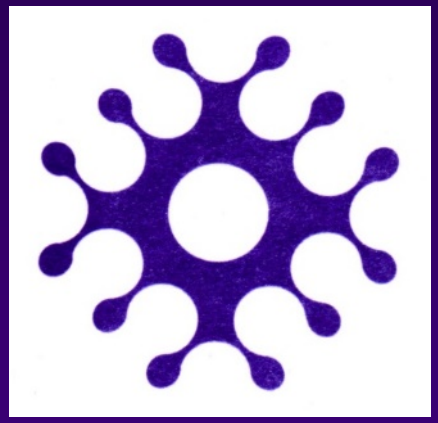
Desired characteristics of HPV tests for cervical cancer screening and clinical management



Mario Poljak

Faculty of Medicine, University of Ljubljana, Slovenia

HPV tests for agreed indications for HPV testing in current clinical practice

HPV tests for epidemiological and vaccine-related studies

HPV tests for different research purposes

*****

two most important parameters <u>which define the purpose of the HPV test</u>

(i) set of targeted HPV types

(ii) level of analytical sensitivity

Ideal HPV Test

for major agreed indications for HPV testing in current clinical practice

HPV test should:

- detect all HPV infections that are associated with, or will develop into high-grade CIN
- differentiate them from transient HPV infections

HPV Test

for major agreed indications for HPV testing in current clinical practice

Broad genotype coverage

High analytical sensitivity

High analytical specificity

HPV Test

for major agreed indications for HPV testing in current clinical practice

Broad genotype coverage	BALANCED = ARTIFICIALLY REDUCED
High analytical sensitivity	BALANCED = ARTIFICIALLY REDUCED
High analytical specificity	NECESSARY

HPV Test

for major agreed indications for HPV testing in current clinical practice

Broad genotype coverage	BALANCED = ARTIFICIALLY REDUCED
High analytical sensitivity	BALANCED = ARTIFICIALLY REDUCED
High analytical specificity	NECESSARY

High clinical sensitivity !!!!

High clinical specificity !!!!

CIN2+/CIN3+

Ideal HPV Test

for major agreed indications for HPV testing in current clinical practice

optimal balance between clinical sensitivity and clinical specificity for CIN2+

aim to minimize redundant/excessive follow-up procedures for hr-HPV positive women with transient hr-HPV infections and/or without cervical lesions

*****

HPV DNA assay with very high analytical sensitivity yields a large number of clinically insignificant positive results resulting in unnecessary follow-up, diagnostics procedures and treatment of healthy women

HPV tests ?

Papillomavirus Research 2015; doi:10.1016/j.pvr.2015.06.006.

European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination

Lawrence von Karsa [a,*], Marc Arbyn [b], Hugo De Vuyst [c], Joakim Dillner [d], Lena Dillner [e], Silvia Franceschi [f], Julietta Patnick [g], Guglielmo Ronco [h], Nereo Segnan [h], Eero Suonio [a], Sven Törnberg [i], Ahti Anttila [j]

HPV test choice

cervical cancer screening program should adopt a HPV primary test for use only if it has been validated by demonstrating reproducible, consistently high sensitivity for CIN2+ and CIN3+ lesions, and only minimal detection of clinically irrelevant, transient HPV infection

HPV tests (neither commercial nor in-house tests) that have not been clinically validated should not be used in clinical practice

Commercially available HPV molecular tests - periodical inventories -

2010

Poljak M, Kocjan BJ. Commercially available assays for multiplex detection of alpha human papillomaviruses. Exp Rev Anti Infect Ther 2010; 8: 1139-62.

2012

Poljak M, Cuzick J, Kocjan BJ, Iftner T, Dillner J, Arbyn M. Nucleic acid tests for the detection of alpha human papillomaviruses. Vaccine 2012; Suppl 30: F100-6.

2015

Poljak M, Kocjan BJ, Oštrbenk A, Seme K. Commercially available molecular tests for human papillomaviruses (HPV): 2015 update. J Clin Virol 2016; 76: (Suppl 1): S3-S13.

