Quality review, validation and availability of HPV tests

Commercially available HPV tests in 2020

Mario Poljak, Marc Arbyn

Faculty of Medicine, University of Ljubljana, Slovenia
Scientific Institute of Public Health, Brussels, Belgium

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Commercially available alpha-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.

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- not a simple addition of newly developed tests to the old list of HPV tests
- the existence of all HPV 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 – September 2019 and January 2020
  - abstracts from main HPV-related conferences (2015-2020)
  - internal files
  - the Chinese National Medical Products Administration (formerly the China Food and Drug Administration) was consulted to obtain a list of HPV tests approved by agency

- conservative estimate – very likely haven't identified all HPV tests currently available
- omission of any particular commercially available HPV test was unintentional

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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)

Human papillomavirus 16, 18 (DNA-Technology LLC, Moscow, Russia)	DISTINCT TEST
Human papillomavirus 18, 45, 39, 59	VARIANT
Human papillomavirus 16, 31, 33, 35, 58, 52, 67	VARIANT
Human papillomavirus 6, 11	VARIANT

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2020

254 distinct commercial HPV assays
(and 425 variants)
on the global market

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Main groups of available commercial HPV molecular 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

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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 ( ) (more details in Table 2), compliance with international consensus guidelines for primary cervical cancer screening in women 30 years and older ( ), evaluation within VALGENT initiative ( ) and WHO prequalification status ( ).

27 pages document…

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

- Hybrid Capture 2 (HC2) HPV DNA Test (Qiagen Gaithersburg, Inc., MD, USA)
- EIA Kit HPV GP HR (Diassay, Ev Rijswijk, Netherlands)
- Cervista HPV HR Test (Hologic, Madison, WI, USA)
- CareHPV Test (Qiagen Gaithersburg, Inc., MD, USA)
- 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, Strasberg, 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)

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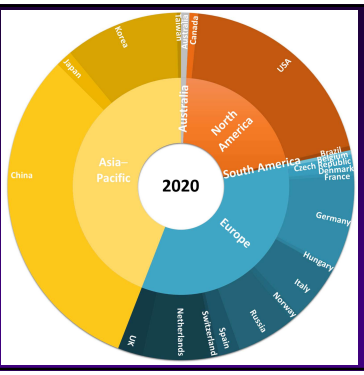

Poljak M at al. Clin Microbiol Infect 2020; in press.

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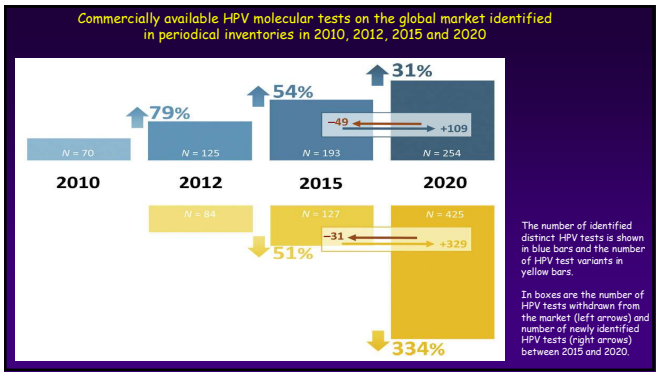

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102/254 (40.3%) of HPV tests with at least one peer reviewed publication

BUT

- 46/254 (18.2%) of HPV tests with published performance evaluation (analytical and/or clinical)
- 56/254 (22.1%) of HPV tests cross-sectional descriptive studies only - no data for key test performance characteristics (sensitivity, specificity, reproducibility)

- "test A versus test B" approach with no reference standard
- ad hoc collections of heterogeneous clinical samples without follow-up
- various target population (including several non-genital)

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

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No.	DNA chip assay	Bioneer result
1	16	16
3	others	53
2	54	54
4	Others	33
Negative sample	Negative	Negative

The clinical evaluation of the *AccuPower*® HPV Genotyping Kit was performed on 5 clinical samples. *AccuPower*® HPV Genotyping Kit is more sensitive than DNA chip assay.

http://eng.bioneer.com/diagnostic/HumanMDxkits/HPV-Genotyping-technical.aspx

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Main problems and limitations:

- manufacturers' webpages in local languages only
- lack of transparent webpages
- indirect marketing
- exclusive orientation towards the Western market
- situation on emerging markets poorly understood
- publication bias

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Sample extraction part ?

the majority of HPV tests currently on the market are not complete diagnostic assays:
- sample extraction part not included in the kit
- recommended nucleic acid extraction methodology not even mentioned in manufacturer's instructions
- only a minority of HPV tests on the market have internal controls

the extraction of DNA/RNA is an invaluable part of the HPV testing procedure !

manufacturers should validate sample extraction procedure for each of the recommended sample collection devices and clinical sample types

list of validated sample collection devices and specimen types should be provided in the manufacturer's instructions

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Issues to be resolved…

we need complete HPV diagnostic assays including sample extraction procedure !

manufacturers should put more effort into evaluating their HPV products

manufacturers should seek advice from established HPV researchers in the very early phase of development on:
- how to properly design a novel test
- define intended use of future test (clinical, epidemiological, research…)
- how to evaluate test performance that the HPV community will accept evaluation/validation results

manufacturer-independent evaluations and publication of results in peer-reviewed journals are crucial

evaluation on alternative clinical specimens needed (self-collected cervicovaginal lavage specimens, vaginal swabs, other self-collected samples, tissues, urine…)

more competitively priced HPV tests needed !

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COVID-19-related problems arising

- unprecedented health and economic impact of the COVID-19 pandemic
- extraordinary demand on a global scale for sampling devices, reagents, consumables, and diagnostic instruments needed for timely diagnosis of SARS-CoV-2 infection
- manufacturer's shift toward new niche market with unprecedented market growth opportunity
- serious COVID-19-related supply chain problems (reagents and consumables)
- shifted interest of public, agencies and medical journals ("nothing is important but COVID-19")
- temporary pause of cervical cancer screening programs
- "new normal"; unclear future; potential of second, third, fourth… waves of pandemic

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Conclusions

- 254+ commercial HPV assays (and 425+ variants) on the global market
- 60% of HPV tests on the global market without a single peer-reviewed publication
- 81% of HPV tests on the market without published performance evaluation (analytical and/or clinical) in peer-reviewed journals
- great majority of performance evaluations not in line with standards agreed in the HPV community
- several clinically unvalidated HPV assays are used worldwide in daily practice - only a small subset of HPV tests on the market has validated clinical performance
- serious COVID-19-related supply chain problems arising

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only clinically validated HPV tests should be used in cervical cancer screening

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