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Review

A review of the clinical performance of the Aptima HPV assay

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ABSTRACT

This comprehensive review compiles published data from 62 original articles comparing different HPV tests and one meta-analysis on the clinical performance of the Aptima HR HPV (AHPV) assay in either screening or referral populations as well as for the purpose of test of cure. A number of publications with technical issues were also considered. Besides a brief introduction in the development of E6/E7 mRNA testing, the review summarizes data on analytical sensitivities and specificities, as well as on clinical sensitivity, specificity, NPV and PPV with histological endpoints CIN2+ and CIN3+, where available. Although most studies were of cross-sectional design, five studies with a longitudinal prospective design or component were identified. In addition to the study design, sample size, age and CIN2/3+ prevalence of the respective cohort are listed. This allows direct comparison of the published data in the respective groups. One major outcome of this review is the remarkably stable similar sensitivities of AHPV and HC2 independent from study design for detection of CIN2/3+ combined with a higher specificity of the AHPV. The second outcome was the longitudinal predictive value derived from registry linkage and other prospective studies that would support the applicability of the AHPV test in primary screening with at least a three year screening interval.

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1. Introduction

E6 and E7 oncogene expression of human papillomavirus (HPV) type 16 as a marker for neoplasia has first been described in 1986, when E6/E7 transcripts were detected in a cervical cancer-derived cell line and a biopsy of cervical carcinoma tissue [1]. Nakagawa et al. later demonstrated the ubiquitous presence of E6 and E7 transcripts of a number of high-risk (HR) HPV types in the vast majority of tested neoplastic cervical specimens [2]. Subsequently, a number of studies confirmed that E6/E7 expression represents a key feature of neoplastic progression and correlated a higher mRNA expression with increasing disease severity (summarized by [3]). In 2004 Sotlar et al. published a nested reverse transcription (RT) multiplex PCR protocol for the combined detection of E6/E7 mRNA of the 18HPV types 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66 and 68 [4] and demonstrated its potential as a sensitive diagnostic tool for cervical intraepithelial neoplasia (CIN) [5]. The authors also showed that E6/E7 mRNA measurement is more sensitive in

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detecting disease than HPV DNA detection by conventional PCR with MY09/MY11 and GP5+/GP6+ primers. DNA-based HPV tests can only discriminate between presence and absence of a HPV-specific DNA target-sequence and cannot determine whether an infection is active or even potentially transforming. As E6/E7 mRNA expression only occurs in actively infected cells and gross transcript levels increase during CIN development and progression, it was hypothesized that HPV mRNA measurement might be more specific in detecting high-grade disease. Indeed the first commercially available test that utilizes E6/E7 mRNA detection demonstrated significantly higher clinical specificities than the HPV DNA-based comparative HR HPV type group tests [6–10]. The PreTect™ HPV-Proofer assay (NorChip AS, Klokkarstua, Norway), which was also marketed under the name NucliSENS EasyQ® HPV by bioMérieux (France) is a real-time multiplex assay that uses nucleic acid sequence based amplification (NASBA)—a sensitive transcription-based amplification system (TAS) for the specific in vitro replication of mRNA. The assay employs this technology in conjunction with molecular beacon probes allowing the direct qualitative detection of HPV oncogene expression of the five HR-HPV types 16, 18, 31, 33, and 45 with simultaneous genotype-specific identification. These five target HPV types have been found in only 75.1% of CIN2/3 and 88.5% of squamous cell carcinomas (SCC) [11,12], which is, however, also the reason for the test's inferior sensitivity for detecting cervical disease and the higher specificity in comparison with other HPV tests [9,10,13].

In 2008 another commercially available mRNA detection assay was launched. The APTIMA® HPV Assay (AHPV; Hologic, San Diego, CA) is a target amplification assay utilizing transcription-mediated amplification (TMA) for the qualitative detection of the viral polycistronic E6/E7 mRNA from 14HPV types. In addition, the APTIMA® HPV 16 18/45 Genotype Assay (AHPV-GT; Hologic, San Diego, CA) employs the TMA technology for type-specific detection of HPV types 16 and 18/45.

This review provides an overview of the published literature about both, the AHPV assay and the AHPV-GT test.