2020

Poljak M, Oštrbenk Valenčak A, Gimpelj Domjanič G, Xu, L, Arbyn M. Commercially available molecular tests for human papillomaviruses: a global overview. Clin Microbiol Infect 2020; 26: 1144-50.

- not a simple addition of newly developed tests to the old list of HPV tests
- the existence of all tests double-checked with manufacturers at every update round

- data retrieved from:
  - Medline/Pubmed, Web of Science, Scopus, Bing, Google Scholar, Google without language or period restrictions
  - abstracts from main HPV-related conferences
  - internal files
  - the Chinese National Medical Products Administration (formerly the Chinese FDA)

- conservative estimate – very likely haven't identified all HPV tests currently available

- omission of any particular commercially available HPV test was unintentional

Test (distinct, unique) vs. test variant

particular HPV test was considered a variant if technologically identical or very similar to the original test but targeting different HPV type(s)

Realquality RQ-HPV HR Multiplex (AB Analitica, Padua, Italy)	DISTINCT HPV TEST
Realquality RQ-HPV HR/LR Multiplex (AB Analitica, Padua, Italy)	VARIANT
Realquality RQ-Multi HPV Detection (AB Analitica, Padua, Italy)	VARIANT

2020

254 distinct commercial HPV assays

(and 425 variants)

on the global market

Poljak M at al. Clin Microbiol Infect 2020; 26: 1144-50

Main groups of available commercial HPV tests on the global market in 2020 (tests vs. variants)

	Tests	Variants
hr-HPV DNA screening tests	40	3
hr-HPV DNA screening tests with concurrent or reflex partial genotyping for the main hr-HPV types	41	3
HPV DNA full genotyping tests	91	21
HPV DNA type- or group-specific genotyping tests	38	89
hr-HPV E6/E7 mRNA tests	9	1
in situ hybridization DNA in mRNA based HPV tests	33	308
HPV DNA tests targeting miscellaneous HPV types	2	0
Total	254	425

Poljak M at al. Clin Microbiol Infect 2020; 26: 1144-50

Table S1: hr-HPV DNA screening tests on the global market in January 2020. All variants of a particular distinct HPV test are marked with the same superscript letter. US FDA approval status (FDA) (more details in Table 2), compliance with international consensus guidelines for primary cervical cancer screening in women 30 years and older (MG), evaluation within VALGENT initiative (V) and WHO prequalification status (WHO).

Tests targeting IARC-2009 hr-HPV types plus HPV66 and/or HPV68

- Hybrid Capture 2 (HC2) HPV DNA Test (Qiagen Gaithersburg, Inc., MD, USA) FDA MG V
- EIA Kit HPV GP HR (Diassay, Ev Rijswijk, Netherlands) MG V
- Cervista HPV HR Test (Hologic, Madison, WI, USA) FDA MG
- CareHPV Test (Qiagen Gaithersburg, Inc., MD, USA) WHO
- 13 High-Risk HPV Real-Time PCR Kit (Hybribio, Beijing, China)
- RealLine HPV HCR Screen (Str-Format) (Bioron Diagnostics GmbH, Ludwigshafen, Germany)
- RealLine HPV HCR Screen (Fla-Format) (Bioron Diagnostics GmbH, Ludwigshafen, Germany)
- CareStart HPV Screening Kit – PNT (Access Bio, Inc., Somerset, NJ, USA)
- Diagnostic Kit for Detection of Human Papillomavirus DNA (PCR-Fluorescent) (Genetel Pharmaceuticals, Shenzhen, China)
- Human Papillomavirus (13 Types) Nucleic Acid Test Kit (PCR-Fluorescence) (Taipu Bio (TIB), Xiamen, China)[a]
- ProDx High-Risk HPV (14 Types) DNA qPCR Detection Kit (Promega Biological Products, Shanghai, China)

