View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

REVIEW

Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening?

M. Arbyn[1], **P. J. F. Snijders**[2], **C. J. L. M. Meijer**[2], **J. Berkhof**[3], **K. Cuschieri**[4], **B. J. Kocjan**[5] **and M. Poljak**[5]

1) *Unit of Cancer Epidemiology and Belgian Cancer Centre, Scientific Institute of Public Health, Brussels, Belgium,* 2) *Department of Pathology,* 3) *Department of Clinical Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands,* 4) *Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh, Edinburgh, Scotland, UK and* 5) *Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Abstract

Several countries are in the process of switching to high-risk human papillomavirus (hrHPV) testing for cervical cancer screening. Given the multitude of available tests, validated assays which assure high-quality screening need to be identified. A systematic review was conducted to answer the question which hrHPV tests fulfil the criteria defined by an international expert team in 2009, based on reproducibility and relative sensitivity and specificity compared to Hybrid Capture-2 or GP5+/6+ PCR–enzyme immunoassay. These latter two hrHPV DNA assays were validated in large randomized trials and cohorts with a follow-up duration of 8 years or more. Eligible studies citing the 2009 guideline were retrieved from Scopus (http://www.scopus.com) and from a meta-analysis assessing the relative accuracy of new hrHPV assays versus the standard comparator tests to detect high-grade cervical intraepithelial neoplasia or cancer in primary screening. The cobas 4800 HPV test and Abbott RealTime High Risk HPV test were consistently validated in two and three studies, respectively, whereas the PapilloCheck HPV-screening test, BD Onclarity HPV assay and the HPV-Risk assay were validated each in one study. Other tests which partially fulfil the 2009 guidelines are the following: Cervista HPV HR Test, GP5+/6+ PCR-LMNX, an in-house *E6/E7* RT quantitative PCR and MALDI-TOF (matrix-assisted laser desorption-ionization time-of-flight). The APTIMA HPV assay targeting *E6/E7* mRNA of hrHPV was also fully validated. However, the cross-sectional equivalency criteria of the 2009 guidelines were set up for HPV DNA assays. Demonstration of a low risk of CIN3+ after a negative APTIMA test over a longer period is awaited to inform us about its utility in cervical cancer screening at 5-year or longer intervals.
Clinical Microbiology and Infection © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Keywords: Cervical cancer, cervical cancer screening, diagnostic test accuracy, human papillomavirus, meta-analysis, systematic review, validation of tests
Article published online: 1 May 2015

Corresponding author: M. Arbyn, Belgian Cancer Centre/Unit of Cancer Epidemiology, Scientific Institute of Public Health, J. Wytsmanstreet 14, B1050, Brussels, Belgium
E-mail: marc.arbyn@wiv-isp.be

Introduction

A high level of evidence is currently available indicating that human papillomavirus (HPV)-based cervical cancer screening is more effective and efficient for the prevention of invasive cervical cancer and mortality from cervical cancer than screening with the Papanicolaou test [1,2]. Four European randomized trials have demonstrated that the cumulative incidence of cervical cancer 5 years after a negative HPV test was lower than the incidence 3 years after a normal cytology result [3]. The HPV assays used in these trials were the Hybrid Capture-2 (HC2) and the GP5+/6+ PCR–enzyme immunoassay (EIA). Moreover, longitudinal follow-up data of screened cohorts indicate a very low risk of developing cervical precancer or cancer over 8 years or more in women with a negative HC2 or GP5+/6+ PCR-EIA test [1,4–8]. Therefore, these two HPV assays are considered as fully clinically and epidemiologically validated [9]. HC2 (Qiagen, Gaithersburg, MD, USA) uses captured RNA probes which hybridize with viral DNA of 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Captured HPV RNA/DNA hybrids are identified

Clinical Microbiology and Infection © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved
http://dx.doi.org/10.1016/j.cmi.2015.04.015

by a secondary capture system which ultimately yields a light signal, the intensity of which is related to viral load [10]. HC2 does not involve DNA amplification. The GP5+/6+ PCR-EIA uses a single pair of consensus primers targeting a conserved DNA sequence of 140 bp of the *L1* gene of HPV. After amplification, a hybridization step with type-specific probes followed by EIA-based detection of hybrids can identify 14 HPV types (the same types targeted by HC2, plus HPV 66) [11].

Within the last decade, over 125 HPV assays (and over 80 variants of the original assays) have been developed, but evidence of their clinical utility has been demonstrated for relatively few [1,12,13]. In 2009, an international team of experts proposed criteria for assay validation in primary screening contexts based on minimal relative clinical accuracy of a given HPV assay compared to HC2 or GP5+/6+ PCR-EIA and minimal intra- and interlaboratory reproducibility [9]. The purpose of this review was to verify which tests fulfil these criteria.

Methods

Research question

This systematic review aims to answer the question which HPV tests can be considered to be clinically validated for use in primary cervical cancer screening. The PICOS (population–intervention–comparator–outcome–study) components can be found in the online Supplementary Material.

Literature retrieval

A search was conducted in Scopus (http://www.scopus.com) to identify reports that cited the guideline containing the equivalency criteria of Meijer *et al.* [9]

As a consequence of the preparation of new guidelines on secondary prevention of cervical cancer screening in Europe, and more recently in the Netherlands, Germany and Belgium, previously published meta-analyses on the diagnostic accuracy of HPV testing in primary screening were updated [1,14]. The search string used to retrieve references is provided in the online Supplementary Material. Details on the selection of relevant references can be found in previously published reports [1,15]. Studies were selected when the accuracy for cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) or CIN3+ of HC2 or GP5+/6+ PCR-EIA was compared with cytology and with another high-risk (hr) HPV assay detecting hrHPV types in cervical samples taken from women attending cervical cancer screening. Women with at least one positive screening test had to be verified with the reference standard (colposcopy and histology). Co-test-negative cases were considered as truly negative, accepting the assumption that the probability of missing cervical precancer was very low [1,6].

Finally, the Cervix1 bibliographic database of the Unit of Cancer Epidemiology (IPH Brussels) was consulted. Cervix1 contains almost 30000 literature references largely related to cervical cancer and HPV. The quality of the selected studies was evaluated using the QUADAS-2 checklist [16]. The results of the HPV assays with individual genotyping were assessed for the presence or absence of hrHPV types according to the pool of 13 or 14 high-risk types in common with the types targeted by HC2 (HPV 16, 18, 31, 33, 45, 51, 52, 56, 58, 59, 68) or GP5+/6+ PCR-EIA (same types plus HPV 66).

