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Systematic review

2020 list of human papillomavirus assays suitable for primary cervical cancer screening

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ABSTRACT

Background: Only clinically validated HPV assays can be accepted in cervical cancer screening.
Objectives: To update the list of high-risk HPV assays that fulfil the 2009 international validation criteria (Meijer-2009).
Data Sources: PubMed/Medline, Embase, Scopus, references from selected studies; published in January 2014 to August 2020.
Study eligibility criteria: HPV test validation studies and primary screening studies, involving testing with an index HPV test and a comparator HPV test with reporting of disease outcome (occurrence of histologically confirmed cervical precancer; CIN2+).
Participants: Women participating in cervical cancer screening.
Interventions: Testing with an index and a comparator HPV test of clinician-collected cervical specimens and assessment of disease outcome (<CIN2, CIN2+). Comparator HPV assays were HC2, GP5+/6+ PCR-EIA, recommended in validation guidelines, or tests with consistent previous validations.
Methods: Assessment of relative clinical accuracy (including non-inferiority statistics index vs comparator assay) and test reproducibility in individual studies; random effects meta-analyses of the relative clinical sensitivity and specificity of index vs comparator tests.

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Results: Seven hrHPV DNA tests consistently fulfilled all validation criteria in multiple studies using predefined test positivity cut-offs (Abbott RealTime High Risk HPV, Anyplex II HPV HR Detection, BD Onclarity HPV Assay, Cobas 4800 HPV Test, HPV-Risk Assay, PapilloCheck HPV-Screening Test and Xpert HPV). Another assay (Alinity m HR HPV Assay) was fully validated in one validation study. The newer Cobas 6800 HPV Test, was validated in two studies against Cobas 4800. Other tests partially fulfilled the international validation criteria (Cervista HPV HR Test, EUROArray HPV, Hybribio's 14 High-Risk HPV, LMNX Genotyping Kit GP HPV, MALDI-TOF, RIATOL qPCR and a number of other in-house developed assays) since the non-inferior accuracy was reached after a posteriori cut-off optimization, inconsistent accuracy findings in different studies, and/or insufficient reproducibility assessment. The APTIMA HPV Assay targeting *E6/E7* mRNA of hrHPV was fully validated in one formal validation study and showed slightly lower pooled sensitivity but higher specificity than the standard comparator tests in seven screening studies. However, the current international validation criteria relate to DNA assays. The additional requirement for longitudinal performance data required for non-DNA based HPV assays was not assessed in this review.

Conclusions: Eleven hrHPV DNA assays fulfil all requirements for use in cervical cancer screening using clinician-collected specimens. **Marc Arbyn, Clin Microbiol Infect 2021;27:1083**

Introduction

The main purpose of cervical cancer screening is to detect precursor lesions that can be easily treated to avoid progression to invasive cancer. Strong evidence supports that screening using assays that detect nucleic acids of oncogenic or high-risk human papillomavirus (hrHPV) types are more effective, in terms of reducing the incidence and mortality from this cancer, than screening with cytology [1–3]. Randomized trials conducted in India and South Africa showed that HPV-based primary screening also protects more effectively against future pre-cancer and cancer than visual inspection of the cervix after application of acetic acid [3,4]. Therefore, an increasing number of countries have switched from cytology to HPV-based screening or have decided to implement this change in the near future [5–10].

A recent review revealed that in 2020 at least 254 distinct HPV assays and 425 assay variants are available on the global market [11]. The large majority of them lack any analytical or clinical evaluation published in the peer-reviewed literature and more than 90% have not undergone regulatory evaluation or have not been evaluated following a stringent clinical validation protocol [11].

In our previous systematic review, published in 2015, a list was made of hrHPV DNA assays that fulfil the validation criteria for use in cervical cancer screening [12]. These criteria were defined by an international team of experts in 2009 and are based on cross-sectional evaluation of the relative clinical accuracy of a given HPV assay compared with Hybrid Capture 2 HPV DNA Test (HC2) or GP5+/6+ PCR-EIA and inter- and intra-laboratory reproducibility [13]. In the current paper we update this list and propose elements to improve the current validation guidelines.

Materials and methods

Study question and objective of the systematic review

This systematic review updates our 2015 review and aims to answer the question which HPV tests can be considered as clinically validated for use in primary cervical cancer screening in 2020. The PICOS (Population-Intervention-Comparator-Outcome-Study) components of the study question and the terminology used to describe tests and disease outcomes can be found elsewhere (please see chapters 1 and 2 of the supporting information).

We report a list of studies that assessed the international validation criteria for HPV assays usable in cervical cancer screening and, in addition, we conducted a meta-analysis of the relative accuracy of index HPV tests versus comparator HPV tests to detect underlying pre-cancer.

Literature retrieval

A new literature search for relevant references was set up to complete the list of validated HPV assays that can be used for cervical cancer screening was published in *Clinical Microbiology and Infection* in 2015 (CMI2015) [12]. The new search targeted reports published since January 1, 2014 allowing for some overlap with CMI2015 and was run for the last time on 30 July 2020. The search string used to retrieve references from PubMed (National Library of Medicine) is shown in Textbox of the supporting information. A search was conducted also in Scopus (www.scopus.com) to identify reports that cited the guideline containing the equivalency criteria of Meijer et al. [13] and the CMI2015 report. We also used the Sciensano *cervix1* bibliographic database used for previous systematic reviews and development of guidelines on secondary prevention of cervical cancer prevention [14,15]. Two co-authors independently identified eligible studies (E.P. and L.X.), extracted data and assessed study design and quality. In case of non-resolvable discordance, the first author (M.A.) was consulted. E.P. was not involved in any of the included studies.

Table 1 contains the full names of the HPV tests assessed for cross-sectional clinical performance according to the international validation criteria for cervical cancer screening, whereas in the text we used test's abbreviations only. The evaluated assays and their manufacturers are listed in Table S2.

Criteria for study selection and quality judgement

An obligatory inclusion criterion for all studies was testing of all study participants with an index HPV test and a comparator HPV test. Three types of study design were considered: validation studies following the Meijer [13] or VALGENT [16] protocols, or cross-sectional screening studies addressing clinical accuracy (see Table S1 and chapter 7 of the supporting information for more details of the considered study designs). Screening studies were selected when the accuracy for cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) or CIN3+ of an index HPV DNA or RNA

Table 1
Characteristics of hrHPV tests assessed for cross-sectional clinical performance according to the international validation criteria cervical cancer screening