Tests targeting IARC-2009 hr-HPV types only

- HPV High Risk Screen Real-TM Quant (Sacace, Como, Italy; Nuclear Laser Medicine S.R.L., Milan, Italy)
- HPV High Risk Screen Real-TM Quant 2 x (Sacace, Como, Italy; Nuclear Laser Medicine S.R.L., Milan, Italy)
- AmpliSens HPV HCR Screen-Titre-FRT PCR Kit (Federal State Institution of Science, Moscow, Russia; Ecoli, Bratislava, Slovakia)[b]
  - AmpliSens HPV HCR Screen-Titre-FRT PCR Kit (2x) (Federal State Institution of Science, Moscow, Russia; Ecoli, Bratislava, Slovakia)[b]
  - AmpliSens HPV HCR Screen-Titre-FRT PCR Kit (4x) (Federal State Institution of Science, Moscow, Russia; Ecoli, Bratislava, Slovakia)[b]

Tests targeting IARC-2009 hr-HPV types and additional alpha-HPV types

- Seeplex HPV4A ACE Screening (Seegene, Seoul, Korea)
- STD Kit (Autoimmun Diagnostika GmbH, Strassberg, Germany)
- AmpliSens HPV HCR Screen-Eph PCR Kit (Federal State Institution of Science, Moscow, Russia; Ecoli, Bratislava, Slovakia)
- HPV-DNA Assay Kit (Tofema, Seoul, Korea)
- PapilloScreen (GeneMatrix Co., Seoul, Korea)
- HPV Screen PCR Kit (BioCore, Seoul, Korea)
- Human Papilloma Virus (HPV Common/Double Check) (Genekam Biotechnology, Duisburg, Germany)

27 pages document…

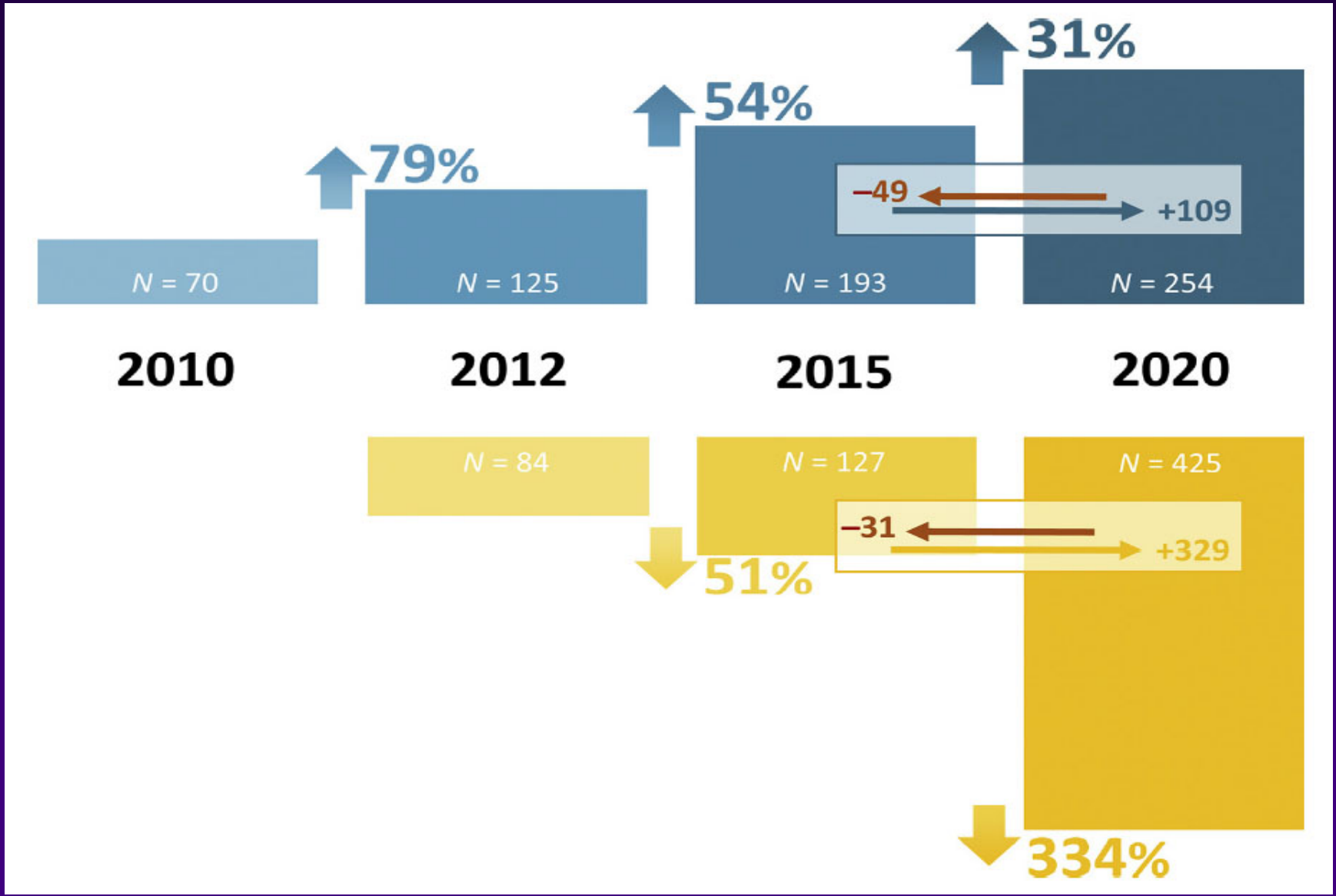
Poljak M at al. Clin Microbiol Infect 2020; 26: 1144-50

