of an imperfect gold standard; cervical cytological findings were considered positive when ASCUS, LSILs, or HSILs were identified (Bethesda classification system) (14). It was found that urine samples had a sensitivity of 68.6% (95% CI, 58.7 to 74.5%), a specificity of 21.2% (95% CI, 10.7 to 32.8%), a positive predictive value (PPV) of 68.0% (95% CI, 59.7 to 75.7%), and a negative predictive value (NPV) of 20.1% (95% CI, 10.2 to 31.6%). Cervical samples had a sensitivity of 57.2% (95% CI, 48.8 to 65.6%), a specificity of 19.1% (95% CI, 48.8 to 65.6%), a PPV of 64.0% (95% CI, 55.4 to 72.2%), and an NPV of 15.1% (95% CI, 6.9 to 24.1%). All urine sample tests had characteristics very similar to those of cervical sample tests (see Table S1 in the supplemental material).

Table 5 presents positivity rates for the generic and type-specific tests, stratified by cytological findings. The stratification results (according to the cytological findings) showed that the tests detected HPV DNA with greater frequency in women with abnormal cytology findings.

DISCUSSION

Considering the role of HPV infection in the development of CC, viral DNA detection has become an important tool for screening at-risk populations (9). The available evidence shows higher HPV infection rates in the presence of immunosuppression caused by HIV than in the general population (80 to 90% versus 22 to 50%), a reduction of low-grade lesion regression rates (27% versus 60%), and a reduction in the time required for the disease to develop (5).

Current screening techniques for detecting HPV DNA have used alternative sample sources (tampon-type devices, self-sampling of vaginal smears, and self-sampled urine) to facilitate inclu-

TABLE 2 Results of generic HPV detection and type-specific distribution for cervical and urine samples

Analysis	Results (n [%]) for[a]:					
	Cervical samples			Urine samples		
	Without infection	Single infection	Multiple infection	Without infection	Single infection	Multiple infection
Generic HPV DNA detection[b]						
GP5+/GP6+	110 (56.7)	16 (8.2)	68 (35.1)	126 (65.0)	27 (13.9)	41 (21.1)
MY09/11	108 (55.7)	18 (9.2)	68 (35.1)	122 (62.9)	26 (13.4)	46 (23.7)
pU1M/2R	115 (59.3)	11 (5.6)	68 (35.1)	133 (68.5)	18 (9.3)	43 (22.2)
Type-specific distribution[c]						
HPV-16	46 (33.8)	11 (8.1)	79 (58.1)	48 (38.7)	21 (16.9)	55 (44.4)
HPV-18	79 (58.1)	4 (2.9)	53 (39.0)	83 (66.9)	4 (3.2)	37 (29.9)
HPV-31	72 (52.9)	6 (4.4)	58 (42.7)	86 (69.4)	3 (2.4)	35 (28.2)
HPV-33	96 (70.6)	1 (0.7)	39 (28.7)	100 (80.7)	2 (1.6)	22 (17.7)
HPV-45	121 (89.0)	2 (1.4)	13 (9.6)	101 (81.5)	6 (4.8)	17 (13.7)
HPV-58	97 (71.3)	4 (2.9)	35 (25.8)	79 (63.7)	7 (5.7)	38 (30.6)

[a] Percentages were calculated by rows for each sample source.
[b] The total number of samples for generic HPV DNA detection was 194.
[c] The total numbers of samples included in the type-specific distribution analyses were 136 for the cervical samples and 124 for the urine samples. Type-specific identification was determined by independent PCRs using type-specific primer sets.

sion of greater numbers of women. Among the various sampling sources available, urine has been considered an economical and easily obtained source for sampling; it has been widely used for detection of other sexually transmitted infections (3), and detection of HPV DNA in self-collected urine samples could facilitate the inclusion of women in regions where gynecological screening coverage is low (23).

This study (the first held in Colombia regarding the frequency of HPV-HIV coinfection) has described infection prevalence regarding HPV detection in cervical and urine samples, and the results showed prevalence rates for both sample sources similar to the data reported in previous studies (24, 25). The urine samples had lower DNA prevalence than the cervical samples; this could

TABLE 3 Agreement in the detection of each viral type in cervical and urine samples according to the results of HPV DNA detection tests

