Accuracy and effectiveness of HPV mRNA testing in cervical cancer screening: a systematic review and meta-analysis

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Summary

Background Cervical cancer screening tests that identify DNA of the main causal agent, high-risk human papillomavirus (HPV) types, are more protective than cervical cytology. We systematically reviewed the literature to assess whether tests targeting high-risk HPV (hrHPV) mRNA are as accurate and effective as HPV DNA-based screening tests.

Methods We did a systematic review to assess the cross-sectional clinical accuracy to detect cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) or 3 or worse (CIN3+) of hrHPV mRNA versus DNA testing in primary cervical cancer screening; the longitudinal clinical performance of cervical cancer screening using hrHPV mRNA versus DNA assays; and the clinical accuracy of hrHPV mRNA testing on self-collected versus clinician-collected samples. We identified relevant studies published before Aug 1, 2021, through a search of Medline (PubMed), Embase, and CENTRAL. Eligible studies had to contain comparative data addressing one of our three clinical questions. Aggregated data were extracted from selected reports or requested from study authors if necessary. QUADAS and ROBINS-1 tools were used to assess the quality of diagnostic test accuracy studies and cohort studies. To assess cross-sectional clinical accuracy of mRNA testing versus DNA testing and clinical accuracy of hrHPV mRNA testing on self-collected versus clinician collected samples, we applied meta-analytical methods for comparison of diagnostic tests. To assess the longitudinal clinical performance of cervical cancer screening using hrHPV mRNA versus DNA assays, we compared the longitudinal sensitivity of mRNA tests and validated DNA tests for CIN3+ and the relative detection of CIN3+ among women who screened negative for hrHPV mRNA or DNA (both used as measures of safety) at baseline and pooled estimates by years of follow-up. A random-effect model for pooling ratios of proportions or risks was used to summarise longitudinal performance.

Findings For the hrHPV mRNA testing with APTIMA HPV Test (APTIMA), the cross-sectional accuracy could be compared with DNA assays on clinician-collected samples in eight studies; longitudinal performance was compared in four studies; and accuracy on self-samples was assessed in five studies. Few reports were retrieved for other mRNA assays, precluding their evaluation in meta-analyses. Compared with validated DNA assays, APTIMA was similarly sensitive (relative sensitivity 0·98 [95% CI 0·95–1·01]) and slightly more specific (1·03 [1·02–1·04]) for CIN2+. The relative sensitivity for CIN3+ was 0·98 (95% CI 0·95–1·01). The longitudinal relative sensitivity for CIN3+ of APTIMA compared with DNA assays assessed over 4–7 years ranged at the study level from 0·91 to 1·05 and in the pooled analysis between 0·95 and 0·98, depending on timepoint, with CIs including or close to unity. The detection rate ratios between 4 and 10 years after baseline negative mRNA versus negative DNA screening were imprecise and heterogeneous among studies, but summary ratios did not differ from unity. In self-collected samples, APTIMA was less sensitive for CIN2+ (relative cross-sectional sensitivity 0·84 [0·74–0·96]) but similarly specific (relative specificity 0·96 [0·91–1·01]) compared with clinician-collected samples.

Interpretation HrHPV RNA testing with APTIMA had similar cross-sectional sensitivity for CIN2+ and CIN3+ and slightly higher specificity than DNA tests. Four studies with 4–7 years of follow-up showed heterogeneous safety outcomes. One study with up to 10 years of follow-up showed no differences in cumulative detection of CIN3+ after negative mRNA versus DNA screening. APTIMA could be accepted for primary cervical cancer screening on clinician-collected cervical samples at intervals of around 5 years. APTIMA is less sensitive on self-collected samples than clinician-collected samples.

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Introduction

Detection of oncogenic DNA or high-risk human papillomavirus (HPV) DNA is more sensitive, but less specific, than cytology for the detection of cervical precancer.[1] Randomised trials have demonstrated that primary screening using HPV DNA tests offer a higher

Research in context

Evidence before this study

Strong evidence indicates that cervical cancer screening with high-risk human papillomavirus (HPV) DNA testing is more effective for the prevention of precancer and cancer than cervical cytology. Since expression of the viral oncoproteins E6 and E7 is required for neoplastic transformation of infected epithelial cells and the development of cervical cancer, it has been postulated that detection of high-risk HPV (hrHPV) E6 and E7 mRNA might be more specific than detection of hrHPV DNA. International guidelines exist for validation of new hrHPV DNA assays, based on demonstration of intra-laboratory and inter-laboratory test reproducibility and non-inferior clinical accuracy to detect cervical precancer compared with two HPV DNA tests with proven high efficacy in randomised trials. For assays targeting molecules other than HPV DNA, additional longitudinal data are needed that demonstrate the safety of these tests (in this study mRNA assays) over a period of 5 years or longer, which is the usual interval in screening with hrHPV DNA assays. Previous reviews have evaluated the accuracy of mRNA assays in triage of women with minor cervical cytological abnormalities and as test of cure after treatment of cervical precancer. We searched Medline, Embase, and CENTRAL for studies published between Jan 1, 2007, and July 31, 2021, without any language restrictions, using the search terms "HPV DNA, HPV mRNA, cervical cancer screening and self-sampling". The search yielded 20 studies.

Added value of this study

In this systematic review and meta-analysis, we have comprehensively addressed three clinical questions relevant to primary cervical cancer screening: the cross-sectional clinical accuracy of hrHPV mRNA versus hrHPV DNA testing for the detection of cervical precancer or cancer in primary cervical cancer screening; the longitudinal clinical performance of cervical cancer screening using hrHPV mRNA assays versus hrHPV DNA assays; and the clinical accuracy of hrHPV mRNA testing on self-collected versus clinician-collected cervical samples. Only one hrHPV mRNA assay (APTIMA) has been evaluated with regards to the three study questions. Our systematic review confirms that APTIMA has similar cross-sectional clinical sensitivity and slightly higher specificity to detect cervical precancer (cervical intraepithelial neoplasia of grade 2 or grade 3 or worse) compared with DNA-based assays. Only four cohort studies, which differed in design, compared the longitudinal performance of APTIMA with comparator HPV DNA assays over a period of 5 years or longer. Longitudinal assay performance was not statistically significantly different, but results were heterogeneous and imprecise. Validated hrHPV DNA assays based on PCR are similarly accurate for the detection of CIN2+ and CIN3+ on self-collected and clinician-collected samples, whereas APTIMA is less sensitive on self-collected samples than on clinician-collected samples.