	hrHPV assay	Nucleic acid targeted	Type of amplification	Genes targeted	Separate genotyping	Internal control for human genes
Standard comparator HPV tests						
	*Hybrid capture 2 HPV DNA test (HC2)	DNA	Signal	Several (undefined)	No (13 hr types in aggregate)	No
	*GP5+/6+ PCR-EIA	DNA	Target	*L1*	No (14 hr types in aggregate)	No
Evaluated index HPV assays						
1	Abbott RealTime High Risk HPV Test [33–35]	DNA	Target (PCR)	*L1*	16,18 and 12 other hr types	*β-globin*
2	Alinity m HR HPV Assay [52]	DNA	Target (PCR)	*L1/E6/E7*	16,18, 45 and 2 aggregates: (A: 31, 33, 52, 58); (B: 35, 39, 51, 56, 59, 66, 68)	*β-globin*
3	AmpFire HPV Screening 16/18/HR [55]	DNA	Target (isothermal) amplification, with real time fluorescence detection	*L1/E6/E7*	Restricted genotyping assay: 16,18 and bulk of 13 hr HPV types (including HPV53) Full genotyping assay identifying all 15 hrHPV types	*β-globin*
4	Anyplex II HPV HR Detection [99]	DNA	Target (RT PCR)	*L1*	Separate identification of 14 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)**	*β-globin*
5	APTIMA HPV Assay [53]	RNA	Target (PCR)	*E6/E7*	No (14 hr types in bulk). Separate typing of 16, 18:45 available as a separate reflex test	No. Assay includes internal controls for non-infectious RNA and DNA
6	BD Onclarity HPV Assay [38]	DNA	Target (PCR)	*E6/E7*	16,18,31,45,51,52; 33/58; 56/59/66; 35/39/68	*β-globin*
7	*care*HPV Test [100]	DNA	Target (PCR)	Several (undefined)	No (14 hr types in aggregate)	No
8	Cervista HPV HR Test [58,59]	DNA	Target (PCR)	*L1/E6/E7*	14 hr types in aggregate. Separate typing of 16, 18 available as a separate reflex test	human *histone 2*
9	CLART HPV4s [56]	DNA	Target (PCR	*L1*	Individual detection of: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	*CFTR*
10	Cobas 4800 HPV Test [42,43]	DNA	Target (PCR)	*L1*	16,18 and 12 other hr types (see HC2 plus 66)	*β-globin*
11	Cobas 6800 HPV Test [54]	DNA	Target (PCR)	*L1*	16,18 and 12 other hr types (see HC2 plus 66)	*β-globin*
12	EUROArray [57]	DNA	Target (PCR)	*E6/E7*	Separate identification of 30 types: 14 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68); 4phr types (26, 53, 73, 82); 12 lr or ur types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89)	*Hsp70*
13	HPV-Risk Assay [46]	DNA	Target (PCR)	*E7*	16, 18 and 13 other hr types (see HC2 plus 66 and 67)	*β-globin*
14	Hybribio 14 High-Risk HPV with 16/18 Genotyping Real-Time PCR Kit [63]	DNA	Target (PCR)	*E region*	16,18 and 12 other hr types (see HC2 plus 66)	*β-globin*
15	INNO-LiPA HPV Genotyping Extra II Assay [64]	DNA	Target (PCR)	*L1*	Separate identification of 32 different HPV types: 13 hrHPV (16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59, 68), 6 phr types (26, 53, 66, 70, 73, 82), 9 lr types (6, 11, 40, 42, 43, 44, 54, 61, 81) plus 4 other types (62, 67, 83, 89)	*HLA-DPB1*
16	Linear Array HPV Genotyping Test [62]	DNA	Target (PCR)	*L1*	Separate typing of 37 types: 14 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68); 4 phr types (26, 53, 73, 82; 10) lr types (6, 11, 40, 42, 43,44, 54, 61, 81), CP6108; and 9 types with undetermined risk (55, 62, 64, 67, 69, 71, 83, 84, IS39)	*β-globin*
17	LMNX Genotyping Kit GP HPV [66]	DNA	Target (PCR)	*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
18	MALDI-TOF [65]	DNA	Target (PCR)	*L1*	Separate typing of hr HPV types 16,18, 31,33,35,39,45,51,52,56,58,59,66,68	*β-globin*
19	OncoTect HPV E6, E7 mRNA Kit [69]	RNA	flow cytometry	*E6/E7*	No	human ectocervical cells for negative control cells HeLa cells for positive control cells
20	PapilloCheck HPV-Screening Test [49]	DNA	Target (PCR)	*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*
21	Pretect HPV-Proofer [68]	RNA	Target (NASBA)	*E7/E7*	Yes (16,18,31,33, 45)	U1 small nuclear ribonucleo-protein-specific mRNA.
22	RIATOL qPCR [60]	DNA	Target (RT PCR)	*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 and 11	*β-globin*
23	SeqHPV [55]	DNA	Targeted NGS	*L1*	Separate typing of hrHPV types 16,18,31,33, 35,39,45,51,52,56,58,59,66,68	*β-globin*
24	Xpert HPV [101]	DNA	Target (RT PCR)	*E6/E7*	Separate identification of HPV16, and four groups of types (HPV18/45, HPV31/33/52/58, HPV51/59, and HPV39/56/66/68)	*hydroxymethylbilane synthase (HMBS)*

ADAT1 gene, adenosine deaminase tRNA specific 1; HPV, human papillomavirus; hr, high-risk; lr, low-risk; phr, potentially high-risk; MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight; NGS, next generation sequencing; qPCR, quantitative PCR; ur, unknown risk; RT, real-time PCR.
**The complementary assay Anyplex II HPV28 enables separate identification of 28 HPV types: 14 high-risk types (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66 and 68), 6 possibly high-risk types (HPV26, 53, 69, 70, 73, and 82) and 8 low-risk types (6, 11, 40, 42, 43, 44, 54, 61). CT: cycle threshold.
* HC2 and GP5+/6+ PCR-EIA are clinically validated in randomized efficacy trials and therefore used as standard comparator HPV tests to validate other HPV assays.

assay detecting hrHPV types was compared with HC2 or GP5+/6+ PCR-EIA in cervical samples taken by a clinician from women attending for cervical cancer screening. Women with at least one positive screening test (including the index and comparator HPV tests, or cytology or visual inspection) had to be verified with the reference standard (colposcopy and histology). Women being negative for all screening tests cases or who consistently had negative results at subsequent screening rounds were considered as truly negative accepting the assumption that the probability of missing cervical precancer was very low [2,17].

We used the QUADAS-2 check list [18] to evaluate quality and design of the accuracy assessment of included screening studies [18] but not for the test validation studies where enrolment of subjects, testing, verification with the reference standard and patient flow were fixed by design.

Listing of studies allowing verification of the validation criteria

According to Meijer guidelines, a candidate HPV test should demonstrate non-inferior sensitivity and specificity to detect CIN2+ compared with a defined comparator tests [13]. HC2 and G5+/6+ PCR EIA are accepted as standard comparator HPV tests since randomized trials have demonstrated that screening using one of these tests provides superior protection against cervical cancer compared to good quality cytology for at least 5 years [1,2]. Since G5+/6+ PCR-EIA is not used and HC2 is less commonly used in current screening practice, we considered the Cobas 4800 as an alternative comparator HPV assay. Cobas 4800 has been consistently validated against the standard comparator HPV tests in multiple studies [12,19,20] and has been evaluated in a regulatory trial against cytology that demonstrated a strong reduction in the longitudinal risk of CIN3+ among Cobas 4800 negative women [21,22]. A representative set of consecutively or randomly collected samples should be evaluated (minimally 60 CIN2+ cases, 800 ≤CIN1 cases) derived from a population-based screening cohort of women aged 30–60 years [13]. For non-inferiority, the p value for a one-sided non-inferiority score test should be lower than 0.05 [23]. The agreed margins for the relative sensitivity and relative specificity (index/standard comparator HPV test) are 0.90 and 0.98, respectively [23]. In addition, the index HPV DNA test should demonstrate high intra- and inter-reproducibility (lower confidence bound ≥87% and kappa ≥0.50). For each retrieved validation study fulfilment of these criteria was checked.

While these cross-sectional validation criteria were developed for HPV DNA assays, we applied them here also to tests that detect hrHPV mRNA. However, longitudinal data are important to fully evaluate hrHPV mRNA tests.

Meta-analysis of the relative accuracy of index vs. comparator HPV tests

A meta-analysis of the relative sensitivity and specificity of the index HPV assay versus comparator HPV tests was conducted using a random-effects model for pooling ratios of proportions [24,25]. Forest plots, with subgroups at the level of individual tests were drawn. Wald-based 90% confidence intervals around the pooled relative accuracy measures were computed which show a statistical coverage that approximates the one-sided non-inferiority testing at $p < 0.05$ [26]. A left 90% CI bound around the relative accuracy exceeding 0.90 (margin for sensitivity) or 0.98 (margin for specificity) confirms non-inferiority.