HPV DNA detection test	HPV type	Agreement (%)	Kappa (95% CI)
GP5+/GP6+	HPV-16	63.4	0.030 (−0.111 to 0.171)
	HPV-18	75.3	−0.024 (−0.152 to 0.104)
	HPV-31	71.1	−0.004 (−0.134 to 0.127)
	HPV-33	87.6	0.141 (−0.056 to 0.338)
	HPV-45	88.1	−0.063 (−0.106 to −0.020)
	HPV-58	80.9	0.136 (−0.038 to 0.310)
MY09/11	HPV-16	65.0	0.074 (−0.072 to 0.219)
	HPV-18	74.2	−0.064 (−0.175 to 0.047)
	HPV-31	72.7	0.017 (−0.117 to 0.150)
	HPV-33	80.9	0.164 (−0.017 to 0.346)
	HPV-45	86.6	−0.068 (−0.112 to −0.023)
	HPV-58	76.3	−0.019 (−0.152 to 0.113)
pU1M/2R	HPV-16	63.9	−0.005 (−0.143 to 0.132)
	HPV-18	78.4	0.135 (−0.026 to 0.296)
	HPV-31	74.2	0.059 (−0.084 to 0.202)
	HPV-33	83.5	0.110 (−0.067 to 0.288)
	HPV-45	92.3	0.077 (−0.139 to 0.293)
	HPV-58	78.4	0.036 (−0.116 to 0.188)

have been related to the biological characteristics of the sample sources, since the samples were not derived from the original site of infection (10). It also may have been related to technical characteristics related to the urine samples, such as contaminants, PCR inhibitors, certain intake conditions, or storage and processing, which also could have affected amplification (12). However, the results obtained in this study support using urine samples for identification of HPV DNA since such samples were generally comparable to cervical samples, which are widely used for HPV detection.

The use of barrier contraceptives has been reported in previous studies as a strategy for the primary prevention of preneoplastic lesions (26). However, the results obtained in this study showed an association between the risk of HPV infection and the use of these contraceptives. These results could be related to lifestyle characteristics, mainly sexual habits, which would favor HPV infection being acquired (27).

HPV DNA detection was favored by the methodological design implemented in this study using three primer sets, as recorded previously; this contributed to making the epidemiological study more robust (16) and facilitated viral DNA detection in a greater number of women. A higher percentage of single infections was detected in urine samples; this could have been related to the normal course of persistent infection involving an increase in the number of viral copies and the start of a cell differentiation program in which one cell layer gradually replaces another (i.e., metaplasia) (28). Such replacement could facilitate cervical cells appearing in urine.

Regarding type-specific viral distribution, it was observed that HPV-16 was the most prevalent in both types of samples, which was consistent with previously reported data for both the general population (20, 29) and the HIV-positive population (30). The distributions of the other tested HPV types varied between the two sample sources, which could have been due to different affinities of some viral types for particular types of epithelium (keratinized or not) (31) or the fact that certain HPV types commonly infect

TABLE 4 Associations between HPV DNA detection in the two types of samples and cytological results

Cytological results[a]	Sample	No. (%) of samples with HPV DNA detected using:			No. (%) of samples with HPV infection[d]	Crude odds ratio (95% CI) for detection results using:			Crude odds ratio (95% CI) for HPV infection[d]
		GP5+/GP6+	MY09/11	pU1M/2R		GP5+/GP6+	MY09/11	pU1M/2R	
Normal (n = 138)	Cervical	49 (35.5)	53 (38.4)	50 (36.2)	92 (66.7)	1.0	1.0	1.0	1.0
	Urine	44 (31.9)	50 (36.2)	36 (26.1)	79 (57.2)	1.0	1.0	1.0	1.0
ASCUS (n = 20)	Cervical	13 (65.0)	12 (60.0)	10 (50.0)	16 (80.0)	3.4 (1.2–9.2)[b]	2.4 (0.9–6.4)	1.8 (0.7–4.6)	2.0 (0.6–6.4)
	Urine	9 (45.0)	10 (50.0)	7 (35.0)	15 (75.0)	1.7 (0.7–4.6)	1.8 (0.7–4.6)	1.5 (0.6–4.1)	2.2 (0.8–6.6)
LSIL (n = 32)	Cervical	19 (59.4)	21 (65.6)	17 (53.1)	25 (78.1)	2.7 (1.2–5.9)[b]	3.1 (1.3–7.0)[b]	2.0 (0.9–4.4)	1.8 (0.7–4.5)
	Urine	14 (43.8)	11 (34.4)	16 (50.0)	27 (84.4)	1.7 (0.8–3.7)	0.9 (0.4–2.1)	2.8 (1.3–6.4)[b]	4.0 (1.4–11.4)[b]
HSIL (n = 4)	Cervical	3 (75.0)	0 (0.0)	2 (50.0)	3 (75.0)	5.4 (0.5–55.4)	0.0 (NC)[c]	1.8 (0.2–13.0)	1.5 (0.2–14.9)
	Urine	1 (25.0)	1 (25.0)	2 (50.0)	3 (75.0)	0.7 (0.1–7.1)	0.6 (0.1–5.8)	2.8 (0.4–21.2)	2.2 (0.2–22.4)

[a] ASCUS, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
[b] Statistically significant.
[c] NC, not calculable. The estimator could not be calculated because one of the fields contained no data during the dispersion analysis.
[d] HPV infection was considered positive when the PCR results using any of the three primer sets were positive.

the lower reproductive tract (32), which favors their detection in urine samples.

