Presejanje s testom HPV:

kateri testi izpolnjujejo merila za uporabo v presejalnih programih?

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HPV

Viral characteristics

- non-enveloped viruses; icosahedral capsid

- remarkably diverse BUT remarkably genetically stable
  (diverged since the origin of humanity only by about 2%)

- classified by the homology of their genome into many genotypes

- genotypes numbered chronologically in order of characterization

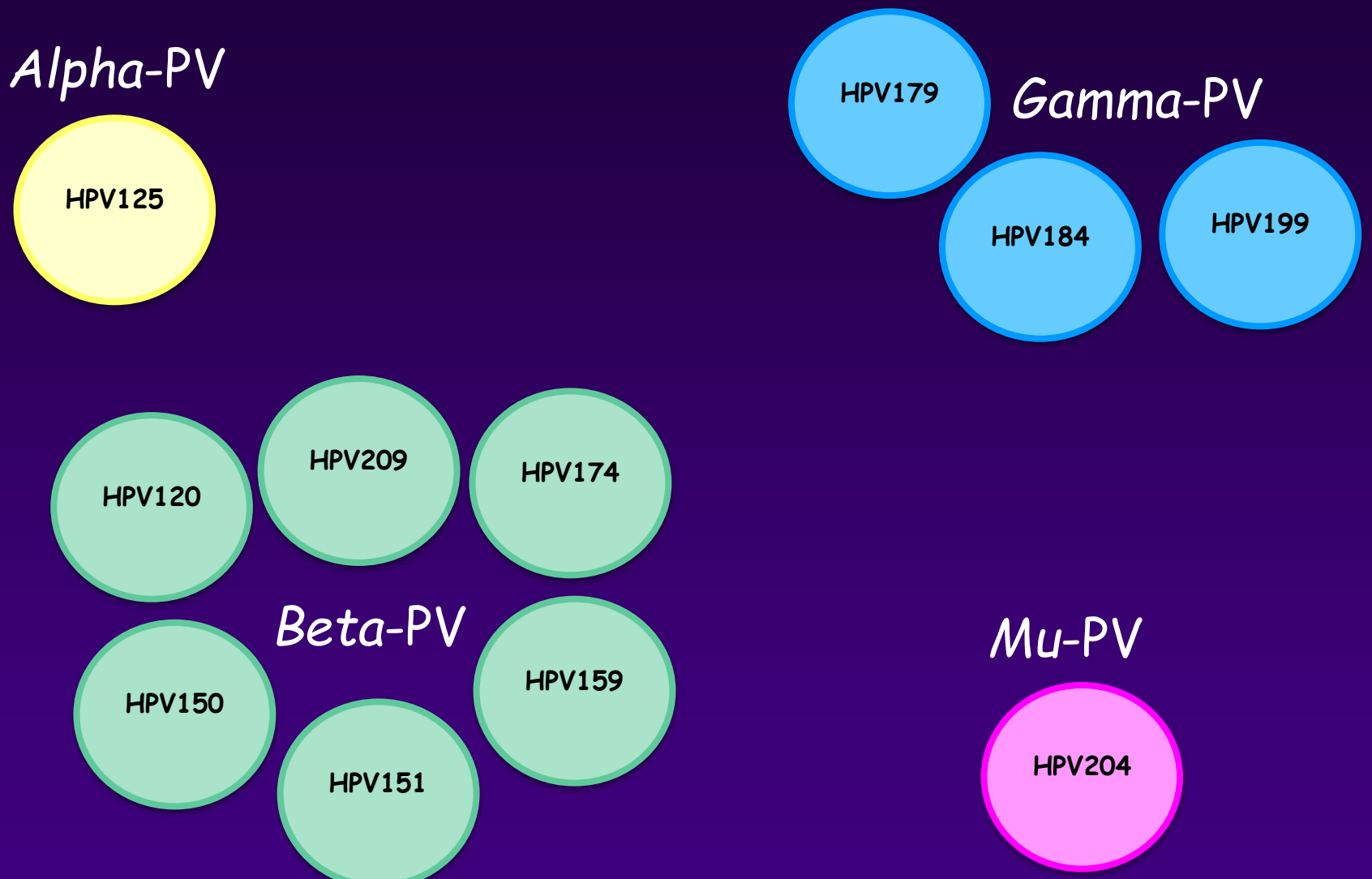
International HPV Reference Center
http://ki.se/en/labmed/international-hpv-reference-center
The PapillomaVirus Episteme (PaVE)
http://pave.niaid.nih.gov/#home
status: 16. 05. 2017