Although criteria have been defined only for the CIN2+ outcome, we have assessed the relative accuracy also for CIN3+.

Results

Selected studies

From the searched databases a total of 2992 references published since 2014 were retrieved from which 32 relevant reports were selected (see PRISMA flow chart in Fig. S1). Addition of 24 references included in our previous review [12], removal of five references present in both the new search and in the previous review and addition of a recent reference from the *cervix1* database, yielded 52 papers included in our review (see list in Table S2).

Evaluated tests, study characteristics

Fifteen studies used the Meijer protocol for validation, thirteen applied the VALGENT protocol whereas the 24 reports contained data from screening studies, among which five applied a stratified sampling design with series of CIN2+ cases and series of ≤CIN1 controls allowing assessment of the Meijer non-inferiority accuracy criteria (see Table S2). Four screening studies included a mixed screening and follow-up population. The standard comparator HPV assays used were HC2 (34 reports) and GP5+/6+ PCR-EIA (12 reports). In five reports, Cobas 4800 and in one other report the Swedish modified GP5+/6+based LMNX [27] (partially validated) were used as comparator HPV test. Forty-two reports evaluated only HPV DNA assays and ten studies evaluated HPV mRNA assays and one of these ten involved testing with hrHPV DNA and mRNA assays.

HC2 targets 13 hrHPV types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) but show cross-reactivity with some non-targeted types [28,29], whereas all the others target at least 14 hrHPV types (the same types as HC2 plus HPV66) with the exception of Pretect HPV Proofer which detects only five hrHPV types (HPV16/18/31/33/45).

The standard comparator HPV assays (HC2, GP5+/6+ PCR-EIA) as well as four evaluated index tests (APTIMA, careHPV, Cervista and OncoTect) identify a pool of targeted high-risk HPV types without individual genotyping data. Eleven assays include limited genotyping by individual detection of HPV16 and HPV18 or individual detection of HPV16 and joint detection of HPV18 and HPV45 with aggregate detection of the other hrHPV genotypes (Abbott RealTime, AmpFire, Cobas 4800, Cobas 6800, DH3 [30], HBRT-H14, HPV-Risk, REALQUALITY RQ-HPV Screen [31], Xpert HPV). Three assays provide extended genotyping (Alinity, BD Onclarity and HPVIR [32]), whereas ten other assays (CLART, EUROArray, GP5+/6+-LMNX, INNO-LiPA, Linear Array, MALDI-TOF, PapilloCheck, Pretect HPV-Proofer, RIATOL qPCR, SeqHPV) provide individual genotyping of all targeted hrHPV types. The most widely evaluated hrHPV mRNA test was APTIMA (8 studies), whereas the other mRNA assays were assessed in only one (OncoTect) or two (PreTect Proofer) studies. Most often, cervical specimens were stored in cytology conserving media: PreservCyt (Hologic) in 38, SurePath (BD) in four studies or another liquid-based cytology in one study. Nucleic acid conserving media were more rarely used ($n = 5$), whereas in three studies multiple media (cell and nucleic acid conserving). A detailed overview of test characteristics can be found in Table 1 and Table S3. Table S6 displays the quality judgements for each QUADAS item in all the 18 included screening

studies. In 17% of the studies patient enrolment was not completely representative for a screening population, in 22% disease outcome was not blinded to the test results, 22% of the studies suffered from potential partial verification bias and in 11% uninterpretable test results and disease outcomes were not presented (see methodological quality graph in Fig. S2). None of the 18 screening studies received a good quality judgement for all QUADAS items. Five screening studies can be considered of good quality (12 of the 13 QUADAS items judged as OK; green in the QUADAS table), whereas at the other side of the quality spectrum 3/18 studies received a bad quality judgement for more than one QUADAS item. The other ten studies may be categorized as medium quality.

Test performance assessed per study

The validation of the accuracy criteria was addressed for 17 index assays (Table 2) whereas the reproducibility was evaluated for 14 index assays (Table 3). The characteristics of the studies applying

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

Evaluated index HPV assay	Study	Index assay		Comparator assay			Index/comparator assay		Non-inferiority test[a]		Validation level[c]
		Absolute		Comparator HPV assay	Absolute		Relative				
		Sensitivity	Specificity		Sensitivity	Specificity	Sensitivity	Specificity	p_{sens}	p_{spec}	
Standard comparator HPV tests											
GP5+/6+ EIA	Meijer, 2009 [13]	98.7%	96.0%	HC2	98.7%	94.1%	1.00	1.02	0.0037	<0.0001	⊕⊕⊕
Evaluated index HPV tests											
PapilloCheck	Hesselink, 2010 [49]	95.8%	96.7%	GP5+/6+ EIA	96.4%	97.7%	0.99	0.99	<0.0001	0.0072	⊕⊕⊕
	Heard, 2016 [50]	96.1%	89.7%	GP5+/6+ EIA	94.1%	90.4%	1.02	0.99	0.0002	0.0970	
Abbott RT	Carozzi, 2011 [33]	96.4%	92.3%	HC2	97.6%	92.6%	0.99	1.00	0.0040	0.0087	
hrHPV test	Poljak, 2011 [34]	100.0%	93.3%	HC2	97.4%	91.8%	1.03	1.02	0.0112	0.0000	⊕⊕⊕
	Hesselink, 2013 [35]	95.6%	92.0%	GP5+/6+ EIA	98.5%	91.8%	0.97	1.00	0.0278	0.0003	
Cobas 4800	Heideman, 2011 [42]	90.0%	94.6%	HC2	91.7%	94.4%	0.98	1.00	0.0216	0.0009	⊕⊕⊕
	Lloveras, 2013 [43]	98.3%	86.2%	HC2	98.3%	85.3%	1.00	1.01	0.0093	0.0012	
	Ejegod, 2020 [20]	92.6%	91.2%	GP5+/6+ EIA	92.6%	89.2%	1.00	1.02	0.0006	<0.0001	
RIATOL	Depuydt, 2012 [60]	93.5%	95.6%	HC2	83.9%	94.4%	1.11	1.01	0.0001	<0.0001	⊕⊕
qPCR	Benoy, 2019 [61]	96.0%	89.5%	GP5+/6+ EIA	96.0%	89.7%	1.00	1.00	0.0006	0.0069	
APTIMA	Heideman, 2013 [53]	95.5%	94.5%	GP5+/6+ EIA	100.0%	93.6%	0.96	1.01	0.0394	0.0002	X
Cervista	Boers, 2014 [58]	89.0%	91.2%	HC2	93.4%	88.8%	0.95	1.03	0.0043	<0.0001	⊕
	Alameda, 2015 [59]	98.4%	85.2%	HC2	100.0%	86.4%	0.98	0.99	0.0122	0.3170[b]	
BD Onclarity	Ejegod, 2014 [38]	92.9%	87.7%	HC2	94.2%	88.8%	0.99	0.99	0.0009	0.0216	
	Cuschieri, 2015 [39]	96.7%	89.6%	HC2	98.4%	89.9%	0.98	1.00	0.0245	0.0155	⊕⊕⊕
	Ejegod, 2016 [40]	96.1%	89.7%	GP5+/6+ PCR	94.1%	90.4%	1.02	0.99	0.0002	0.0970	
	Bonde, 2019 [41]	92.6%	92.6%	GP5+/6+ EIA	92.6%	89.6%	1.00	1.04	<0.0001	<0.0001	
HPV-Risk assay	Hesselink, 2014 [46]	97.1%	94.3%	GP5+/6+ EIA	97.1%	94.1%	1.00	1.00	0.0056	0.0003	
	Polman, 2017 [47]	93.7%	91.8%	HC2	96.1%	89.9%	0.98	1.02	<0.001	<0.001	⊕⊕⊕
	Heideman, 2019 [48]	93.4%	92.6%	GP5+/6+ EIA	92.6%	89.%	1.01	0.99	0.0006	<0.0001	
Anyplex II HPV HR	Hesselink, 2016 [36]	98.3%	93.6%	GP5+/6+ PCR	98.3%	94.1%	1.00	0.99	0.0052	0.0232	
HPV HR	Jung, 2016 [37]	92.5%	81.7%	HC2	87.5%	81.8%	1.06	1.00	0.0067	0.0354	⊕⊕⊕
	Ostrbenk, 2018 [19]	96.9%	94.1%	HC2	95.9%	92.7%	1.01	1.01	0.001	<0.0001	
Xpert HPV	Cuschieri, 2016 [102]	94.1%	90.3%	GP5+/6+ PCR	94.1%	90.3%	1.00	1.00	0.0171	0.0269	⊕⊕
INNO-LiPA	Xu, 2018 [64]	96.9%	87.9%	HC2	95.9%	97.0%	1.01	0.95	0.0002	0.9998	⊖
Linear Array[e]	Xu, 2018 [62]	98.0%	94.3%	HC2	95.9%	92.7%	1.02	1.02	0.0076	<0.0001	⊕⊕
EUROArray	Viti, 2018 [57]	93.7%	89.9%	HC2	96.1%	90.1%	0.98	1.00	0.0076	0.0070	⊕[d]
Cobas 6800	Saville, 2019 [54]	98.3%	88.4%	Cobas 4800	100.0%	89.4%	0.98	0.98	0.0157	.0442	⊕⊕⊕
	Frayle, 2019 [45]	98.3%	92.1%	Cobas 4800	100.0%	92.8%	0.98	0.99	0.0157	0.0056	
Alinity	Ostrbenk, 2020 [52]	100%	92.4%	HC2	95.6%	91.9%	1.05	1.01	0.0006	<0.0001	⊕⊕
HBRT-H14	Xu, 2020 [63]	93.9%	93.7%	GP5+/6+ PCR	95.9%	92.9%	0.98	1.01	0.0159	<0.0001	⊕[d]
CLART	Ejegod, 2020 [56]	92.6%	88.8%	mod GP5+/6+	90.1%	89.1%	1.03	1.00	0.0021	0.0083	⊕
		97.5%	93.8%	PCR LMNX	100%	86.7%	0.98	1.08	0.0127	<0.0001	