The association found between the detection of viral infections in urine samples using PCRs (in combination) and the pU1M/2R primer set and abnormal cytological findings supported the use of this sample source in screening tests. This methodology has advantages, such as convenience regarding sample collection, low costs, and greater acceptability among women (12). It also suggests the usefulness of this sample source in identifying women at risk of developing precancerous lesions, consistent with previous reports (17).

In spite of relatively good agreement between the detection of each viral type in cervical and urine samples according to the results of each HPV DNA detection test, the kappa values were extremely low. This finding could have been due to the paradoxical relationship between kappa values and agreement (as proposed for this type of study), which is dramatically affected by trait prevalence in the population being considered (33), in this case the high frequency of infection found in both sample sources.

The operative characteristics of the tests from the two sample sources led to low results. However, it should be noted, regarding diagnostic performance, that cervical cytological data represent an imperfect gold standard; this could be related to the presence of disease, not the infection, and what is ultimately determined by molecular testing. In spite of this, the tests had similar results with the two sample sources, supporting the use of urine samples for the molecular identification of HPV DNA, since such samples have sensitivity comparable to that found in a prior study for

TABLE 5 Molecular test positivity for detection of HPV infection, stratified by cytological findings

Analysis	HPV DNA detection test or HPV type	Sample	No. (%) with positive results and cytological findings of:			
			Normal	ASCUS	LSIL	HSIL
Generic HPV DNA detection (n = 194)	HPV infection[a]	Cervical	92 (66.7)	16 (80.0)	25 (78.1)	3 (75.0)
		Urine	79 (57.2)	15 (75.0)	27 (84.4)	3 (75.0)
	GP5+/GP6+	Cervical	49 (35.5)	13 (65.0)	19 (59.4)	3 (75.0)
		Urine	44 (31.9)	9 (45.0)	14 (43.8)	1 (25.0)
	MY09/11	Cervical	53 (38.4)	12 (60.0)	21 (65.6)	0 (0.0)
		Urine	50 (36.2)	10 (50.0)	11 (38.4)	1 (25.0)
	pU1M/2R	Cervical	50 (36.2)	10 (50.0)	17 (53.1)	2 (50.0)
		Urine	36 (26.1)	7 (35.0)	16 (50.0)	2 (50.0)
Type-specific distribution[b]	HPV-16	Cervical	64 (69.6)	9 (56.3)	14 (56.0)	17 (60.7)
		Urine	45 (57.0)	9 (60.0)	3 (100.0)	22 (73.3)
	HPV-18	Cervical	39 (42.4)	2 (12.5)	16 (56.0)	2 (66.7)
		Urine	25 (31.7)	8 (53.3)	7 (25.9)	1 (33.3)
	HPV-31	Cervical	38 (41.3)	8 (50.0)	17 (60.0)	1 (33.3)
		Urine	24 (30.4)	7 (46.7)	7 (25.9)	0 (0.0)
	HPV-33	Cervical	24 (26.1)	3 (18.8)	12 (48.0)	1 (33.3)
		Urine	16 (20.3)	2 (13.3)	5 (18.5)	1 (33.3)
	HPV-45	Cervical	7 (7.6)	3 (18.8)	5 (20.0)	0 (0.0)
		Urine	12 (15.2)	5 (33.3)	5 (18.5)	1 (33.3)
	HPV-58	Cervical	24 (26.1)	3 (18.8)	11 (44.0)	1 (33.3)
		Urine	26 (32.9)	6 (40.0)	11 (40.7)	2 (66.7)

[a] HPV infection was considered positive when the PCR results using any of the three primer sets were positive.
[b] Categories involved less than 194 women, given that only women identified as being infected with HPV were included in the type-specific identification reactions, meaning that n values for each diagnostic category varied for the HPV types. Type-specific identification was determined by independent PCRs using type-specific primer sets.

molecular detection in cervical samples obtained from a general population (17). Future studies should involve the development of urine sample validation for detection of HPV DNA.

Even though the samples did not come directly from the viral infection site, there are valid reasons for using urine as a potential alternative for detection of HPV DNA, such as the proximity of the lower urinary tract to the vagina, vulva, and cervix and the susceptibility of the bladder epithelium to HPV infection. It has been suggested that the main source of viral DNA could be contamination by desquamated cells and that the amount of viral DNA in urine could be related to the amount of epithelial cells and the viral load (34).

The data reported in this study have suggested the use of molecular tests not only with directly derived cervical tissue samples but also with self-collected samples such as urine samples. Our results showed that this sample source was useful for identifying women with abnormal cytological findings and presented operative characteristics similar to those found for cervical samples. The intrinsic characteristics (ease of collection, low cost, and greater acceptability by the female population) of urine samples indicated that such samples might be included in future routine screening tests, especially in developing countries where CC rates are high.

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