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Aptima HPV E6/E7 mRNA Test Is as Sensitive as Hybrid Capture 2 Assay but More Specific at Detecting Cervical Precancer and Cancer▽

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Detection of human papillomavirus (HPV) E6/E7 oncogene expression may be more predictive of cervical cancer risk than testing for HPV DNA. The Aptima HPV test (Gen-Probe) detects E6/E7 mRNA of 14 oncogenic types. Its clinical performance was compared with that of the Hybrid Capture 2 DNA test (HC2; Qiagen) in women referred for colposcopy and those routinely screened. Aptima was also compared with the PreTect HPV-Proofer E6/E7 mRNA assay (Proofer; Norchip) in the referral population. Cervical specimens collected in PreservCyt (Hologic Inc.) were processed for HPV detection and genotyping with the Linear Array (LA) method (Roche Molecular Diagnostics, Laval, Quebec, Canada). Histology-confirmed high-grade cervical intraepithelial neoplasia (CIN 2) or worse (CIN 2+) served as the disease endpoint. On the basis of 1,418 referral cases (CIN 2+, $n = 401$), the sensitivity of Aptima was 96.3% (95% confidence interval [CI], 94.4, 98.2), whereas it was 94.3% (95% CI, 92.0, 96.6) for HC2. The specificities were 43.2% (95% CI, 40.2, 46.2) and 38.7% (95% CI, 35.7, 41.7), respectively ($P < 0.05$). In 1,373 women undergoing routine screening (CIN 2+, $n = 7$), both Aptima and HC2 showed 100% sensitivity, and the specificities were 88.3% (95% CI, 86.6, 90.0) and 85.3% (95% CI, 83.5, 87.3), respectively ($P < 0.05$); for women ≥30 years of age ($n = 845$), the specificities were 93.9% (95% CI, 92.3, 95.5) and 92.1% (95% CI, 90.3, 93.9), respectively ($P < 0.05$). On the basis of 818 referral cases (CIN 2+, $n = 235$), the sensitivity of Aptima was 94.9% (95% CI, 92.1, 97.7) and that of Proofer was 79.1% (95% CI, 73.9, 84.3), and the specificities were 45.8% (95% CI, 41.8, 49.8) and 75.1% (95% CI, 71.6, 78.6), respectively ($P < 0.05$). Both Aptima and Proofer showed a higher degree of agreement with LA genotyping than HC2. In conclusion, the Aptima test is as sensitive as HC2 but more specific for detecting CIN 2+ and can serve as a reliable test for both primary cervical cancer screening and the triage of borderline cytological abnormalities.

Persistent infection with oncogenic human papillomavirus (HPV) is the underlying cause of cervical cancer (34, 42), and therefore, testing for oncogenic HPV infection could serve as an accurate means of detecting women at risk for cervical cancer. There are also indications that testing for HPV might be the most effective method of cervical cancer screening in developing countries (32). Moreover, HPV testing would be warranted as a primary screening tool in the era of HPV vaccination (13). Numerous studies have established that testing for HPV DNA is significantly more sensitive than Pap cytology for the detection of high-grade cervical intraepithelial neoplasia (CIN 2) or worse (CIN 2+, i.e., CIN 2, CIN 3, squamous cell carcinoma, endocervical adenocarcinoma *in situ*, and endocervical adenocarcinoma) (1, 4, 15, 20, 27, 30) and is recommended in primary cervical cancer screening and for the triage of borderline cytological abnormalities (33, 43, 44). However, HPV testing lacks specificity due to the ubiquitous and transient nature of HPV infection in women, and therefore, the positive predictive value (PPV) tends to be lower than that obtained by cytology (10, 15). The above observation nonetheless has been based on HPV DNA testing, with most studies utilizing the Hybrid Capture 2 DNA test (HC2; Qiagen) (10, 15). While HC2 is highly sensitive for the detection of 13 high-risk oncogenic types targeted by the test (10, 11, 15), it is also known to cross-react with untargeted nononcogenic types, thus potentially contributing to a reduction in the test's specificity (5, 28, 31).

The oncogenic process in cervical cancer is initiated and mediated by the upregulation of HPV E6/E7 oncoproteins, and thus, overexpression of these oncoproteins is a marker for an increased risk of cervical cancer (26, 38, 45). Therefore, detection of E6/E7 oncogene expression could be more specific and a better predictor of cervical cancer risk than the detection of HPV DNA, and E6/E7 oncogenic expression can be detected by testing for E6/E7 mRNA transcripts (38). There are presently two E6/E7 mRNA-based tests developed and commercialized, with the indication that the detection of E6/E7 mRNA could improve the specificity of HPV testing (12, 17, 18, 23).

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The Aptima HPV assay (Gen-Probe) is a recently developed qualitative nucleic acid amplification test that detects E6/E7 mRNA collectively from 14 high-risk oncogenic types, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68. There is indication that the Aptima test has clinical sensitivity similar to that of HC2 but improved clinical specificity over that of HC2, which is designed to detect DNA of the same HPV types as Aptima except for type 66 (12, 40). The PreTect HPV-Proofer test (Proofer; Norchip) is the other E6/E7 mRNA-based assay, which detects E6/E7 mRNA individually from five high-risk oncogenic types, HPV-16, -18, -31, -33, and -45, and this has been in use in Europe for some time. There have been several studies on the clinical performance of Proofer in Europe (16, 17, 18, 19, 22, 23, 24), with a single North American study in Canada (29). Proofer has been shown to be significantly more specific than both Aptima and HC2, but it lacks sensitivity for the detection of CIN 2+ (29, 40).