Table 2 is ranked according the year of the first publication.

[a] p values for non-inferiority of the evaluated index assay compared with the comparator HPV assay.

[b] We corrected an error in Alameda, 2015 [59], which was due to switching of + and − columns and rows. The corrected data showed that the non-inferiority test was not significant for specificity.

[c] Validation level for the test accuracy criterion as proposed by Meijer et al. 2009 [13]. ⊕⊕⊕⊕ validated in large randomized controlled trials with cancer incidence as an outcome; considered as standard comparator HPV tests; ⊕⊕⊕: fully validated in multiple studies; ⊕⊕ fully validated in one study; ⊕ partially validated. X not evaluated since not a hrHPV DNA assay.

[d] Validated only after a posteriori cut-off optimization.

[e] Commercially not available anymore from 01 January 2020.

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

Evaluated Assay	Study	Intra-laboratory			Inter-laboratory			Validation level[a]
		Reproducibility (hrHPV)			Reproducibility (hrHPV)			
		Overall	LCIB	Kappa	Overall	LCIB	Kappa	
PapilloCheck[b]	Hesselink, 2010 [49]	97.6%	96.3%	0.941	94.0%	92.1%	0.842	⊕⊕
Abbott RealTime	Carozzi, 2011 [33]	98.5%	97.3%	0.969	—	—	—	⊕⊕
	Poljak, 2011 [34]	100.0%	99.5%	1.000	100.0%	99.5%	1.000	
	Hesselink, 2013 [35]	99.8%	99.1%	0.996	98.4%	97.2%	0.965	
Alinity	Ostrbenk, 2020 [52]	96.7%	94.8%	0.92	98.7%	97.3%	0.97	⊕⊕
Cobas 4800	Heideman, 2011 [42]	98.3%	97.2%	0.963	94.6%	92.8%	0.882	⊕⊕
	Lloveras, 2013 [43]	98.3%	97.2%	0.963	98.4%	97.2%	0.962	
Cobas 6800	Saville, 2019 [54]	98.4%	97.2%	0.962	98.2%	96.9%	0.957	⊕⊕
	Frayle, 2019 [45]	99.0%	98.1%	0.976	99.1%	98.1%	0.978	
RIATOL qPCR	Depuydt, 2012 [60]	98.7%	97.8%	0.956	—	—	—	⊕
APTIMA	Heideman, 2013 [53]	96.0%	94.4%	0.893	95.1%	93.3%	0.865	⊕⊕
Cervista	Boers, 2014 [58]	91.9%	89.7%	0.829	90.7%	88.4%	0.807	⊕⊕
	Alameda, 2015 [59]	94.9%	93.0%	0.890	96.5%	94.9%	0.907	
BD Onclarity	Ejegod, 2014[c] [38]	98.6%	97.5%	0.967	98.4%	97.2%	0.962	⊕⊕
	Ejegod 2016[d] [40]	97.4%	95.9%	0.935	96.8%	95.2%	0.921	
HPV-Risk	Hesselink, 2014 [46]	99.5%	97.3%	0.987	99.2%	97.2%	0.981	⊕⊕
Anyplex HR	Hesselink, 2016 [36]	96.0%	94.4%	0.909	96.8%	95.3%	0.927	⊕⊕
	Jung, 2016 [37]	98.0%	96.7%	0.980	97.4%	96.0%	0.974	
Xpert HPV	Cuschieri, 2016 [102]	96.9%	95.0%	0.924	97.8%	96.2%	0.948	⊕⊕
EUROArray	Viti, 2018 [57]	98.4%	96.9%	0.96	94.5%	92.1%	0.85	⊕⊕
CLART	Ejegod, 2020 [56]	95.0%	93.2%	0.87	89.4%	87.1%	0.69	⊕⊕

hrHPV, high-risk human papillomavirus; LCIB, lower 95% confidence interval bound; RT, real time; qPCR, quantitative polymerase chain reaction.
[a] Validation level for the reproducibility criterion: ⊕⊕ high intra- and inter-laboratory reproducibility confirmed; ⊕ high intra-laboratory reproducibility confirmed. Tests as defined by Meijer et al. 2009 [13].
[b] Data on intra- and inter-laboratory reproducibility of the PapilloCheck were retrieved from an online source (http://www.pathology.nl) [103].
[c] Validated on ThinPrep specimens.
[d] Evaluated on SurePath specimens.

the Meijer protocol are summarized in Table S4, whereas the characteristics of the VALGENT studies are described in chapter 7 of the supporting information.

For eight assays (Abbott RealTime [33–35], Anyplex HR [19,36,37], BD Onclarity [38–41], Cobas 4800 [20,42,43], Cobas 6800 [44,45], HPV-Risk [46–48], PapilloCheck [49,50] and Xpert HPV [51, 102]) non-inferior sensitivity and specificity for CIN2+ was demonstrated consistently in at least two studies, using the manufacturer defined test-cut-off and in addition sufficiently high intra- and inter reproducibility was documented. Alinity [52] and APTIMA [53] also fulfilled all criteria each in a single validation study, however the latter was evaluated also in multiple screening studies (see below). In all studies, the relative sensitivity and specificity was evaluated using one of the two standard comparator HPV tests, with exception of Cobas 6800, AmpFire and SeqHPV assays, which were compared with the validated Cobas 4800 [45,54,55], and CLART, which was compared with the non-validated modified GP5+/6+ LMNX [56].