HPV-216

212 official HPV genotypes

HPV-46, HPV-55, HPV-64 and HPV-79 did not meet the criteria as a unique HPV

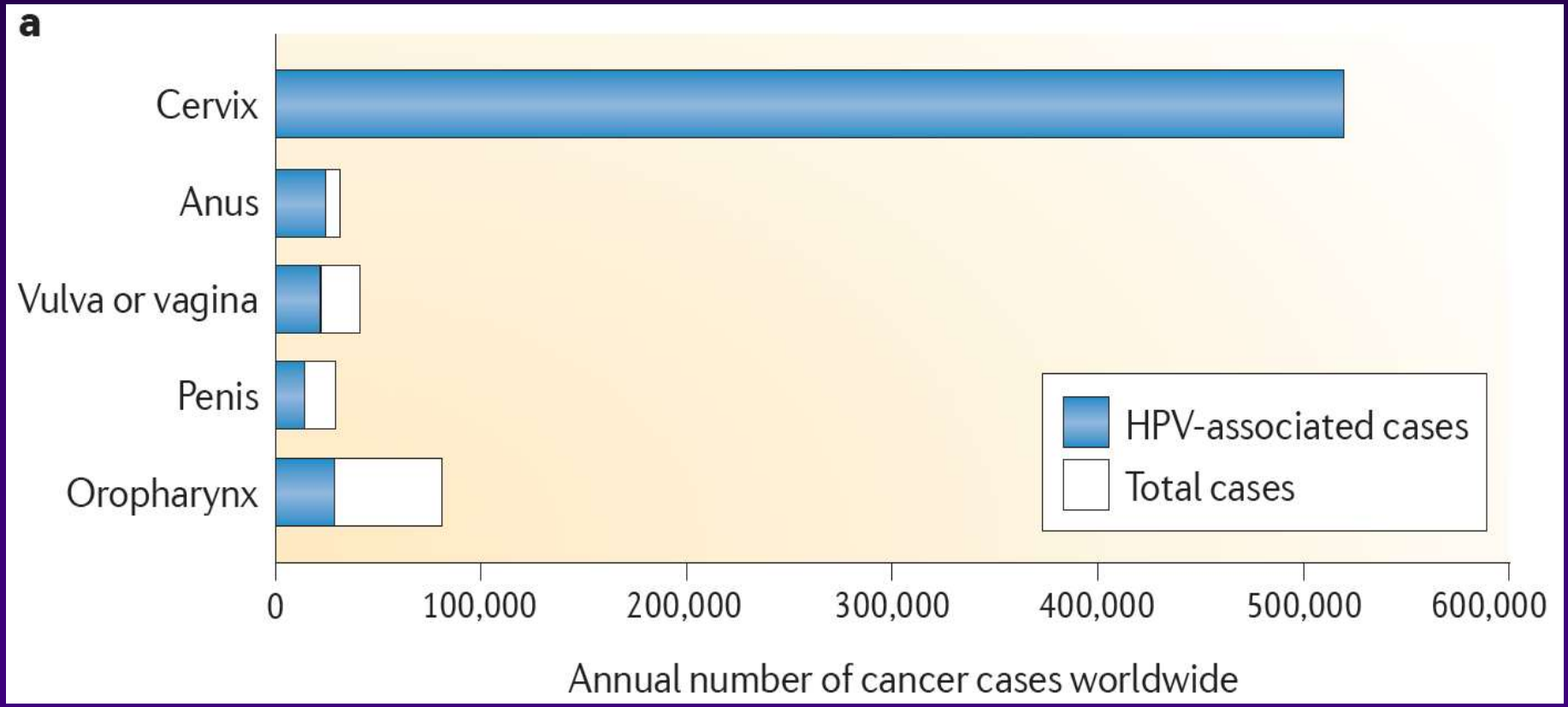
HPV types characterized in Slovenia



Genus	HPV Genotypes
Alpha-HPV	HPV-2, HPV-3, HPV-6, HPV-7, HPV-10, HPV-11, HPV-13, HPV-16, HPV-18, HPV-26, HPV-27, HPV-28, HPV-29, HPV-30, HPV-31, HPV-32, HPV-33, HPV-34, HPV-35, HPV-39, HPV-40, HPV-42, HPV-43, HPV-44, HPV-45, HPV-51, HPV-52, HPV-53, HPV-54, HPV-56, HPV-57, HPV-58, HPV-59, HPV-61, HPV-62, HPV-66, HPV-67, HPV-68, HPV-69, HPV-70, HPV-71, HPV-72, HPV-73, HPV-74, HPV-77, HPV-78, HPV-81, HPV-82, HPV-83, HPV-84, HPV-85, HPV-86, HPV-87, HPV-89, HPV-90, HPV-91, HPV-94, HPV-97, HPV-102, HPV-106, HPV-114, HPV-117, HPV-125, HPV-160, HPV-177
Beta-HPV	HPV-5, HPV-8, HPV-9, HPV-12, HPV-14, HPV-15, HPV-17, HPV-19, HPV-20, HPV-21, HPV-22, HPV-23, HPV-24, HPV-25, HPV-36, HPV-37, HPV-38, HPV-47, HPV-49, HPV-75, HPV-76, HPV-80, HPV-92, HPV-93, HPV-96, HPV-98, HPV-99, HPV-100, HPV-104, HPV-105, HPV-107, HPV-110, HPV-111, HPV-113, HPV-115, HPV-118, HPV-120, HPV-122, HPV-124, HPV-143, HPV-145, HPV-150, HPV-151, HPV-152, HPV-159, HPV-174, HPV-182, HPV-185, HPV-195, HPV-196, HPV-198, HPV-209
Gamma-HPV	HPV-4, HPV-48, HPV-50, HPV-60, HPV-65, HPV-88, HPV-95, HPV-101, HPV-103, HPV-108, HPV-109, HPV-112, HPV-116, HPV-119, HPV-121, HPV-123, HPV-126, HPV-127, HPV-128, HPV-129, HPV-130, HPV-131, HPV-132, HPV-133, HPV-134, HPV-135, HPV-136, HPV-137, HPV-138, HPV-139, HPV-140, HPV-141, HPV-142, HPV-144, HPV-146, HPV-147, HPV-148, HPV-149, HPV-153, HPV-154, HPV-155, HPV-156, HPV-157, HPV-158, HPV-161, HPV-162, HPV-163, HPV-164, HPV-165, HPV-166, HPV-167, HPV-168, HPV-169, HPV-170, HPV-171, HPV-172, HPV-173, HPV-175, HPV-176, HPV-178, HPV-179, HPV-180, HPV-181, HPV-183, HPV-184, HPV-186, HPV-187, HPV-188, HPV-189, HPV-190, HPV-191, HPV-192, HPV-193, HPV-194, HPV-197, HPV-199, HPV-200, HPV-201, HPV-202, HPV-203, HPV-205, HPV-210
Mu-HPV	HPV-1, HPV-63, HPV-204
Nu-HPV	HPV-41

