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Generic Microtiter Plate Assay for Triaging Clinical Specimens Prior to Genotyping of Human Papillomavirus DNA via Consensus PCR▽

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A generic human papillomavirus (HPV) probe assay was compared to the Linear Array to detect HPV DNA in 1,013 clinical specimens. The sensitivity, specificity, and negative predictive value of the assay were 99.5% (95% confidence interval [CI], 98.4% to 99.9%), 58.6% (95% CI, 53.9% to 63.1%), and 98.9% (95% CI, 96.5% to 99.8%), respectively. This assay conveniently identifies HPV-positive specimens.

Human papillomavirus (HPV) genotyping is labor-intensive and costly. An assay using a generic HPV probe to select only samples containing HPV DNA for a genotyping assay would be desirable. A generic probe mix was shown to have excellent sensitivity to detect HPV DNA in samples collected from HIV-seropositive women (7). The performance of this HPV generic probe assay in samples from HIV-seronegative women and in extragenital samples is unknown. In the current study, we report results obtained from a digoxigenin-labeled generic probe assay in microwell plates (DIG-MWP assay) to detect HPV DNA in clinical samples collected from various sampling sites in the course of several epidemiological studies.

Overall, 1,013 clinical specimens (509 self-collected vaginal swabs, 69 endocervical brushings, 121 penile and scrotal scrapings [2], 140 fingertip brushings [3], and 174 oral samples [5, 8]) were included in this evaluation. These samples were collected from 739 participants (598 women, 141 men) participating in several epidemiological studies (2, 6, 9), a population-based HPV prevalence study, and a case control study of head and neck cancers. Informed consent was obtained from each participant in the course of the parent studies. All studies had the approval of the ethics committees of the institutions involved.

All samples were tested blindly concurrently in two assays for detection of HPV DNA amplified with biotinylated PGMY primers. DNA was extracted from these samples with Gentra Puregene or Master Pure (Epicentre, Madison, WI). Five microliters of extracted DNA was tested with the Linear Array (LA) HPV genotyping assay (Roche Diagnostics, Laval, Canada), according to a standard protocol (4). The DIG-labeled HPV generic probe was synthesized by amplification of HPV types 11, 16, 18, and 51 using type-specific primers, as described previously (7). Generic probe detections were performed in MWPs using the commercially available PCR-enzyme-linked immunosorbent assay (ELISA) DIG detection kit (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer's instructions with the following modification: 20 μl of denatured amplified DNA was hybridized in 200 μl of hybridization buffer with 20 μl of the denatured generic HPV probe pool for 3 h at 42°C. A specimen was considered positive if the corrected A_{405} was greater than 0.5, negative if the value was less than 0.2, and borderline in the range between 0.2 and 0.499.

Overall, 962 of 1,013 samples (95.0%; 95% confidence interval [CI], 93.4% to 96.2%) were considered to be positive for β-globin in LA. Of the 51 samples with a low cellular yield, 27 (52.9%; 95% CI, 39.5% to 66.0%) contained at least one HPV type. Considering only the 989 samples that contained β-globin DNA and/or HPV DNA, 569 (57.5%; 95% CI, 54.4% to 60.6%) were HPV positive by the LA, of which 306 contained more than 1 HPV genotype. The distribution of HPV genotypes detected in the 569 HPV-positive samples with LA is provided in Table 1.

As indicated in Table 2, the sensitivity, specificity, positive predictive value, and negative predictive value of the DIG-MWP generic probe assay with respect to HPV results with LA for detection of HPV DNA in 1,013 samples were 99.5% (95% CI, 98.4% to 99.9%), 58.6% (95% CI, 53.9% to 63.1%), 75.5% (95% CI, 72.3% to 78.4%), and 98.9% (95% CI, 96.5% to 99.8%), respectively. The sensitivities of the DIG-MWP generic probe assay were similar across sampling sites. However, the specificity of the assay was greater for hand and oral specimens. The overall agreement between the HPV DNA detection assays was 81.5% (95% CI, 79.0 to 83.8%), for a kappa value of 0.61 (95% CI, 0.55 to 0.66). Specimens with borderline results with the DIG-MWP HPV generic probe (n = 140)

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TABLE 1. Distribution of HPV genotypes detected in 569 HPV-positive samples analyzed with the Linear Array[a]

HPV type	No. (%) of samples
High-risk types	
16	115 (20.2)
18	26 (4.6)
26	0 (0.0)
31	51 (9.0)
33	6 (1.1)
34 (64)	1 (0.2)
35	16 (2.8)
39	53 (9.3)
45	14 (2.5)
51	60 (10.6)
52	34 (6.0)
53	64 (11.3)
56	44 (7.7)
58	33 (5.8)
59	34 (6.0)
66	52 (9.1)
67	29 (5.1)
68	20 (3.5)
69	0 (0.0)
70	17 (3.0)
73	36 (6.3)
82 (IS*39* and W13b)	19 (3.3)
Low/unknown-risk types	
6	53 (9.3)
11	4 (0.7)
40	27 (4.8)
42	72 (12.7)
44 (55)	30 (5.3)
54	42 (7.4)
61	32 (5.6)
62	71 (12.5)
71	0 (0.0)
72	15 (2.6)
81	17 (3.0)
83	20 (3.5)
84	53 (9.3)
89 (CP6108)	93 (16.3)

[a] Of the 1,013 samples tested with the Linear Array, 569 tested positive for HPV DNA. The percentage of positivity for each type was calculated based on 569 samples. Types in parentheses are the new designations for HPV genotypes. The distribution of HPV genotypes was done according to Bouvard et al. (1).

accounted for 76.1% (95% CI, 69.4% to 81.7%) of the 184 falsely positive samples. In comparison, only 27 of 566 (4.8%; 95% CI, 3.3% to 68.8%) true-positive samples with the DIG-MWP HPV generic probe generated borderline results ($P < 0.0001$; chi-square test). When samples with borderline results were considered negative for HPV, the specificity of the DIG-MWP generic probe assay increased but the sensitivity decreased, except for oral samples. Considering samples with borderline results to be negative also resulted in a greater agreement between tests.