As there are a number of other HPV tests developed and currently available for comparative studies and clinical application, additional data on the relative clinical performance of Aptima would be useful. In this regard, a further assessment of the clinical performance of Aptima in comparison with that of HC2 is warranted, as the latter has been extensively validated and is recommended as a reference test to evaluate any newly developed HPV tests (21). Notwithstanding the differences between Aptima and Proofer, there have been limited data comparing the clinical performance of these two E6/E7 mRNA-based tests. Further, the relative performance of these tests from the standpoint of genotype specificity has not been analyzed using a standardized genotyping method. The aim of the present study was to assess the clinical performance of Aptima for the detection of CIN 2+ in comparison with that of HC2 and Proofer along with genotyping using a standardized commercially available method. This study was carried out utilizing cervical specimens from a random sample of women with a history of abnormal cytology referred for colposcopic assessment as well as those routinely screened, as indicated in the recently published guidelines for validating HPV tests (21). The data in this paper present the relative clinical sensitivity, specificity, and predictive values of Aptima for the detection of CIN 2+ on the basis of cross-sectional data.

(Preliminary data of this study were presented in part at the EUROGIN Meeting, Nice, France, 2008 [29a].)

MATERIALS AND METHODS

Study population. The study population was comprised of a random sample of women referred for colposcopy for further assessment of cervical cancer risk and follow-up and those routinely screened. The colposcopy referral population consisted of women either newly diagnosed with abnormal Pap cytology of any grade who were referred for colposcopy or those with a history of abnormal cytology who were being followed up in colposcopy clinics according to the routine standard of care. The referral cases were enrolled from five tertiary care centers in five provinces across Canada for a longitudinal study. The prompting Pap test had been performed using conventional cytology at various sites served by the referral centers. Women 15 years of age or older who had had any grade of cytological abnormality within the previous 2 years and who had not received treatment were eligible. The routine screen participants were those receiving their normal Pap cytology screening in two centers. Those consenting to participate in the study signed a written informed consent. The study was approved by the institutional ethics review boards of all participating study centers.

The study population was comprised of 1,418 referral cases and 1,373 routinely screened women for the Aptima-HC2 study. The mean age of the referral group participants was 30.6 years (standard deviation [SD], 10.35 years; range, 15 to 80 years; median, 27 years). The proportion of women <30 years of age was 59%. The interval of time between the initial cytological diagnosis and enrollment at the colposcopy referral visit ranged from 1 to 3 months for new cases and 6 to 24 months for colposcopy follow-up cases with a history of abnormal cytology. The mean age of the routine screen participants was 36.3 years (SD, 13.75 years; range, 16 to 81 years; median, 35 years). In this population, the proportion of women <30 years of age was 38.3%. The Aptima-Proofer comparative study was limited to a proportion of the referral population and did not include the routine screen population.

Study procedures and testing methods. Upon enrollment, a single cervical specimen was collected from all participants using a Cervex broom-type brush (Rovers Medical Devices, Oss, Netherlands) and suspended into PreservCyt collection medium (Hologic Inc., Marlborough, MA), according to the manufacturer's instructions. For the referral group, cervical specimens were taken immediately prior to colposcopic examination. All specimens were couriered to the Public Health Laboratory, St. John's, Newfoundland, Canada, for processing. Liquid-based cytology (LBC) was performed using the ThinPrep (Hologic Inc.) method in a central laboratory, and the results were reported according to the 2001 Bethesda system (37). Only the LBC results obtained at the time of enrollment were used for study purposes.

Residual PreservCyt samples from the referral group were tested simultaneously with Aptima, HC2, and Proofer, followed by HPV genotyping using the Linear Array (LA) HPV genotyping test. Aptima, HC2, and Proofer were carried out using fresh specimens within 2 weeks of collection, and LA genotyping was performed with frozen aliquots. Fresh residual PreservCyt samples from the routine screen population were tested simultaneously with Aptima and HC2. The procedures used for these tests are briefly summarized below. Researchers and their technologists performing these tests were blinded to results obtained in the other tests and also to cytology, colposcopy, and histology results.

Aptima. Aptima (Gen-Probe Inc., San Diego, CA) detects HPV E6/E7 mRNA from 14 high-risk oncogenic types, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68, collectively from liquid cervical specimens. The assay incorporates an internal control to control for nucleic acid capture, amplification, and detection, as well as operator or instrument error. An aliquot of 1 ml of each PreservCyt sample was transferred to 2.9 ml buffered detergent solution, and a 400-μl aliquot of the mixture was then tested on a semiautomated Direct Tube Sampling (DTS) system (Gen-Probe), according to the manufacturer's instructions. Assay results are interpreted on the basis of the signal-to-cutoff ratio (S/CO) for the analyte, and specimens with S/CO values of ≥0.5 were considered positive.