Implications of all the available evidence

APTIMA could be accepted for primary cervical cancer screening on clinician-collected cervical samples in programmes with screening intervals of around 5 years. Considering the paucity of longitudinal evidence of performance over longer intervals, monitoring of interval cancers occurring among hrHPV mRNA-negative women is recommended. mRNA testing on self-samples is not acceptable. Research on alternative sample handling protocols for mRNA testing on self-samples aiming to approve clinical sensitivity might identify safe methods that are insufficiently validated at present.

level and longer duration of protection against invasive cervical cancer than cytology-based screening.[1,2] An additional advantage of HPV testing is that it can be used on self-collected vaginal samples. The accuracy of high-risk HPV (hrHPV) DNA testing on vaginal self-collected samples is similar to hrHPV DNA testing on clinician-collected cervical samples for the detection of cervical precancer if a clinically validated PCR-based assay is used.[3,4] Offering self-sampling kits to women who do not participate regularly in screening typically increases response rates compared with conventional invitation reminders to contact their physician or nurse for the collection of a cervical specimen.[4] International expert consensus recommendations require demonstration of high intra-laboratory and inter-laboratory reproducibility and non-inferior sensitivity and specificity for the outcome of cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) compared with standard comparator assays.[5] The 2020 list of clinically validated hrHPV DNA assays includes 11 assays that fulfil all validation criteria.[6]

Infection with oncogenic HPV types can also be detected by identifying their mRNA transcripts. Numerous HPV DNA assays have been developed; however, only a small number detect viral mRNA.[7] The APTIMA HPV Test (referred to as APTIMA hereafter; Hologic, Bedford, MA, USA), which detects E6 and E7 mRNA of 14 hrHPV types in aggregate, has been approved by the US Food and Drug Administration (FDA) for cervical cancer screening in combination with cytology.[8] However, HPV mRNA testing alone, without concomitant cytology, is currently not approved by the FDA for screening. Few studies have evaluated the longitudinal performance of HPV mRNA testing. Furthermore, the international expert recommendations for evaluation of HPV screening tests were developed based on the performance of HPV DNA assays that have been widely evaluated in randomised trials versus cytology and registry-based studies, with several years of follow-up to evaluate long-term safety. Evaluation of screening tests with targets other than HPV DNA requires demonstration of non-inferior accuracy

compared with standard comparator tests, longitudinal safety, and preferentially also good performance on self-samples.[5,9]

In this systematic review and meta-analysis, we aimed to assess the cross-sectional clinical accuracy of hrHPV mRNA versus hrHPV DNA testing for the detection of cervical precancer or cancer in primary cervical cancer screening; the longitudinal clinical performance of cervical cancer screening using hrHPV mRNA assays versus hrHPV DNA assays; and the clinical accuracy of hrHPV mRNA testing on self-collected versus clinician-collected cervical samples. The outcomes of this review are crucial for decision makers and stakeholders who develop guidelines and recommendations for cervical cancer screening. For example, the preliminary results from this meta-analysis were used to support new WHO guidelines for mRNA-based HPV screening. The methods used in this systematic review and meta-analysis might also contribute to new validation criteria for non-hrHPV DNA-based screening assays.[6,10]

Methods

Search strategy and selection criteria

We did a systematic review and meta-analysis including comparative studies on the performance of hrHPV DNA and mRNA assays in cervical cancer screening. We searched Medline (PubMed), Embase, and CENTRAL databases from Jan 1, 2007, to July 31, 2021, without language restrictions. We also used Scopus to identify citations from our previously published meta-analysis on validation of HPV tests and on accuracy of HPV testing on self-samples.[3,4,6,9] Additionally, we did specific searches to target new hrHPV mRNA assays included in a 2020 inventory of HPV tests currently on the market (appendix pp 7–9).[7] The clinical questions, search terms, and corresponding population, intervention or exposure, comparator, outcome, study design (PICOS) components are detailed in the appendix (pp 2–6). For PICOS-1, we assessed relative cross-sectional accuracy of hrHPV DNA versus RNA assays. For PICOS-2, we assessed longitudinal performance of hrHPV RNA versus DNA assays. For PICOS-3, we assessed relative cross-sectional accuracy of hrHPV RNA assays on self-collected versus clinician-collected samples. To assess PICOS-1, eligible studies had to apply an index test (a hrHPV mRNA assay) and a comparator test (a hrHPV DNA assay) on cervical samples, distinguishing hrHPV DNA assays that are clinically validated or not for use in cervical screening.[6] For PICOS-2, we included data from screening cohorts with paired DNA and mRNA testing at baseline or data from separate cohorts tested with mRNA or DNA assays with follow-up over two or more screening rounds separated by an interval of at least 3 years with CIN3+ rates reported at each screening round. For PICOS-3, eligible studies had to apply the same hrHPV mRNA test on vaginal self-samples (index) and clinician-collected cervical samples (comparator) collected from the same women. The reference or gold standard test was based on histologically confirmed detection of CIN2+ or CIN3+. For the evaluation of cross-sectional performance (PICOS-1 and PICOS-3), eligible studies had to contain data enabling extraction or computation of the number of true and false positive and negative results for both index and comparator tests, used on the same participants.