Equivalency criteria

In 2009 an international expert committee proposed that a new hrHPV DNA assay should be at least as accurate as the HC2 or GP5+/6+ PCR-EIA, and should be highly reproducible in order to be eligible for use in cervical cancer screening. The candidate test should demonstrate a relative sensitivity and specificity to detect CIN2+ compared to one of the aforementioned standard comparator tests of $\geq$0.90 and $\geq$0.98, respectively [9]. A representative set of consecutively collected samples should be selected (minimally 60 CIN2+ cases, 800 $\leq$CIN1 cases) derived from a population-based screening cohort of women aged 30 to 60 years [9]. The p value for a noninferiority score test should be lower than 0.05 [17]. In addition, a high inter- and intrareproducibility (lower confidence bound [LCIB] $\geq$87%) should be reached. For each retrieved validation study, fulfillment of these criteria was checked.

Meta-analysis

A meta-analysis of the relative sensitivity and specificity of the new HPV assay versus one of the two standard comparator HPV tests was conducted using a random-effects model for pooling ratios of proportions [18]. Forest plots, with subgroups at the level of individual tests, were drawn. Whether the confidence interval (CI) included unity and the LCIB was equal to or exceeded 0.90 for sensitivity or 0.98 for specificity was assessed to determine noninferiority of the new test compared to the standard comparator test.

In order to assure comparable inference, 90% CIs around the relative accuracy measures were computed which show a statistical coverage similar to that of the one-sided non-inferiority testing at $p < 0.05$.

Although equivalency criteria have been defined only for the CIN2+ outcome, we have extracted the relative accuracy values also at the level of CIN3+.

Results

One hundred ninety-seven references were retrieved in Scopus; 11 of them contained data that fitted the Meijer validation

 © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved,

TABLE 1. Characteristics of hrHPV tests validated using cross-sectional equivalence performance criteria

	hrHPV assay	Nucleic acid targeted	Type of amplification	Genes targeted	Separate genotyping	Internal control for human genes
Standard comparator tests						
*	HC2	DNA	Signal	Whole viral genome	No	No
*	GP5+/6+ PCR-EIA	DNA	Target	*L1*	No	No
HPV assays validated according to the Meijer protocol						
1	APTIMA HPV assay [26]	RNA	Target	*E6/E7*	No (a prototype identifying HPV16, 18–45 exists)	No. Assay includes internal controls for non-infectious RNA and DNA.
2	Abbott RealTime High Risk HPV test [20–22]	DNA	Target	*L1*	16,18 & 12 other hr types	*β-globin*
3	BD Onclarity HPV assay [30]	DNA	Target	*E6/E7*	16,18,31,45,51,52; 33–58; 56–59–66; 35–39–68	*β-globin*
4	Cervista HPV HR Test [27,28]	DNA	Signal	*L1/E6/E7*	14 hr types. Separate typing of 16, 18 available as a reflex test.	human *histone 2*
5	cobas 4800 HPV test [23,24]	DNA	Target	*L1*	16,18 & 12 other hr types (see HC2 plus 66)	*β-globin*
6	qPCR(E6/E7) [25]	DNA	Target	*E6/E7*	Separate typing of hrHPV types 16,18,31,33, 35,39,45,51,52,56,58,59,66,68; phr type 53 and lr types 6 & 11.	*β-globin*
7	HPV-Risk assay [29]	DNA	Target	*E7*	16, 18 and 13 other hr types (see HC2 plus 66 & 67)	*β-globin*
8	PapilloCheck HPV-screening test [19]	DNA	Target	*E1*	Separate typing of 16,18,31,33,35,39,45,51,52,53,56,58,59,66,68. As well as phr types 70,73,82 and lr types 6,11,40,42,43,44.	*ADAT1*
HPV assays assessed in cross-sectional studies in a primary screening setting or according to the VALGENT protocol						
1	GP5+/6+-LMNX [41]	DNA	Target	*L1*	Separate typing of hr HPV types 16,18,31,33,35,39,45,51,52,56,58,59,66,68; Detects also phr types: 26,53,73,82.	Human DNA fragment located on chromosome 14
2	*care*HPV Test [38]	DNA	Signal	Whole viral genome	No	No
3	MALDI-TOF [40]	DNA	Target	*L1*	Separate typing of hr HPV types 16,18,31,33,35,39,45,51,52,56,58,59,66,68.	*β-globin*
4	Pretect HPV-Proofer [55]	RNA	Target	*E7/E7*	Yes (16,18,31,33,45)	U1 small nuclear ribonucleo-protein-specific mRNA.

hrHPV, high-risk human papillomavirus; *ADAT1* gene, adenosine deaminase, tRNA specific 1; phr, potentially high-risk type; qPCR, quantitative PCR; MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight.
* HC2 and GP5+/6+ PCR-EIA are clinically validated in randomised efficacy trials and therefore used as standard comparator tests to validate other HPV assays.

Clinical Microbiology and Infection © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved,

TABLE 2. Sensitivity and specificity of hrHPV assays validated for cervical cancer screening, relative sensitivity and specificity of the evaluated hrHPV assays compared to the standard comparator tests (HC2 or GP5+/6+ PCR-EIA)

Evaluated assay	Study	Evaluated assay: Absolute sensitivity	Evaluated assay: Absolute specificity	Comparator assay	Comparator: Absolute sensitivity	Comparator: Absolute specificity	Evaluated/comparator assay: Relative sensitivity	Evaluated/comparator assay: Relative specificity	Non-inferiority test*: p_{sens}	Non-inferiority test*: p_{spec}	Validation level‡
GP5+/6+ EIA	Meijer, 2009 [9]	98.7%	96.0%	HC2*	98.7%	94.1%	1.00	1.02	0.0037	<0.0001	⊕⊕⊕⊕
PapilloCheck	Hesselink, 2010 [19]	95.8%	96.7%	GP5+/6+ EIA	96.4%	97.7%	0.99	0.99	<0.0001	0.0072	⊕⊕
Abbott RT hrHPV test	Carozzi, 2011 [20]	96.4%	92.3%	HC2	97.6%	92.6%	0.99	1.00	0.0040	0.0087	⊕⊕⊕
	Poljak, 2011 [21]	100.0%	93.3%	HC2	97.4%	91.8%	1.03	1.02	0.0112	0.0000	
	Hesselink, 2013 [22]	95.6%	92.0%	GP5+/6+ EIA	98.5%	91.8%	0.97	1.00	0.0278	0.0003	
cobas 4800	Heideman, 2011 [23]	90.0%	94.6%	HC2	91.7%	94.4%	0.98	1.00	0.0216	0.0009	⊕⊕⊕
	Lloveras, 2013 [24]	98.3%	86.2%	HC2	98.3%	85.3%	1.00	1.01	0.0093	0.0012	
qPCR(*E6/E7*)	Depuydt, 2012 [25]	93.5%	95.6%	HC2	83.9%	94.4%	1.11	1.01	0.0001	<0.0001	⊕⊕
APTIMA	Heideman, 2013 [26]	95.5%	94.5%	GP5+/6+ EIA	100.0%	93.6%	0.96	1.01	0.0394	0.0002	x
Cervista	Boers, 2014 [27]	89.0%	91.2%	HC2	93.4%	88.8%	0.95	1.03	0.0043	<0.0001	⊕
	Alameda, 2015 [28]	98.4%	85.2%	HC2	100.0%	86.4%	0.98	0.99	0.0122	0.3170†	
BD Onclarity	Ejegod, 2014 [30]	92.9%	87.7%	HC2	94.2%	88.8%	0.99	0.99	0.0009	0.0216	⊕⊕
HPV-Risk assay	Hesselink, 2014 [29]	97.1%	94.3%	GP5+/6+ EIA	97.1%	94.1%	1.00	1.00	0.0056	0.0003	⊕⊕