HC2. HC2 (Qiagen, Mississauga, Ontario, Canada) detects DNA of 13 high-risk oncogenic types, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68, collectively through hybridization of a RNA probe cocktail with the target DNA, capture of the resultant RNA-DNA hybrids by antibodies specific for the hybrids, and detection of the antibodies with a chemiluminescence substrate. This test was performed with 4 ml of PreservCyt samples using conversion buffer, according to the manufacturer's instructions. Specimens with relative light unit/cutoff ratio (RLU/CO) values of ≥1.0 were considered positive.

Proofer. Proofer (Norchip, Klokkarstua, Norway) is a real-time multiplex nucleic acid sequence-based amplification assay (NASBA) for isothermal amplification and detection of E6/E7 mRNA from five high-risk oncogenic types, HPV-16, -18, -31, -33, and -45, using molecular beacon probes. Five milliliters of PreservCyt sample was processed for the extraction of HPV RNA using a Magnapure instrument (Roche Molecular Diagnostics), and HPV E6/E7 mRNA was detected according to the manufacturer's instructions and as previously described (17, 23). An FLx900I fluorescence reader (Bio-Tek, Winooski, VT) was used for the detection of the accumulated mRNA product with PreTect analysis software (Norchip) for analyzing the fluorescence profiles. To verify the integrity of RNA in the specimen, the test includes a primer set and a probe directed against the human U1 small nuclear ribonucleoprotein-specific mRNA. Standardized artificial oligonucleotides corresponding to the respective viral sequences are provided in the test kit, and these were included as positive controls for each of the five HPV types targeted by the test. Water was used as the negative control.

LA genotyping test. The LA assay (Roche Molecular Diagnostics, Laval, Canada) is a qualitative nucleic acid amplification test that detects the L1 DNA sequence of 36 high- and low-risk mucosal HPV types (HPV-6, -11, -16, -18, -26, -31, -33, -34, -35, -39, -40, -42, -44, -45, -51, -52, -53, -54, -56, -58, -59, -61, -62, -66, -67, -68, -69, -70, -71, -72, -73, -81, -82, -83, -84, and -89), with genotyping of specific HPV types (9). The LA assay uses PCR for the amplification of the target sequence, with the β-globin DNA sequence coamplified to control for sample

TABLE 1. Comparison of Aptima and HC2 for detection of cervical intraepithelial lesions in referral population by histological grade[a]

Diagnostic index	Histological category	Aptima		HC2	
		No. of relevant results/total no. tested (%)	95% confidence interval	No. of relevant results/total no. tested (%)	95% confidence interval
Sensitivity	CIN 2+ (n = 401)	386/401 (96.3)	94.4, 98.2	378/401 (94.3)	92.0, 96.6
	CIN 2 (n = 120)	112/120 (93.3)	88.8, 97.8	106/120 (88.3)	82.6, 94.0
	≥CIN 3 (n = 281)	274/281 (97.5)	95.7, 99.3	272/281 (96.8)	94.7, 98.9
Specificity	≤CIN 1 (n = 1,017)	439/1,017 (43.2)	40.2, 46.2	394/1,017 (38.7)[b]	35.7, 41.7
	CIN 1 (n = 366)	93/366 (25.4)	20.9, 29.9	81/366 (22.1)[b]	17.8, 26.4
	Negative (n = 651)	346/651 (53.1)	49.3, 56.9	313/651 (48.1)[b]	44.3, 51.9
Positive predictive value	CIN 2+ (n = 401)	386/964 (40.0)	36.9, 43.1	378/1,001 (37.8)	34.8, 40.8
Negative predictive value	≤CIN 1 (n = 1,017)	439/454 (96.7)	95.1, 98.3	394/417 (94.5)	92.3, 96.7

[a] n = 1,418.
[b] Specificities for detection of CIN 2+ between Aptima and HC2 significant at $P < 0.05$.

adequacy and DNA quality. The biotinylated amplicons generated are denatured and hybridized to an array containing immobilized probes for 36 HPV genotypes. HPV DNA was extracted from 250 μl of PreservCyt sample using an AmpliLute liquid medium extraction kit (Roche Molecular Diagnostics), according to the manufacturer's instructions. Extracted DNA was then tested using the LA assay as previously described (9). Because the HPV-52-specific probe cross-reacts with types 33, 35, and 58, samples positive with the HPV-52-specific probe were further tested with a validated real-time PCR assay specific for type 52 (8). Only the samples reactive in the HPV-52 real-time PCR assay were considered HPV-52 positive.

Colposcopy and histology. Participating obstetrician/gynecologist specialists at the study sites carried out colposcopy and, if warranted, cervical biopsy on the day of patient enrollment according to the standard of care. In some cases, biopsy specimens were taken in subsequent follow-up visits, and in such instances, histology results for biopsy specimens taken no later than 6 months following enrollment were included in the study analysis. Cervical histology was read and reviewed independently by pathologists at the respective study sites, and the results served as the disease endpoint for study purposes. The pathologists were blinded to HPV results.