See Online for appendix

Data extraction

Assessment of study eligibility and data extraction from selected studies were performed independently by MA and MS or EP and in case of disagreement, discussed until consensus was reached. The absolute number of true and false positive and negative results for index and comparator tests were extracted or computed. For PICOS-2, we extracted the total number of women testing positive and negative for hrHPV mRNA and DNA at baseline and who had subsequent screening visits; and the cumulative number of CIN3+ cases detected at baseline and in follow-up by baseline screening test results. Our meta-analyses included only aggregated data.

The study design and quality of reports contributing to PICOS-1 and PICOS-3 were evaluated using the QUADAS-2 checklist,[11] whereas studies addressing PICOS-2 were evaluated using the Cochrane tool for assessment of randomised interventions and the ROBINS-1 tool for assessment of non-randomised interventions.[12,13] When data were not directly extractable for the published reports, we requested them from the respective authors.

To define test positivity for mRNA and DNA assays, we used the cutoff proposed by the manufacturer of assays (relative light units >1 for HC2 [Qiagen, Hilden, Germany][14] and >3× optical density of the negative control for GP5+/6+[15] PCR with enzyme immunoassay [EIA] identification of hrHPV types [Diassy, Rijkswijk, the Netherlands]). The cutoffs for the mRNA assays are defined in the appendix (pp 7–9). Accuracy was determined for the disease outcomes of cervical precancer, defined as cervical intraepithelial neoplasia of CIN2+ or grade 3 or worse (CIN3+) including adenocarcinoma in situ. We followed the PRISMA guidelines for reporting of meta-analyses and recommendations for observational cross-sectional (in particular on cross-sectional diagnostic test accuracy[16,17]) and cohort studies.[18]

Statistical analysis

We fitted a generalised linear mixed model for pooling of the accuracy data; a binomial distribution modelled the within-study variation and a bivariate normal distribution modelled the covariation between logit sensitivity and logit specificity.[19,20] The model included two covariates: an indicator variable for the comparator test and an indicator variable for the different index tests. The model allows for extreme observations (zero or 100%).

The relative cross-sectional sensitivity and specificity estimates of mRNA index tests versus DNA comparator tests on clinician samples in screening (PICOS-1) and of mRNA on self-collected versus clinician-collected specimens (PICOS-3) were computed as the ratio of the marginal (average of predicted) sensitivities and specificities. We used the Stata procedure *metadta* for meta-analysis of diagnostic test accuracy data.[21] This procedure requires at least four studies for pooling. When fewer than four studies were available, we used a fixed-effects model for pooling of ratios of proportions.[21,22] For relative sensitivity and specificity estimates, when the upper 95% CI bound was higher than or equal to unity, we could conclude that there was no evidence that screening with mRNA is less accurate than screening with DNA assays for the detection of cervical precancer in screening. Variability was assessed by the τ^2 as recommended for meta-analysis of diagnostic data.[21]

We did a sensitivity analysis and a subgroup meta-analysis to verify the impact of inclusion of informative studies that did not fulfil all inclusion criteria and to assess the effect of the comparator HPV DNA assay on relative accuracy.

For PICOS-2, two longitudinal performance parameters were assessed: the longitudinal relative sensitivity (ratio of the proportion of baseline mRNA-positive results and the proportion of baseline DNA-positive results) among patients with CIN3+ identified during the whole study period (baseline and subsequent screening episodes); and the cumulative detection rate ratios of CIN3+ over time among women who tested hrHPV mRNA-negative versus hrHPV DNA-negative at baseline. A relative longitudinal sensitivity not lower than unity and a relative detection rate ratio not higher than unity indicated that screening with mRNA was not less safe than screening with DNA assays. Reported cumulative detection data were completed with estimates derived from published cumulative incidence or Kaplan-Meier graphs using DigitizeIt.

Only direct evidence of comparisons was included in the meta-analyses. 95% CIs excluding unity or p values less than 0·05 indicated significant difference. All statistical analyses were performed with Stata (version 16.0).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We identified 1235 articles, of which 20 were relevant. The flowcharts explaining study retrieval and selection, and the list of selected studies can be found in the appendix (pp 10–13) We retrieved data for four of six identified hrHPV mRNA assays (APTIMA, PreTect-Proofer [NorChip, Klokkarstua, Norway], OncoTect [IncellDx, Menlo Park, CA, USA], Optimygene [Optipharm, Osong, South Korea]; appendix pp 7–9). Data on the cross-sectional relative accuracy of hrHPV mRNA versus hrHPV DNA assays was extracted from 12 studies (eight for APTIMA,[23–30] two for PreTect-Proofer,[26,31] one for OncoTect,[32] and one for Optimygene[33]). Five reports[30,34–37] from four studies compared the longitudinal performance of APTIMA with validated hrHPV DNA tests (HC2 or cobas 4800 [Roche, Pleasanton, CA, USA]). Five reports evaluated the relative accuracy of APTIMA on self-collected specimens versus clinician-collected specimens[28,38–41] (appendix pp 12–13). The study quality and other study characteristics are described in the appendix (pp 14–26). The technical hrHPV DNA assays used as comparator are described in the appendix (p 27).

29 677 women were enrolled in eight studies assessing the cross-sectional accuracy of APTIMA versus hrHPV DNA assays in primary cervical cancer screening, with sample sizes ranging from 908 women[27] to 9451 women.[29] The range of good quality judgments (QUADAS score Y) is summarised for 13 QUADAS items in the appendix (pp 14–15). For the eight APTIMA studies, the quality score was good in 84% (87 of 104 judgments [13 QUADAS items in eight studies yields 104 judgments]). Unclear study quality scores (QUADAS score U) were noted in nine (9%) of 104 judgements and low quality (QUADAS score N) in eight (8%) of the judgements. The study quality was judged as low for the following items: inclusion of participants from a non-screening setting (one study[25]), non-verification of participants without disease (one study[27]), non-blinding of the reference test (three studies[24,28,29]), partial verification (two studies[23,26]), and non-reporting of invalid outcomes (one study[23]).