* p values for non-inferiority of the evaluated assay compared to the comparator assay.
† We corrected an error in Alameda, 2015 [28] (due to switching of + and − columns and rows). The corrected data showed that the non-inferiority test was not significant for specificity.
‡Validation level for the test accuracy criterion as proposed by Meijer *et al* 2009 [9].
⊕⊕⊕⊕ validated in large randomized controlled trials with cancer incidence as an outcome; considered as standard comparator tests.
⊕⊕⊕: fully validated in multiple studies.
⊕⊕ fully validated in one study.
⊕ partially validated.
X not evaluated since not a hrHPV DNA assay.

protocol (Supplementary Table S1, Supplementary Fig. S1) [19–29]. A 12th reference was retrieved from the Cervix1 database [30]. Eight candidate hrHPV assays were evaluated (PapilloCheck HPV-screening test [19]; Abbott RealTime High Risk HPV test [Abbott RT hrHPV test] [20–22]; cobas 4800 HPV test [23,24]; an in-house quantitative PCR [qPCR] targeting the *E6* or *E7* HPV genes [25]; APTIMA HPV assay [26]; Cervista HPV HR Test [27,28]; BD Onclarity HPV assay [30]; and the HPV-Risk assay [29]) (Supplementary Table S2). The test characteristics are listed in Table 1. Details about the study design and characteristics are provided in Supplementary Table S3. Data on intra- and interlaboratory reproducibility of the PapilloCheck HPV-Screening test were retrieved from an online source (http://www.pathology.nl) [31].

Using the Meijer validation protocol, five HPV DNA assays (PapilloCheck HPV-Screening test; Abbott RT hrHPV test; cobas 4800 HPV test; BD Onclarity HPV assay; HPV-Risk assay) and the APTIMA assay, targeting *E6/E7* mRNA, fulfilled all the equivalency criteria (Tables 2 and 3). For the Abbott RT hrHPV test and the cobas 4800 HPV test, respectively, three [20–22] and two studies [23,24] consistently confirmed equivalency for accuracy and high reproducibility. The Cervista HPV HR Test also fulfilled all the criteria in one study [27], but in another study, inferior specificity compared to HC2 could not be rejected ($p_{n.inf}$ 0.3170) [28]. The in-house *E6/E7* qPCR fulfilled the criteria for accuracy and intralaboratory reproducibility, but interlaboratory reproducibility was not reported [25].

The meta-analysis of the relative accuracy for CIN2+ included (besides the 12 aforementioned validation studies) 10 additional reports evaluating HPV tests in primary screening [21,32–40] as well as one validation study designed according to the VALGENT protocol (see chapter 6 of Supplementary Materials) [41]. In total, 22 comparisons with HC2 and 4 with GP5+/6+ PCR-EIA were noted involving 12 assays. The four assays not included in the retrieval of the Meijer validation studies were *care*HPV Test [38,39], Pretect HPV-Proofer (*E6/E7* mRNA assay) [36], MALDI-TOF (matrix-assisted laser desorption-ionization time-of-flight) [40] and an alternative GP5+/6+ PCR with Luminex-based identification of separate HPV types (LMNX Genotyping Kit HPV GP) [41] (Table 1). For the MALDI-TOF, only accuracy for CIN3+ was reported, but data for the end point of CIN2+ was provided by the author upon request [40]. Supplementary

TABLE 3. Intra- and inter-laboratory reproducibility of hrHPV assays validated for cervical cancer screening

Evaluated Assay	Study	Intra-laboratory reproducibility (hr HPV DNA)			Inter-laboratory reproducibility (hrHPV)			Validation Level‡
		Overall	LCIB	Kappa	Overall	LCIB	Kappa	
PapilloCheck	Hesselink, 2010 [19]	97.6%	96.3%	0.941	94.0%	92.1%	0.842	⊕⊕
Abbott RT hrHPV test	Carozzi, 2011 [20]	98.5%	97.3%	0.969	–	–	–	⊕⊕
	Poljak, 201 [21]1	100.0%	99.5%	1.000	100.0%	99.5%	1.000	
	Hesselink, 2013 [22]	99.8%	99.1%	0.996	98.4%	97.2%	0.965	
cobas 4800	Heideman, 2011 [23]	98.3%	97.2%	0.963	94.6%	92.8%	0.882	⊕⊕
	Lloveras, 2013 [24]	98.3%	97.2%	0.963	98.4%	97.2%	0.962	
qPCR(*E6/E7*)	Depuydt, 2012 [25]	98.7%	97.8%	0.956	–	–	–	⊕
APTIMA	Heideman, 2013 [26]	96.0%	94.4%	0.893	95.1%	93.3%	0.865	⊕⊕
Cervista	Boers, 2014 [27]	91.9%	89.7%	0.829	90.7%	88.4%	0.807	⊕⊕
	Alameda, 2015 [28]	94.9%	93.0%	0.890	96.5%	94.9%	0.907	
BD Onclarity	Ejegod, 2014 [30]	98.6%	97.5%	0.967	98.4%	97.2%	0.962	⊕⊕
HPV-Risk assay	Hesselink, 2014 [29]	99.5%	97.3%	0.987	99.2%	97.2%	0.981	⊕⊕

hrHPV: high-risk human papillomavirus; LCIB: lower 95% confidence interval bound; RT:real time; qPCR: quantitative polymerase chain reaction.
‡Validation level for the reproducibility criterion: ⊕⊕ high intra- and inter-laboratory reproducibility confirmed; ⊕ high intra-laboratory reproducibility confirmed. Tests are validated as proposed by Meijer *et al* 2009 [9].

