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Comparison of PapType to Digene Hybrid Capture 2, Roche Linear Array, and Amplicor for Detection of High-Risk Human Papillomavirus Genotypes in Women with Previous Abnormal Pap Smears

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PapType human papillomavirus (HPV) assay was compared to Hybrid Capture 2 (HC2), Amplicor (Amp), and Linear Array (LA) HPV tests in 894 women undergoing management for a high-grade Pap smear abnormality. The sensitivity in detection of underlying high-grade histological diagnosis by PapType was 90.3% and by HC2 was 79.8%, while by Amp and LA it was 92.4% and 91.6%, respectively. The specificities were 52.5%, 55.3%, 49.4%, and 51.7% for PapType, HC2, Amp, and LA, respectively.

One of the utilities of the human papillomavirus (HPV) DNA testing in clinical practice is test of cure following local ablative or excisional therapy for precursor cervical cancer lesions of high-grade cervical intraepithelial neoplasia 2 and 3 (CIN2 and CIN3).

In recent times, an increasing number of assays have been developed and are available for diagnostic and clinical use (7). Evaluation of such tests is important in order to assess not only their sensitivities and specificities compared to other more established commercial assays but also their clinical utility in detecting high-risk (HR) HPV genotypes and association to dysplastic or neoplastic changes in patients requiring treatment. Target amplification assays, such as Roche Amplicor (Amp) and Linear Array (LA), have a higher sensitivity for detection of high-grade lesion in the test-of-cure algorithm than the signal amplification-based assay, the Digene Hybrid Capture 2 (HC2) HPV test (3).

In this study, the performance of the PapType HPV test in the detection of underlying CIN2 and CIN3 (Genera Biosystems Limited, Melbourne, Australia) was compared to those of the HC2 (using a threshold of 5,000 copies), AMP, and LA HPV assays. The PapType HPV test is a PCR-based qualitative *in vitro* diagnostic (IVD) test for the simultaneous detection and genotyping of 14 HR HPV genotypes and 2 low-risk (LR) HPV genotypes with an analytical sensitivity of 500 copies per reaction (PapType package insert). We utilized archival PreservCyt clinical specimens from a large cohort study aimed to evaluate the utility of HPV DNA in follow-up of women being treated for histologically proven high-grade dysplasia (3, 5).

Stored frozen (unthawed) DNA previously extracted from PreservCyt samples using MagNA Pure LC (6) was tested using the PapType high-risk HPV detection and genotyping kit (Genera Biosystems Limited) according to the manufacturer's instructions and as described previously (8). The Paptype HPV test was performed with 5 μl of DNA in a final reaction volume of 20 μl. All testing was performed blinded to the results of HC2, Amp, and LA. The PapType HPV test results were subsequently compared to those from histology, HC2, Amp, and LA HPV tests, which were performed and reported previously (3, 5). Statistical analyses were performed using 2-by-2 contingency tables, with two-sided *P* values calculated using Fisher's exact test. Agreement between tests was assessed by Cohen's kappa statistic. Confidence intervals (95% CI) for proportions were calculated using a two-tailed test and assuming a random sample of the population. Confidence intervals for HPV genotypes detected were calculated by the Pearson test using a Poisson distribution.

Overall, 894 clinical specimens, comprising cytological scrapes with known contemporaneously collected histological results, were available for retesting with the PapType HPV test. Cytology clinical samples were obtained prior to treatment of patients. Overall, 10 specimens had negative internal controls and HPV and were removed from further analyses. Among the remaining 884 specimens, HR HPV positivity values determined by each of the four HPV tests were 73.0% (PapType), 65.6% (HC2), 75.5% (Amp), and 74.1% (LA). The levels of concordance between PapType and other HPV tests for the absence/presence of HR HPV types was considered good (HC2, 84.5% agreement, $\kappa = 0.638$) to very good (Amp, 94.6% agreement, $\kappa = 0.858$; LA, 95.9% agreement, $\kappa = 0.895$) (Table 1). Both HPV genotyping tests (PapType and LA) were compared for detection of 13 mutual HR HPV genotypes by the respective assays. Samples positive for HPV-33, -35, and -58 by LA were evaluated for the presence of HPV-52 by real-time PCR assay as described previously (4). The PapType test identified 645 specimens as having HR genotypes (irrespective of LR HPV genotypes) (73.0%), 8 had only HPV-6 and/or -11 genotypes (0.9%), 16 had HPV-66 genotypes (1.8%), and 215 were HPV negative (24.3%), compared to LA with 655 specimens with HR genotypes (74.1%), 8 with only HPV-6 and/or -11 genotypes (0.9%), and 221 HPV negative/other types (including HPV-66) (25.0%) (Table 1).

