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Analytical characterization of the APTIMA® HPV Assay

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ABSTRACT

Background: Human papillomavirus (HPV) testing has improved the sensitivity for the detection of cervical pre-cancer and cancer as compared to Pap testing. Several HPV tests are commercially available and most target the DNA from 13 or 14 high-risk HPV types. The APTIMA® HPV Assay however, detects HPV E6/E7 mRNA from 14 high-risk types of HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
Objective: To determine the analytical performance characteristics of the APTIMA HPV Assay.
Study Design: Analytical sensitivity, analytical specificity, reproducibility, and the effect of potentially interfering substances was determined for the APTIMA HPV Assay on both the DTS (semi-automated) and TIGRIS DTS (fully automated) systems.
Results: The 95% detection limit for both systems was between 17 and 488 copies/reaction, depending on the HPV type. The assay did not cross-react with normal flora and opportunistic organisms that may be found in cervical samples, or low-risk HPV types. Spermicides, anti-fungal and anti-itch medications, whole blood, glacial acetic acid, and most lubricants did not interfere with assay performance. Those lubricants containing polyquaternium 15 did interfere with assay performance. Inter-instrument, inter-operator, inter-lot, and inter-run signal variability were <10% for >99% of the data. Intra-run variability was <15%, except for those samples with concentrations at or below the 95% detection limit of the assay.
Conclusions: Based upon the analytical sensitivity, analytical specificity, and low variability, the APTIMA HPV Assay showed excellent performance and robustness.

1. Abbreviations

CE: Conformité Européenne
CI: confidence interval
CIN: cervical intraepithelial neoplasia
%CV: percent coefficient of variation
DNA: deoxy nucleic acid
DTS: direct tube sampling
E: early
HC2: Digene Hybrid Capture 2 High Risk HPV DNA Test
HPV: human papillomavirus
L: late
mL: milliliter
Pap: Papanicolaou
RNA: ribonucleic acid
S/CO: signal to cutoff ratio
STM: specimen transport medium
v/v: volume/volume
w/v: weight/volume
WHO: World Health Organization

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2. Introduction

Cervical cancer is the most common cancer affecting women in developing countries and the second most common cause of female cancer mortality worldwide.[1] Almost all (99.8%) cases of cervical cancer are caused by human papillomavirus (HPV), which is contracted by sexual transmission.[2]

HPV is a common DNA virus that infects skin or mucosal cells.[3] There are more than 100 types of HPV, of which approximately 40 are sexually transmitted.[4–6] However, 14 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are associated with an increased risk of developing cervical cancer and are considered high-risk HPV types.[7] Persistent

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infection with one or more of the 14 high-risk HPV types is the principal cause of cervical intraepithelial neoplasia (CIN), the precursor of cervical cancer.[8] Other HPV types are associated with a low risk of developing cervical cancer and/or the development of genital warts.[3,6]

Papillomaviruses are small, non-enveloped, double-stranded circular DNA viruses comprised of 8 genes: the early (E) genes and the late (L) genes.[3] The proteins encoded by the E6 and E7 genes bind to cellular pRB and p53 proteins, respectively, disrupting their functions. This interaction results in the alteration of cell cycle regulatory pathways, which leads to cellular transformation.[3] Thus, the E6 and E7 genes are believed to be the major requirement for the development of CIN and cervical cancer progression.

The Papanicolaou (Pap) test has been the main diagnostic tool to screen for cervical cancer for many years, however it has limited sensitivity, and can fail to detect cancerous cells in as many as one-third of routine Pap test screenings.[9] HPV DNA testing has been found to be more sensitive than the Pap test in detecting CIN 2 and CIN 3 pre-cancerous cervical cells.[10–13]

Currently, the Digene Hybrid Capture® 2 HPV DNA Test (HC2, Qiagen, Germantown, MD) and the Hologic Cervista™ HPV HR test (Hologic Incorporated, Bedford, MA) are the only United States Food and Drug Administration (US FDA) cleared tests. However, several HPV tests that detect HPV DNA or RNA are currently available in Europe and have been CE-marked (Conformité Européenne). HC2, the Roche AMPLICOR® HPV Test (Amplicor, Roche Molecular Diagnostics, Pleasanton, CA), and the Abbott RealTi*m*e High Risk HPV assay (Abbott Molecular, Abbott Park, IL) are all tests that detect the DNA from 13 or 14 high-risk HPV types. The NorchipPreTect HPV-Proofer Test HPV-Proofer (Norchip AS, Klokkarstua, Norway) detects the E6/E7 mRNA of 5 high-risk HPV types. While the performance of the Abbott test has not been extensively evaluated in the field, the performance of the HC2, Amplicor and Proofer tests is not optimal. The HC2 and Amplicor tests are very sensitive for detection of HPV DNA, however they do not discriminate between progressive and transient infections and therefore are not very specific for detecting high-grade lesions. The Proofer mRNA test is more specific than the two DNA assays but lacks sensitivity.[14]

The present study was conducted to determine the analytical performance of the CE-marked Gen-Probe® APTIMA HPV Assay. The APTIMA HPV Assay detects HPV E6/E7 mRNA which provides an important advantage because E6/E7 mRNA expression correlates with increased cervical lesion severity and, therefore, is a better indicator of disease progression than simply the presence of HPV DNA.[15–17] In addition, the assay detects 14 high-risk types, which provides better sensitivity than the Proofer test, which only detects 5 high-risk types.