Data analysis. The clinical performance of the HPV tests was assessed on the basis of histological diagnosis, with CIN 2+ serving as the disease endpoint. The cross-sectional HPV data based on a single cervical brush specimen collected at the time of enrollment and histology results for cervical biopsy specimens obtained either at enrollment or during follow-up for up to 6 months were utilized in this evaluation. Sensitivity, specificity, and predictive values were calculated using the conventional contingency tables, and 95% confidence intervals (CIs) were computed using the binomial method. McNemar's chi-square test was used to test the differences between sensitivities and specificities, as they were calculated using the same set of samples. Accuracy of HPV detection was calculated as the percentage of the correct results by the respective HPV test compared to the histology results. The HPV results were also studied analytically with LA genotyping results. A significance level of 0.05 was used in comparing performance characteristics.

RESULTS

Comparison of Aptima and HC2 for detecting CIN 2+ in referral population. The clinical performance of Aptima in comparison with that of HC2 was assessed with cervical specimens collected from 1,418 women referred for colposcopy. Of these, 401 (28.3%) women had CIN 2+, which included 13 cases of invasive cervical cancer. There were 366 women with CIN 1 and 651 having either a normal colposcopy result with no biopsy or negative histology, for a total of 1,017 women with a diagnosis of ≤CIN 1 (Table 1). Overall, Aptima was positive for 964 (68%) of the total of 1,418 women and 386 of the 401 women with CIN 2+, for a sensitivity of 96.3%. HC2 was positive for 1,001/1,418 women (70.6%) and 378/401 women with CIN 2+, for a sensitivity of 94.3%. A further analysis of the tests' sensitivities for CIN 2 and ≥CIN 3 is shown in Table 1. The sensitivity values were not significantly different between the two tests. In terms of specificity, Aptima was negative for 439 of the 1,017 women with ≤CIN 1, for a specificity of 43.2%, and HC2 was negative for 394/1,017 women with ≤CIN 1, for a specificity of 38.7% ($P < 0.05$). Specificities based on CIN 1 and negative histology also showed significantly higher values for Aptima (Table 1). Table 2 provides the details of test results for the 13 cases of invasive cervical cancer. The overall agreement between Aptima and HC2 was 89.6% (1,271/1,418; kappa, 0.75). Considering only the 401 women with CIN 2+, the agreement between Aptima and HC2 was 95% (381/401); there were 372 positive and 9 negative results for both tests, and 20 samples yielded discordant results. Of the nine CIN 2+ cases testing negative by both tests, LA genotyping identified the oncogenic types targeted by both tests in four and nononcogenic types in two, and LA genotyping was negative for three. Among the 20 discordant results, there were 14 Aptima positive ($Aptima^+$) and HC2-negative ($HC2^-$) specimens, 12 of which contained the genotypes targeted by both tests and 2 were not tested by LA genotyping. The remaining six specimens with discordant results were Aptima negative ($Aptima^-$) and HC2 positive ($HC2^+$), and five of these contained the genotypes targeted by both tests and one contained untargeted nononcogenic types.

Aptima and HC2 results were further analyzed for agreement with LA genotyping results for the total of 1,418 specimens. LA genotyping was performed on 940 of the 964 $Aptima^+$ specimens, of which 936 tested positive for HPV DNA. Of these, 918 (98.1%) contained at least 1 of the 14 types targeted by Aptima. In the remaining 18, the following types, alone or in combination, were detected, in descending order of frequency: 70, 54, 42, 53, 55, 82, 61, 62, 67, 6, 83, and 89. In comparison, LA genotyping was performed on 975 of the 1,001 $HC2^+$ specimens, of which 969 tested positive for HPV DNA. Of these, 907 (93.6%) contained at least 1 of the 13 types targeted by HC2. In the remaining 62 (6.4%), the following

TABLE 2. Comparison of LBC, Aptima, HC2, Proofer, and LA genotyping results for invasive cervical cancer

Case no.	Age (yr)	LBC finding[a]	Aptima result[b]	Aptima S/CO	HC2 result[b]	HC2 RLU/CO	Proofer result[c]	Proofer genotype	LA genotype(s)	Histology[a]
1	77	UNS	Positive	13.55	Positive	31.58	Positive	33	33	SCC
2	46	ASCUS	Positive	14.22	Negative	<1	Positive	45	16, 45	SCC
3	75	UNS	Positive	10.29	Positive	27.35	NT[d]		31	SCC
4	39	ASCUS	Positive	10.85	Positive	771.23	NT		16	SCC
5	43	ASCUS	Positive	11.21	Positive	13.96	NT		16, 61	SCC
6	24	HSIL	Positive	12.27	Positive	292.38	Positive	16	16, 39, 53	SCC
7	28	LSIL	Positive	11.56	Positive	718.77	Positive	16	16	SCC
8	42	HSIL	Positive	11.49	Positive	3.32	Positive	16	16	SCC
9	30	HSIL	Positive	16.98	Positive	3.13	Positive	45	45	SCC
10	45	ASCUS	Positive	18.78	Positive	138.93	NT		16	SCC
11	51	ASCUS	Positive	13.62	Positive	42.88	NT		52, 58	SCC
12	34	LSIL	Positive	11.94	Positive	1.22	Positive	18	18, 62	SCC
13	32	ASC-H	Positive	17.52	Positive	541.52	Positive	18	18	Adenocarcinoma