The relative sensitivity of APTIMA for CIN2+ compared with the standard hrHPV DNA assays (HC2 in seven studies,[23–26,28–30] GP5+/6+ PCR in one study[27]) varied between 0·94 and 1·13 among individual studies[23,29] and, for the relative specificity for CIN grade lower than 2, the range of variation was 1·01 to 1·08 (figure 1, appendix p 28).[23,27–30] The pooled relative sensitivity of APTIMA for the detection of CIN2+ compared with the DNA comparator tests did not differ from unity (0·98 [95% CI 0·95–1·01]), whereas the relative specificity exceeded unity (1·03 [95% CI 1·02–1·04], appendix pp 28–29). In six studies[23,24,26,28–30] comparing the sensitivity and specificity of APTIMA with HC2 for the detection of CIN3+, the pooled relative sensitivity was 0·98 (95% CI 0·95–1·01; table 1; appendix pp 28, 30). Inter-study heterogeneity was moderate to low (τ^2 of 0·69 and 0·18 for sensitivity and specificity for CIN2+, respectively; appendix p 31).

OncoTect was compared with a hrHPV DNA assay in one study (n=2049).[32] Inclusion criteria were poorly documented and only a small number of women included in the study had disease outcomes (n=260) and

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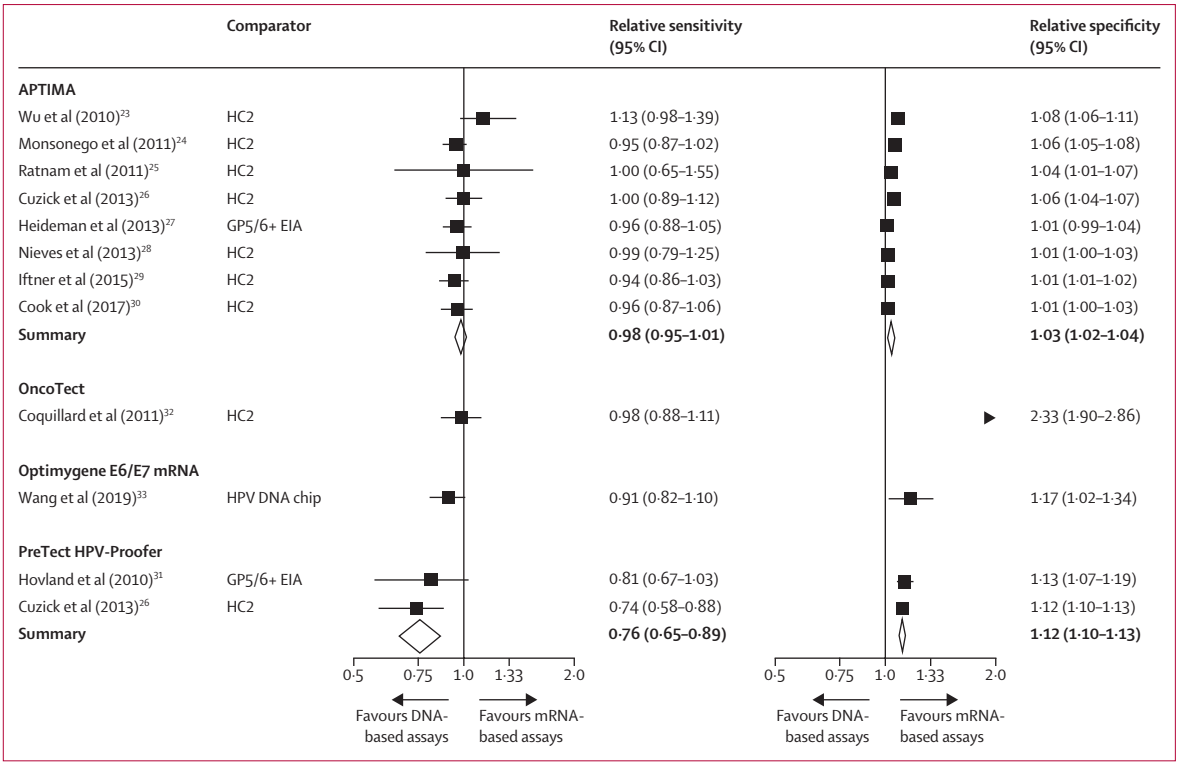

Figure 1: **Relative sensitivity and specificity of high-risk human papillomavirus testing with mRNA versus DNA assays for the detection of CIN2+ in cervical cancer screening, using clinician-collected specimens**

The pooled relative accuracy estimate is shown as a diamond, with its width representing the 95% CI. The outlying specificity estimate in Coquilard et al (2011) is shown as an arrow, because its CI is outside the range of the x-axis. CIN2+=cervical intraepithelial neoplasia of grade 2 or worse.

	Number of studies	Pooled relative sensitivity (95% CI)		Pooled relative specificity <CIN2 (95% CI)
		CIN2+	CIN3+	
HrHPV RNA *vs* hrHPV DNA assays on cervical clinician-collected specimens (PICOS-1)				
APTIMA	8; 5*	0·98 (0·95–1·01)	0·98 (0·95–1·01)	1·03 (1·02–1·04)
Pretect HPV-Proofer	2; 1*	0·76 (0·65–0·89)	0·69 (0·44–0·86)	1·12 (1·10–1·13)
HrHPV mRNA assays on self-collected vaginal specimens *vs* cervical clinician-collected specimens (PICOS-3)				
APTIMA	5	0·84 (0·74–0·96)	0·64 (0·43–0·93)	0·96 (0·91–1·01)

CIN2+=cervical intraepithelial neoplasia of grade 2 or worse. CIN3+=cervical intraepithelial neoplasia of grade 3 or worse. CIN=cervical intra-epithelial neoplasia. HPV=human papillomavirus. hrHPV=high-risk HPV. *Number of studies for the CIN3+ outcome.