Table S4 summarizes the methodologic quality of the ten included screening studies, which was overall moderate to good with average negative scores for the 13 QUADAS-2 items varying between 0% and 15%, equivocal scores varying between 0% and 31% and positive scores varying between 54% and 100%.

The relative sensitivity and specificity values for the outcome of CIN2+ of the eight tests validated according to the Meijer protocol (Table 2) were similar to those observed in primary screening studies. The 90% CI intervals always included unity. Moreover, the LCIBs for the relative sensitivity (pooled for tests evaluated in multiple studies or individual if a test was evaluated in only one study) consistently exceeded 0.90. Also, the LCIBs for specificity were ≥0.98 (Table 4, Figs. 1 and 2). Two further tests, one evaluated within primary screening and the other according to the VALGENT protocol, met the accuracy criteria, namely the MALDI-TOF (which had a relative sensitivity of 0.97 [90% CI, 0.94–1.00] and specificity of 1.09 [90% CI, 1.01–1.16]) [40] and the GP5+/6+-LMNX (which had a relative sensitivity of 0.98 [90% CI, 0.93–1.03] and specificity of 1.00 [90% CI, 0.97–1.02]) [41] (See Supplementary Material, chapter 6). The *care*HPV Test and the Pretect HPV-Proofer showed significantly inferior sensitivity: *care*HPV Test/HC2 = 0.86 (90% CI, 0.79–0.94); Pretect HPV-Proofer/HC2 = 0.74 (90% CI, 0.63–0.88). However, the specificity of the latter tests was similar or higher than for the standard comparators.

The relative accuracy for the outcome of CIN3+ is detailed in Table 4 and Supplementary Figs. S2 and S3.

Discussion

Besides HC2 and GP5+/6+ PCR-EIA validated in randomized trials, the Abbott RT hrHPV test, cobas 4800 HPV test, BD Onclarity HPV assay, HPV-Risk assay and the PapilloCheck HPV-Screening test can also be considered as clinically validated hrHPV DNA assays usable for primary HPV-based cervical cancer screening. For all these assays, noninferior sensitivity and specificity for the outcome CIN2+ and sufficient intra- and inter-laboratory reproducibility has been demonstrated in at least one validation study following the Meijer validation protocol. The Cervista HPV HR Test can be added to this list, although one study failed to show noninferior specificity [28]. However, the pooled relative specificity compared to HC2 was not significantly different from unity (90% CI, 0.98–1.04 with LCIB ≥0.98). One in-house quantitative RT-PCR targeting *E6/E7* DNA sequences of 17 HPV genotypes [25] also fulfilled three of the Meijer criteria, but its interlaboratory reproducibility has not been assessed [42]. The meta-analysis revealed two further assays (the GP5+/6+ PCR with Luminex identification of high-risk types and the MALDI-TOF) which were noninferior to the standard comparator tests with respect to clinical accuracy. Given the absence of formal checking of reproducibility, these latter two may be considered partially validated.

Testing for viral mRNA and need for longitudinal data

The APTIMA assay detects *E6* or *E7* transcripts of 14 high-risk types. This test showed consistently similar study-specific and

TABLE 4. Relative accuracy of other HPV tests compared to HC2 (at RLU*≥1) or GP5+6+ PCR–EIA to find underlying CIN2+ or CIN3+ in primary screening

Comparison	Outcome	Relative sensitivity (90% CI)	Relative specificity (90% CI)	No. of studies
APTIMA/HC2	CIN2+	0.98 (0.95–1.01)	1.04 (1.02–1.07)	6
Abbott RT hrHPV test/HC2	CIN2+	0.99 (0.96–1.03)	1.02 (1.01–1.02)	3
BD Onclarity/HC2	CIN2+	0.99 (0.96–1.03)	0.99 (0.98–1.00)	2
Cervista/HC2	CIN2+	0.98 (0.93–1.03)	1.01 (0.98–1.04)	2
cobas 4800/HC2	CIN2+	1.00 (0.97–1.03)	0.99 (0.98–1.00)	3
*care*HPV Test/HC2	CIN2+	0.86 (0.79–0.94)	1.01 (0.99–1.03)	2
MALDI-TOF/HC2	CIN2+	0.97 (0.94–1.00)	1.09 (1.01–1.16)	1
Pretect HPV-Proofer/HC2	CIN2+	0.74 (0.63–0.88)	1.12 (1.10–1.13)	1
qPCR (*E6/E7*)/HC2	CIN2+	1.12 (1.02–1.22)	1.01 (1.00–1.03)	1
APTIMA/GP5+/6+ EIA	CIN2+	0.96 (0.92–1.01)	1.01 (0.99–1.03)	1
Abbott RT hrHPV test/GP5+/6+ EIA	CIN2+	0.97 (0.92–1.02)	1.00 (0.98–1.03)	1
GP5+/6+-LMNX/GP5+/6+ EIA	CIN2+	0.98 (0.93–1.03)	1.00 (0.97–1.02)	1
HPV-Risk assay/GP5+/6+ EIA	CIN2+	1.00 (0.95–1.05)	1.01 (0.99–1.02)	1
APTIMA/HC2	CIN3+	0.98 (0.95–1.02)	1.04 (1.02–1.07)	5
Abbott RT hrHPV test/HC2	CIN3+	0.99 (0.93–1.05)	1.02 (1.01–1.03)	2
BD Onclarity/HC2	CIN3+	1.00 (0.92–1.09)	0.99 (0.97–1.00)	1
Cervista/HC2	CIN3+	0.97 (0.94–1.01)	1.03 (1.02–1.04)	1
cobas 4800/HC2	CIN3+	1.00 (0.92–1.09)	0.99 (0.98–1.00)	1
*care*HPV Test/HC2	CIN3+	0.91 (0.78–1.06)	1.02 (1.00–1.05)	1
MALDI-TOF/HC2	CIN3+	0.96 (0.93–1.00)	1.02 (1.01–1.03)	1
PapilloCheck/HC2	CIN2+	0.99 (0.96–1.03)	0.99 (0.98–1.00)	1
Pretect HPV-Proofer/HC2	CIN3+	0.71 (0.52–0.94)	1.12 (1.10–1.13)	1
GP5+/6+-LMNX/GP5+/6+ EIA	CIN3+	1.00 (0.96–1.04)	1.00 (0.97–1.04)	1