3. Materials and methods

3.1. APTIMA HPV Assay technology

The APTIMA HPV Assay (Gen-Probe Incorporated, San Diego, CA) is a multiplex nucleic acid test that detects HPV E6/E7 mRNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The assay provides a qualitative result (positive/negative) for the presence/absence of these high-risk HPV types, but does not determine the specific HPV type present in the specimen.

The APTIMA HPV Assay involves three main steps, which take place in a single tube: (1) capture of the target mRNA using HPV-specific capture oligomers and magnetic microparticles; (2) target mRNA amplification using transcription-mediated amplification;[18,19] and (3) detection of the amplification products (amplicons) using the Hybridization Protection Assay.[20]

3.2. Specimen processing

One milliliter of a ThinPrep (Hologic Incorporated, Bedford, MA) liquid Pap specimen is transferred to an APTIMA Specimen Transfer tube (Gen-Probe Incorporated, San Diego, CA) containing 2.9 mL of GEN-PROBE Specimen Transport Medium (STM). Four hundred microliters of the diluted liquid Pap specimen is tested in the APTIMA HPV Assay.

3.3. Controls and calibrators

An internal control transcript is added to each reaction to verify the performance of each step of the assay: capture, amplification and detection. The signal detected from the internal control amplicon is differentiated from that of the HPV target by the Dual Kinetic Assay. This method allows for differentiation of the kinetics of light emission from different labels on the probes.[21]

One positive calibrator (in vitro transcript in a buffered solution) and one negative calibrator (buffered solution) are tested in triplicate at the beginning of each run. They are used to determine the validity of the run and to establish the assay cutoff values for the internal control and analyte signals. The signal observed for each reaction is then compared to the cutoff values. Those reactions with an analyte signal to cutoff (S/CO) ratio $\geqslant$1.00 are considered positive for HPV. Those samples with an analyte S/CO ratio <1.00 must have an internal control signal greater than or equal to the internal control cutoff value to be considered a valid negative result.

A positive control (lysed, inactivated HPV-positive cultured cells) and a negative control (lysed, inactivated HPV-negative cultured cells) are also processed as separate samples and used to determine run validity.

3.4. Instrumentation

The assay can be performed on the semi-automated Direct Tube Sampling (DTS) systems, comprised of a TECAN EVO pipettor (optional), a GEN-PROBE SB100 dry heat block and vortexer, a Target Capture system and a LEADER HC+ luminometer, or on the fully automated TIGRIS DTS system (Gen-Probe Incorporated, San Diego, CA). The throughput is approximately 180 specimens for 1 operator in about 5 to 6 hours for the DTS systems and approximately 1000 specimens in about 14 hours for the TIGRIS DTS system.

3.5. Analytical sensitivity study design

The analytical sensitivity of the assay was determined by spiking individual HPV-negative clinical Pap specimens (in PreservCyt solution) with HPV in vitro transcripts, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, or HPV-infected cells obtained from ATCC, SiHa (HTB-35), HeLa (CCL-2), ME180 (HTB-33), and MS751 (HTB-34), at various concentrations. SiHa cells (HPV 16) have been reported to contain 1–2 copies of integrated viral genome,[22] HeLa cells (HPV 18) 10–50 copies,[22] ME180 cells (HPV 68) 2 copies,[23] and MS751 cells (HPV 45)