2. AHPV methodology

The Aptima HPV assay involves three consecutive steps, which take place in a single tube: target capture specimen processing; target amplification by transcription-mediated amplification (TMA) [14] and detection of the amplification products by the hybridization protection assay (HPA) [15]. The assay incorporates an internal control for nucleic acid capture, amplification, and detection, as well as operator or instrument error. After cell lysis the target mRNA is isolated from the specimen by sequence-specific capture oligomers that also contain a deoxyadenosine tail. During the hybridization step, capture oligomers bind to specific nucleotide sequences of target E6/E7 mRNA molecules. Oligomer-target complexes are then captured by decreasing the temperature of the reaction to room temperature, which allows hybridization of the deoxyadenosine region of the capture oligomer to poly-deoxythymidine molecules covalently attached to magnetic particles. After target capture, the HPV mRNA is amplified via TMA, which involves the two enzymes MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase generates a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase then produces multiple copies of RNA amplicons from the DNA copy template. Detection of the RNA amplicons is achieved by the hybridization protection assay (HPA) using single-stranded nucleic acid probes complementary to the amplicon. After inactivating unhybridized probes, RNA:DNA hybrids are detected as photon signals by luminometric measurements. Internal control signals are discriminated from the HPV signals by employing probes with different light emission kinetics (flasher vs glower). The dual kinetic assay (DKA) then differentiates between the signals from both labels. Results are reported as relative light units (RLU).

The AHPV assay may be run on the semi-automated direct tube sampling (DTS®) system (Hologic) as well as on the TIGRIS® DTS™ (Hologic) or the PANTHER® platform (Hologic), which fully automate the target capture specimen processing, TMA and DKA detection steps.

3. Aptima HPV Assay vs Aptima HPV 16 18/45 Genotype Assay

In 2011 the US Food and Drug Administration (FDA) approved the AHPV assay for usage in women 30 years and older, and for women between 21 and 29 years of age with ASC-US, in order to determine the requirement of additional follow-up and diagnostic or treatment procedures (http://www.fda.gov/medicaldevices/productsandmedicalprocedures/deviceapprovalsandclearances/recently-approveddevices/ucm278520.htm). The AHPV group test has been tailored for combined detection of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. These fourteen types have been classified as group 1 (carcinogenic), group 2A (probably carcinogenic) or group 2B (possibly carcinogenic) carcinogenic for humans by the IARC [16]. In contrast, the AHPV-GT assay has been designed for type-specific detection of HPV type 16 and for combined detection of HPV types 18 and 45. These types have been shown to be prevalent in 94% of cervical adenocarcinoma cases [17]. The AHPV-GT assay received approval from the FDA in 2012 for application in women with AHPV-positive test results. (http://www.fda.gov/medicaldevices/productsandmedicalprocedures/deviceapprovalsandclearances/recently-approveddevices/ucm325771.htm). Both tests have also been labelled for marketability within the European Economic Area. The CE mark indicates conformity of a given test with European law, constitutes no approval and is therefore different from the FDA approval process. The FDA guidelines for market approval of in vitro diagnostic devices for the detection of HPV require that the respective test is both safe and effective by evaluating analytical and clinical performance data (http://www.fda.gov/RegulatoryInformation/Guidances/ucm181509.htm).

4. Analytical performance

Analytical performance of the AHPV assay has been thoroughly evaluated for samples collected in Cytyc ThinPrep® Pap test PreservCyt® Solution (Hologic Inc., Bedford, MA; hereafter: PreservCyt) [18,19]. As a result the analytical sensitivity of the AHPV assay was less than 200 copies of HPV transcripts per reaction for most target types [18]. Analytical sensitivity for the detection of E6/E7 mRNA was much higher than for E6/E7 DNA, and DNA detection was 1000–3000 times lower as compared to the HC2 test [19]. Indeed the analytical sensitivity for the detection of HPV 16, 18 and 45 was higher for the AHPV assays than for the Hybrid Capture 2 (HC2) test (Hilden, Germany) [20]. The analytical specificity was 99% with virtually no cross-reactivity with low-risk (LR) HPV types or other pathogens and selected species of the normal microbiological Human flora, but cross-reactivity with less defined types HPV 26, 53 and 82 regarding carcinogenicity has been described [21]. Furthermore, interference with assay performance was excluded for substances frequently applied to the genital area [18,22] and has only been observed for polyquaterium 15, a compound found in few personal lubricants [18]. A sufficient analytical performance was also recently confirmed for the AHPV-GT assay [23].