[a] UNS, unsatisfactory; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells of undetermined significance, favor high grade; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.
[b] On the basis of a total of 1,418 referral specimens tested.
[c] On the basis of a subset of 818 referral specimens tested.
[d] NT, not tested due to lack of sufficient specimen.

types, alone or in combination, were detected, in descending order of frequency: 53, 66, 62, 70, 42, 54, 6, 55, 61, 89, 73, 67, 82, 40, 83, 84, 72, and 81. Since HPV-66 is a type included in Aptima but not in HC2, its prevalence was specifically analyzed. Type 66 was detected in 21 CIN 2+ cases but always in conjunction with multiple-type infections involving other oncogenic HPV types. All 21 CIN 2+ cases tested positive with both Aptima and HC2. Type 66 was also detected in 81 cases of ≤CIN 1; 68 were multiple-type infections and 13 were single-type infections. Aptima and HC2 were positive for 56 and 62 of the 68 cases of ≤CIN 1 with multiple-type infections, respectively, and 9 and 10 of the 13 cases of ≤CIN 1 with single-type infections, respectively.

The Aptima and HC2 results were also analyzed taking into account cytology results, as shown in Table 3. The proportion of cases of CIN 2+ at histology increased with increasing severity of cytological abnormalities, i.e., 28.9% for atypical squamous cells of undetermined significance (ASCUS) category, 29.9% for low-grade squamous intraepithelial lesions (LSILs), and 75.0% for high-grade squamous intraepithelial lesions (HSILs). The comparison of the sensitivities of the tests for detection of CIN 2+ in each of the cytological categories showed no significant differences. However, the specificity of Aptima was significantly higher than that of HC2 for detection of CIN 2+ in the ASCUS and LSIL categories (Table 3).

Comparison of Aptima and Proofer for detecting CIN 2+ in referral population. Due to the lack of sufficient volume of residual PreservCyt samples, the performance of Aptima could be compared with that of Proofer on only 818 of the referral case specimens. Of these, histology identified 235 (28.7%) cases of CIN 2+, which included 8 cases of invasive cervical cancer. There were 204 women with CIN 1 and 379 with either a normal colposcopy finding with no biopsy or negative histology, for a total of 583 with a diagnosis of ≤CIN 1. Aptima showed significantly higher sensitivity and significantly lower specificity than Proofer across histological grades (Table 4). However, Proofer detected all eight cases of invasive cervical cancer, and this included the case which tested negative by HC2 (Table 2). The overall agreement between Aptima and Proofer was 71.1% (582/818). The agreement between the two

TABLE 3. Correlation of Aptima and HC2 results with cytological and histological diagnoses[a]

Cytology	Histology	No. of specimens			
		Aptima		HC2	
		Positive (% sensitivity)	Negative (% specificity)	Positive (% sensitivity)	Negative (% specificity)
Negative (n = 467)	≤CIN 1 (n = 398)	196	202 (50.8)	205	193 (48.5)
	≥CIN 2 (n = 69)	62 (89.9)	7	58 (84.1)	11
ASCUS[b] (n = 419)	≤CIN 1 (n = 298)	161	137 (46.0)	178	120 (40.3)[c]
	≥CIN 2 (n = 121)	116 (95.9)	5	114 (94.2)	7
LSIL (n = 324)	≤CIN 1 (n = 227)	165	62 (27.3)	181	46 (20.3)[c]
	≥CIN 2 (n = 97)	95 (97.9)	2	93 (95.9)	4
HSIL (n = 124)	≤CIN 1 (n = 31)	27	4 (12.9)	27	4 (12.9)
	≥CIN 2 (n = 93)	92 (98.9)	1	92 (98.9)	1

[a] n = 1,334. Eighty-four cases with unsatisfactory Pap smears were excluded from the total of 1,418.
[b] Includes 22 cases of ASCUS, favor high grade (ASC-H), and two cases of atypical glandular cells.
[c] Specificities for detection of CIN 2+ between Aptima and HC2 significant at $P < 0.05$

TABLE 4. Comparison of Aptima and Proofer tests for detection of cervical intraepithelial lesion in referral population by histological grade[a]

Diagnostic index	Histological category	Aptima		Proofer	
		No. of relevant results/no. tested (%)	95% confidence interval	No. of relevant results/no. tested (%)	95% confidence interval
Sensitivity	CIN 2+ (n = 235)	223/235 (94.9)	92.1, 97.7	186/235 (79.1)[b]	73.9, 84.3
	CIN 2 (n = 63)	58/63 (92.1)	85.4, 98.8	40/63 (63.5)[b]	51.6, 75.4
	≥CIN 3 (n = 172)	165/172 (95.9)	92.9, 98.9	146/172 (84.9)[b]	79.5, 90.3
Specificity	≤CIN 1 (n = 583)	267/583 (45.8)	41.8, 49.8	438/583 (75.1)[c]	71.6, 78.6
	CIN 1 (n = 204)	54/204 (26.5)	20.4, 32.6	135/204 (66.2)[c]	59.7, 72.7
	Negative (n = 379)	213/379 (56.2)	51.2, 61.2	303/379 (79.9)[c]	75.9, 83.9
Positive predictive value	CIN 2+ (n = 235)	223/539 (41.4)	37.2, 45.6	186/331 (56.2)	50.9, 61.5
Negative predictive value	≤CIN 1 (n = 583)	267/279 (95.7)	93.3, 98.1	438/487 (89.9)	87.2, 92.6