Table 1
Analytical specificity panel

Description	Concentration (per mL of test sample)	Description	Concentration (per mL of test sample)
HPV low risk types		**Bacteria/Fungi/Protozoa** (cont'd)	
HPV 6	2.5×10^6 copies	*Gardnerella vaginalis*	1×10^8 CFU
HPV 11	2.5×10^6 copies	*Haemophilus ducreyi*	1×10^8 CFU
HPV 42	2.5×10^6 copies	*Klebsiella pneumoniae*	1×10^8 CFU
HPV 43	2.5×10^6 copies	*Lactobacillus acidophilus*	1×10^8 CFU
HPV 44	2.5×10^6 copies	*Lactobacillus crispatus*	1×10^8 CFU
HPV 53	2.5×10^6 copies	*Lactobacillus delbrueckii* ssp. *bulgaricus*	1×10^8 CFU
HPV 61	2.5×10^6 copies	*Lactobacillus jensenii*	1×10^8 CFU
HPV 71	2.5×10^6 copies	*Listeria monocytogenes*	1×10^8 CFU
HPV 81	2.5×10^6 copies	*Mobiluncus curtisii*	2×10^7 CFU
Adenovirus 2	1×10^6 vp	*Mycobacterium smegmatis*	1×10^8 CFU
Cytomegalovirus	3.3×10^1 TCID 50	*Mycoplasma fermentans*	5×10^7 CFU
Epstein–barr virus	4×10^7 vp	*Mycoplasma genitalium*	1×10^8 CFU
HIV-1	1×10^6 copies	*Mycoplasma hominis*	5×10^7 CFU
Herpes simplex virus 1	2.5×10^5 TCID 50	*Neisseria gonorrhoeae*	1×10^8 CFU
Herpes simplex virus 2	5×10^4 TCID 50	*Neisseria meningitidis*	1×10^8 CFU
SV40	1.2×10^4 TCID 50	*Peptoniphilus lacrimalis*	1×10^8 CFU
Bacteria/Fungi/Protozoa		*Peptostreptococcus anaerobius*	1×10^8 CFU
Acinetobacter lwoffii	1×10^8 CFU	*Propionibacterium acnes*	1×10^8 CFU
Actinomyces israelii	1×10^8 CFU	*Proteus mirabilis*	1×10^8 CFU
Alcaligenes faecalis	1×10^8 CFU	*Proteus vulgaris*	1×10^8 CFU
Atopobium vaginae	5×10^7 CFU	*Providencia stuartii*	1×10^8 CFU
Bacillus cereus	1×10^8 CFU	*Pseudomonas aeruginosa*	1×10^8 CFU
Bacteroides fragilis	1×10^8 CFU	*Ruminococcus productus*	1×10^8 CFU
Bacteroides ureolyticus	1×10^8 CFU	*Serratia marcescens*	1×10^8 CFU
Bifidobacterium adolescentis	1×10^8 CFU	*Staphylococcus aureus*	1×10^8 CFU
Bifidobacterium breve	1×10^8 CFU	*Staphylococcus epidermidis*	1×10^8 CFU
Campylobacter fetus-fetus	1×10^8 CFU	*Staphylococcus saprophyticus*	1×10^8 CFU
Clostridium difficile	6×10^7 CFU	*Streptococcus agalactiae*	1×10^8 CFU
Clostridium perfringens	1×10^8 CFU	*Streptococcus pyogenes*	1×10^8 CFU
Corynebacterium genitalium	1×10^8 CFU	*Streptococcus sanguinis*	1×10^8 CFU
Corynebacterium xerosis	1×10^8 CFU	*Ureaplasma urealyticum*	1×10^8 CFU
Enterobacter cloacae	1×10^8 CFU	*Candida albicans*	1×10^8 CFU
Enterococcus faecalis	1×10^8 CFU	*Chlamydia trachomatis*	2×10^4 TCID 50
Escherichia coli	1×10^8 CFU	*Neisseria gonorrhoeae* and *Chlamydia trachomatis*	5×10^8 CFU, 1.5×10^4 TCID 50
Finegoldia magna	1×10^8 CFU	*Trichomonas vaginalis*	1×10^7 cells
Fusobacterium nucleatum	1×10^8 CFU		

vp = viral particle; CFU = colony-forming unit; TCID = tissue culture infectious dose.

1 integrated copy of the HPV viral genome.[24] Each of the cell lines has also been reported to express E6/E7 mRNA. The individual specimens were diluted 1:2.9 with STM. Thirty replicates of each concentration were tested with two lots of reagent (total n = 60) in both the DTS and TIGRIS DTS systems. For each concentration, the positivity rate was calculated and the predicted 95% detection limit was determined using Probit regression analysis for each HPV type on each system. Probit analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.6. Analytical specificity study design

The analytical specificity of the assay was determined by spiking PreservCyt solution with various organisms or nucleic acid at various concentrations including: 9 low-risk HPV in vitro transcripts; 53 species of cultured bacteria and mycoplasma (found in the normal vaginal flora, intestinal flora, or in genito-urinary infections); 1 yeast/fungus; 1 protozoan; and 7 common human viruses (Table 1). The spiked PreservCyt solutions were diluted 1:2.9 with STM. Twelve replicates of each panel member were tested with one reagent lot on both the DTS and TIGRIS DTS systems. Twenty-four replicates of a control panel of PreservCyt solution were also tested on both systems. The assay specificity was calculated as follows: (number of valid replicates tested − number of positive replicates)/(number of valid replicates tested). The 95% confidence interval was calculated using the Score method.

Table 2
Interference panel

Product category	Product brand
Lubricant	KY® Sensual Mist™ Personal Lubricant (v/v)
	KY® Warming™ Jelly Personal Lubricant (w/v)
	KY® Warming Liquid Personal Lubricant (v/v)
	Astroglide® Personal Lubricant & Moisturizer (v/v)
	Target® Lubricating Liquid Personal Lubricant (v/v)
Spermicide	Ortho Options® Gynol II® Vaginal Contraceptive Jelly Original Formula (w/v)
	Ortho Options® Gynol II®Vaginal Contraceptive Jelly Extra Strength (w/v)
	Ortho Options™ Delfen® Vaginal Contraceptive Foam (w/v)
	Encare® Vaginal Contraceptive (w/v)
	Ortho Options® Conceptrol® Vaginal Contraceptive (w/v)
Anti-fungal/anti-itch medication	Vagisil® Anti-Itch Crème Maximum Strength (w/v)
	Monistat® Soothing Care® Itch Relief Cream (w/v)
	Monistat® 3 Combination Pack (w/v)
	Target® Tioconazole 1 Vaginal Antifungal (w/v)
	Target® Miconazole 3 Vaginal Antifungal combination pack (w/v)
Glacial Acetic Acid	N/A (v/v)
Whole Blood	N/A (v/v)

v/v = volume/volume; w/v = weight/volume.