HPV, human papillomavirus; EIA, enzyme immunoassay; CI, confidence interval; qPCR, quantitative PCR; MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight.
*RLU: relative light units.

pooled sensitivity and superior specificity for CIN2+ compared to HC2 (pooled relative sensitivity of 0.98, 90% CI, 0.95–1.01; pooled relative specificity of 1.04, 90% CI, 1.02–1.07). Moreover, in a validation study using GP5+/6+ PCR-EIA as comparator, noninferior accuracy and excellent reproducibility was evidenced [26]. The similar relative sensitivity and superior specificity of APTIMA compared to HC2, observed in primary screening, is in agreement with the findings from a previous meta-analysis on its performance in triage of women with minor cytologic abnormalities [42]. In both triage of atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL), APTIMA was as sensitive for detection of CIN2+ as HC2. However, the former was significantly more specific than HC2 for excluding cervical precancer (ratio = 1.19 [95% CI, 1.08–1.31] or 1.37 [95% CI, 1.22–1.54, in triage of ASC-US or LSIL, respectively]) [42]. The equivalence criteria for tests in primary screening, based on relative cross-sectional accuracy, are only valid for testing for hrHPV DNA and not for other molecular markers (such as viral RNA, methylation of protein markers) [9]. These other markers should reach the same level of evidence required for the original HPV DNA screening tests: the demonstration of a low cumulative incidence of CIN3+ after a baseline negative screening test result over a period of at least 5 years similar to that after a negative screening result with a validated hrHPV DNA assay [1]. It should be remarked that recently a low longitudinal risk of CIN3+ (<0.3%) was demonstrated up to 3 years after a negative APTIMA test which was similar to the risk after a negative HC2 test [43]. This means that screening at 3-year intervals after a negative APTIMA test could be considered acceptable. For screening with APTIMA at intervals of 5 years or more, as proposed for validated hrHPV DNA tests, more data over longer periods will provide additional reassurance. For hrHPV DNA assays, for which equivalent cross-sectional accuracy as HC2 or GP5+/6+ PCR-EIA are accepted as sufficient evidence to allow their use in screening, the generation of longitudinal data remain useful and corroborate the evidence level. In the final report of the ATHENA trial, it was shown that the cumulative risk of CIN3+ after a negative cobas 4800 HPV test result was 0.3% (95% CI, 0.1–0.7). For comparison, after negative cytology and after a co-negative cytology–cobas 4800 HPV test result, the 3-year risks of CIN3+ were 0.8% (95% CI, 0.5–1.1) and 0.3% (95% CI, 0.1–0.6), respectively [44]. A low risk of CIN2+ was also demonstrated in the second screening round 3 years after a negative baseline Abbott RT hrHPV test (0/1647 = 0.0% vs. 1/1635 = 0.06% after a negative HC2) [45].

Other performance criteria

Besides accuracy, other fundamental assay characteristics should be taken into account when the choice of the screening test is considered. These would include the high throughput capacity, automation, costs, applicability on samples taken by the women themselves and the ability to perform ancillary

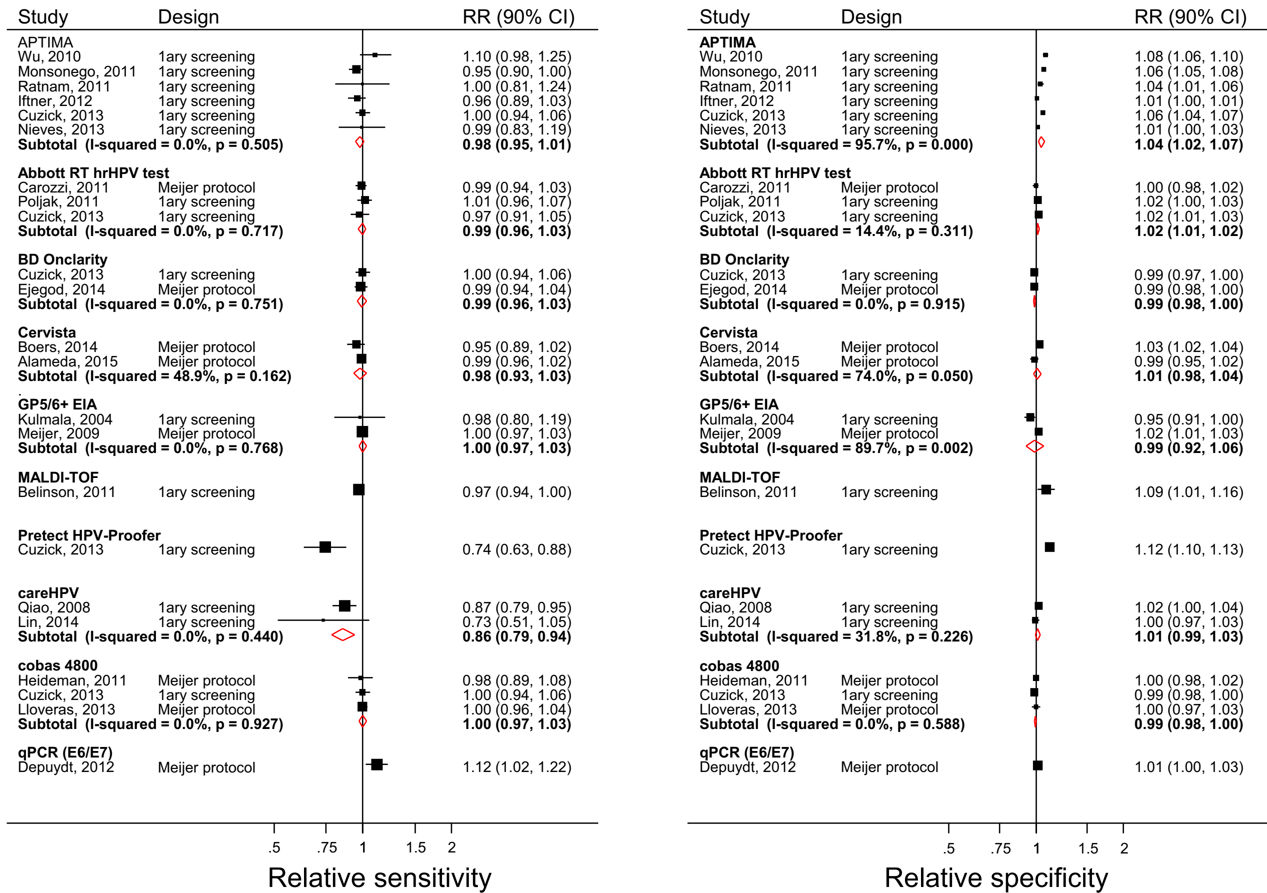

FIG. 1. Relative sensitivity (left) and specificity (right) of hrHPV DNA or RNA assays compared to HC2 to detect CIN2+ in cervical cancer screening.