[a] n = 818.
[b] Sensitivities for detection of CIN 2+ between Aptima and Proofer significant at $P < 0.05$.
[c] Specificities for detection of CIN 2+ between Aptima and Proofer significant at $P < 0.05$.

tests for the detection of CIN 2+ was 81.7% (192/235); there were 183 positive results and 9 negative results for both tests, and 43 specimens yielded discordant results. Of the nine CIN 2+ cases testing negative by both tests, LA genotyping detected at least one of the genotypes targeted by Aptima in four and nononcogenic types in three, and two were negative. However, none of these specimens contained the five types targeted by Proofer. The analysis of the 43 discordant results is shown in Table 5.

Aptima and Proofer results were further analyzed for agreement with LA genotyping results on the total of 881 specimens. Of the 331 Poofer-positive (Proofer$^+$) specimens, LA genotyping identified at least one of the five types targeted by Proofer in 320 (96.7%). Considering type-specific results, there was a 96.1% (318/331) agreement between Proofer and LA genotyping. Of the 235 CIN 2+ cases, there were 200 (85.1%) containing at least one of the five types targeted by Proofer, and 197 (98.5%) and 182 (91.0%) of these were positive by Aptima and Proofer, respectively. Of the 18 Proofer-negative CIN 2+ cases in this subset, Aptima was positive for 17. Among the 35 CIN 2+ cases that did not have the five types targeted by Proofer, LA genotyping detected other oncogenic types in 24 (77.4%) of the 31 tested, with the most common types being 52, 51, 58, and 39. The Aptima and Proofer results were also analyzed taking into account the cytology results, as shown in Table 6. The comparison of sensitivities and specificities of these tests for the detection of CIN 2+ in each of the cytological grades showed significant differences, with Aptima showing a higher sensitivity and a lower specificity than Proofer (Table 6).

Comparison of Aptima and HC2 for detecting CIN 2+ in routine screened population. In the 1,373 women participating in routine cervical cancer screening, 7 were found to have CIN 2+, including a case of adenocarcinoma. Both Aptima and HC2 were positive in all seven cases of CIN 2+. The remaining 1,366 with ≤CIN 1 included 30 with CIN 1 and 1,336 with either normal colposcopy findings with no biopsy or negative histology. The specificities on the basis of the results obtained with the 1,366 women with ≤CIN 1 were 88.4% (95% CI, 86.6, 90.0) for Aptima and 85.2% (95% CI, 83.3, 87.1) for HC2 ($P < 0.05$). As this study population comprised women 16 to 81 years of age and HPV-based screening is indicated for women ≥30 years, specificity was reassessed on the basis of the results for women in this age group (n = 845). Aptima showed a specificity of 93.9% (95% CI, 92.3, 95.5), whereas HC2 showed a specificity of 92.1% (95% CI, 90.3, 93.9) ($P < 0.05$). The corresponding figures for women <30 years of age (n = 521) were 79.5% (95% CI, 75.8, 82.8) and 74.1% (95% CI, 70.3, 77.9), respectively ($P < 0.05$).

DISCUSSION

The main goal of this study was to compare the clinical performance of the Aptima mRNA assay with that of HC2 for the detection of cervical precancerous lesions and cancer in

TABLE 5. Analysis of 43 Aptima-Proofer discordant results in CIN 2+ cases

Result	n[a]	No. of specimens with the following LA genotyping result:					
		Positive for Aptima		Positive for Proofer		Negative for Proofer targeted types	Not tested[b]
		Targeted types	Untargeted types	Targeted types	Untargeted types		
Aptima$^+$/Proofer$^-$	40	19	18	17	2	18	3
Aptima$^-$/Proofer$^+$	3	3		3			

[a] n, total number of specimens tested.
[b] Not tested due to lack of sufficient specimen.

TABLE 6. Correlation of Aptima and Proofer results with cytological and histological diagnoses[a]

Cytology	Histology	No. of specimens			
		Aptima		Proofer	
		Positive (% sensitivity)	Negative (% specificity)	Positive (% sensitivity)	Negative (% specificity)
Negative (n = 222)	≤CIN 1 (n = 193)	76	117 (60.6)	36	157 (81.3)[b]
	≥CIN 2 (n = 29)	25 (86.2)	4	21 (72.4)[d]	8
ASCUS[c] (n = 226)	≤CIN 1 (n = 167)	90	77 (46.1)	42	125 (74.9)[b]
	≥CIN 2 (n = 59)	54 (91.5)	5	44 (74.6)[d]	15
LSIL (n = 226)	≤CIN 1 (n = 161)	112	49 (30.4)	48	113 (70.2)[b]
	≥CIN 2 (n = 65)	63 (96.9)	2	50 (76.9)[d]	15
HSIL (n = 91)	≤CIN 1 (n = 23)	20	3 (13.0)	11	12 (52.2)[b]
	≥CIN 2 (n = 68)	67 (98.5)	1	58 (85.3)[d]	10