3.7. Interference study design

Assay sensitivity and specificity in the presence of various gynecological and feminine hygiene substances (Table 2), as well as whole blood and glacial acetic acid, was determined by spiking each substance (at 1% and 10% volume/volume [v/v] or weight/volume [w/v]) into PreservCyt solution and PreservCyt solution containing HPV 16-infected SiHa cells (at approximately 3 cells per reaction). The PreservCyt solutions were diluted 1:2.9 in STM and twelve replicates of each panel member tested on both the DTS and TIGRIS DTS systems. A control panel comprised of PreservCyt solution, with and without HPV-infected SiHa cells, was also tested (n = 24) on each system. The assay specificity was calculated as follows for the HPV-negative panels: (number of valid replicates tested − number of positive replicates)/(number of valid replicates tested). The assay sensitivity was calculated as follows for the HPV-positive panels: (number of valid positive replicates)/(number of valid replicates tested). The 95% confidence intervals were determined using the Score method.

3.8. Reproducibility study design

The reproducibility of the assay was determined by testing a panel of 16 samples in triplicate in two runs with two lots of reagents, on three instruments (for each of the DTS and TIGRIS systems), by three operators at one site (108 total replicates). The panel of 16 samples consisted of: 6 HPV-negative samples (3 samples were STM [panel members 1, 8 and 12] and 3 were pools of residual HPV-negative liquid Pap specimens in PreservCyt solution [panel members 4, 9 and 16]); 4 low positive HPV samples in STM at the approximated 95% detection limit (samples contained 0.15 HeLa cell/reaction [panel member 3], 1 SiHa cell/reaction [panel member 2], HPV 16 in vitro transcript at 30 copies/reaction [panel member 10] or HPV 18 in vitro transcript at 30 copies/reaction [panel member 11]); and 6 moderately positive HPV samples in STM at greater than or equal to 3 times the 95% detection limit (samples contained 1 HeLa and 10 SiHa cells/reaction [panel member 7], 1 ME180 cells/reaction [panel member 5], 1 MS751 cells/reaction [panel member 6], HPV 16 in vitro transcript at 100 copies/reaction [panel member 13], HPV 18 in vitro transcript at 100 copies/reaction [panel member 14] or HPV 16 and 18 in vitro transcripts at 100 copies/reaction [panel member 15]). The percent agreement with the expected results was calculated. The inter-instrument, inter-operator, inter-lot, inter-run, and intra-run variabilities were determined by calculating the percent coefficient of variation (%CV) for each analyte S/CO.

4. Results

4.1. Analytical sensitivity

Analytical sensitivity results (95% detection limit) for both the DTS and TIGRIS DTS systems are presented in Table 3. Depending upon the high-risk HPV type, the analytical sensitivity of the assay in the semi-automated DTS systems was between 38 and 488 HPV mRNA copies/reaction; HPV types 31, 33, 35, 39, 45, 59, and 66 had predicted 95% detection limits less than 100 mRNA copies/reaction; HPV types 16, 18, 56, and 68 had predicted 95% detection limits between 100 and 200 mRNA copies/reaction; and HPV types 51, 52, and 58 had predicted 95% detection limits between 300 and 500 mRNA copies/reaction.

In the fully automated TIGRIS DTS system, the analytical sensitivity was between 17 and 275 HPV mRNA copies/reaction. HPV types 16, 31, 33, 35, 39, 45, 56, 58, 59, and 68 had predicted 95% detection limits less than 100 mRNA copies/reaction; HPV types 18, 51, and 66 had predicted 95% detection limits between 100 and 200 mRNA copies/reaction; and only HPV type 52 had a predicted 95% detection limit between 200 and 300 mRNA copies/reaction. This showed that the APTIMA HPV Assay had a slightly higher analytical sensitivity on the TIGRIS DTS system than on the DTS systems.