254 distinct commercial HPV assays in 2020

- HPV DNA - based 95%
- HPV mRNA - based 5%
- HPV protein - based < 1%

Poljak M at al. Clin Microbiol Infect 2020; 26: 1144-50

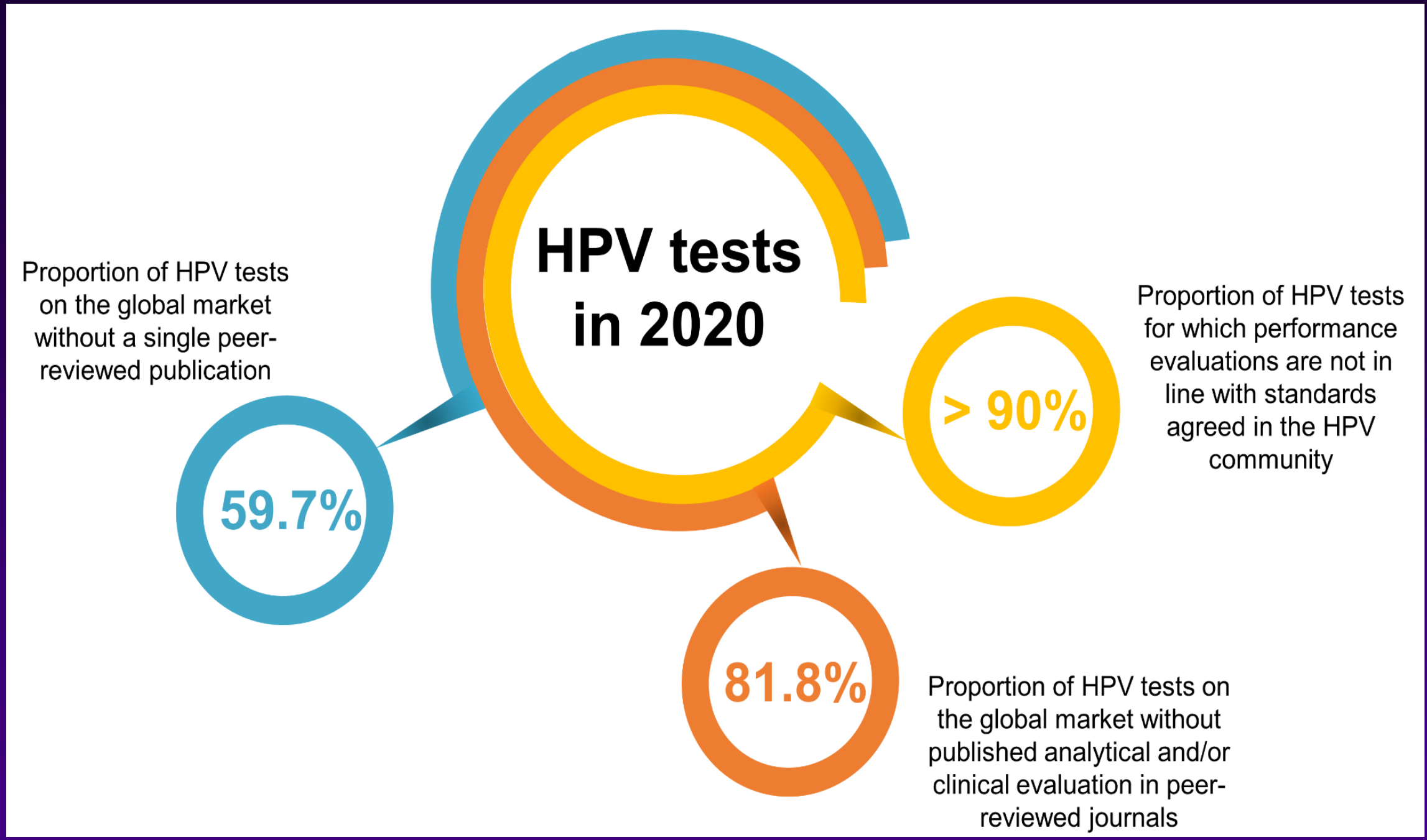
Commercially available HPV tests on the global market identified in periodical inventories in 2010, 2012, 2015 and 2020