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TABLE 1 Concordance between HPV risk types detected using PapType, Amp, and LA HPV tests[a]

HR HPV result	No. (%) of specimens by PT		% absolute agreement (95% CI)	Kappa value
	HR HPV/positive	LR HPV/negative		
HC2			84.5 (82.5–86.5)	0.638
Positive	544	36[b]		
Negative	101[c]	203[d]		
Amp			94.6 (93.3–95.8)	0.855
Positive	632	35[e]		
Negative	13[f]	204[g]		
LA			95.9 (94.8–97.0)	0.895
HR	632	23[h]		
LR/negative	13[i]	216[i,k]		
Total	645 (73.0)	239 27.(0)		

[a] PT, PapType; HC2, Hybrid Capture 2; Amp, Amplicor; LA, Linear Array; HR, high risk; LR, low risk; 95% CI, 95% confidence intervals; HR HPV positive, presence of ≥1 of the 13 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Agreement between tests was assessed by Cohen's kappa statistic.
[b] Includes 11 PT-LR/HC2-pos (1 HPV-6, 10 HPV-66) types.
[c] Includes 26 multiple types; 23 HPV-16, 11 HPV-31, and 10 HPV-18 types; and other HR types.
[d] Includes 13 PT-LR/HC2-neg (6 HPV-66, 3 HPV-6, 4 HPV-11) types.
[e] Includes 10 PT-LR/AMP+ (8 HPV-66, 2 HPV-6) types.
[f] Includes 13 PT-HR/AMP-neg (3 HPV-52, 2 HPV-16, 2 HPV-45, 2 HPV-68, 1 HPV-18, 1 HPV-31, 1 HPV-51, 1 HPV-51/56) types.
[g] Includes 14 PT-LR/AMP-neg (8 HPV-66, 4 HPV-11, 2 HPV-6) types.
[h] Includes 6 PT-LR/LA+ (1 HPV-6/51 [LA] versus HPV-6 [PT], 5 multiple HR-HPV [LA] versus HPV-66 [PT]) and 17 PT-Neg/LA-HR (4 HPV-51, 3 HPV-16, 3 HPV-59, 2 HPV-52, 1 HPV-31, 1 HPV-39, 1 HPV-58, 1 HPV-18/51, 1 HPV-51/58).
[i] Includes 1 PT-HR (HPV-16)/LA-LR (HPV-6) and 12 PT-HR/LA-Neg (4 HPV-16, 2 HPV-31, 2 HPV-68, 1 HPV-18, 1 HPV-45, 1 HPV-51, 1 HPV-52).
[j] Includes 12 matches for LR (PT versus LA) (8 HPV-66, 3 HPV-6, and 1 HPV-11) and 4 PT-neg/LA-LR (3 HPV-6, 1 HPV-66).
[k] Includes 6 PT-LR/LA-neg (3 HPV-11, 3 HPV-66) and 194 negative matches.

When comparing the detection of HR HPV to histological diagnosis (using CIN2+ as a measure of clinically significant disease) (Table 2), PapType demonstrated a clinical sensitivity of 90.3% (95% CI of 88.4 to 92.3) and specificity of 52.5% (95% CI of 49.2 to 55.8) for the detection of HR HPV infections. The PapType, LA, and Amp HPV tests correctly identified more histologically confirmed cervical disease (CIN2+) than the HC2 HPV test. The overall match between the HR HPV test result and histological diagnosis was 75.0% (95% CI of 72.6 to 77.4) for both the PapType and Amp HPV tests, 69.9% (95% CI of 67.4 to 72.5) for HC2, and 75.5% (95% CI of 73.1 to 77.8) for the LA HPV test. The clinical sensitivity and specificity of the PapType HPV test were comparable to those of both Amp and LA HPV tests (the 95% confidence intervals overlap), while the sensitivity was greater than that of the HC2 test (Table 2). One limitation of this study was lack of consensus review of histology diagnosis, which may contribute to differences seen in detection of high grade. There was no significant difference in the number of each of the HPV genotypes detected by the PapType or LA HPV tests. However, PapType detected a higher number of HPV-33 infections and fewer HPV-51 infections. These differences were classified as not statistically significant (Fig. 1).

This study demonstrated that 90.3% of women with a histological diagnosis of CIN2 or higher were positive for HR HPV by the PapType HPV test. This level of detection was comparable to both the LA (91.6%) and Amp HPV (92.4%) tests and greater than the HC2 HPV test (79.8%). Indeed, these 884 samples have previously been shown to contain HPV genotypes other than the 13 common HR genotypes in 131 (14.8%) samples (5). This study showed lower sensitivities across the board for all the test compared to those reported earlier, as only a subset of those original samples were tested in this evaluation.