5. Suitability of different collection media

The majority of clinical and analytical data are based on samples collected in PreservCyt medium and only few studies are available for other transport media. Additional performance data are for example available for samples collected in Digene specimen transport medium (D-STM), which was found to be acceptable for AHPV testing with the restriction to non-denatured samples [19]. Direct comparison data of the suitability of different transport media are only available from one study. Chernesky et al. compared AHPV test performances using three samples from each of 580 women, collected in either PreservCyt, TriPath Imaging® SurePath® Preservative Fluid (Becton Dickinson Co., Franklin Lakes, NJ; hereafter: SurePath) and the Aptima specimen collection and transport kit (SCT) [24]. Agreement between the test results using different collection media was excellent. However, the results obtained from SurePath specimens yielded fewer positive events compared to the two comparative media, which is evidenced by a significantly lower kappa-value for SurePath vs SCT ($\kappa = 0.72$, 95% confidence interval (CI): 0.66–0.78) in comparison to PreservCyt vs SCT ($\kappa = 0.82$, 95%CI: 0.77–0.86) and a lower sensitivity for CIN2+ in SurePath versus PreservCyt (Table 1). The slightly inferior performance of AHPV using SurePath samples was also demonstrated in a large study comparing various HPV detection assays in two sets of specimens collected in either PreservCyt or SurePath medium [25]. The authors reported a marginally reduced clinical sensitivity relative to the performance of the HC2 test in SurePath than in PreservCyt samples. A similar effect on the clinical sensitivity of the AHPV assay was seen in the HORIZON study, where SurePath samples were collected and tested by four different HPV tests [26,27]. In the latter study, the AHPV assay was found to have a significantly lower clinical sensitivity in detecting CIN2+ and CIN3+ in comparison to the HC2 test (Fig. 1). The most probable reason for this inferior performance using SurePath samples is the fact, that it contains a fixative agent, which cross-links cellular proteins and nucleic acids, leading to an aggravation of the target capture reaction due to less easily accessible mRNA [28]. Moreover, the manufacturer-recommended treatment of SurePath samples with Proteinase K prior to performing the AHPV assay in order to reverse the cross-linking process appears insufficient, considering recently published data [24,26,27]. A more efficient pre-treatment protocol was recently CE marked [29]. More importantly, the use of SurePath medium has also been reported to interfere with DNA-based HPV test performances including the HC2 test, which is why the FDA has so far approved the application of SurePath medium only for cytology [30, http://www.fda.gov/downloads/aboutfda/reportsmanualsforms/reports/ucm472777.pdf+surepath&client=FDAgov&proxystylesheet=FDAgov&output=xml_no_dtd&site=FDAgov&ie=UTF-8&access=p&oe=UTF-8]

6. Clinical performance

An ideal HR-HPV test for identification of HPV-associated disease that needs treatment has to combine high clinical sensitivity with high clinical specificity. Clinical sensitivity is different from the analytical sensitivity of a given test system, as a test with a good clinical sensitivity usually has a higher cut-off for being positive than the analytical detection threshold that separates signal from noise. Therefore, clinical trials are needed to define the cut-off for the clinical sensitivity, which separates latent and clinically irrelevant infections from transforming infections with underlying high grade disease. However, as HPV infections are quite common and not all HPV infections cause disease and have a high tendency for spontaneous regression, the positive predictive value, which is the likelihood that a test-positive person has disease, is for all HPV tests rather low. As cervical cancer is the rare outcome of a common infection with HR HPV types, in a well screened routine screening population the negative predictive value, which is the percentage of all people, who test negative and have no disease, is high for all screening tests including cytology. The prevalence of disease in the screened population, however, has a major impact on the clinical specificity. In a routine screening population of women aged 30 years and older with usually up to 1% of CIN2+ lesions, the specificity of the AHPV test is well above 85%. In referral populations, the much higher prevalence of disease causes a dramatic decrease in clinical specificity which might reach levels of 20%. This happens due to the increased prevalence, which diminishes the denominator in the calculation for the specificity (all women without disease). Therefore, it is important not to compare PPV and clinical specificity (which are directly linked to each other) from studies using screening populations with those from populations where individuals were referred because of abnormal cytology, or had a test of cure. The FDA guidelines for market approval have been backed by the "Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older" published by Meijer et al., which sets standards for the respective new HPV DNA test's clinical performance in relation to the gold-standard HC2 or GP5+/6+ test [31]. According to these criteria, a validation study has been performed in comparison to GP5 + 6+ PCR confirming AHPV's high intra-laboratory reproducibility over time (96.0%) and a 96.7% inter-laboratory agreement of the AHPV assay [32]. Earlier, the AHPV assay performed robustly in an inter-instrument, -operator, -lot and -run reproducibility study [18]. Heideman et al. also confirmed the non-inferior clinical performance [32]. In addition, clinical sensitivity, specificity and negative and positive predictive values of the AHPV test have been thoroughly evaluated in comparison to liquid based cytology (LBC) and other HPV nucleic acid tests and respective studies are summarized in Table 1. HPV testing in general is an established tool for the triage of borderline cytology results and national cervical cancer screening guidelines have been adapted accordingly in many countries [33,34]. Hence, the majority of comparison studies have been performed in referral populations (Table 1). On the other hand, HPV testing becomes increasingly valuable for primary cervical cancer screening, as shown by the large number of randomized controlled trials (RCTs) providing evidence that HPV testing reduces CIN3+ and cervical cancer in the second screening round [35–39] and with the large US-based Athena trial [40,41] leading to the FDA-approval of the cobas 4800HPV test (Roche Molecular Systems, Pleasanton, CA) with concurrent HPV16/18 genotyping for application in primary screening (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm394773.htm, [9]).