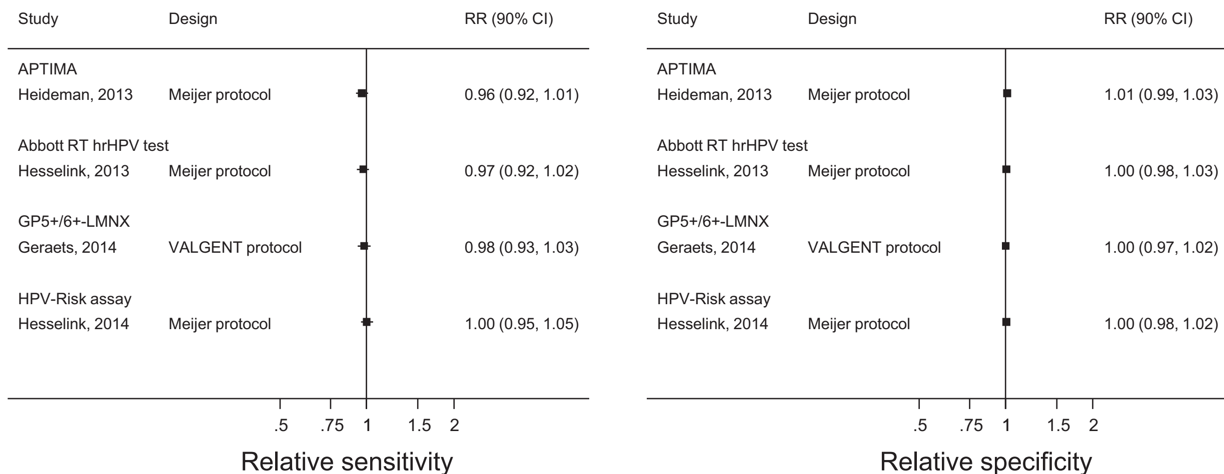

FIG. 2. Relative sensitivity (left) and specificity (right) of hrHPV DNA or RNA assays compared to the GP5+/6+ PCR-EIA to detect CIN2+ in cervical cancer screening.

triage tests [1,46,47]. A substantial price reduction of the test may be obtained by centralized purchasing tenders addressed to manufacturers, appealing for a cost-effective, clinically validated HPV assay to be used in a screening programme [48]. The potential for ancillary or *a priori* separate genotyping, the range of targeted genotypes and the targeted virus gene are other factors that may play a role in the determination of the preferred HPV assay. It has been shown that in certain rare cases of cervical cancer the *L1* gene might be disrupted, causing false-negative screening results when using tests that target this region [49,50]. However, recent genome-wide profiling of HPV integration in human DNA of cervical cancer patients has revealed a variety of disruptions in the HPV genome which may also involve *E6* and *E7* genes [51]. The requirement of *E6/E7* activity for the development and the maintenance of cervical cancer suggests that possible disruptions in *E6/E7* genes would be accompanied with the presence of virus copies that are not disrupted in *E6/E7*. Still, another recent study of a series of HC2-negative cervical cancers from Spain showed that in cases which were *L1*-PCR negative, *E7*-based PCR was also often negative [52]. These HPV-negative cancers had a poorer prognosis than HPV-positive cases [52]. The proportion of such HPV-negative cancers is low but currently not well known.

Comprehensive genotyping of biopsies from cervical cancer cases as well as previous archived cytology specimen stored in a biobank may offer important tools for quality assurance and monitoring safety within HPV-based screening programmes [53]. Furthermore, it must be remarked that criteria discussed in this review only concern HPV testing of a specimen collected by direct scraping of the surface of the cervix by a clinician. It has been shown that HPV testing using signal amplification assays (even with methods clinically validated on clinician collected material) is less sensitive and specific on self-collected compared to clinician-collected samples [46,47]. PCR-based systems validated on clinician-based cervical samples, on the other hand, so far have shown similar clinical accuracy on self-collected and clinician-collected specimen [46,47].

Equivalency criteria of the Meijer guideline

An important issue in the validation process of HPV tests is the origin of the samples and the status of the cervical cell specimen included in the series of cases with disease (CIN2+) or nodisease (≤CIN1). As stated in the Meijer guideline, samples should be derived from a screening population of women aged 30 or older. This condition was fulfilled in eight [19–23,26,27,29] but not in four other validation studies [24,25,28,30]. The method of selection of CIN2+ cases was also variable, with one study including only specimens from women with CIN3+ [19] and another including samples from women referred to colposcopy [30]. The choice of relative accuracy targets makes the validation process more robust and less vulnerable to selection biases induced by inclusion of non-screening specimens. A more important bias may be induced by arbitrary selection, and the best way to avoid this is to impose continuous selection of samples (the first occurring cases and controls in a comprehensive screening database linked to an outcome). The accuracy of ascertainment of nondisease (≤CIN1) may influence the relative specificity. In most of the retrieved validation studies, ascertainment of controls was not well specified. In one study, it was noted that the control specimens were selected from women with normal cytology and with no cytologic abnormality within 12 months [22]. It cannot be excluded that some CIN2+ may have been missed, resulting in outcome misclassification. An improvement of the Meijer guideline may be to restrict control specimen to women who had negative cytology results in two subsequent screening rounds as proposed in the VALGENT protocol [41]. Furthermore, the policy used to screen the target population (cytology alone, combined cytology and HPV testing, HPV testing alone) and the verification of screen test positive results may influence the validation of HPV assays. For instance, in VALGENT-2 studies [41], diagnostic assessment was only triggered by a cytology result of high-grade squamous intraepithelial lesion or repeated ASC-US or LSIL. A certain amount of underdiagnosis cannot be excluded, giving some advantage to a less sensitive but more specific HPV test. For this reason, the VALGENT-2 protocol foresees verifying occurrence of CIN2+ cases in the next screening round. Conversely, in VALGENT-1 and in the validation study of the qPCR (*E6/E7*), diagnostic assessment was performed after combined HPV and cytology screening where cytologists had previous knowledge of the results of the HPV test, possibly resulting in some overdiagnosis which might have generated an advantage for analytically sensitive assays [54]. Furthermore, the performance validation metric may need adaptation to be applicable and relevant for immunized populations.