EUROArray initially did not reach sufficient sensitivity but subsequently fulfilled all the three validation criteria after cut-off optimization [57]. Cervista showed non-inferior sensitivity and reproducibility [58,59]. However while specificity was non-inferior in one study [58] this was not demonstrated in the other study [59]. The in-house RIATOL qPCR test showed non-inferior sensitivity and specificity compared with HC2 and intra-laboratory reproducibility in one internal evaluation study [60] and non-inferior sensitivity and specificity in a VALGENT study [61]. However, inter-laboratory reproducibility has not been evaluated and the VALGENT study defined (by design) the test positivity cut-off a posteriori. Identification of 13 hrHPV types with the Linear array was neither less sensitive nor less specific than HC2 but reproducibility was not documented [62]. HBRT-H14 did fulfil the accuracy validation criteria after cut-off optimization but reproducibility was not assessed [63]. A biobank-based study nested in the CHIMUST screening study demonstrated non-inferior sensitivity and specificity of the AmpFire and SeqHPV compared with Cobas 4800, but no reproducibility information was reported [55].

Identification of 13 hrHPV types with INNO-LiPA was sufficiently sensitive but less-specific for CIN2+ than HC2 [64].

Meta-analysis

The meta-analysis of the relative accuracy of index versus comparator tests for CIN2+ included (besides the aforementioned validation studies) also the reports evaluating HPV tests in screening studies (see Figs. 1–3 and Table 4). Estimates of the relative accuracy for outcome CIN3+ are shown in Figs S3–S6 and Table S7.

Fig. 1 shows the pooled relative sensitivity and specificity for CIN2+ compared with an established comparator HPV test for the nine index hrHPV DNA assays that showed non-inferior accuracy and sufficient intra- and inter-reproducibility (Tables 2 and 3). The pooled relative sensitivity varied between 0.97 and 1.05, with the 90% LCIB always exceeding 0.90. The pooled relative specificity for < CIN2 varied from 1.00 to 1.02, with LCBI always ≥0.98. The pooled relative sensitivity estimated for outcome CIN3+ ranged between 0.98 and 1.02 with the lowest 90% LCIB being 0.93 (Fig. S3).

Fig. 2 displays the relative accuracy estimates of 10 hrHPV DNA assays that did not fulfil all validation criteria with at the bottom three in-house assays assessed internally (see Table S9, which indicates which criterion was not fulfilled). The pooled relative sensitivity and specificity for CIN2+ varied in the range 0.97–1.05 and 0.99–1.09, respectively with 90% LCIBs in the ranges 0.93–0.98 and 0.97–1.01, respectively.

The pooled relative sensitivity of APTIMA for CIN2+ was marginally below unity for CIN2+ (ratio: 0.97, 90% CI 0.949–0.995) and not significantly different from unity for CIN3+ (ratio 0.98, 90%

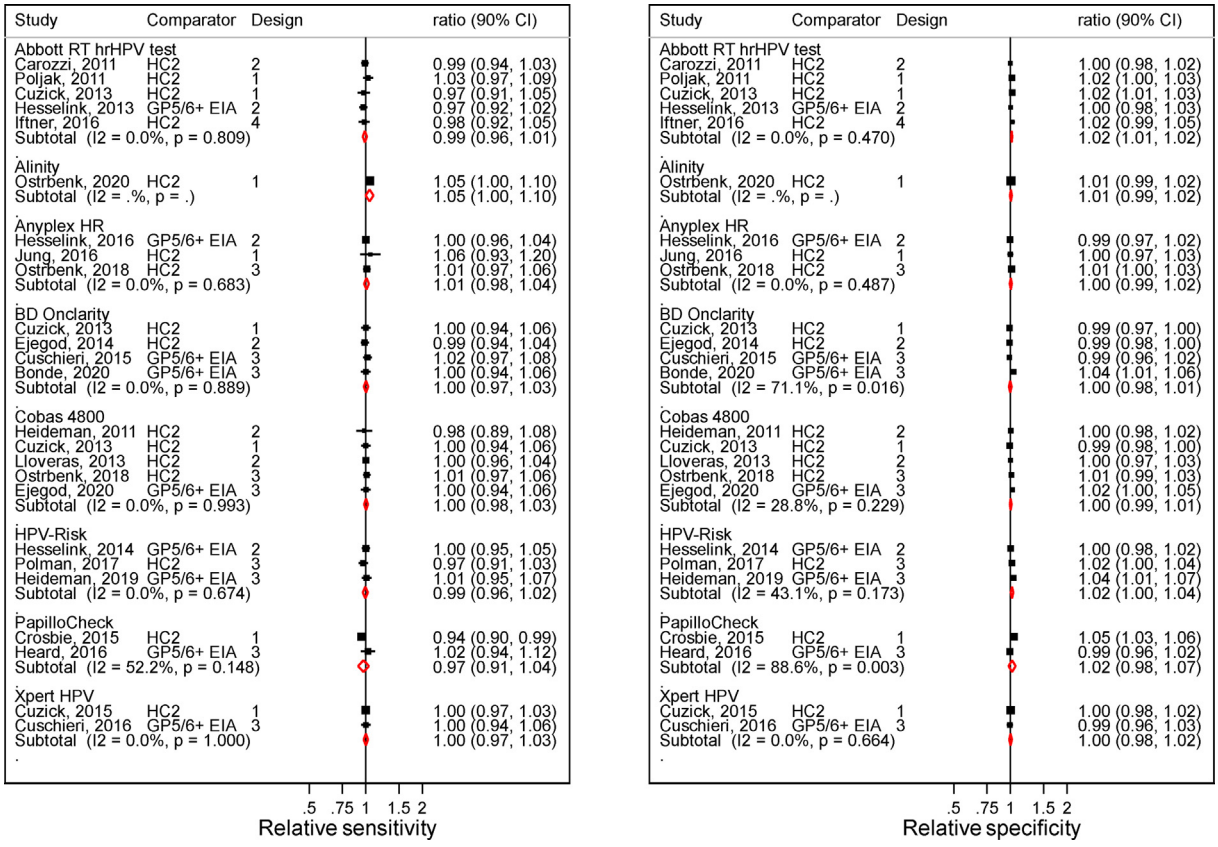

Fig. 1. Relative sensitivity (left) and specificity (right) of fully clinically validated high-risk human papillomavirus (hrHPV) DNA assays compared with established comparator HPV tests to detect CIN2+ in cervical cancer screening, by study design: 1 = cross-sectional screening study; 2 = according to the Meijer protocol; 3 = according to the VALGENT protocol; 4 = cross-sectional study mixed screening/clinical population.