A number of comparison studies using the AHPV test have been conducted in routine screening populations (Table 1). Another population, which benefits from HPV testing are women that have completed treatment for prevalent CIN or cancer and few studies have been reported for AHPV in test-of-cure populations (Table 1). Despite these diverse populations and varying study designs, the AHPV test consistently shows similar sensitivities for CIN2+ or CIN3+. In comparison with other HPV tests, the AHPV test repeatedly has equivalent clinical sensitivity, but superior specificity for the detection of cervical disease (CIN2+/CIN3+) (Table 1). This is especially evident when the comparison is restricted to the gold-standard HC2 test, where the majority of available studies report an insignificantly lower to equal sensitivity and a significantly higher specificity for the AHPV test (Fig. 1). These results are supported by meta-analysis data [8] and previous review reports [9,42–45]. The only deviations are reported from studies using SurePath samples (Moss et al. [25], Rebolj et al. [27] and Rebolj et al. [26]) as described above (Table 1 and Fig. 1).

Table 1
Summary of studies reporting clinical performance data on the AHPV assay stratified by study population.

Citation	Population			Endpoint		Test	Sensitivity	Specificity	NPV	PPV	Collection medium	
	Type	N[a]	Age	Type	*N* (rate)						Type	Split sample
Screening population												
Wu et al. [61] (SHENCCAST I)	Screening	2000	25–59	CIN2+	27 (0.014)	HC2	88.9	84.5			PreservCyt	Partially
						AHPV	100	91.2			PreservCyt	
						LBC	66.7	95.5			SurePath	
Ratnam et al. [62]	Screening	1373	16–81	CIN2+	7 (0.005)	HC2	100	85.2			PreservCyt	Yes
						AHPV	100	88.4				
Monsonego et al. [63] (FASE)	Screening	4429	20–65	CIN2+	101 (0.023)	HC2	96.7[b]	86.4[b]			PreservCyt	Yes
						AHPV	92.0[b]	91.8[b]				
						LBC	69.1[b]	91.9[b]				
				CIN3+	27 (0.006)	HC2	95.3[b]	84.9[b]				
						AHPV	95.7[b]	90.3[b]				
						LBC	73.3[b]	90.8[b]				
Monsonego et al. [64] (FASE)		5006		Absolute and relative risk analysis between HC2, AHPV and LBC								
Nieves et al. [65] (MECCS II)	Screening	2049	30–50	CIN3+	16 (0.008)	HC2	100	92.2		9.2	PreservCyt	Yes
						AHPV	100	93.5		10.7		
						LBC	87.5	94.1		10.5		
Cuzick et al. [46]	Screening	6000	20–66	CIN2+	40 (0.007)	HC2	97.5	85.4		4.3	PreservCyt	Yes
						rtHPV	95.0	87.2		4.7		
						BD HPV	97.5	84.3		4.0		
						Cobas	97.5	84.5		4.1		
						AHPV	97.5	90.2		6.3		
				CIN3+	19 (0.003)	HC2	100			2.1		
						rtHPV	94.7			2.2		
						BD HPV	100			2.0		
						Cobas	100			2.0		
						AHPV	100			3.1		
Rebolj et al. [28] (Horizon)	Screening	5070	16–89	Agreement analysis between HC2 and AHPV								
Rebolj et al. [66] (Horizon)	Screening	5064	Unknown	Agreement analysis between HC2, cobas, CLART and AHPV							SurePath	Yes
Rebolj et al. [26] (Horizon)	Screening	1278	23–29	CIN2+	68 (0.053)	HC2	94.0	72.0				
						AHPV	85.0	77.0				
						Cobas	99.0	62.0				
						CLART	99.0	66.0				
				CIN3+	44 (0.034)	HC2	95.0	71.0				
						AHPV	82.0	75.0				
						Cobas	98.0	61.0				
						CLART	100	65.0				
Munson et al. [67]	Screening	4056	≥20	Agreement analysis between Cervista and AHPV							PreservCyt	Yes
Pyne et al. [23]	Screening	967	>30	Analytical performance comparison between HC2 and AHPV							PreservCyt	Yes
Iftner et al. [52] (GAST)	Screening	9451	30–60	CIN2+	90 (0.010)	HC2	93.2[b]	94.9[b]	99.9[b]	17.9[b]	PreservCyt	Yes
						AHPV	87.8[b]	96.1[b]	99.8[b]	21.1[b]		
						LBC	39.5[b]	98.4[b]	99.3[b]	22.7[b]		
				CIN3+	43 (0.005)	HC2	100[b]					
						AHPV	90.9[b]					
						LBC	49.8[b]					
Reid et al. [58] (CLEAR)	Screening, 3-year f-up	10,509	30–89	CIN2+	47 (0.004)	HC2	63.6	94.8			PreservCyt	Yes
						AHPV	55.3	96.3				
				CIN3+	23 (0.002)	HC2	81.8	94.7				
						AHPV	78.3	96.2				
Referral population												
Szarewski et al. [68] (Predictors 1)	Referral	953	18–67	CIN2+	(∼0.280)	HC2	99.6	28.4		36.1	PreservCyt	Yes
						Amplicor	98.9	21.7		33.5		
						AHPV	95.2	42.2		39.9		
						LA	98.2	32.8		37.7		