Table 1: **Pooled relative sensitivity for CIN2+ and CIN3+ and specificity for less than CIN2 of hrHPV mRNA testing versus hrHPV DNA testing on cervical specimens and high-risk-HPV mRNA testing on self-collected vaginal specimens versus cervical clinician-collected samples**

the study had a QUADAS quality score of Y for only two of 13 items. The sensitivity for CIN2+ of OncoTect was similar to HC2, whereas the specificity was higher; findings for CIN3+ were similar (figure 1; appendix p 30).

The Optimygene assay was evaluated against a non-validated hrHPV DNA assay in only one study (n=220).[33] The sensitivity of the Optimygene assay for CIN2+ was lower (but not significantly) and the specificity higher than the hrHPV DNA assay (relative sensitivity 0·91 [95% CI 0·82–1·10]; relative specificity 1·17 [95% CI 1·02–1·34]; figure 1).

PreTect-Proofer (assay restricted to only five HPV types) was compared with standard comparator hrHPV DNA assays in two studies (one with the GP5+/6+ as comparator [n=267][31] and another with the HC2 as comparator [n=5747][26]). In both comparisons, the sensitivity of PreTect-Proofer was lower than the respective hrHPV DNA test and the specificity for outcome CIN2+ was higher; pooled relative sensitivity was 0·76 (95% CI 0·65–0·89) and pooled relative specificity was 1·12 (90% CI 1·10–1·13; figure 1). Inter-study heterogeneity was visibly small (figure 1; appendix p 32). Accuracy of PreTect-Proofer for CIN3+ was assessed in only one study and that showed similar results as for CIN2+[26] (appendix pp 30).

Subgroup meta-analyses of the relative accuracy of hrHPV mRNA testing with APTIMA versus DNA testing stratified by DNA comparator test identified no differences, with the exception of that the gain in specificity of APTIMA for the outcome less than CIN2 was not significant when compared with GP5+/6+ PCR (appendix p 29). The relative accuracy of APTIMA versus DNA assays was similar when assays other than the standard hrHPV DNA tests were used as the comparator (appendix pp 27, 37). In a sensitivity analysis, addition of

two excluded but informative studies yielded a slightly lower sensitivity for CIN2+ of mRNA testing with APTIMA versus hrHPV DNA with HC2 (relative sensitivity 0·97 [95% CI 0·95–1·00]; appendix p 38).

Among four longitudinal studies,[34–37,42] including 29 702 women, three contained data enabling assessment of the relative longitudinal sensitivity with paired HPV mRNA and DNA testing on all specimens.[34,35,42] One study was a biobank-based study in which archived cervical specimens collected 4 months to 7 years before the diagnosis of CIN3+ were tested with APTIMA and with the cobas 4800 HR-HPV DNA test.[34] The other two studies were prospective screening cohorts, in which all specimens were tested for hrHPV mRNA (APTIMA) and DNA (HC2) at baseline.[35,42]

Three studies allowed assessment of the cumulative detection of CIN3+ after negative mRNA tests and after negative DNA tests, with paired mRNA and DNA testing in two studies,[35,42] and one other involving separate testing (one cohort screened with an mRNA test and the other screened with a DNA test[36]).

The risk of bias was high in the Italian two-cohort study[36] because of differential testing (separate populations screened with HC2 and APTIMA) and potential differences in the quality of follow-up, and in the Swedish biobank study (the longitudinal sensitivity decreased over time until 4 years, but remained stable thereafter; appendix p 16). A score of moderate study quality (indicated as yellow in the ROBINS-I table in the appendix [p 16]) was noted in two studies,[34,36] twice in one study,[42] and five times in another study.[35]

The longitudinal relative sensitivity of mRNA testing versus DNA testing for CIN3+, documented for periods spanning 4–7 years, varied between 0·91[35] and 1·05.[42] The 95% CIs usually included unity or were close to unity (table 2, appendix p 34). We pooled the relative longitudinal sensitivity from two studies for follow-up periods of 4 years (0·98 [95% CI 0·95–1·01]; $p_{heterogeneity}$=0·36)[34,42] and 6 years (0·95 [95% CI 0·91–1·01]; $p_{heterogeneity}$=0·23; appendix p 32).

Two studies provided plots with the cumulative detection rates of CIN3+ after baseline negative mRNA- or DNA-negative screen test results, over a follow-up period up to 6 years[35] and 10 years[37] (appendix pp 33–34). The cumulative detection rates and respective ratios at different timepoints, were extracted, computed from data received from the authors or estimated from the digitised cumulative incidence plot (table 3). In the GAST trial,[35] the detection rate ratio exceeded unity at 5 years (unstable estimate, with a wide CI) and at 6 years of follow-up (detection rate ratio 1·43 [95% CI 0·80–2·56]). In the Italian cohort,[36] which tested mRNA and DNA assays in different populations, the cumulative detection rate of CIN3+ observed 5 years after negative baseline screening was 0·22% in the cohort screened with the mRNA test and 0·45% in the cohort screened with HC2, yielding a detection rate ratio of 0·49 (95% CI 0·35–0·69). In the FOCAL trial,[37,42] the detection rate ratio never differed from unity. At 10 years of follow-up, the cumulative rates of CIN3+ were 1·22% (after hrHPV mRNA-negative test at baseline) and 1·27% (after hrHPV DNA-negative test at baseline), yielding a detection rate ratio of 0·96 (95% CI 0·61–1·51). The pooled detection rate ratios for different follow-up periods at 5 and 6 years of follow-up are shown in the appendix (p 35).