Table 3
APTIMA HPV Assay analytical sensitivity

HPV type	95% detection limit (95% CI), number of copies/reaction	
	DTS systems	TIGRIS DTS system
HPV 16	106 (71–181)	45 (33–71)
HPV 18	116 (81–189)	123 (87–196)
HPV 31	38 (26–64)	24 (17–38)
HPV 33	39 (27–67)	47 (33–74)
HPV 35	43 (31–67)	56 (39–92)
HPV 39	41 (29–67)	17 (12–28)
HPV 45	54 (37–88)	69 (47–115)
HPV 51	488 (335–804)	195 (150–283)
HPV 52	357 (256–559)	275 (197–431)
HPV 56	127 (89–202)	83 (61–127)
HPV 58	301 (193–541)	93 (67–144)
HPV 59	98 (72–151)	73 (51–121)
HPV 66	64 (46–101)	145 (107–222)
HPV 68	143 (100–232)	46 (35–70)
SiHa cells	1.073 (0.738–1.768)	1.206 (0.814–2.035)
HeLa cells	0.06 (0.043–0.093)	0.096 (0.066–0.159)
ME180 cells	0.033 (0.024–0.05)	0.05 (0.036–0.079)
MS751 cells	0.052 (0.035–0.087)	0.08 (0.053–0.139)

CI = confidence interval.

Table 4
APTIMA HPV Assay analytical specificity

Samples/Organisms	No. of sample types tested	DTS systems			TIGRIS DTS system		
		No. of replicates[a]	Specificity (95% CI)	Average S/CO (SD)	No. of replicates[a]	Specificity (95% CI)	Average S/CO (SD)
Control	N/A	24	100% (90–100%)	0.01 (0.03)	24	100% (90–100%)	0.00 (0.00)
Low-risk HPV	9	108[b]	99% (95–100%)	0.02 (0.12)	108	99% (95–100%)	0.11 (1.15)
Bacteria/fungi/protozoa	55	660	100% (99–100%)	0.00 (0.03)	660	99% (98–100%)	0.01 (0.19)
Viruses	7	84	100% (96–100%)	0.00 (0.02)	84	99% (94–100%)	0.07 (0.60)

SD = standard deviation; N/A = not applicable.
[a] 12 replicates were tested per sample. [b] 4 invalid replicates were excluded from the analysis.

The assay also detected between 0.03 and 1.2 HPV-infected cells per reaction of SiHa, HeLa, ME180 and MS751 cultured cells in the DTS and TIGRIS DTS systems, suggesting that each infected cell contained multiple HPV mRNA copies (Table 3).

4.2. Analytical specificity

Analytical specificity and cross-reactivity of the APTIMA HPV Assay with organisms described in Table 1, on both the DTS and TIGRIS DTS systems, are presented in Table 4. The overall analytical specificity of the assay was equal to or greater than 99% in both systems for each group tested. In the DTS systems, HPV 44 yielded one false positive result out of 12 replicates (n = 104 total replicates for all low-risk HPV types). In the TIGRIS DTS system, testing of HPV 42, *Alcaligenes faecalis*, *Enterococcus faecalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and adenovirus 2 yielded false positive results in one out of 12 replicates of each tested. The average analyte S/CO values were all <0.11, well below the assay analyte cutoff of 1.00. Thus, while a few replicates yielded false positive results, no single organism consistently yielded false positive results in either system, suggesting that these were random events rather than true cross-reactivity.

4.3. Interference

Interference results are presented in Table 5 (sensitivity) and Table 6 (specificity) for both the DTS and TIGRIS DTS systems. When lubricants lacking polyquaternium 15, or spermicides, anti-fungal or anti-itch medications, whole blood, or glacial acetic acid were present in the samples at 1% and 10%, the assay sensitivity was 100% (95% CIs ranged from 90% to 100%; see Table 5) and the specificity was 100% (95% CIs ranged from 90% to 100%; see Table 6) in the DTS and TIGRIS DTS systems, demonstrating that these substances did not interfere with assay performance. Only one substance, polyquaternium 15 (present in two brands of lubricants: Astroglide Personal Lubricant [BioFilm, Inc, Vista, CA] and Target Lubricating Liquid [Target Corporation, Minneapolis, MN]) interfered with assay performance. In the HPV-negative panels containing these two lubricants, no false positive results were observed, however the percentage of invalid reactions was high, 36% and 35%, for the DTS and TIGRIS DTS systems respectively. Similarly,

Table 5
APTIMA HPV Assay analytical sensitivity with samples containing gynecological and feminine hygiene substances

Sample	DTS systems			TIGRIS DTS system		
	No. of replicates tested	Sensitivity (95% CI)	Average S/CO (SD)	No. of replicates tested	Sensitivity (95% CI)	Average S/CO (SD)
Control	32	100% (94–100%)	11.16 (0.85)	44	100% (94–100%)	11.29 (0.44)
Lubricants [a]	72	100% (96–100%)	11.08 (0.39)	72	100% (96–100%)	10.70 (0.38)
Lubricants [b]	72 [c]	100% (82–100%)	7.04 (3.57)	78 [d]	89% (75–96%)	8.96 (3.20)
Spermicides [e]	120	100% (98–100%)	11.07 (0.46)	120	100% (98–100%)	11.16 (0.43)
Anti-fungal/Anti-itch medications [f]	120	100% (98–100%)	10.83 (0.93)	120	100% (98–100%)	11.07 (0.35)
Whole blood	24	100% (90–100%)	8.94 (1.90)	24	100% (90–100%)	8.90 (2.35)
Glacial acetic acid	24	100% (90–100%)	11.46 (0.25)	24	100% (90–100%)	11.11 (0.34)