Table 1 (*Continued*)

Citation	Population Type	N[a]	Age	Endpoint Type	*N* (rate)	Test	Sensitivity	Specificity	NPV	PPV	Collection medium Type	Split sample
						CLART	80.9	37.1		33.0		
						LBC	93.8	58.1		47.3		
				CIN3+	(~0.200)	HC2	99.5	25.4		25.6		
						Amplicor	99.5	19.7		23.5		
						AHPV	97.4	38.8		28.9		
						LA	99.0	29.6		26.9		
						CLART	83.9	36.0		24.0		
						LBC	95.9	53.2		34.2		
Szarewski et al. [47] (Predictors 2)		1099		CIN2+	359 (0.327)	HC2	96.3	19.5		37.4		
						rtHPV	93.3	27.3		38.2		
						BD HPV	95.0	24.2		37.8		
						Cobas	95.2	24.0		37.6		
						AHPV	95.3	28.8		39.3		
						LBC	88.9	58.1		50.7		
				CIN3+	224 (0.204)	HC2	98.7			24.0		
						rtHPV	97.3			24.7		
						BD HPV	97.8			24.2		
						Cobas	97.3			23.9		
						AHPV	97.8			25.1		
						LBC	92.9			33.1		
Mesher et al. [69] (Predictors 1 + 2)		1228		CIN2+	203 (0.165)	HC2	96.0	23.3		20.1		
						rtHPV	95.0	31.7		22.2		
						BD HPV	94.0	25.9		21.2		
						Cobas	94.9	25.0		21.2		
						AHPV	94.1	34.7		22.3		
				CIN3+	97 (0.079)	HC2	100			10.1		
						rtHPV	100			11.2		
						BD HPV	100			9.4		
						Cobas	100			9.4		
						AHPV	99.0			11.2		
Dockter et al. [70]	Referral	753	Unknown	CIN2+	141 (0.187)	HC2	95.0	47.4	97.6	29.4	PreservCyt	Yes
						AHPV	90.8	56	96	32		
				CIN3+	87 (0.115)	HC2	98.9	44.4	99.7	18.9		
						AHPV	97.7	53	99.4	21		
Reuschenbach et al. [71]	Referral	275	28–44	CIN2+	161 (0.585)	HC2	91.5	63.4	83.5	78.5	PreservCyt	Yes
						AHPV	88.4	71.2	80.6	81.9		
				CIN3+	110 (0.4)	HC2	96.4	49.1	95.3	55.8		
						AHPV	95.5	56.4	94.9	59.3		
Ratnam et al. [62]	Referral	1418	15–80	CIN2+	401 (0.282)	HC2	94.3	38.7	94.5	37.8	PreservCyt	Yes
						AHPV	96.3	43.2	96.7	40.0		
Clad et al. [72]	Referral	424	Unknown	CIN2+	252 (0.594)	HC2	91.3	61.0			PreservCyt	Yes
						AHPV	91.7	75.0				
						LBC	84.9	66.3				
				CIN3+	163 (0.384)	HC2	95.7	46.0				
						AHPV	98.2	56.3				
						LBC	93.9	54.4				
Ovestad et al. [48]	Referral ASCUS, LSIL	528	26–69	CIN2+	47 (0.089)	*AHPV*	98.0	38.0			PreservCyt	Yes
						Amplicor	100	18.0				
						Cobas	96.0	26.0				
Waldstrom and Ornskov [73]	Referral LSIL	405	16–65	CIN2+	67 (0.165)	*AHPV*	92.5	38.2	96.3	22.9	PreservCyt	Yes
				CIN3+	31 (0.077)	*AHPV*	93.9	35.5	98.5	11.4		
Waldstrom and Ornskov [55]	Referral ASCUS; registry f-up on *N* = 325 for ≥15 months	369	30–69	CIN2+	48 (0.130)	*AHPV*	87.5	78.0	97.3	40.8	PreservCyt	Yes
						LA[c]	93.8	64.3	98.3	31.3		
				CIN3+	27 (0.073)	*AHPV*	92.6	73.8	99.1	24.3		
						LA[c]	92.6	60.1	98.9	17.4		
Waldstrom et al. [54]	Referral LSIL, 5 year registry f-up	469	16–65	CIN2+	87 (0.186)	*AHPV*	92.0	36.1	95.2	24.7	PreservCyt	Yes
				CIN3+	46 (0.098)	*AHPV*	95.7	33.8	98.6	13.6		
Stoler et al. [74] (CLEAR)	Referral ASCUS	939	≥21	CIN2+	91 (0.097)	*AHPV*	86.8	62.9	97.8	20.1	PreservCyt	Yes
						HC2	88.8	55.8	97.7	18.7		
				CIN3+	40 (0.043)	*AHPV*	90.2	60.2	99.3	9.4		
						HC2	92.3	53.3	99.3	8.5		