The meta-analysis evaluating the cross-sectional relative accuracy of HPV mRNA assays on self-collected vaginal samples versus cervical clinician-collected samples included five studies,[28,38–41] comprising 3183 women (inter-study range 37[28] to 2049[39]). The evaluated mRNA test was APTIMA in all five studies. One study enrolled healthy women attending screening,[28] three studies enrolled patients from a colposcopy clinic,[38–40] and another study

	Follow-up duration, years	Data source	Longitudinal sensitivity, %		Relative sensitivity (95% CI)
			mRNA	DNA	
FOCAL					
Cook, 2018[42]	4	Authors	87·60%	83·60%	1·05 (0·90–1·22)
Swedish biobank study					
Forslund, 2018[34]	4	Authors	81·9%	84·0%	0·97 (0·94–1·01)
Forslund, 2018[34]	5	Authors	81·3%	84·2%	0·97 (0·94–1·00)
Forslund, 2018[34]	6	Authors	81·1%	83·8%	0·97 (0·94–1·00)
Forslund, 2018[34]	7	Authors	81·1%	83·8%	0·97 (0·94–1·00)
GAST					
Iftner, 2019[35]	5	Reported	82·4%	87·8%	0·91 (0·82–1·00)

CIN3+=cervical intraepithelial neoplasia of grade 3 or worse.

Table 2: **Longitudinal sensitivity of high-risk HPV mRNA versus DNA testing to detect cumulatively identified CIN3+ by duration of follow-up**

	Duration of follow-up, years	Data source	Detection rate, %		DRR (95% CI)
			mRNA	DNA	
FOCAL					
Cook, 2018[42]	4	Authors	0·10%	0·13%	0·76 (0·18–3·09)
Strang, 2021[37]	5	Estimated*	0·15%	0·20%	0·77 (0·23–2·59)
Strang, 2021[37]	6	Estimated	0·20%	0·25%	0·81 (0·28–2·40)
Strang, 2021[37]	7	Estimated	0·26%	0·31%	0·85 (0·23–2·20)
Strang, 2021[37]	10	Reported	1·22%	1·27%	0·96 (0·61–1·51)
GAST					
Iftner, 2019[35]	5	Estimated	0·14%	0·04%	3·27 (1·04–10·28)
Iftner, 2019[35]	6	Authors	0·31%	0·22%	1·43 (0·80–2·56)
Italian two-cohort study					
Zorzi, 2020[36]	5	Reported	0·22%	0·45%	0·49 (0·35–0·69)

CIN3+=cervical intraepithelial neoplasia of grade 3 or worse. hrHPV=high-risk human papillomavirus. DRR=detection rate ratio. *Estimated from cumulative detection rate plot.

Table 3: **Cumulative detection rates and DRRs of CIN3+ among women who were hrHPV mRNA-negative or hrHPV DNA-negative at baseline**

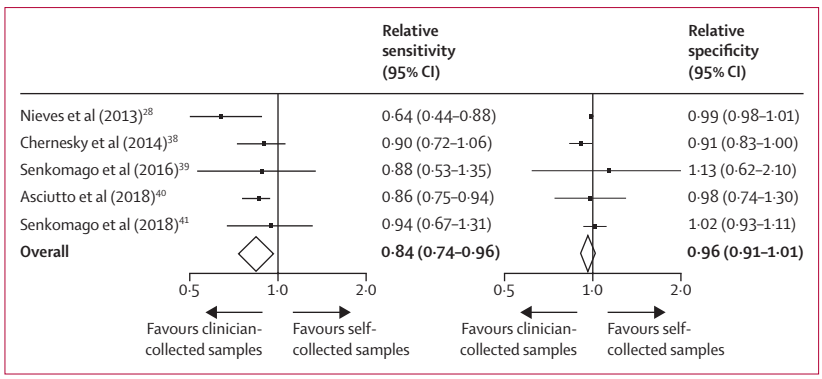

Figure 2: **Relative sensitivity and specificity of high-risk human papillomavirus mRNA testing to detect CIN2+ on vaginal self-collected cervical specimens versus clinician-collected cervical specimens**
The pooled relative accuracy estimate is shown as a diamond, with its width representing the 95% CI. CIN2+=cervical intraepithelial neoplasia of grade 2 or worse.

included female sex workers.[41] Each study used a particular device for self-sampling. The self-sample was transferred into a commercial medium used for liquid-based cytology in one study,[28] whereas the other studies used a storage medium specifically developed by the test manufacturer for testing APTIMA on vaginal self-samples[38–41] (appendix pp 24–26).

The range of good quality judgments (QUADAS score=Y; appendix pp 18–19) for 13 QUADAS items, varied among studies from four of 13[41] to 12 of 13.[40] For the five studies combined, a good quality score was given in 60% (39 of the 65 [13 × 5] judgements). Unclear and low study quality scores (QUADAS score=U and N, respectively) were noted in 31% (20 of 65) and 9% (6 of 65) of the judgements. The study quality was judged as low (QUADAS score N) for the following items: exclusion of participants not documented (two studies[38,39]), non-blinding of the reference test (one study[28]), partial verification (one study[41]), withdrawals not explained (one study[41]), and non-reporting of invalid outcomes (one study[39]).

The sensitivity for CIN2+ of APTIMA on self-collected samples was lower than for clinician-collected samples (relative sensitivity 0·64–0·94) in all studies, whereas relative specificities were lower in three studies [28,38,40] and higher in two studies[39,41] (range 0·91–1·13). The pooled relative sensitivity for CIN2+ was significantly lower than unity (0·84 [95% CI 0·74–0·96]) whereas the relative specificity did not differ from unity: 0·96 [95% CI 0·91–1·01]; figure 2). For CIN3+, the sensitivity of APTIMA was lower on self-collected samples than for clinician-collected samples (0·64 [95% CI 0·43–0·93]; appendix p 36).