The number of identified distinct HPV tests is shown in blue bars and the number of HPV test variants in yellow bars.

In boxes are the number of HPV tests withdrawn from the market (left arrows) and number of newly identified HPV tests (right arrows) between 2015 and 2020.

Poljak M at al.
Clin Microbiol Infect 2020; 26: 1144-50



Poljak M at al. Clin Microbiol Infect 2020; 26: 1144-50

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

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

Regulatory approvals

US Food and Drug Administration (FDA) approval

Academic validations

- International guidelines (Meijer's criteria)
- Valgent 1-4
- Academic multi-test comparisons

Regulatory approvals US Food and Drug Administration (FDA) approval

HPV testing only

cobas 4800 HPV Test, cobas 6800/8800 HPV Test, cobas 5800 HPV Test (Roche)

BD Onclarity HPV assay (BD)

Alinity m High Risk HPV Assay (Abbott)

Co-testing

Hybrid Capture 2 (hc2) HPV DNA Test (Qiagen)

Cervista HPV HR Test + Cervista HPV 16/18 Test (Hologic)

APTIMA HPV Assay + APTIMA HPV 16 18/45 genotype assay (Hologic)

cobas 4800 HPV Test, cobas 6800/8800 HPV Test, cobas 5800 HPV Test (Roche)

BD Onclarity HPV assay (BD)

Alinity m High Risk HPV Assay (Abbott)

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

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- Academic multi-test comparisons

Int. J. Cancer: **124,** 516–520 (2009)

FAST TRACK

Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older

Chris J.L.M. Meijer[1]*, Johannes Berkhof[2], Philip E. Castle[3], Albertus T. Hesselink[1], Eduardo L. Franco[4], Guglielmo Ronco[5], Marc Arbyn[6,7], F. Xavier Bosch[8], Jack Cuzick[9], Joakim Dillner[10], Daniëlle A.M. Heideman[1] and Peter J.F. Snijders[1]

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[2]*Department of Clinical Epidemiology and Biostatistics, VU University Medical Center, 1007 MB Amsterdam, The Netherlands*
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[4]*Division of Cancer Epidemiology, McGill University, Montreal, Canada*
[5]*Unit of Cancer Epidemiology, Centro per la prevenzione Oncologica, Turin, Italy*
[6]*Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium*
[7]*ECCG (European Cooperation on Development and Implementation of Cancer Screening and Prevention Guidelines), IARC, Lyon, France*
[8]*Servei d'epidemiologia, Institut Catala d'Oncologia (ICO), Hospitalet del llobregat, Barcelona, Spain*
[9]*Queen Mary's School of Medicine and Dentistry and Cancer Research UK, London, United Kingdom*
[10]*Department of Medical Microbiology University Hospital, Lund University, Malmö, Sweden*

relative clinical accuracy compared to either of two HPV tests which demonstrated lower cumulative incidence of cervical cancer 5 years after a negative HPV test than 3 years after a normal cytology in four large European randomized trials