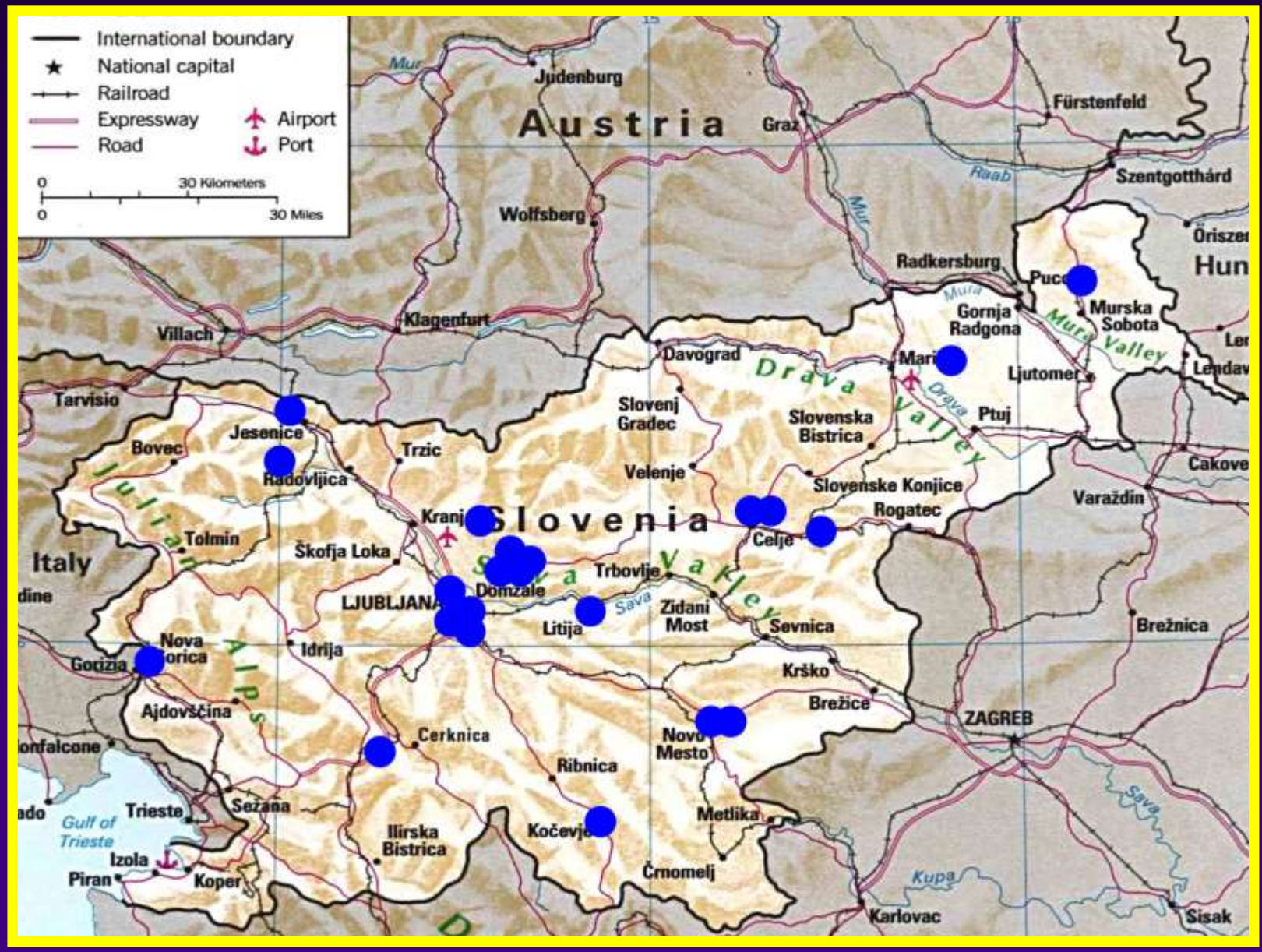
High-risk alpha HPV genotypes

HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39
HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59



Schiller JT, Lowy DR. Nat Rev Microbiol 2012;10:681-92.

Slovenian Primary Cervical Cancer Screening Cohort Study



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Maja Primic Žakelj

Transport

Miha Pirc (100,304 km)