Castle et al. [49,59] (CLEAR)		988		CIN2+	94 (0.095)	*AHPV*	89.4	63.1	98.3	20.3		
						Cobas	89.4	59.3	98.1	18.8		
				CIN3+	42 (0.043)	*AHPV*	95.2	60.5	99.7	9.7		
						Cobas	92.9	56.8	99.4	8.7		
Rebolj et al. [27] (Horizon)	≥ASCUS	367	Unknown	CIN2+	119 (0.324)	HC2	96	25			SurePath	Yes
						Cobas	95	31				
						CLART	94	36				
						AHPV	89	40				
				CIN3+	84 (0.229)	HC2	95	22				
						Cobas	94	27				
						CLART	93	32				
						AHPV	87	35				
Persson et al. [21]	ASCUS/LSIL, 4 years registry f-up	219	23–60	Triage analysis between LA and AHPV							PreservCyt	Yes
Binnicker et al. [50]	≥ASCUS	350	Unknown	CIN2+	81 (0.231)	HC2	97.5	27.1	97.3	28.7	PreservCyt	Yes
						Cobas	91.4	31.2	92.3	28.6		
						AHPV	91.4	42.0	94.2	32.1		
				CIN3+	41 (0.117)	HC2	95.1	23.6	97.3	14.2		
						Cobas	95.1	28.8	97.8	15.1		
						AHPV	95.1	38.2	98.3	17.0		
Cuschieri et al. [75]	Referral	1336	19–64	CIN2+	81 (0.061)	*AHPV*	88.9	42.1			PreservCyt	Yes
						HC2 @ c/o = 1	91.4	41.6				
						HC2 @ c/o = 2	91.4	43.9				
						HC2 @ c/o = 10	85.2	51.9				
				CIN3+	21 (0.016)	AHPV	100	38.5				
						HC2 @ c/o = 1	100	37.6				
						HC2 @ c/o = 2	100	39.6				
						HC2 @ c/o = 10	100	47.7				
Moss et al. [25]	ASC, LSIL	5455 6816	Unknown	Relative performance analysis between HC2, Cervista, rtHPV, COBAS and AHPV							PreservCyt SurePath	Partially Partially
Guo et al. [76]	Referral ASCUS LSIL	411	21–69	CIN2+	72 (0.175)	HC2	100	6.2			PreservCyt	Yes
						AHPV	87.5	32.7				
				CIN3+	17 (0.041)	HC2	100	5.3				
						AHPV	94.1	30.2				
Johansson et al. [53]	ASCUS, LSIL, treated and untreated; 4.5 years registry f-up	ASCUS (211) LSIL (131)	35–87	CIN2+ ASCUS	61 (0.289)	*AHPV*	96.7	12.7	90.5	31.0	SurePath	No
				CIN2+ LSIL	45 (0.344)		97.8	5.8	83.0	35.2		
				CIN3+ ASCUS	23 (0.109)		100	12.7	100	14.9		
				CIN3+ LSIL	20 (0.153)		100	5.8	100	19.8		
Test of cure												
Persson et al. [56]	Test of cure; average 3.6 years registry f-up	143	21–56	CIN2/HSIL	5 (0.035)	LA	100	80.9	100	21.2	PreservCyt	Yes
						AHPV	57.1	93.4	97.7	30.8		
						LBC	85.7	87.5	99.2	26.1		
Cubie et al. [57]	Test of cure; National screening program; average 13.2 months f-up	1020	Unknown	CIN2+	23 (0.023)	HC2 @ c/o = 1	100	79.0	100	10.0	Unknown	Unknown
						HC2 @ c/o = 2	96.0	82.0	100	11.0		
						Cervista	96.0	75.0	100	8.0		
						AHPV	91.0	84.0	100	12.0		
						rtHPV	100	78.0	100	9.0		
						Cobas	100	75.0	100	8.0		
Other studies												
Heideman et al. [32]	843 <CIN2, 69CIN2+	912	30–60	CIN2+	69 (0.076)	*AHPV*	94.2	94.5			PreservCyt	Yes
Nolte and Rivbeiro-Nesbitt [77]	Samples were selected by Cervista positivity (50% positive, 50% negative) from a screening population	208	Unknown	Agreement analysis between Cervista and AHPV							PreservCyt	Yes
Nakayama et al. [78]	Mixed	410	20–76	HSIL	50 (0.121)	HC2	98.0	72.4	99.6	33.1	PreservCyt	Yes
						Amplicor	96.0	71.2	99.2	32.2		
						AHPV	96.0	76.3	99.3	36.1		
Chernesky et al. [24]	Mixed	580	18–63	CIN2+	30 (0.052)	*AHPV*	96.6	66.2	99.7	13.3	PreservCyt	No
						AHPV	93.3	70.9	99.5	14.9	SurePath	