Int. J. Cancer: **124,** 516–520 (2009)

FAST TRACK

Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older

Chris J.L.M. Meijer[1*], Johannes Berkhof[2], Philip E. Castle[3], Albertus T. Hesselink[1], Eduardo L. Franco[4], Guglielmo Ronco[5], Marc Arbyn[6,7], F. Xavier Bosch[8], Jack Cuzick[9], Joakim Dillner[10], Daniëlle A.M. Heideman[1] and Peter J.F. Snijders[1]

Requirements for HPV tests in primary cervical screening

1. A clinical sensitivity for CIN2+ not less than 90% of the clinical sensitivity of the hc2 in women of at least 30 years.

2. A clinical specificity for CIN2+ not less than 98% of the clinical specificity of the hc2 in women of at least 30 years of age.

3. Intra-laboratory reproducibility and inter-laboratory agreement with a lower confidence bound not less than 87%.

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

Regulatory approvals

US Food and Drug Administration (FDA) approval

Academic validations

- International guidelines (Meijer's criteria)
- Valgent 1-4
- Academic multi-test comparisons

The VALGENT

(clinical VALidation of human papillomavirus GENotyping Tests)

protocol provides a comprehensive design to validate and compare HPV tests using residual archived cervical cell samples

samples from 1,000-1,300 screened women

enrichment with 300 pathological samples:

- 100 x ASC-US
- 100 x LSIL
- 100 x HSIL

follow-up from the 1,300-1,600 women will identify:

- 70–130 CIN2+ cases allowing computation of clinical sensitivity
- 800-1,200 subjects without CIN, allowing computation of clinical specificity

VALGENT 1

5 HPV assays - samples derived from a Belgian biobank

VALGENT 2

6 HPV assays - samples derived from Scottish HPV archive

VALGENT 3

14 HPV assays – samples derived from Slovenian national cohort

VALGENT 4

11 HPV assays – samples from Copenhagen, Denmark

Clin Microbiol Infect 2015;21:817-26

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

Clin Microbiol Infect 2021;17:1083-95

Marc Arbyn [1, 2, *], Marie Simon [3], Eliana Peeters [1], Lan Xu [1, 4], Chris J.L.M. Meijer [5], Johannes Berkhof [6], Kate Cuschieri [7], Jesper Bonde [8], Anja Ostrbenk Vanlencak [9], Fang-Hui Zhao [10], Remila Rezhake [1, 10, 11], Murat Gultekin [12], Joakim Dillner [13], Silvia de Sanjosé [14], Karen Canfell [15, 16], Peter Hillemanns [17], Maribel Almonte [18], Nicolas Wentzensen [19, †], Mario Poljak [9, †]

[1] *Unit of Cancer Epidemiology, Belgian Cancer Centre, Scientific Institute of Public Health, Brussels, Belgium*
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[3] *Haute Autorité de Santé, Saint Denis, France*
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[14] *National Cancer Institute, Rockville, USA*
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[16] *School of Public Health, University of Sydney, Sydney, Australia*
[17] *Departments of Gynaecology and Obstetrics, Hannover Medical School, Germany*
[18] *International Agency for Research on Cancer, Lyon, France*
[19] *Division of Cancer Epidemiology and Genetics, National Cancer, Institute, Bethesda, MD, USA*

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

Marc Arbyn, Marie Simon, Silvia de Sanjosé, Megan A Clarke, Mario Poljak, Remila Rezhake, Johannes Berkhof, Victoria Nyaga, Murat Gultekin, Karen Canfell, Nicolas Wentzensen

Lancet Oncol **2022; 23: 950–60**

hrHPV mRNA testing with APTIMA HPV Test had similar cross-sectional sensitivity for CIN2+ and CIN3+ and slightly higher specificity than validated HPV DNA tests