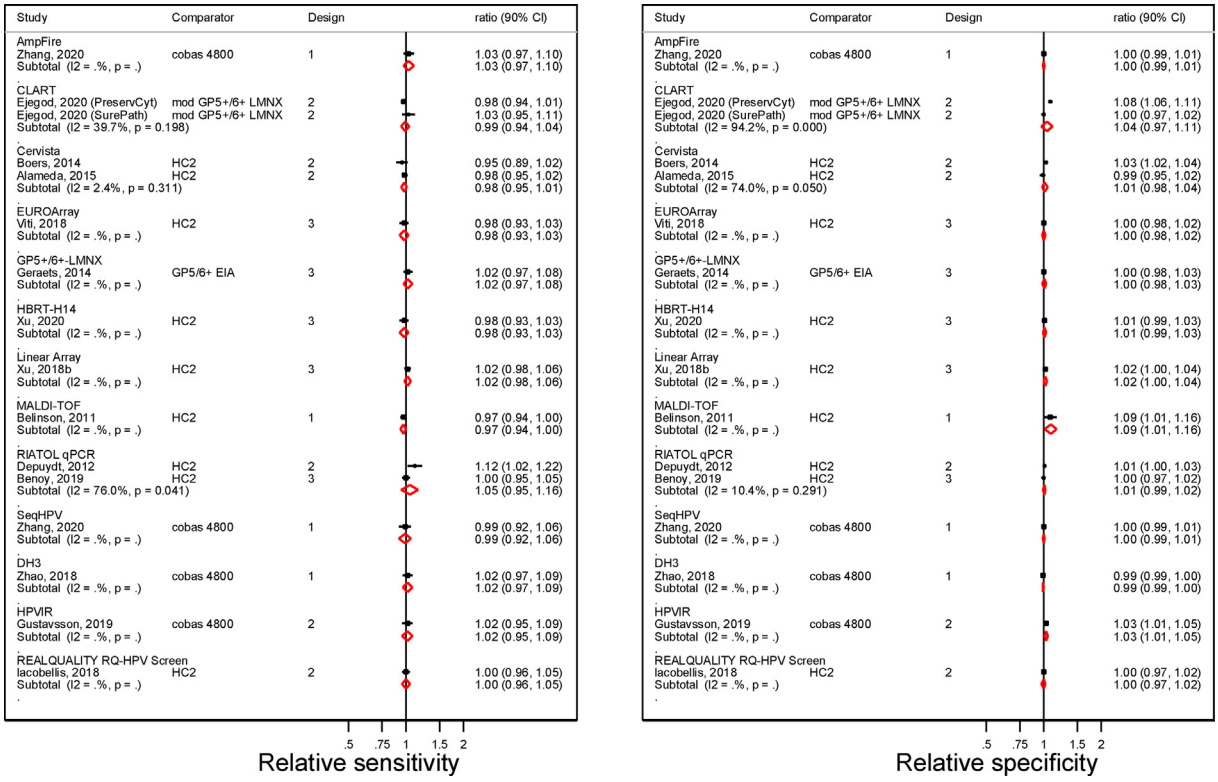

Fig. 2. Relative sensitivity (left) and specificity (right) of partially clinically validated or internally validated in-house high-risk human papillomavirus (hrHPV) DNA assays compared with comparator HPV tests to detect CIN2+ in cervical cancer screening by study design: 1 = cross-sectional screening study; 2 = according to the Meijer protocol; 3 = according to the VALGENT protocol; 4 = cross-sectional study mixed screening/clinical population.

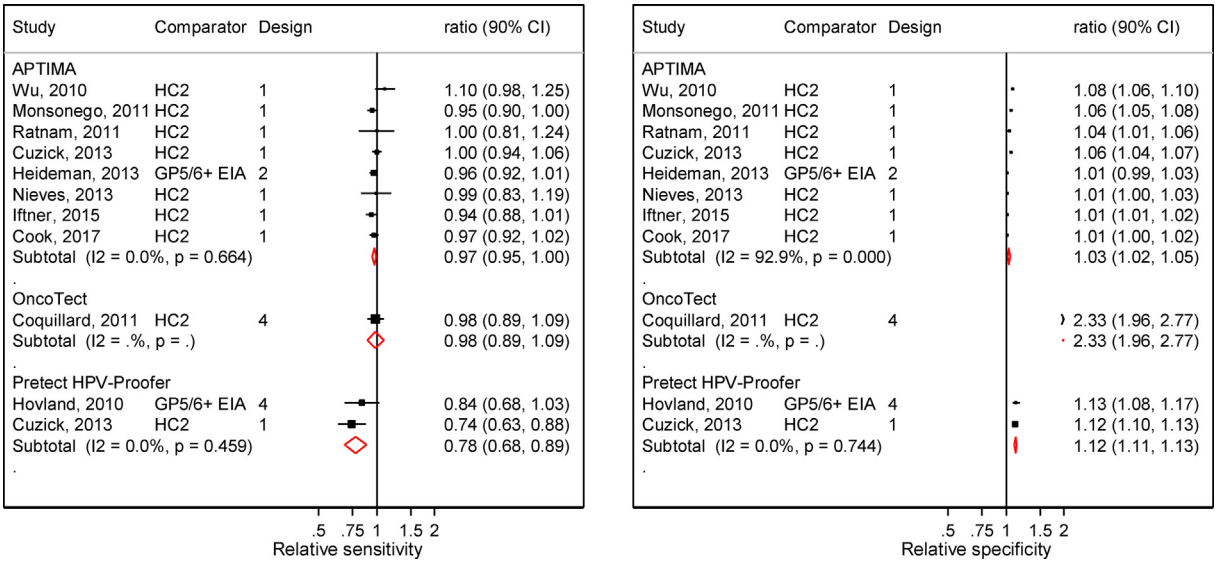

Fig. 3. Relative sensitivity (left) and specificity (right) of HPV mRNA assays compared with validated comparator hrHPV DNA tests to detect CIN2+ in cervical cancer screening, by study design: 1 = cross-sectional screening study; 2 = according to the Meijer protocol; 3 = according to the VALGENT protocol; 4 = cross-sectional study mixed screening/clinical population.

Table 4
Relative accuracy of other HPV tests compared with a standard HPV comparator to identify underlying CIN2+ in primary screening, separated by validation status