CIN: cervical intraepithelial neoplasia, LA: Linear Array HPV Genotyping test (Roche), AHPV: Aptima HPV assay (Hologic), HC2: digene HC2High-Risk HPV DNA Test (Qiagen), rtHPV: RealTime High Risk HPV test (Abbott), LBC: liqid based cytology, Cobas: cobas HPV test (Roche), CLART: Clinical Array Technology HPV2 assay (Genomica), Amplicor: AMPLICOR® Human Papillomavirus Test (Roche), BD HPV: Onclarity™ HPV Assay (BD), Cervista: Cervista HPV HR test (Hologic), ASCUS: atypical squamous cells of undetermined significance, LSIL: low grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion, NPV: negative predictive value, PPV: positive predictive value, f-up: follow-up.

[a] Included in analysis.

[b] Verification bias adjusted.

[c] The LA test was considered positive when one or more of the 14 AHPV target HPV types was found.

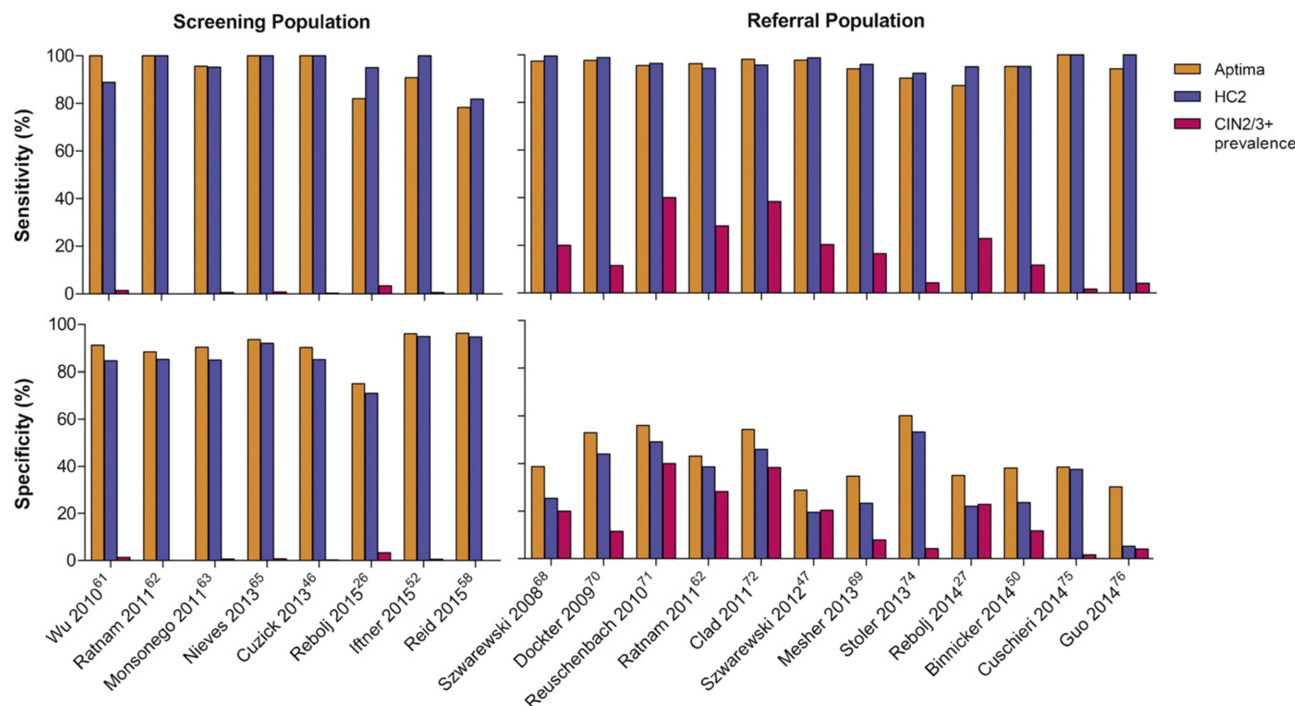

Fig. 1. Clinical sensitivities and specificities for CIN2/3+ detection of the AHPV (orange bars) and HC2 (purple bars) HPV tests from previously published studies on screening and referral populations. CIN2/3 prevalence of the respective study population is shown by magenta-colored bars.

Notably, the clinical performance of the AHPV test is equivalent to the performance of the cobas HPV test [46–50]—the only FDA-approved HPV test for primary screening to date in the USA. Thus, the AHPV test might be considered suitable for primary cervical cancer screening. Of special interest is the observation by two independent groups showing that usage of the AHPV versus the HC2 test in women with abnormal results would lead to a reduced rate of colposcopy referrals of 21% or 23%, respectively [51,52]. However, despite fulfilling the criteria for HPV DNA test requirements, HPV mRNA based tests are required to undergo further validation studies demonstrating a low cumulative CIN3+ incidence rate over time following a negative baseline test result [9,42]. So far, first longitudinal data for the AHPV test