Discussion

For one hrHPV mRNA assay (APTIMA), the cross-sectional accuracy and longitudinal performance on clinician-collected samples could be compared with clinically validated hrHPV DNA assays in 12 studies, of which four provided data for two or more screening rounds with follow-up of 5 years or longer. The cross-sectional relative accuracy of APTIMA on clinician-collected cervical samples was compared with APTIMA on self-collected vaginal samples in five studies. APTIMA on cervical samples showed similar cross-sectional and longitudinal sensitivity and higher cross-sectional specificity for cervical precancer than DNA comparator tests. However, on self-collected samples, APTIMA was less sensitive than on clinician-collected samples. Few eligible studies were retrieved that evaluated other mRNA assays, therefore, no conclusive interpretation can be made. Therefore, we will focus the discussion mainly on the APTIMA assay.

APTIMA was approved for use in primary cervical cancer screening in conjunction with cervical cytology by the US FDA in 2011. At present, APTIMA is recommended as the preferred standalone screening test in a small number of countries or regions (south Sweden since 2017, Wales [UK] and Basque Country [Spain] since 2018, Scotland [UK] since 2020) and it is accepted as one of the allowed standalone HPV screening tests in Australia, France, England (UK), Nigeria, and Zambia.

WHO 2021 guidelines for cervical cancer screening focused on screening using validated hrHPV DNA tests.[43] Subsequently, an extension on screening using hrHPV mRNA assays was published in late 2021, based on a preliminary version of this meta-analysis supplemented with extensive modelling of the relative harms, benefits, and cost-effectiveness of HPV DNA versus HPV mRNA screening.[44] The resulting WHO recommendations are that HPV mRNA testing on clinician-collected specimens might be used in primary screening at 5-year intervals, as opposed to HPV DNA assays that are recommended to be used at 5–10-year intervals. The recommendation regarding screening every 5 years for mRNA testing was made because of the heterogeneous and scarce evidence for comparative safety intervals longer than 5 years.

Most of the available hrHPV mRNA assays, with the exception of PreTect-Proofer and Optimygene, do not include amplification and identification of human mRNA in the target specimen as an internal control of specimen adequacy. Although several validated HPV DNA assays include specimen adequacy controls, HC2 (one of the two standard comparator hrHPV DNA assays) does not include such a control. Several validated HPV DNA assays generate a quantified metric of the signal strength, whereas all mRNA assays considered in this review generated a qualitative (present or absent) output, precluding cutoff optimisations. APTIMA and OncoTect do not identify HPV types separately, whereas the other mRNA assays have extended or full genotyping capacity, which can be used for triage of hrHPV-positive women. The APTIMA HPV 16 18/45 Genotype assay (Hologic) identifies the three most carcinogenic HPV types, but a separate test is required.

mRNA is a less stable molecule than DNA and might therefore be more susceptible to degradation in extreme storage conditions or long transport times. However, the

sensitivity of APTIMA was not lower than hrHPV DNA testing with HC2 in a Scottish study assessing triage of women with minor cytological abnormalities in which study specimens were derived from a cervical cytology biobank.[45] The Swedish biobank study included in our review demonstrated the feasibility of APTIMA testing on archived specimens stored for up to 7 years.[34] However, optimised biobank conditions (storage of cell pellets at –20°C or –80°C shortly after collection) might not reflect real-world situations.

Use of APTIMA on self-collected vaginal samples identified fewer CIN2+ lesions than on clinician-collected samples. This reduction in sensitivity could potentially be compensated for by preheating the self-specimen for 1 h up to 90°C. Borgfeldt and Forslund showed that application of this procedure resulted in a sensitivity gain (85·3% to 95·3%; relative increase with a factor 1·11).[46] However, well designed diagnostic test accuracy studies (eg, VALHUDES[47]) are needed to confirm these findings.

HPV mRNA assays differ from most established HPV DNA assays with respect to the type of nucleic acid and the HPV genome region they target, which might affect clinical performance. All HPV mRNA assays assessed in this review target transcripts of the HPV *E6* or *E7* genes, whereas the comparator tests target DNA sequences from the *L1* region. E6 and E7 expression increases when cellular transformation occurs at the transition from HPV infection to cervical precancer. Therefore, it has been proposed that detection of HPV E6 or E7 mRNA might indicate transforming infections whereas detection of viral DNA only indicates presence of the virus.[48] Detection of the oncogene transcripts could therefore be expected to result in higher specificity of E6 and E7 mRNA assays.[49,50] However, it has been shown that PreTect-Proofer and APTIMA also detect HPV DNA.[51,52] The higher observed clinical specificity of PreTect-Proofer is to likely to be due to restriction of the assay to only five hrHPV types.[31,52] The slightly higher specificity of APTIMA compared with the HPV DNA assays might be due to a reduction in analytical sensitivity, which might explain the lower clinical sensitivity of APTIMA on self-samples taken from the vagina where hrHPV load is lower than in cervical scrapes. It has been suggested that viral genome disruption in the *L1* region might affect sensitivity of HPV DNA tests targeting that region.[53] However, a 2021 review found no differences in clinical accuracy for CIN2+ or CIN3+ lesions between assays targeting DNA from *E6*, *E7*, or other viral genes.[6] A 2022 assessment of multiple hrHPV DNA assays used on cytology or tissue specimens from patients with precancer or cancer of diverse anogenital or oropharyngeal origin identified a high level of concordance between HPV DNA tests targeting *L1* or *E6* and *E7* genes.[53]

We did not include two informative studies comparing APTIMA with HC2 in the first meta-analysis because they did not fulfil all eligibility criteria: the Danish HORIZON study[54] was excluded due to absence of specificity data; and the CLEAR study[8] was excluded because it included only women with negative cervical cytology resulting in a cohort that was not representative of an overall screening population. Inclusion of these two studies in our first meta-analyses, using a simple random-effect model for pooling of proportions, would have resulted in a slightly lower relative sensitivity for CIN2+ compared with the main meta-analysis without affecting the relative specificity for CIN3+. A Swedish trial in which women eligible for screening were randomly assigned into self-sampling and midwife-sampling groups found the detection rate ratio of CIN2+ with APTIMA was near to unity for both groups.[55] This trial was not incorporated in our meta-analysis addressing PICOS-3, since the study did not evaluate performance of APTIMA in paired self-collected and clinician-collected samples. The finding of similar CIN2+ rates between the groups might be explained by imbalances between study groups (differential participation, age composition, and test-positivity rates) rather than similar test performance.