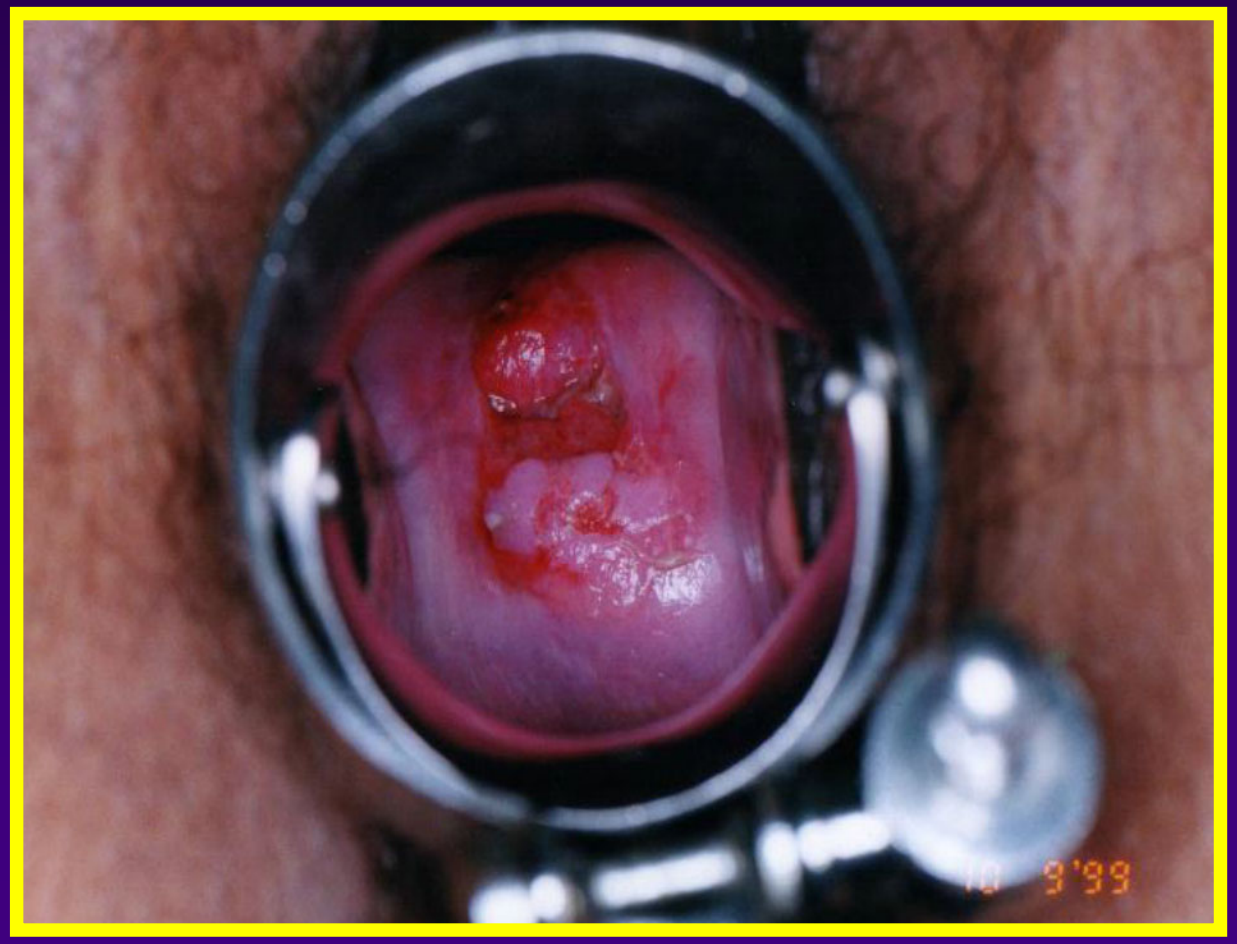
four studies with 4–7 years of follow-up showed heterogeneous safety outcomes

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 HPV Test could be accepted for primary cervical cancer screening on clinician collected cervical samples at intervals of around 5 years