are available from studies using national registries as follow-up strategy [21,53–57]. These studies cover a range of 6 months to 5 years of passive follow-up periods. All but one [56] of these studies found that a positive AHPV result at baseline is an excellent predictor for future CIN2+ or CIN3+ development in referral [21,53–55] or test-of-cure [57] populations. In order to fit to current cervical cancer screening intervals based on the aforementioned randomized controlled trials [35–39], longitudinal data are needed from screening settings for a period of at least three to five years [42]. Recently, the first longitudinal screening study was published in which AHPV was evaluated as adjunctive screening test in women 30 years and older. As a result, the CLEAR trial demonstrated that AHPV testing has a similar sensitivity for detection of CIN3+ and a significantly higher specificity than the HC2 test. More importantly, the study showed that women with NILM cytology and with a negative AHPV or HC2 test result at baseline have a very similar low risk (<0.3%) of developing CIN3+ after three years [58]. These data confirm that the AHPV test is suitable for primary cervical cancer screening at 3-years intervals.

Concerning the AHPV-GT test, only one clinical evaluation study has been performed to date. The CLEAR trial demonstrated that the AHPV-GT test is both reliable and effective in cervical cancer risk stratification in a referral population [49,59,60].

7. Conclusions

In summary, this comprehensive review of published literature on the clinical performance of the Aptima HPV test shows remarkably similar sensitivity combined with superior specificity for CIN2/3+ detection in comparison to the gold-standard HPV DNA-based test HC2 or GP5+/6+ PCR and throughout very different study designs.

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Conflict of interest

None.

Ethical approval

None.

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