SD = standard deviation.
[a] Brands tested: KY Sensual Mist; KY Warming Jelly; and KY Warming Liquid.
[b] Brands tested: Astroglide Personal Lubricant and Target Brand Lubricating Liquid containing polyquaternium 15.
[c] 60 invalid replicates were excluded from the analysis.
[d] 41 invalid replicates were excluded from the analysis.
[e] Brands tested: Gynol II Vaginal Contraceptive Original Formula; Gynol II Vaginal Contraceptive Extra Strength; Delfen Vaginal Contraceptive Foam; Encare Vaginal Contraceptive; Conceptrol Vaginal Contraceptive.
[f] Brands tested: Vagisil Maximum Strength; Monistat Soothing Care; Monistat 3 Combination Pack; Target Brand Tioconazole 1; Target Brand Miconazole 3.

Table 6
APTIMA HPV Assay specificity with samples containing gynecological and feminine hygiene substances

Sample	DTS systems			TIGRIS DTS system		
	No. of replicates tested	Sensitivity (95% CI)	Average S/CO (SD)	No. of replicates tested	Sensitivity (95% CI)	Average S/CO (SD)
Control	24	100% (90–100%)	0.01 (0.03)	24	100% (90–100%)	0.00 (0.00)
Lubricants [a]	72	100% (96–100%)	0.00 (0.01)	72	100% (96–100%)	0.00 (0.00)
Lubricants [b]	72 [c]	100% (94–100%)	0.00 (0.00)	78 [d]	100% (95–100%)	0.00 (0.00)
Spermicides [e]	120	100% (98–100%)	0.00 (0.00)	122	100% (98–100%)	0.00 (0.00)
Anti-fungal/Anti-itch medications [f]	120 [g]	100% (98–100%)	0.00 (0.01)	120	100% (98–100%)	0.00 (0.00)
Whole blood	24 [g]	100% (89–100%)	0.00 (0.00)	24	100% (90–100%)	0.00 (0.00)
Glacial acetic acid	24	100% (90–100%)	0.00 (0.00)	24	100% (90–100%)	0.00 (0.00)

SD = standard deviation.
[a] Brands tested: KY Sensual Mist; KY Warming Jelly; and KY Warming Liquid.
[b] Brands tested: Astroglide Personal Lubricant and Target Brand Lubricating Liquid containing polyquaternium 15.
[c] 26 invalid replicates were excluded from the analysis.
[d] 25 invalid replicates were excluded from the analysis.
[e] Brands tested: Gynol II Vaginal Contraceptive Original Formula; Gynol II Vaginal Contraceptive Extra Strength; Delfen Vaginal Contraceptive Foam; Encare Vaginal Contraceptive; Conceptrol Vaginal Contraceptive.
[f] Brands tested: Vagisil Maximum Strength; Monistat Soothing Care; Monistat 3 Combination Pack; Target Brand Tioconazole 1; Target Brand Miconazole 3.
[g] One invalid replicate was excluded from the analysis.

for the HPV-positive panel members containing these two lubricants, the invalid rates were 83% and 53% for the DTS and TIGRIS DTS systems, respectively, and the positivity rates were 100% and 89% for the remaining valid reactions for the DTS and TIGRIS DTS systems, respectively. Upon titration of these substances, the APTIMA HPV Assay yielded 100% sensitivity and 100% specificity when the substances were present at concentrations less than or equal to 0.1% v/v (data not shown). The average analyte S/CO values for all substances were similar to the control panel values, except for HPV-positive whole blood samples, which were lower than the control on both instrument platforms. However, the decrease in signal did not impact the accuracy of the assay result (100% positive). The average analyte S/CO values in the HPV-negative samples were all <0.01, well below the assay cutoff of 1.00.

4.4. Reproducibility

Assay reproducibility is presented in Table 7. In the DTS systems, the overall agreement between replicate results was $\geqslant$97.2%. The inter-instrument, inter-operator, inter-lot and inter-run variability for HPV-negative and HPV-positive (HPV 16,18, SiHa, HeLa, ME180, MS751) panel members was quite low, <5% (inter-instrument), <5% (inter-operator), $\leqslant$7% (inter-lot), and $\leqslant$10% (inter-run). The intra-run and total