Individual HR HPV genotypes detected by the PapType HPV test matched those detected by the LA HPV test in 78.5% of cases, with a further 15.6% having a partial match. Among those samples testing HPV positive by one/both genotyping tests ($n = 690$), 500 (72.5%) demonstrated identical HPV-type profiles, which is comparable with similar studies (1, 2, 9). Comparing samples positive for HPV-16 by either PapType or LA ($n = 321$) showed 301 (93.8%) to be HPV-16 positive by both tests (as a single or multiple infection), with 12 being HPV-16 positive by LA and negative by PapType and 8 being HPV-16 negative by LA and positive by PapType.

As this study population focused on women with abnormal Pap smears, representing a population with inflated disease prevalence, the clinical performance shown here should not be extrapolated to the general screening population. However, this study provided the capacity to assess the PapType assay using samples

TABLE 2 Correlation between histological diagnosis and the results for high-risk HPV obtained using the PapType, LA, Amp, and HC2 HPV tests[a]

HR-HPV result	Histological diagnosis (no. of specimens)						% sensitivity or specificity (95% CI)[c]	Positive or negative predictive value (95% CI)[c]
	Normal	CIN1	CIN2	CIN3	Cancer[b]	≥CIN2		
PT+	64	106	168	289	18	475	90.3 (88.4–92.3)	73.6 (70.7–76.5)
PT−	104	84	34	17	0	51	52.5 (49.2–55.8)	78.7 (76.0–81.4)
HC2+	59	101	147	259	14	420	79.8 (77.2–82.5)	72.4 (69.5–75.4)
HC2−	109	89	55	47	4	106	55.3 (52.0–58.6)	65.1 (62.0–68.3)
Amp+	65	116	175	293	18	486	92.4 (90.6–94.1)	72.9 (69.9–75.8)
Amp−	103	74	27	13	0	40	49.4 (46.1–52.7)	81.6 (79.0–84.1)
LA+	63	110	174	290	18	482	91.6 (89.8–93.5)	73.6 (70.7–76.5)
LA−	105	80	28	16	0	44	51.7 (48.4–55.0)	80.8 (78.2–83.4)
Total	168	190	202	306	18	526		

[a] PT, PapType; HC2, Hybrid Capture 2; AMP, Amplicor; LA, Linear Array; 95% CI, 95% confidence intervals; HR HPV positive, presence of ≥1 of the 13 HR HPV types.
[b] Cancer includes both adenocarcinoma *in situ* and squamous cell carcinoma.
[c] Sensitivity and specificity were determined by comparison of detection of >CIN2 to detection of high-risk HPV on PapType, HC2, and LA HPV tests.

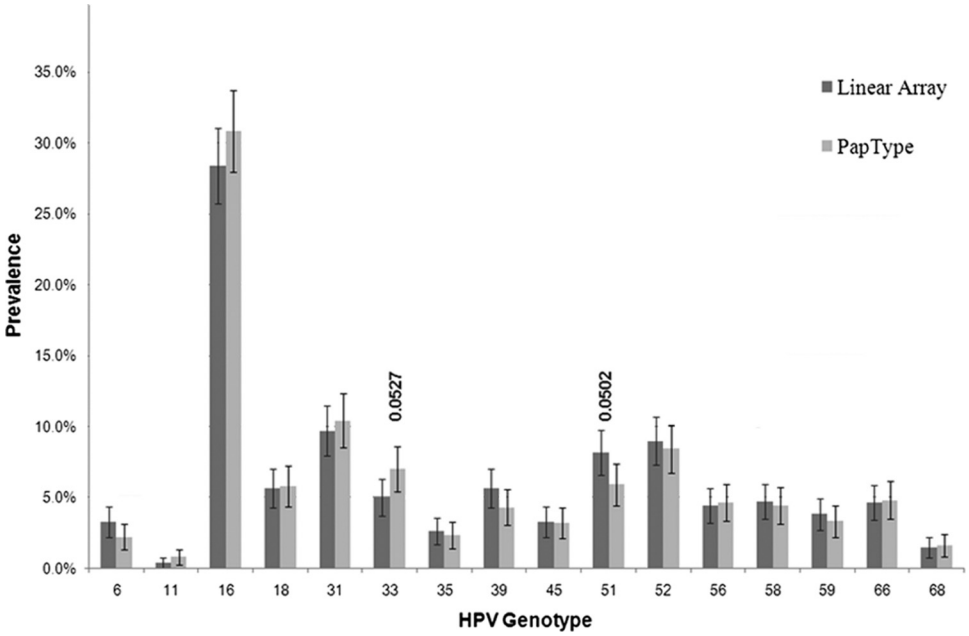

FIG 1 Total number of HR HPV genotypes detected by the PapType and LA HPV tests.

from women with heightened levels of disease, providing a valuable cohort for sensitivity testing.

This study demonstrates the ability of the PapType HPV test to accurately detect HR HPV in cervical samples. It shows a clinical sensitivity equivalent to LA and better than HC2 for the test-of-cure patient population studied.

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