[a] n = 765. Fifty-three cases with unsatisfactory Pap smears were excluded from the total of 818.
[b] Specificities for detection of CIN 2+ between Aptima and Proofer significant at $P < 0.05$.
[c] Includes 19 cases of ASCUS, favor high grade (ASC-H), and 1 case of atypical glandular cells.
[d] Sensitivities for detection of CIN 2+ between Aptima and Proofer significant at $P < 0.05$.

both colposcopy referral and routinely screened populations. The Aptima assay was also compared with Proofer in the referral population. We chose referral patients because this population selects out women with the highest prevalence of cervical oncogenic HPV infection and the associated cervical precancerous lesions and cancer. This allowed us to demonstrate the relative sensitivity of Aptima with a large number of CIN 2+ cases within a limited sample size of the study population and to also assess its performance for the triage of borderline cytological abnormalities. On the other hand, the majority of women undergoing routine cervical cytology screening do not have HPV infection and thus have negligible prevalent cervical lesions, and this served as an ideal population with which to define the relative specificity of the Aptima test. With a sample size of 1,418 referral women with 401 cases of CIN 2+ (CIN ≥3, n = 281), this study is the largest cross-sectional study to date to assess the performance of Aptima in comparison with that of HC2 and the second study to compare Aptima with Proofer in this population. In addition, our study is one of the few studies to assess the performance of Aptima in a routinely screened population.

The Aptima-HC2 comparative study with the referral population showed a slightly higher sensitivity for Aptima than HC2, i.e., 96.3% versus 94.3%, for the detection of CIN 2+. Recently, published studies with similar referral populations showed a sensitivity of 95.5% for Aptima versus 99.6% for HC2 in the United Kingdom (40) and sensitivities of 90.8% and 95.0%, respectively, in France (12). A study in Germany has shown similar results with cervical specimens tested from cytologically abnormal cases stored for up to 3 years (5a). While our observed sensitivity rate in a Canadian population was generally in agreement with those from the studies described above, it should be noted that the manufacturer has recently revised the test's cutoff from ≥1.0, which was used in the above studies, to the cutoff of ≥0.5 that we used in our study. The slightly increased sensitivity of Aptima over that of HC2 that we observed may be, to some extent, attributable to this lower test cutoff. It is also worth noting that Aptima was positive for all 13 cases of cervical cancer, compared to 12 detected by HC2. Our study showed that the specificity of Aptima was significantly higher than that of HC2, i.e., 43.2% and 38.7%, respectively, consistent with the findings of other studies (12, 40). The higher specificity of Aptima could be attributable to the targeting of mRNA as well as the absence of cross-reactivity. Our data indicated that the lower specificity of HC2 is due in part to its cross-reactivity with untargeted nononcogenic types. The HC2 cross-reactivity rate of 6.4% that we observed in the present study is similar to the rate of 6.5% noted in a previous study (29) and the rate of 7.9% reported in a larger study (5). Regardless, the overall agreement of Aptima with HC2 was good, reaching 95.0% for the detection of CIN 2+.

The Aptima-Proofer comparative study utilizing specimens from a subset of the referral population showed a significantly higher sensitivity for Aptima than Proofer, i.e., 94.9% and 79.1%, respectively, and a lower specificity for Aptima than Proofer, i.e., 45.8% and 75.1%, respectively. In a study similar to ours conducted in the United Kingdom, Proofer showed a sensitivity of 73.6% and a specificity of 73.1% for detecting CIN 2+ (40). The higher sensitivity and lower specificity of Aptima compared with those of Proofer are attributable to the wider spectrum of HPV types targeted by Aptima and the higher prevalence of oncogenic types other than the five targeted by Proofer in ≤CIN 1 lesions. Conversely, the lower sensitivity and higher specificity of Proofer are mainly due to the limited number of HPV genotypes targeted by Proofer, as previously reported (29). While a higher specificity of an HPV test is desirable, targeting only the most common oncogenic types in cervical cancer may have adverse clinical implications. Regardless, Proofer, targeting the five most common oncogenic types in cervical cancer, still might identify the majority of women whose cervical lesions are likely to progress, and there is an indication that Proofer is a better predictor of CIN 2+ than DNA-based tests (17, 19, 24). Further, as opposed to Aptima, Proofer provides simultaneous type-specific HPV identification, which is useful for risk stratification and better clinical management, especially for infections with HPV types 16 and 18 (14).

