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Validated HPV tests usable in cervical cancer screening on clinician-collected cervical specimens

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An increasing number of countries and international cancer organisations recommend screening using clinically validated tests that identify nucleic acid sequences of carcinogenic HPV types.

HPV testing and clinical validation of HPV assays

Ample evidence indicates that cervical cancer screening using assays targeting carcinogenic human papillomavirus (HPV) types, followed by management of HPV-positive women offers stronger protection against future cancer of

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the cervix uteri than cytology-based screening[1,2]. Therefore, an increasing number of countries and international cancer organisations have switched or are in the phase of switching to virological screening and recommend screening using clinically validated tests that identify nucleic acid sequences of carcinogenic HPV types[3]. Whereas hundreds of different HPV assays are commercially available, only a small minority of them have demonstrated fulfilment of the international validation criteria defined by Meijer et al in 2009[4-6]. The clinical validation of a new index HPV assay for use in cervical cancer screening requires that the new assay demonstrates non-inferior sensitivity and non-inferior specificity to detect cervical intraepithelial neoplasia of grade 2 (CIN2) or worse compared to a first or second-generation comparator test. The benchmarks for relative sensitivity and specificity (index versus comparator assay) are ≥0.90 and ≥0.98, respectively[6]. Practically this means that the left 90% confidence interval bound (CIB) around the relative sensitivity or relative specificity is not lower than these benchmarks.

The clinical validation of a new index HPV assay for use in cervical cancer screening requires that the new assay demonstrates non-inferior sensitivity and non-inferior specificity to detect cervical intraepithelial neoplasia of grade 2 or worse compared to a first or second-generation comparator test.

Initially, only Hybrid Capture 2® (HC2, Qiagen, Gaithersburg, MD, USA) and GP5+/6+ PCR-EIA® (Diassay, Rijkswijk, the Netherlands) were accepted as comparator tests since these assays were evaluated in population-based randomised trials that proved lower cumulative incidence of invasive cancer than cytology. Since these two assays are no longer widely used as most laboratories have moved to newer-generation HPV tests, new criteria for second-generation comparators have been defined. Four assays fulfil the second-generation standard comparator criteria: RealTime High-Risk HPV Test® (Abbott, Wiesbaden, Germany), Cobas 4800 HPV Test®(Roche Molecular System, Pleasanton, CF, USA), Onclarity HPV Assay® (BD Diagnostics, Sparks, MD, USA); and Anyplex II HPV HR Detection® (Seegene, Seoul, South Korea) (Arbyn 2024, submitted). Besides fulfilling the criteria of non-inferior clinical accuracy, sufficient intra- and inter-laboratory reproducibility of the index HPV assay should be documented where the lower 95% CIB ≥87% and kappa ≥0.5[6].

The international validation criteria outlined above concern only HPV DNA assays applied to cervical specimens. Assays that target other molecules than HPV DNA sequences also have to provide evidence of the longitudinal safety, which means documentation of a similar or lower risk of CIN3+ after a negative test result compared to the risk after a negative result of a validated HPV DNA test, over a period of at least five years[5].

In this article we update previous lists of clinically validated HPV tests[5,7]. Systematic reviews and meta-analyses on the accuracy for CIN2+ of new HPV tests compared to a first or second-generation comparator assay were extended in time to cover reports published up to April 2024. The systematic review also incorporates recently published data concerning intra- and inter-reproducibility of new assays and longitudinal performance of HPV mRNA assays.

The updated list contains 19 HPV DNA tests that fulfil the cross-sectional validation criteria and one HPV mRNA test, which has also demonstrated non-inferior longitudinal performance compared to validated DNA assays.

Compared to the latest list incorporated in the 2023 ESGO Book of Gynaecologic Oncology, containing 16 validated assays, four additional assays that fulfil international validation criteria can be added: OncoPredict HPV QT® (Hiantis, Milano, Italy), RIATOL HPV genotyping qPCR assay® (AML, Antwerp, Belgium), Allplex HPV HR Detection assay® (Seegene, Seoul, South Korea)

Table 1

List of validated HPV nucleic acid tests that can be used in cervical cancer screening on cervical clinician-collected specimens (as of April 2024)