Table 7
APTIMA HPV Assay reproducibility

Panel no.	Sample type	Number of replicates	DTS systems								TIGRIS DTS system							
			Agreement (%)	Mean S/CO (SD)	Inter-inst %CV	Inter-op %CV	Inter-lot %CV	Inter-run %CV	Intra-run %CV	Total %CV	Agreement	Mean S/CO (SD)	Inter-inst %CV	Inter-op %CV	Inter-lot %CV	Inter-run %CV	Intra-run %CV	Total %CV
1	Neg	108	100	0.00 (0.00)	0.0	1.5	1.9	0.7	5.8	6.3	100	0.00 (0.00)	0.0	1.5	0.0	0.0	4.4	4.7
4	Neg	108[a]	100	0.00 (0.01)	2.1	1.8	0.2	0.7	6.6	7.2	100	0.00 (0.05)	0.0	2.2	0.8	0.0	5.0	5.6
8	Neg	108	100	0.00 (0.00)	0.0	2.2	1.1	1.5	6.1	6.8	100	0.00 (0.00)	0.5	2.4	0.0	0.0	4.6	5.2
9	Neg	108[a]	97.2	0.06 (0.32)	0.0	3.6	0.0	1.3	7.5	8.4	100	0.02 (0.13)	0.7	2.7	1.3	0.0	6.6	7.3
12	Neg	108	100	0.00 (0.01)	0.0	0.0	1.3	1.0	7.6	7.8	100	0.00 (0.00)	0.6	2.3	0.9	0.0	4.9	5.6
16	Neg	108	99.1	0.03 (0.30)	2.3	1.7	0.0	1.1	7.5	8.1	100	0.00 (0.00)	0.0	1.5	0.0	0.0	4.4	4.7
2	Low Pos	108	98.1	10.68 (2.07)	2.6	0.0	4.1	0.0	19.0	19.6	100	10.95 (0.58)	1.3	0.0	0.9	0.8	5.1	5.4
3	Low Pos	108	99.1	10.65 (2.43)	4.7	0.0	2.5	3.0	22.3	23.1	100	9.78 (2.71)	4.1	12.9	0.0	0.0	25.4	28.7
10	Low Pos	108	98.1	10.61 (1.78)	0.0	0.0	0.0	0.0	16.8	16.8	99.1	10.71 (1.57)	0.0	1.7	0.0	0.0	14.6	14.7
11	Low Pos	108	98.1	9.04 (3.07)	0.0	4.1	0.0	10.0	32.6	34.3	99.1	9.43 (2.31)	4.4	7.2	4.0	0.0	23.3	25.2
5	Mod Pos	108[b]	100	8.84 (0.67)	1.8	0.8	2.3	0.0	7.2	7.8	100	9.07 (1.20)	3.7	4.5	6.7	0.0	11.4	14.4
6	Mod Pos	108	100	15.75 (1.09)	2.4	2.6	7.0	0.9	3.9	8.7	100	15.44 (1.23)	0.6	0.0	8.8	0.0	5.0	10.1
7	Mod Pos	108[b]	100	22.90 (2.17)	3.2	0.0	0.0	0.0	9.1	9.7	100	22.15 (1.83)	1.7	2.5	0.0	0.0	7.9	8.5
13	Mod Pos	108	100	10.99 (0.46)	1.4	0.8	0.2	0.0	3.9	4.2	100	11.18 (0.36)	1.5	1.2	0.2	0.0	2.8	3.4
14	Mod Pos	108	100	12.22 (1.58)	2.6	0.0	0.0	0.0	12.8	13.0	100	11.63 (1.44)	0.0	3.9	0.0	1.9	11.8	12.6
15	Mod Pos	108	100	23.37 (2.53)	2.8	1.5	0.0	0.6	10.5	11.0	100	22.97 (1.34)	2.2	0.9	0.8	0.0	5.4	6.0

S/CO = signal to cutoff ratio; SD = standard deviation; inst = instrument; op = operator; %CV = percent coefficient of variance; Neg = negative; Pos = positive

[a] One invalid replicate on the TIGRIS DTS system; was not retested, excluded from the analysis.

[b] One invalid replicate on the DTS systems; was not retested, excluded from the analysis.

variability was slightly higher, especially for the low positive panel members. The negative panel member intra-run and total variability was <10%; the low positive panel member intra-run and total variability was <35%; and the moderate positive panel member intra-run and total variability was <13%.

In the TIGRIS DTS system, the overall agreement between replicate results was ⩾99.1%. The inter-instrument, inter-operator, inter-lot and inter-run variability for HPV-negative and HPV-positive panel members was quite low, <5% (inter-instrument), <13% (inter-operator), <9% (inter-lot), and <2% (inter-run). The intra-run and total variability was slightly higher, especially for the low positive panel members. The negative panel member intra-run and total variability was <8%; the low positive panel member intra-run and total variability was <29%; and the moderate positive panel member intra-run and total variability was <15%.