Of the three samples falsely negative by the DIG-MWP HPV generic probe assay, 2 were self-collected vaginal swabs and 1 was a fingertip sample. One of these samples was β-globin negative. Each of the false-negative samples contained one HPV genotype (type 39, 62, or 89). They generated optical densities in the DIG-MWP generic probe assay of 0.099, 0.105, and 0.179, respectively. These samples remained negative after retesting with the DIG-MWP generic probe assay. Bands for each HPV type detected in LA were at the limit of visibility.

TABLE 2. Clinical performance of the DIG-MWP generic probe on 1,013 specimens with respect to Linear Array results[a]

Specimen type	No. of samples with DIG-MWP/LA result of:				Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Agreement (%) (95% CI)	Kappa (95% CI)
	+/+	+/−	−/+	−/−						
All (n = 1,013)										
Borderline +	566	184	3	260	99.5 (98.4–99.9)	58.6 (53.9–63.1)	75.5 (72.3–78.4)	98.9 (96.5–99.8)	81.5 (79.0–83.9)	0.61 (0.55–0.66)
Borderline −	539	44	30	400	94.7 (92.6–96.3)	90.1 (86.9–92.6)	92.5 (90.0–94.4)	93.0 (90.2–95.1)	92.7 (90.9–94.2)	0.85 (0.79–0.91)
Cervicovaginal (n = 578)										
Borderline +	394	100	2	82	99.5 (98.1–99.9)	45.1 (38.0–52.3)	97.5 (95.4–98.7)	97.6 (91.2–99.5)	82.4 (79.0–85.3)	0.52 (0.45–0.59)
Borderline −	384	50	12	132	97.0 (94.7–98.3)	72.5 (65.6–78.5)	88.5 (85.1–91.2)	91.7 (85.9–95.3)	89.3 (86.5–91.6)	0.73 (0.66–0.82)
Hand (n = 140)										
Borderline +	64	17	1	58	98.5 (91.0–99.9)	77.3 (66.6–85.4)	79.0 (68.8–86.6)	98.3 (90.2–100.0)	87.1 (80.5–91.8)	0.75 (0.58–0.91)
Borderline −	63	8	2	67	96.9 (88.8–99.8)	89.3 (80.1–94.7)	88.7 (79.1–94.4)	97.1 (89.4–99.8)	92.9 (87.2–96.2)	0.86 (0.69–1.00)
Male genitalia (n = 121)										
Borderline +	75	20	0	26	100.0 (94.2–100.0)	56.5 (42.2–69.8)	79.0 (69.6–86.0)	100.0 (95.3–100.0)	75.2 (66.8–82.1)	0.62 (0.45–0.78)
Borderline −	73	13	2	33	97.3 (90.2–99.8)	71.7 (57.4–82.8)	84.9 (75.7–91.1)	94.3 (80.4–99.4)	87.6 (80.4–92.5)	0.72 (0.55–0.90)
Oral (n = 174)										
Borderline +	33	47	0	94	100.0 (87.6–100.0)	66.7 (58.5–73.9)	41.3 (31.1–52.2)	100.0 (95.3–100.0)	73.0 (65.9–79.1)	0.43 (0.31–0.55)
Borderline −	33	8	0	133	100.0 (87.6–100.0)	94.3 (89.0–97.3)	80.5 (89.0–97.3)	100.0 (96.6–100.0)	95.4 (91.1–97.8)	0.86 (0.72–1.00)

[a] Positive results with the DIG-MWP assay were stratified as indicated by considering samples with borderline results either positive (borderline +) or negative (borderline −). Sampling sites are explained in detail in the text. The LA result was considered positive if one or more genotypes were detected. PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

The DIG-MWP generic assay consistently detected 10 copies of purified HPV DNA for HPV16, -18, -31, -33, -35, -45, -52, and -66, as previously described (7).

Although the sensitivities of the DIG-MWP HPV generic probe method were similar in both studies, the agreement between the genotyping assay and the DIG-MWP HPV generic probe assay in the initial report reached 93%, a level slightly higher than the level of 81% obtained in the current study (7). More than 75% of samples falsely positive with the DIG-MWP HPV generic probe assay generated borderline results in the current evaluation. In the initial report, one-half of the samples with borderline results were reclassified outside the equivocal zone after retesting (7). Thus, retesting samples with borderline results could have increased the level of agreement between assays in this study. Furthermore, the samples falsely positive with the DIG-MWP HPV generic probe could contain HPV DNA that was undetected with LA because of genomic variations at probe binding sites, or the genotype(s) may not have been included in the probe fixed on the array of LA. Regardless of the reason, the somewhat lower specificity is not problematic since this test is used mainly for triage and definitive results are based on a genotyping assay.

Our findings support the use of the DIG-MWP generic HPV assay as a convenient triage procedure to identify HPV-positive samples for subsequent genotyping. The cost of the DIG-MWP generic HPV assay was less than $5.00 (Canadian currency) per test. The same PCR products are tested in LA and the generic HPV assays. The DIG-MWP HPV generic probe assay can also be utilized in extragenital samples from men and women.

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