Our study of the referral population utilized LA genotyping to assess false-negative and discordant results with the HPV

tests. It should be noted that while LA genotyping is a standardized commercially available test, the failure of this test to detect some or all of the targeted HPV types in a sample has been reported (41) and cannot be ruled out in our study. Our data indicated a better overall agreement of both Aptima and Proofer results with LA genotyping results (98.1% and 96.7%, respectively) than HC2 results with LA genotyping results (93.6%). The lower agreement of HC2 is attributable to its cross-reactivity with untargeted nononcogenic types, as noted above. In contrast, Aptima$^+$/Proofer$^-$ results were attributable to oncogenic types not targeted by Proofer. In a small proportion of Aptima- and Proofer-positive specimens, LA genotyping identified nononcogenic types exclusively. It is not known whether this is due to the difference in the tests' targets or the limitation of LA genotyping to detect coexisting oncogenic types. The genotypic analysis highlights the relative analytical specificities of the three tests, with Aptima showing a better specificity. Our data also provided confirmation that the five types targeted by Proofer accounted for 85.1% of CIN 2+ cases in the North American setting, and this is similar to the 82.1% fraction we previously reported (29) and consistent with the recognized prevalence rates (2, 6, 36). This implies that the remaining CIN 2+ fraction is attributable to other oncogenic types. LA genotyping confirmed that a majority of Proofer-negative CIN 2+ cases, in fact, contained oncogenic HPV types not targeted by that assay; most of these tested positive by Aptima. If we restrict Proofer sensitivity analysis to the five types targeted by the test, the sensitivity of Proofer would improve significantly, reaching 91% compared with a sensitivity of 98.5% for Aptima (data not shown). The above difference in sensitivity may be attributable to a higher threshold of the Proofer cutoff for the detection of E6/E7 mRNA, as previously reported (29). Further, we specifically analyzed our genotypic data to determine the relevance of including type 66 in the Aptima test. The data indicated that type 66 was detected only in association with other oncogenic types in CIN 2+ cases and as a single type only in ≤CIN 1 cases, the majority of which tested positive by Aptima and also by HC2 due to its known cross-reactivity. Considering these results and the high probability of regression of CIN 1 lesions, our data appear to suggest that the inclusion of type 66 in the Aptima test is more likely to reduce specificity than increase sensitivity. While this is a speculation based on a limited number of cases and requires verification, it is worth noting that although type 66 is considered an oncogenic type and indicated for inclusion in HPV tests (7, 21, 39), there is only limited evidence for its oncogenic potential in cervical cancer (3).

Our analysis of the relative performance of Aptima stratified by cytological grades in the referral population provides data on the potential application of this test for the triage of borderline cytological abnormalities. Due to its higher specificity, Aptima would reduce colposcopy referrals by nearly 6.0% compared to HC2 for women with ASCUS cytology while retaining the same level of sensitivity as HC2 for the detection of CIN 2+. This reduction would remain about the same if these tests are used for women with LSIL cytology as well (Table 3). A similar analysis of Aptima with Proofer indicated that the Aptima referral rate would be significantly higher than the Proofer referral rate due to the lower specificity of Aptima compared with that of Proofer. But the higher specificity of Proofer and the attendant greater reduction in referral rate is achieved at the cost of lowered sensitivity (Table 4). This is to be taken into account when balancing sensitivity and specificity and could be influenced by specific needs and settings.

We chose to include women <30 years of age in our routine screened population to further define the potential for increased specificity of an mRNA-based test as this age group is known to have a higher prevalence of transient HPV infection. Aptima showed a significantly higher specificity than HC2 in this age group as well as those ≥30 years of age, while it maintained the same level of sensitivity as HC2 for detecting CIN 2+. This has significance in population-based adjunct or primary HPV screening, considering that even a small reduction in test specificity can result in a large number of women being unnecessarily targeted for intensified clinical management. A recent French study based on 4,429 routinely screened women 20 to 65 years of age has reported sensitivities of 95.7% and 95.3% for Aptima and HC2, respectively, for the detection of CIN 3 or worse, with Aptima showing a significantly higher specificity, i.e., 90.31% versus 84.9% (25). A study in China based on 2,097 routinely screened women 25 to 29 years of age has shown similar results (44a).

The recently published guidelines for validating new HPV assays for primary cervical screening advise that this be conducted with a representative population-based screening cohort of women ≥30 years of age and that the sensitivity of a candidate test be at least 90% of the sensitivity of HC2 and achieve a statistical power of over 99% when 100 cases of CIN 2+ are tested (21). Although there were only a very few CIN 2+ cases in our routine screened population due to the limited sample size, there were 401 cases of CIN 2+ in the referral population, with Aptima showing sensitivity equal to that of HC2 for detecting CIN 2+. Considering that ≥CIN 3 is a better marker of risk of progression to invasive cervical cancer than ≤CIN 2 (21, 35), an equivalent sensitivity of Aptima was also observed on the basis of 281 cases of ≥CIN 3 (Table 1). Regarding specificity, the guidelines advise that a candidate test should have specificity at least 98% of the specificity of HC2 and that this be demonstrated with a minimum random sample of 800 women ≥30 years of age to achieve a power of 80%. In our sample size of 845 routinely screened women in this age group, Aptima showed a significantly higher specificity than HC2. It should also be noted that the performance indices of HC2 in our studies of both referral and routine screened populations were consistent with those previously reported (10, 21, 40). In conclusion, our data indicate that the Aptima test is as sensitive as HC2 but more specific for detecting CIN 2+ and has the potential to serve as a reliable test for both primary cervical cancer screening and the triage of borderline cytological abnormalities.

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