ASSAY	MANUFACTURER	GENOTYPING CAPACITY	NUMBER OF TYPES	GENOTYPING DETAIL‡	HUMAN GENE¥	STORAGE MEDIA
A. Standard comparator hrHPV DNA tests (validated in population-based randomised trials), used as comparator in validation studies:						
A1. Hybrid Capture 2 HPV DNA Test	Qiagen, Gaithersburg, MD, USA	None	13	16/18/31/33/35/39/45/51/52/56/58/59/68	No	PC,SP
A2. GP5+/6+ PCR-EIA	Diassay, Rijkswijk, the Netherlands	None	14	16/18/31/33/35/39/45/51/52/56/58/59/66/68	No	PC,SP
B. hrHPV DNA tests validated consistently in multiple studies against standard comparator tests:						
B1. Alinity m HR HPV Assay	Abbott, Wiesbaden, Germany	Extended	14	16,18,45,31/33/52/58,35/39/51/56/59/66/68	Yes	PC
B2. Anyplex II HPV HR Detection	Seegene, Seoul, South Korea	Full	14	16,18,31,33,35,39,45,51,52,56,58,59,66,68	Yes	PC
B3. Cobas 4800 HPV Test	Roche Molecular System, Pleasanton, CF, USA	Limited	14	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC,SP
B4. HPV-Risk Assay	Self-Screen BV, Amsterdam, The Netherlands	Limited	15	16,18,31/33/35/39/45/51/52/56/58/59/66/67/68	Yes	PC,SP
B5. NeuMoDX HPV assay	Qiagen, Ann Arbor, MI, USA	Limited	15	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC
B6. Onclarity HPV Assay	BD Diagnostics, Sparks, MD, USA	Extended	14	16,18,31,45,51,52,33/58,35/39/68,56/59/66	Yes	PC,SP
B7.PapilloCheck HPV-Screening Test	Greiner Bio-One, Frickenhausen, Germany	Full	24	06,11,16,18,31,33,35,39,40,42,43,45,44/55,51,52,53,56,58,59,66,68,70,73,82	Yes	PC
B8. RealTime High Risk HPV Test	Abbott, Wiesbaden, Germany	Limited	14	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC
B9. Xpert HPV	Cepheid, Sunnyvale, CA, USA	Extended	14	16,18/45,31/33/35/52/58,51/59,39/56/66/68	Yes	PC
C. hrHPV DNA test validated consistently in multiple studies against alternative comparator test:						
C1. Cobas 6800 HPV Test	Roche Molecular System, Pleasanton, CF, USA	Limited	14	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC
D. hrHPV DNA tests evaluated in only one study against standard comparator tests:						
D1. CLART HPV4S	GENOMICA SAU, Madrid, Spain	Full	16	06,11,16,18,31,33,35,39,45,51,52,56,58,59,66,68	Yes	PC,SP
D2. OncoPredict HPV Screening	Hiantis Srl, Milan, Italy	Limited	13	16,18,31/33/35/39/45/51/52/56/58/59/68	Yes	PC
D3. REALQUALITY RQ-HPV Screen	AB ANALITICA, Padua, Italy	Limited	14	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC
E. hrHPV mRNA test:						
E1. APTIMA HPV Assay	Hologic, Bedford, MA, USA	None*	14	16/18/31/33/35/39/45/51/52/56/58/59/66/68	No	PC
F. Added since the last international publication of the list of clinically validated HPV tests						
F1. OncoPredict HPV QT	Hiantis Srl, Milan, Italy	Full	12	16,18,31,33,35,39/45,51,52,56,58,59	Yes	PC
F2. RIATOL HPV genotyping qPCR assay	AML, Antwerp, Belgium	Full	17	06,11,16,18,31,33,35,39,45,51,52,53,56,58,59,66,68	Yes	PC
F3. Allplex HPV HR Detection assay	Seegene, Seoul, South Korea	Full	14	16,18,31,33,35,39,45,51,52,56,58,59,66,68	Yes	PC
F4. Vitro HPV Screening Assay	Vitro S. A., Sevilla, Spain	Limited**	14	16,18,31/33/35/39/45/51/52/56/58/59/66/68	Yes	PC

* Another mRNA assay (APTIMA HPV16, 18/45, Hologic) can identify HPV16 and HPV18/45

** HPV Direct Flow Chip (Vitro S.A): provides full genotyping if Vitro HPV Screening shows other hrHPV (not HPV16/18)

‡ A slash "/" means that HPV types are identified as an aggregate; a comma "," means that HPV types or groups of types are identified separately

A HPV genotype in green does not belong to the IARC group I "carcinogenic types" or to group IIA "probably carcinogenic types" (Bouvard Lancet Oncol 2009[9])

¥ Amplification of human gene, which is an internal quality indicator that the specimen contains human cells

PC PreservCyt (Hologic, Bedford, MA, USA)

SP SurePath (BD Diagnostics, Sparks, MD, USA)

and Vitro HPV Screening assay® (Vitro S, Sevilla, Spain). The results are summarized in Table 1. The updated list contains 19 HPV DNA tests that fulfilled the cross-sectional validation criteria and one HPV mRNA test, which also demonstrated non-inferior longitudinal performance compared to validated DNA assays[8]. Three assays target only an aggregate of 13 to 14 high-risk HPV types and do not provide any genotyping detail; eight assays provide limited genotyping capacity enabling separate identification of the most carcinogenic types HPV16 and HPV18 (with or without HPV45); three assays provide extended genotyping (more genotyping detail compared to tests with limited genotyping capacity) whereas six assays enable separate genotyping of all targeted types. Two test manufacturers produced assays with limited or no genotyping, but provide a second reflex assay allowing for more genotype granularity when the initial assay is positive. ■

CONFLICTS OF INTERESTS

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