APTIMA HPV Test is less sensitive on self-collected samples than clinician-collected samples

- 254+ commercial HPV assays (and 425+ variants) on the market in 2020
- 2 + 15 HPV assays fulfil cross-sectional criteria for primary screening



HPV tests for agreed indications for HPV testing in current clinical practice

HPV tests for epidemiological and vaccine-related studies

HPV tests for different research purposes

*****

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(i) set of targeted HPV types

(ii) level of analytical sensitivity

HPV tests for major agreed indications for HPV testing in current clinical practice

Broad genotype coverage	BALANCED = ARTIFICIALLY REDUCED
High analytical sensitivity	BALANCED = ARTIFICIALLY REDUCED
High analytical specificity	NECESSARY

HPV tests for epidemiological & vaccine-related studies

Broad genotype coverage	DESIRED
High analytical sensitivity	NECESSARY
High analytical specificity	NECESSARY

How to evaluate commercial HPV tests for epidemiological and vaccine-related studies ?

head-to-head comparison with one or more tests that scored the highest in the WHO HPV LabNet proficiency panels should be used as a evaluation standard

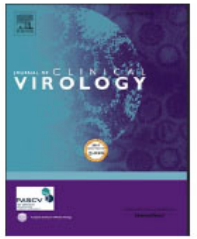
genotyping proficiency panels containing defined amounts of the international standards and candidate standards for clinically most important HPV types

Journal of Clinical Virology 154 (2022) 105237

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv



Improving human papillomavirus (HPV) testing in the cervical cancer elimination era: The 2021 HPV LabNet international proficiency study

Laila Sara Arroyo Mühr [a], Carina Eklund [a], Camilla Lagheden [a], Ola Forslund [b], Karin Dahlin Robertsson [c], Joakim Dillner [a,d,*]

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[b] Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, Lund, 221 85, Sweden
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ARTICLE INFO

Keywords:
Human papillomavirus
Quality assurance
International standards
Cervical cancer
Cancer eradication

ABSTRACT

Background: Proficient Human Papillomavirus (HPV) genotyping services are essential to support HPV and cervical cancer elimination strategies, in particular to support HPV vaccine research.
Objectives: To perform a global HPV genotyping proficiency study, with evaluation in relation to previous proficiency studies.
Study design: The proficiency panel contained 44 coded samples (40 samples containing one or more purified HPV types (HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59/68a/68b) in human DNA, 1 human DNA control and 3 DNA extraction controls). Proficiency required detection of both single and multiple infections of 50 International Units of HPV 16/18, of 500 genome equivalents for other HPV types and no false positivity.
Results: One hundred and thirty-two laboratories submitted 211 datasets. Most assays used (182/211 datasets) were commercially available. An all-time high of 75% of the datasets were 100% proficient. One or more false positives were found in 17.5% of datasets. Among laboratories who participated in the 2019 proficiency study, full proficiency increased from 25% in 2019 to 60% in 2021. The high overall proficiency was mostly attributable to a large number of new laboratories, which used similar assays.
Conclusions: The worldwide deterioration in comparability and reliability of HPV testing found in 2019 is now reversed and an overall increase in proficiency is found.

HPV testing for men?

HPV testing for partners of HPV-positive women?

HPV testing for men?

HPV testing for partners of HPV-positive women?

Fighting misperception

analytically more sensitive HPV tests are NOT better than tests with clinically validated cut-offs (analytically less sensitive HPV tests)

HPV tests that cover large number of genotypes are NOT better than tests that target only 12-14 high risk HPV genotypes

higher price ≠ better and clinically more useful HPV test

(frequent) testing with analytically highly sensitive HPV tests that cover many genotypes as follow-up after "treatment" with topical products of dubious and/or unproven medical efficacy represent misuse of HPV testing and should not be performed

only clinically validated HPV tests should be used in cervical cancer screening programs and for clinical management

only a minority of HPV tests on the market are properly clinically validated

great majority of HPV tests on the market (and offered by labs) lack proof of clinical validation and these HPV tests should not be used for clinical management