Comparison	Out-come	Relative sensitivity (90% CI)	Relative specificity (90% CI)	No. of studies
Fully validated hrHPV DNA tests				
Abbott RealTime/HC2 or GP5+/6+ EIA	CIN2+	0.99 (0.96–1.01)	1.02 (1.01–1.02)	5
Alinity/HC2	CIN2+	1.05 (0.99–1.10)	1.01 (0.99–1.02)	1
Anyplex HR/HC2 or GP5+/6+ EIA	CIN2+	1.01 (0.96–1.04)	1.00 (0.99–1.02)	3
BD Onclarity/HC2 or GP5+/6+ EIA	CIN2+	1.00 (0.97–1.03)	1.00 (0.98–1.01)	4
Cobas 4800/HC2 or GP5+/6+ EIA	CIN2+	1.00 (0.98–1.03)	1.00 (0.99–1.01)	5
Cobas 6800/Cobas 4800	CIN2+	0.98 (0.96–1.01)	0.99 (0.97–1.01)	2
HPV-Risk/HC2 or GP5+/6+ EIA	CIN2+	0.99 (0.96–1.02)	1.02 (1.00–1.04)	3
PapilloCheck/HC2 GP5+/6+ EIA	CIN2+	0.97 (0.91–1.04)	1.02 (0.98–1.07)	2
Xpert HPV/HC2 or GP5+/6+ EIA	CIN2+	1.00 (0.97–1.03)	1.00 (0.98–1.02)	2
Partially validated tests				
AmpFire/Cobas 4800	CIN2+	1.03 (0.97–1.10)	1.00 (0.99–1.01)	1
Cervista/HC2	CIN2+	0.98 (0.95–1.01)	1.01 (0.98–1.04)	2
CLART/mod GP5+/6+ LMNX (sp)	CIN2+	1.03 (0.95–1.11)	1.00 (0.97–1.02)	1
CLART/mod GP5+/6+ LMNX (pc)	CIN2+	0.98 (0.94–1.01)	1.08 (1.06–1.11)	1
EUROArray/HC2[a]	CIN2+	0.98 (0.93–1.03)	1.00 (0.98–1.03)	1
GP5+/6+-LMNX/GP5+/6+ EIA	CIN2+	1.02 (0.97–1.08)	1.00 (0.98–1.03)	1
HBRT-H14/HC2[a]	CIN2+	0.98 (0.93–1.03)	1.01 (0.99–1.03)	1
Linear Array/HC2	CIN2+	1.02 (0.98–1.06)	1.02 (1.00–1.04)	1
MALDI-TOF/HC2	CIN2+	0.97 (0.94–1.00)	1.09 (1.01–1.16)	1
RIATOL qPCR/HC2[b]	CIN2+	1.05 (0.95–1.16)	1.01 (0.99–1.02)	2
SeqHPV/Cobas 4800	CIN2+	0.99 (0.92–1.06)	1.00 (0.99–1.01)	1
Internally validated in-house hrHPV DNA tests				
DH3/HC2	CIN2+	1.02 (0.95–1.09)	1.03 (1.01–1.05)	1
HPVIR/Cobas 4800	CIN2+	1.02 (0.95–1.09)	1.03 (1.01–1.05)	1
REALQUALITY RQ-HPV Screen /HC2	CIN2+	1.00 (0.96–1.05)	1.00 (0.97–1.02)	
HPV RNA tests				
APTIMA/HC2 or GP5+/6+ EIA	CIN2+	0.97 (0.95–1.00)	1.03 (1.02–1.05)	8
Pretect HPV-Proofer/HC2	CIN2+	0.78 (0.68–0.89)	1.12 (1.11–1.13)	2
OncoTect/HC2	CIN2+	0.98 (0.89–1.09)	2.33 (1.96–2.77)	1
APTIMA/HC2 or GP5+/6+ EIA	CIN2+	0.97 (0.95–1.00)	1.03 (1.02–1.05)	8
hrHPV DNA tests not reaching validation criteria for cervical cancer screening				
*care*HPV Test/HC2[c]	CIN2+	0.86 (0.79–0.94)	1.01 (0.99–1.03)	2
INNO-LiPA/HC2[c]	CIN2+	1.01 (0.97–1.06)	0.95 (0.93–0.97)	1

HPV, human papillomavirus; EIA, enzyme immunoassay; CI, confidence interval; qPCR, quantitative PCR; MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight; sp, evaluation on SurePath samples; pc, evaluation on PreservCyt samples.

[a] Validation included cut-off optimization.

[b] The second study included cut-off optimization.

[c] *care*HPV showed relative sensitivity and INNO-LiPA lower specificity which were significantly lower than 1 compared with the standard comparator HPV tests.

CI 0.95–1.02) with significantly increased specificity for < CIN2 (ratio: 1.03, 90% CI 1.02–1.05) (see Figs. 3 and Fig. S5).

The *care*HPV Test and the Pretect HPV-Proofer showed pooled relative sensitivities for CIN2+ significantly below 1: *care*HPV Test/HC2 = 0.86 (90% CI 0.78–0.94); Pretect HPV-Proofer/HC2 = 0.78 (90% CI 0.68–0.89), see Figs. 3 (bottom) and Fig. S6). However, the specificity of the latter tests were similar or higher compared with the standard comparators. INNO-LiPA II Extra was not less sensitive for CIN2+ or CIN3+ but less specific than HC2 (relative specificity for CIN2+ of 0.95, 90% CI 0.93–0.97).

The relative sensitivity and specificity estimates did not differ by choice of the comparator assay (Table S8).

Discussion

HC2 and GP5+/6+ PCR-EIA have been validated through randomized trials and are therefore considered as standard comparator HPV tests in validation studies. In addition, seven other hrHPV DNA tests consistently show non-inferior accuracy compared with these two standard comparator tests as well as high intra- and inter-laboratory reproducibility as evidenced in multiple studies: Abbott RealTime, Anyplex HR, Cobas 4800, BD Onclarity, HPV-Risk, PapilloCheck and Xpert HPV. A recent study showed that these validation criteria were also fulfilled for the Alinity and two other reports demonstrated similar findings, for the Cobas 6800. However, for the latter assay, the Cobas 4800 was used as an alternative comparator HPV test.

A series of other hrHPV DNA assays fulfilled some but not all the Meijer validation conditions (see Table S9). Additional evaluation of the currently lacking reproducibility and/or a confirmatory study considering the new optimized cut-offs could add more assays to the list of fully validated tests. In-house assays which are not commercially available and which lack registration and approval by a regulatory body, cannot be added to the list of validated assays accepted for general use in screening beyond the laboratory performing the test internally. There would always be a concern about constant availability and stability of an assay over time when it is not a commercial quality-controlled kit.

The BD Onclarity and the Cobas 4800, 6800 and 8800 assays are approved by the US Food and Drug Administration for cervical cancer screening by HPV testing alone. FDA approved also HC2, Cervista and APTIMA but only in co-testing with cytology (Table S9) [67].

Assays targeting other molecules than viral DNA

APTIMA, which qualitatively detects E6 or E7 mRNA of 14 high-risk types, fulfilled the cross-sectional Meijer criteria for hrHPV DNA assays but additional longitudinal safety data are needed to evaluate the duration of reassurance provided by a negative test [12,13]. The cumulative incidence of CIN3+ after a negative mRNA test at screening should not be higher than after a negative clinically validated hrHPV DNA test over a period corresponding with the interval recommended in HPV-based screening programmes (usually 5 years). The evaluation of longitudinal performance of APTIMA is the object of an ongoing systematic review.

The Pretect HPV-Proofer, another mRNA assay that identifies the five most oncogenic HPV types, was significantly more specific (on average 12% higher) but was also significantly less sensitive (on average 22%) for detection of CIN2+ than the standard hrHPV DNA tests. These differences in accuracy can be fully explained by the number of HPV types targeted by Pretect HPV-Proofer [68]. OncoTect quantifies intracellular E6 or E7 transcripts using flow cytometry. One study compared OncoTect with HC2 within a mixed population although detail on the nature of the population was lacking. Reproducibility was confined only to intra-run correlation coefficient on 30 specimens) [69].

Longitudinal safety evidence is also needed for other molecular markers, not based on detection of hrHPV DNA, such as viral or host-methylation profiles or over-expression of certain viral or human proteins.

Alternative comparator HPV tests

At the time of the development of the Meijer validation criteria, HC2 and GP5+/6+ PCR were the main HPV tests used in secondary cervical cancer prevention or research. However, these tests do not provide genotyping, and other validated assays with limited, extended or full genotyping capacity have shown increased volumes and market share and are used in triage algorithms. Moreover, the requirement to use only the current standard comparators would increase the cost of future assay validation and consume specimens of precious validation panels. In this light, a substantial discussion as to the acceptance of new comparator HPV tests is needed. Table S8 showed complete absence of heterogeneity in relative accuracy by use of different comparator HPV tests. Alinity, validated against HC2, showed very similar relative accuracy when compared with Cobas 4800 or Abbott RealTime [52]. The latter test is used by WHO as their comparator in prequalifiation studies [70].