5. Discussion

5.1. Analytical sensitivity

For most high-risk HPV types the APTIMA HPV Assay was capable of detecting less than 200 copies of HPV in vitro transcript per reaction (Table 3), which is equivalent to less than 800 copies/mL of the test sample and less than 2000 copies/mL in a liquid Pap specimen. The analytical sensitivity was between 300 and 500 copies/reaction for HPV 51, 52 and 58, which are rare types.[25] The TIGRIS DTS system yielded a slightly higher analytical sensitivity than the DTS systems, which was significant for some types as noted by the 95% confidence intervals (Table 3), with 13 of 14 HPV types yielding an analytical sensitivity of less than 200 copies/reaction. These results demonstrate sensitive detection of each of the 14 high-risk types that the assay was designed to detect, especially those that are more prevalent in cervical cancer: HPV types 16, 18, 45, 31 and 33.[6]

In most HPV infected cell lines (HeLa, ME180, MS751), 95% detection limits were well below 1 cell/reaction. For SiHa cells, the 95% detection limit was approximately 1.2 cells/reaction. In comparison, the HPV-Proofer assay is reported to have an analytical sensitivity of 10 to 100 cells/reaction for SiHa cells and 1 to 5 cells/reaction for HeLa cells, depending upon the presence of background cells.[26] The HC2 test has an analytical sensitivity of approximately 1 picogram of HPV DNA per milliliter of specimen, which represents approximately 5000 DNA copies/reaction.[27,28] The Amplicor HPV test has a limit of detection of 100 to 240 DNA copies/mL.[29] The optimal limit of detection of HPV DNA and RNA in clinical samples is not known, however E6/E7 mRNA is considered to be a better marker for the detection of disease (high-grade cervical lesions) than the presence of HPV DNA.[15–17]

5.2. Analytical specificity

No cross-reactivity was observed in the APTIMA HPV Assay with the variety of low risk HPV types, bacteria, protozoa, and fungi that were tested in this study (normal and opportunistic vaginal, intestinal and genitor-urinary infections): no organism repeatedly yielded false positive results and the analytical specificity of the assay was 99% (Table 4). Similarly, no cross-reactivity has been reported in the Amplicor HPV test.[29] The HC2 assay, on the other hand, has been reported to cross-react to some degree with HPV types 6, 11, 40, 42, 53, 54, 82, 84

and 73,[27] which are all low-risk HPV types, which may result in unnecessary referral to colposcopy for patients infected with these types.

5.3. Interference

Substances such as lubricants not containing polyquaternium 15, spermicides, anti-fungal medications, anti-itch medications, whole blood, and glacial acetic acid did not interfere with assay performance when present individually in the sample at 1% or 10%. While the APTIMA HPV Assay detects the mRNA from 14 high-risk HPV types, HPV 16-infected SiHa cells were tested as a representative HPV type for this study. Polyquaternium 15, a substance present in two brands of lubricants evaluated in the study (Astroglide Personal Lubricant and Target Lubricating Liquid), interfered with the performance of the assay when present in a sample at a concentration of more than 0.1%, resulting in an increase in the number of invalid reactions (Tables 5 and 6). Possible interference has also been reported for the HC2 and Amplicor tests with some types of anti-fungal cream and contraceptive jelly.[27,29]

5.4. Reproducibility

Inter-instrument, inter-operator, inter-lot, and inter-run variability was less than 10% (for more than 99% of the conditions evaluated for each panel member), showing that the APTIMA HPV Assay is robust in both the DTS and TIGRIS DTS systems (Table 7). Intra-run variability for S/CO values was >15%, but <35%, for the low positive panel members. The increased intra-run variability is expected for these panel members since the concentration is set at the assay's 95% detection limit. Nevertheless, despite a higher variability in S/CO values for these samples, the agreement between all replicate data was >98%. Four representative HPV types, HPV 16, HPV 18, HPV 68 and HPV 45, either in vitro transcripts or infected cell lines, were evaluated as representative types to assess APTIMA HPV Assay reproducibility. While consistent results were observed between reagent lots, operators and instruments, only one site performed the testing for the study.

5.5. DTS Systems versus TIGRIS DTS System

Overall, the analytical performance of the APTIMA HPV Assay was slightly better in the TIGRIS DTS system than in the DTS systems: the analytical sensitivity in the TIGRIS DTS system was 17 to 275 mRNA copies/reaction compared with 38 to 488 mRNA copies/reaction in the DTS systems; moreover, the overall variability of the S/CO value was generally lower in the TIGRIS DTS system (%CV ranging from 3.4% to 28.7%, with only 2 of 16 samples having a %CV >15%) compared with the DTS systems (%CV ranging from 4.2% to 34.3%, with 4 of 16 samples having a %CV >15%). This slight difference in performance is likely due to the complete automation of the TIGRIS DTS system, which eliminates minor operator variability that is sometimes present with the DTS system.

With an analytical sensitivity of approximately 20 to 500 HPV mRNA copies/reaction, an analytical specificity $\geqslant$99%, an inter-instrument, inter-operator, inter-lot, inter-run variability of <10%, and the absence of interference from most feminine hygiene products, the APTIMA HPV Assay showed excellent analytical performance and robustness. The APTIMA HPV Assay also has several advantages over the other HPV tests: (1) it detects HPV E6/E7 mRNA, which may be a better marker of advanced disease than the HPV DNA detected by HC2 and Amplicor tests; (2) the limit of HPV mRNA detection is lower than that reported for the Proofer test,[26] (3) unlike the HC2 test, it does not cross-react with low-risk HPV types tested in the current study, and (5) it is compatible with a fully automated processing system. Studies evaluating assay performance with clinical specimens are on-going.

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