Addressing all these pitfalls and potential biases will make the development of future validation guidelines challenging. Nevertheless, the current Meijer criteria have been of considerable benefit in identifying a group of HPV tests with a good balance between clinical sensitivity and specificity, particularly given the expanding choice of assays.

Conclusion

The guideline based on reproducibility and equivalent accuracy defined by Meijer *et al.* [9] is a milestone in HPV-based cervical cancer screening. The hrHPV DNA assays fully matching these criteria and which can be recommended

today in HPV-based cervical cancer screening using clinician-collected cervical samples are the following: HC2, GP5+/6+ PCR-EIA, Abbott RT hrHPV test, cobas 4800 HPV test, PapilloCheck HPV-Screening test, BD Onclarity HPV assay and the HPV-Risk assay. The mRNA-based APTIMA assay also fully matches the criteria, although further longitudinal data are awaited. Other HPV DNA tests which partially fulfil these guidelines are the following: Cervista HPV HR Test, GP5+/6+ PCR-LMNX, an in-house *E6/E7* qPCR and MALDI-TOF. This list of tests recommendable in HPV–based cervical cancer screening requires regular updating as evidence accrues.

Transparency declaration

MA, HB, CJLMM, MP and PJFS were supported by the seventh framework program of DG Research of the European Commission through the COHEAHR Network (grant 603019). MA received funding the National Institute of Public Health and the Environment (Bilthoven, the Netherlands), the German Guideline Program in Oncology (German Cancer Aid project 110163) and the Belgian Health Care Knowledge Centre (Brussels, Belgium). The research in the laboratory of PJFS and CJLMM is also supported by grants from the Dutch Cancer Society (KWF2007-3771, KWF2009-4413, KWF2009-4522 and KWF2010-4668), Zorgonderzoek Nederland-Medische Wetenschappen (ZON-MW) and the European Research Council (ERC advanced 2012-AdG, proposal 322986 Mass-care). KC received research grants from Abbott, Hologic, Cepheid and Becton Dickinson. CM has been on the speaker's office of GSK, Merck, Qiagen and Roche and has stock in self-Screen and Diassy. MP's institution received research grants from Abbott. PS has been on the speaker's bureau of Roche, Gen-Probe, Abbott and Qiagen and has minority stock in Self-screen.

Acknowledgements

We acknowledge J. Dillner (Karolinska Institute, Stockholm, Sweden) for reviewing the article, and J. Belinson (Preventive Oncology International Inc. and The Cleveland Clinic, Women's Health Institute, Cleveland, OH, USA) for provision of data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.04.015.

References

[1] Arbyn M, Ronco G, Anttila A, et al. Evidence regarding HPV testing in secondary prevention of cervical cancer. Vaccine 2012;30(Suppl. 5):F88–99.
[2] Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural India. N Engl J Med 2009;360:1385–94.
[3] Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014;383:524–32.
[4] Dillner J, Rebolj M, Birembaut P, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008;337:a1754.
[5] Katki HA, Schiffman M, Castle PE, et al. Benchmarking CIN 3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. J Low Genit Tract Dis 2013;17:S28–35.
[6] Castle PE, Glass AG, Rush BB, et al. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. J Clin Oncol 2012;30:3044–50.
[7] Elfstrom KM, Smelov V, Johansson AL, et al. Long term duration of protective effect for HPV negative women: follow-up of primary HPV screening randomised controlled trial. BMJ 2014;348:g130.
[8] Vink MA, Bogaards JA, Meijer CJ, Berkhof J. Primary human papillomavirus DNA screening for cervical cancer prevention: can the screening interval be safely extended? Int J Cancer 2015. In press.
[9] Meijer CJLM, Castle PE, Hesselink AT, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009;124:516–20.
[10] Lorincz AT. Methods of DNA hybridisation and their clinical applicability to human papillomavirus detection. In: Franco EL, Monsonego J, editors. New developments in cervical cancer screening and prevention; 1997. p. 325–37. Oxford: Blackwell Science.
[11] Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6+-mediated PCR–enzyme immunoassay method for rapid detection of 14 high risk and 6 low risk human papillomavirus types in cervical scrapings. J Clin Microbiol 1997;35:791–5.
[12] Poljak M, Cuzick J, Kocjan BJ, Iftner T, Dillner J, Arbyn M. Nucleic acid tests for the detection of human papillomaviruses. Vaccine 2012;30(Suppl. 5):F100–6.
[13] Poljak M, Kocjan BJ. Commercially available assays for multiplex detection of alpha human papillomaviruses. Expert Rev Anti Infect Ther 2010;8:1139–62.
[14] Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006;24(Suppl. 3):S78–89.
[15] Arbyn M, Haelens A, Desomer A, et al. Cervical cancer screening program and human papillomavirus (HPV) testing, part II: update on HPV primary screening. KCE Rep 2015;238:1–282.
[16] Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.
[17] Tang NS, Tang ML, Chan IS. On tests of equivalence via non-unity relative risk for matched-pair design. Stat Med 2003;22:1217–33.
[18] Dersimonian R, Laird NM. Meta-analysis in clinical trials. Controlled Clin Trials 1986;7:177–88.
[19] Hesselink AT, Heideman DA, Berkhof J, et al. Comparison of the clinical performance of PapilloCheck human papillomavirus detection with that of the GP5+/6+-PCR–enzyme immunoassay in population-based cervical screening. J Clin Microbiol 2010;48:797–801.