Other performance, logistical and economic factors

Besides clinical accuracy and reproducibility, addressed in validation studies or screening trials, the choice of a screening test is also determined by the underlying risk of disease [71] as well as several other characteristics, such as: availability of the assay, reagents and consumables (which might be particular challenging in period of the COVID-19 pandemic, see below); need for particular nucleic acid extraction procedures, through-put capacity and turnaround time (time span between arrival of the specimen and result communication); costs; applicability on self-collected vaginal samples or on urine; the requirement of equipped certified laboratories; operational ease; the possibility of point-of care testing and the capacity to provide intrinsic triage information allowing risk stratification (partial, extended or full genotyping, viral load/signal strength). A comprehensive overview of logistical, regulatory, managerial, training and quality control aspects of the choice of HPV assays, procurement, sample collection (device, preservation medium), transport (duration and conditions) of specimens to the laboratory, pre-analytical handling, testing and result communication has been synthesized in a recent WHO document [72].

Most of the HPV assays validated until now for screening require a well-equipped laboratory and well-trained personnel to perform the HPV tests. There are two hrHPV DNA assays, one using a hybrid capture technology and another cartridge-based, that are prequalified by the WHO for hrHPV testing in field conditions in low resource countries [70]. A promising less costly and rapid assay with applicability in medium-level laboratories is the AmpFire which uses isothermal LAMP amplification of 15 hrHPV types, with limited or full genotyping capacity, that does not require DNA extraction or particular storage media [55]. Point-of-care hrHPV testing is particularly relevant for screen and treat strategies, where detection for a limited number of the most carcinogenic types at low viral load cut-off and detection of the other types at higher viral load cut-off may offer a balance of slightly reduced sensitivity offset by reduced harms [73].

HPV negative cervical cancer, spectrum of HPV types, targeted viral genes

Occurrence of HPV-negative cervical cancers, although very rare [74,75], has been an object of concern with respect to screening with HPV testing alone, and is sometimes used as an argument of screening with co-testing (HPV and cytology) [76]. However, the very small difference in longitudinal cumulative incidence of CIN3+ and cancer after negative HPV versus after negative co-testing indicates minor gains for considerable extra costs and harms associated with co-testing [77–80]. Inclusion of additional, possibly carcinogenic HPV genotypes (IARC group II: types 26, 53, 67, 73, 87) in HPV screening tests would yield substantial losses in test specificity without tangible benefit in cancer protection [81,82].

Some virologists prefer HPV assays targeting the oncogenes *E6/E7* genes, since the *L1* gene may be interrupted due to viral integration [83]. However, there is little evidence from large scale studies that L1 disruption affects sensitivity. In our meta-analysis, the relative sensitivity for CIN2/3+ compared with the standard comparator HPV tests was very similar for PCRs targeting *L1* or *E6/7* (Figs S7 and S8). 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 of the performance of HPV tests in screening programmes [84].

Availability of HPV assays for screening, impact of the SARS CoV-2 pandemic

The dramatic outbreak of the COVID-19 pandemic, in January 2020, has triggered unprecedented demands on a global scale for sampling devices, reagents, consumables, test platforms and laboratory personal needed for the timely diagnosis of SARS-CoV-2. A recent survey of the American Society of Microbiology among CLIA-certified labs showed an average of 41% testing capacity devoted to COVID-19 and 71% of labs noted shortage of supplies for diagnosis of sexually transmitted infections, including HPV [85]. Nevertheless, COVID-19 may also create new opportunities for more efficient screening, including HPV testing on self-samples [86,87] and may generate the development of innovative tools such as rapid testing systems including low-cost portable instruments (for instance based on microfluidic devices, isothermal amplification or CRISPR-based technology) [88–90], which may be converted in the future to affordable screening assays for HPV, with applicability in low resource settings [91]. The validation of at least some of these new assays will be a major challenge in order to cover the world-wide need for good-quality tests required to respond to the WHO cervical cancer elimination goal [92]. The global test supply needed to reach 70% screening coverage aimed by WHO is estimated to be in the range of 1.4–1.5 billion HPV assays over a time span of five years (more precise estimates will be published soon, personal communication: O. Demke and N. Broutet, WHO, 2020).

Limitations of the review

Our review assesses only cross-sectional test performance for CIN2/3+ but not screening effectiveness. However, given the strong evidence of protection against future cancer by screening with two hrHPV DNA tests (HC2 and GP5+/6 + PCR EIA), non-inferior accuracy of other hrHPV DNA tests compared with these standard comparator HPV assays maybe considered sufficient for validation of other hrHPV DNA tests usable in screening [13]. By accepting this principle, long lasting expensive trials can be avoided. We did not evaluate the sensitivity for the outcome cancer. Since the fraction of cancers among the group of CIN2/3+ is small, demonstration of similar accuracy for precancer cannot preclude different performance for cancer detection. Nonetheless, we should not consider this necessarily as a flaw. Indeed, sensitivity of screening tests on *clinical* specimens collected at or shortly before the cancer diagnosis implies severe selection bias [93]. Cumulative incidence of cancer after negative screen tests over periods corresponding with recommended screening intervals (interval cancers) [94] and retesting of archived *screening* samples with multiple tests are pertinent quality control measures that may provide useful information on longitudinal performance of the HPV tests used in screening [94].

We could apply a formal quality judgement using the QUADAS framework only for the screening studies. Patient selection bias, partial verification and un-blinded interpretation of disease outcome were noted in several studies that may generate bias in absolute accuracy estimates. However, estimates of relative accuracy are more robust and less prone biases since balanced over the index and comparator HPV test.

Diverse QUADAS issues are fixed by design in the test validation studies. Nonetheless, the spectrum of a screening population is absent in studies following the Meijer protocol whereas in VALGENT, a screening population can be simulated by weighting the screening and enrichment populations. Since relative accuracy estimates did not differ by study design, we may conclude that the overall pooling across the three study types was justified.

Update and extension of HPV test validation guidelines

The international validation criteria [13] were pivotal in objective decision making regarding the choice of HPV tests allowed in screening programmes [15,95]. However, the current guidelines concern only hrHPV DNA tests on cervical samples and there is clear need to update and extend them. Currently an expert team is preparing new criteria that will include HPV genotyping, acceptance of standard comparator HPV tests other than HC2 and GP5+/6+ PCR-EIA, and HPV testing on vaginal self-collected specimens (vaginal self-samples, urine). Recent meta-analyses indicated that HPV tests based on a principle of signal amplification (for instance HC2, *care*HPV) are less sensitive and specific on vaginal self-samples whereas PCR-based hrHPV DNA assays, validated on cervical specimens, were as sensitive and nearly as specific on vaginal compared with cervical samples [96,97]. However, criteria specific for combinations of HPV tests on self-samples still have to be defined [98]. Table S11 contains 14 items that need to be addressed in future validation guidelines.

Conclusion

The guideline based on reproducibility and non-inferior accuracy defined by Meijer et al. [13] represented a milestone in HPV-based cervical cancer screening. However, the guidelines need updating to incorporate assays that target other molecules than viral DNA and comprise genotyping and sample handling procedures. The hrHPV DNA assays fully matching the current criteria and which can be recommended today in HPV-based cervical cancer screening using clinician-collected cervical samples are: HC2, GP5+/6+ PCR-EIA, Abbott RealTime, Alinity, Anyplex HR, Cobas 4800, PapilloCheck, Onclarity, HPV-Risk and Xpert HPV. By accepting Cobas 4800, as alternative comparator, we can add Cobas 6800 also to the list of validated assays. This list of tests requires regular updating as evidence accrues.

Transparency declaration

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Author contributions

Study design: M.Ar.; Assistance in study design: N.W., M.P. Literature retrieval, data extraction from literature: M.Ar., E.P., R.R., L.X. Edition of manuscript: M.Ar, N.W., M.P. Critical review of the manuscript: M.S., C.J.L.M.M., J.B., K.C., J.B., A.O.V., R.R., F.H.Z., M.G., J.D., S.D.S., K.C., P.H., M.Al.

Appendix A. Supplementary data

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