A strength of our meta-analysis was the use of state-of-the-art statistical methods for pooling of diagnostic test accuracy studies, which account for the correlation between sensitivity and specificity and allowing for paired test comparisons and inclusion of covariate information.[21]

The choice to evaluate the HPV mRNA assays against the standard hrHPV DNA comparator assays (HC2 and GP5+/6+ PCR) recommended in international validation guidelines facilitates bridging to previous meta-analyses done with the purpose of establishing lists of HPV tests that fulfil requirements for cervical cancer screening.[5,6] However, HC2 and GP5+/6+ PCR are not frequently used in current screening programmes. The cross-sectional relative clinical accuracy of APTIMA would have been similar if we had used newer HPV DNA assays, which are used in current screening programmes.

The direction and magnitude of the relative cross-sectional accuracy estimates were fairly consistent, precise, and in agreement with systematic reviews on triage of women with minor cytological lesions,[45,56] whereas longitudinal performance estimates were heterogeneous and imprecise (based on a small number of studies, with two studies comprising ten CIN3+ cases or fewer detected after negative baseline screening). The Italian two-cohort study was the only study that assessed the incidence of cervical cancer 5 years after negative APTIMA or negative HC2 results (detection rate ratio 0·51 [95% CI 0·01–4·22]).[36] However, the study did not conduct paired testing of both assays in the same women, but rather compared two different regions each using one of the two tests. Differences in the study populations might translate to different detection ratios and thus the results need to be interpreted cautiously.

HC2 might cross-react with possibly hrHPV types with very low carcinogenic potential[57,58] and this might result in underestimation of the useful relative sensitivity of APTIMA versus HC2, but increases the relative

specificity. However, in a subgroup meta-analysis, no differences were identified in relative sensitivity of APTIMA versus HC2 and APTIMA versus GP5+/6+ PCR (a test that is less prone to type cross-reaction). The clinical specificity of APTIMA was higher than HC2, but was not more specific than GP5+/6+ EIA.

In the three longitudinal studies that compared the cumulative CIN3+ incidence 5–6 years after negative APTIMA versus negative HC2 at baseline, we observed relative risks that ranged from 0·49[36] to 1·43.[35] On a relative scale, these differences seem large, however, the absolute CIN3+ risks after negative APTIMA were low. The estimated absolute risks of CIN3+ after negative APTIMA baseline screening were 2·2/1000 after 5 years in the Italian two-cohort study and 2·0/1000 and 3·1/1000 after 6 years in the FOCAL and GAST trials, respectively. The risks after negative HC2 baseline screening were 4·5/1000 (Italian two-cohort), 2·5/1000 (FOCAL), and 2·2/1000 (GAST). The absolute differences (risk after negative APTIMA minus risk after negative APTIMA [–2·3/1000 for the Italian two-cohort,[36] –0·5/1000 for FOCAL,[37] and +0·9/1000 for GAST[35]]) are small. These low CIN3+ rates suggest that screening with APTIMA at 5-year intervals might be safe.

To conclude, on clinician-collected specimens, HPV mRNA testing using APTIMA has similar cross-sectional sensitivity and slightly higher specificity for both CIN2+ and CIN3+ compared with validated hrHPV DNA assays.

There is no evidence indicating that APTIMA is less safe in screening with 5 year or shorter intervals. However, longitudinal relative performance indicators are imprecise, heterogeneous, and based on few studies. In all three eligible studies, the risk of CIN3+ 5 years after negative APTIMA was not higher than 3/1000. hrHPV mRNA testing with APTIMA is less sensitive on self-collected vaginal samples than on clinician-collected samples. APTIMA could therefore be accepted for primary screening on cervical clinician-collected samples but not on vaginal self-collected samples.

Contributors

MA designed the systematic review and meta-analyses. MA, MS, SdS, MAC, and NW formulated the clinical questions. MA and MS did study selection and data extraction. MA and MS verified all extracted data. MA performed the statistical analyses. MA and MS edited the draft manuscript and KC wrote part of the Discussion. VN developed the statistical *metadta* procedure used for the pooling of accuracy data and provided statistical advice. All coauthors critically revised and approved the manuscript. All authors had full access to all of the data and the corresponding author had the final responsibility to submit for publication.

Declaration of interests

MA and VN are employees of Sciensano, which received support from VALGENT and VALHUDES researcher-induced frameworks for comparison and validation of HPV tests used on clinician-collected and self-collected samples. KC is co-Principal Investigator of an investigator-initiated trial of cervical screening, Compass, run by the VCS Foundation, which is a government-funded not-for-profit charity; the VCS Foundation has received equipment and a funding contribution from Roche Molecular Diagnostics. KC is also co-principal investigator on a major investigator-initiated implementation programme, Elimination of Cervical Cancer in the Western Pacific, which will receive support from the Minderoo Foundation and the Frazer Family Foundation and equipment donations from Cepheid. JB received support from the National Institute for Public Health and the Environment for the HPV Self-sampling Ct project and VIS modelling project. MAC was member of the US National Cancer Institute Steering Committee that prepared Enduring Guidelines for Cervical Cancer Screening and Management. The employer of MP received free-of-charge reagents from Qiagen, Seegene, Abbott, and Roche. MG worked on a project of Roche Molecular Diagnostics as an advisor, and received payment for lectures or presentations from Qiagen GmBH and Roche Molecular diagnostics. MS, SdS, RR, and NW declare no competing interests.

Data sharing

Only aggregated data without any identifiable information were used for this meta-analysis. The diagnostic accuracy data extracted from eligible studies are available in the appendix (p 42).

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