[20] Carozzi FM, Burroni E, Bisanzi S, et al. Comparison of clinical performance of Abbott RealTime High Risk HPV Test with that of Hybrid Capture 2 Assay in a screening setting. J Clin Microbiol 2011;49:1446–51.
[21] Poljak M, Ostrbenk A, Seme K, et al. Comparison of clinical and analytical performance of the Abbott RealTime High Risk HPV Test to the performance of Hybrid Capture 2 in population-based cervical cancer screening. J Clin Microbiol 2011;49:1721–9.
[22] Hesselink AT, Meijer CJ, Poljak M, et al. Clinical validation of the Abbott RealTime High Risk (HR) HPV assay according to the guidelines for HPV DNA test requirements for cervical screening. J Clin Microbiol 2013;51:2409–10.
[23] Heideman DA, Hesselink AT, Berkhof J, et al. Clinical validation of the cobas(R) 4800 HPV test for cervical screening purposes. J Clin Microbiol 2011;49:3983–5.
[24] Lloveras B, Gomez S, Alameda F, et al. HPV testing by cobas HPV test in a population from Catalonia. PLoS One 2013;8:e58153.
[25] Depuydt CE, Benoy IH, Beert JF, Criel AM, Bogers JJ, Arbyn M. Clinical validation of a type-specific real time quantitative human papillomavirus PCR to the performance of Hybrid Capture 2 for the purpose of cervical cancer screening. J Clin Microbiol 2012;50:4073–7.
[26] Heideman DA, Hesselink AT, van Kemenade FJ, et al. The APTIMA HPV assay fulfills the cross-sectional clinical and reproducibility criteria of international guidelines for HPV test requirements for cervical screening. J Clin Microbiol 2013;51:3653–7.
[27] Boers A, Wang R, Slagter-Menkema L, et al. Clinical validation of the Cervista HPV HR test according to the international guidelines for human papillomavirus test requirements for cervical cancer screening. J Clin Microbiol 2014;52:4391–3.
[28] Alameda F, Garrote L, Mojal S, et al. Cervista HPV HR Test for cervical cancer screening: a comparative study in the Catalonian population. Arch Pathol Lab Med 2015;139:241–4.
[29] Hesselink A, Berkhof J, van der Salm ML, et al. Clinical validation of the HPV-Risk assay: a novel, real-time PCR assay for the detection of high-risk human papillomavirus DNA by targeting the *E7* region. J Clin Microbiol 2014;52:890–6.
[30] Ejegod DM, Serrano I, Cuschieri K, et al. Clinical validation of the BD Onclarity™ HPV Assay using a non-inferiority test. J Med Microbiol Diagn 2014;S3: 03:1–4. http://dx.doi.org/10.4172/2161-0703.S3-003.
[31] Dutch Society of Pathology (Nederlandse Vereniging voor Pathologie). Approval PapilloCheck (Goedkeuring Papillocheck). Available at: http://www.pathology.nl.
[32] Wu R, Belinson SE, Du H, et al. Human papillomavirus messenger RNA assay for cervical cancer screening: the Shenzhen Cervical Cancer Screening Trial I. Int J Gynecol Cancer 2010;20:1411–4.
[33] Monsonego J, Hudgens MG, Zerat L, et al. Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid based cytology in primary cervical cancer screening (the FASE study). Int J Cancer 2011;129:691–701.
[34] Ratnam S, Coutlee F, Fontaine D, et al. Aptima HPV *E6/E7* mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. J Clin Microbiol 2011;49:557–64.
[35] Iftner T. Comparison of Aptima and HC2 in a routine screening trial in Germany with follow up. Paper presented at: 28th International Conference of the Papillomavirus Society, CP-303; San Juan, Puerto Rico. 2012.
[36] Cuzick J, Cadman L, Mesher D, et al. Comparing the performance of six human papillomavirus tests in a screening population. Br J Cancer 2013;108:908–13.
[37] Nieves L, Enerson CL, Belinson S, et al. Primary cervical cancer screening and triage using an mRNA human papillomavirus assay and visual inspection. Int J Gynecol Cancer 2013;23:513–8.
[38] Qiao YL, Sellors JW, Eder PS, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. Lancet Oncol 2008;9:929–36.
[39] Lin CQ, Chen F, Liu B, et al. A parallel study of *care*HPV and Hybrid Capture 2 human papillomavirus DNA testing for cervical cancer screening in rural China. J Virol Methods 2014;202:73–8.
[40] Belinson JL, Wu R, Belinson SE, et al. A population-based clinical trial comparing endocervical high-risk HPV testing using Hybrid Capture 2 and Cervista from the SHENCCAST II study. Am J Clin Pathol 2011;135:790–5.
[41] Geraets D, Cuschieri K, Koning M, et al. Clinical evaluation of a GP5+/6+-based Luminex assay having full high-risk HPV genotyping capability and an internal control. J Clin Microbiol 2014;52: 3996–4002.
[42] Arbyn M, Roelens J, Cuschieri K, et al. The APTIMA HPV assay versus the Hybrid Capture II test in triage of women with ASC-US or LSIL cervical cytology: a meta-analysis of the diagnostic accuracy. Int J Cancer 2013;132:101–8.
[43] Reid JL, Wright Jr TC, Stoler MH, et al. HPV oncogenic mRNA testing for cervical cancer screening; baseline and longitudinal 2 results from the CLEAR Study 3. Am J Clin Pathol 2015. in-press.
[44] Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol 2015;136:189–97.
[45] Poljak M, Ostrbenk A, Seme K, Jancar N, Vrtacnik-Bokal E. 3-year longitudinal data of the clinical performance of Abbott RealTime High Risk HPV test in the cervical cancer screening settings—interim results. Paper presented at: 29th International Papillomavirus Conference and Clinical Workshop; Seattle, WA, USA. 2014.
[46] Arbyn M, Verdoodt F, Snijders PJF, et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol 2014;15:172–83.
[47] Arbyn M, Castle P. Offering self-sampling kits for HPV testing to reach women who do not attend in the regular cervical cancer screening program. Cancer Epidemiol Biomarkers Prev 2015;24:769–72.
[48] Dillner J. Primary human papillomavirus testing in organized cervical screening. Curr Opin Obstet Gynecol 2013;25:11–6.
[49] Tjalma WA, Depuydt CE. Cervical atypical glandular cells and false negative HPV testing: a dramatic reality of the wrong test at the right place. Eur J Gynaecol Oncol 2014;35:117–20.
[50] Arbyn M, Tommasino M, Depuydt C, Dillner J. Are twenty human papillomavirus types causing cervical cancer? J Pathol 2014;234: 431–5.
[51] Hu Z, Zhu D, Wang W, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. Nat Genet 2015;47:158–63.
[52] Rodriguez-Carunchio L, Soveral I, Steenbergen RD, et al. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG 2015;122:119–27.
[53] Arbyn M, Van Veen EB, Andersson K, et al. Cervical cytology biobanking in Europe. Int J Biol Markers 2010;25:117–25.
[54] Schmitt M, Depuydt CE, Benoy I, et al. Viral load of high-risk human papillomaviruses as reliable clinical predictor for the presence of cervical lesions. Cancer Epidemiol Biomarkers Prev 2013;22: 406–14.
[55] Hovland S, Arbyn M, Lie AK, et al. A comprehensive evaluation of the accuracy of cervical pre-cancer detection methods in a high-risk area in East Congo. Br J Cancer 2010;102:957–65.