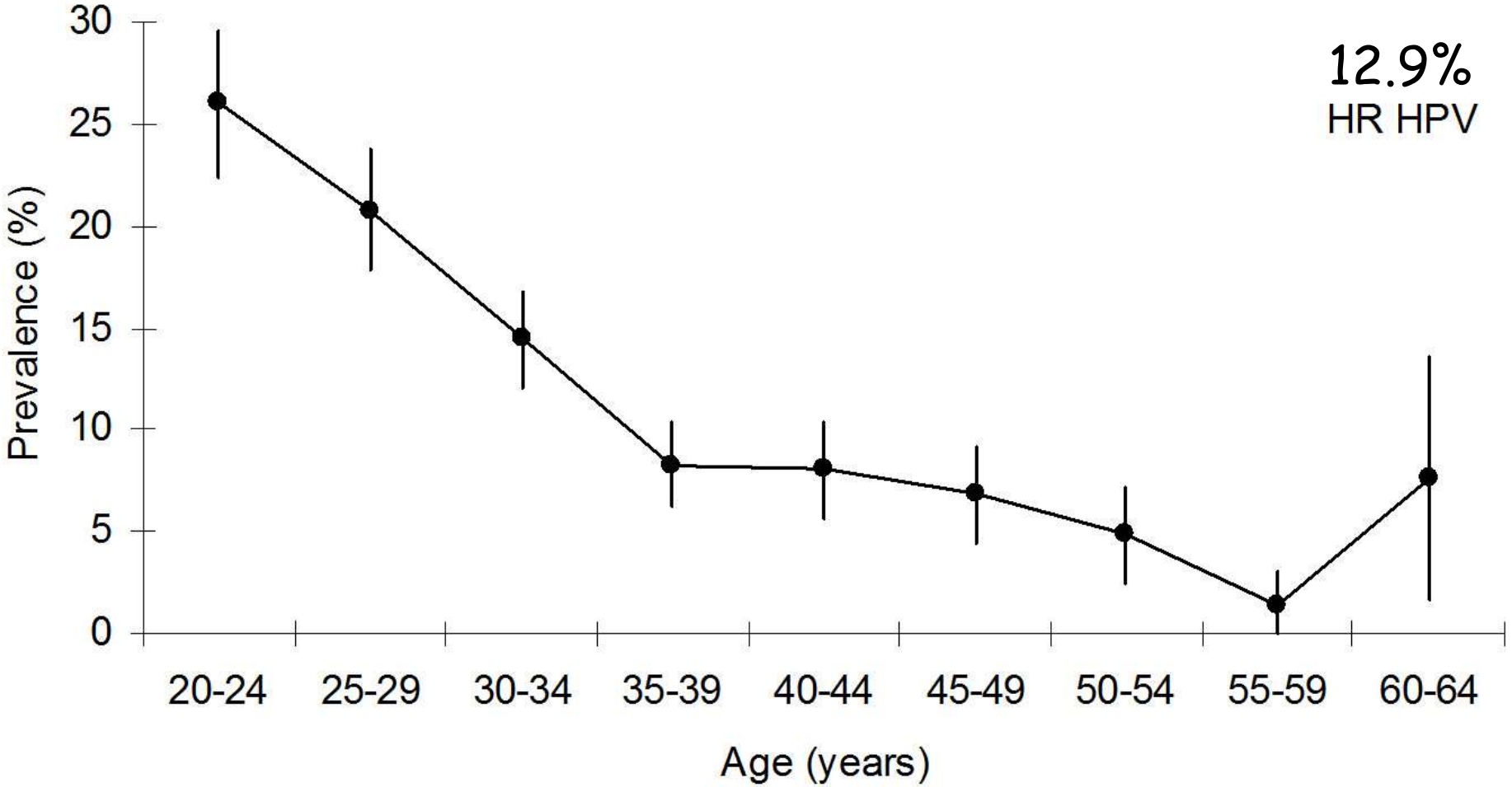
Slovenian Primary Cervical Cancer Screening Cohort Study

First screening round

4,507 unselected women aged 20-64 years, who attended routine PAP screening with local gynecologists

(Dec 2009-Aug 2010)

Prevalence of infection with 14 hr-HPV types with 95% confidence intervals according to age among 4,431 women screened for cervical cancer, Slovenia, 2010



Vaccine 2012; 30: 116-120.

Cross-sectional comparison of clinical sensitivity for the detection of CIN2+ and clinical specificity for the detection of lesions < CIN2

TOTAL COHORT

- 4,432 women (all women 20-65 years old)
  - cases (CIN2+): 57 women
  - controls: 4,375 women

cytology sensitivity for CIN2+: 66.2 % (95% CI, 53.6-76.9)

cytology sensitivity for CIN3+: 80.6 % (95% CI, 61.9-91.9)

HPV test sensitivity for CIN2+: 98.2 % (95% CI, 90.6-100.0)

Slovenian Primary Cervical Cancer Screening Cohort Study

First screening round

4,507 unselected women aged 20-64 years, who attended routine PAP screening with local gynecologists

(Dec 2009-Aug 2010)

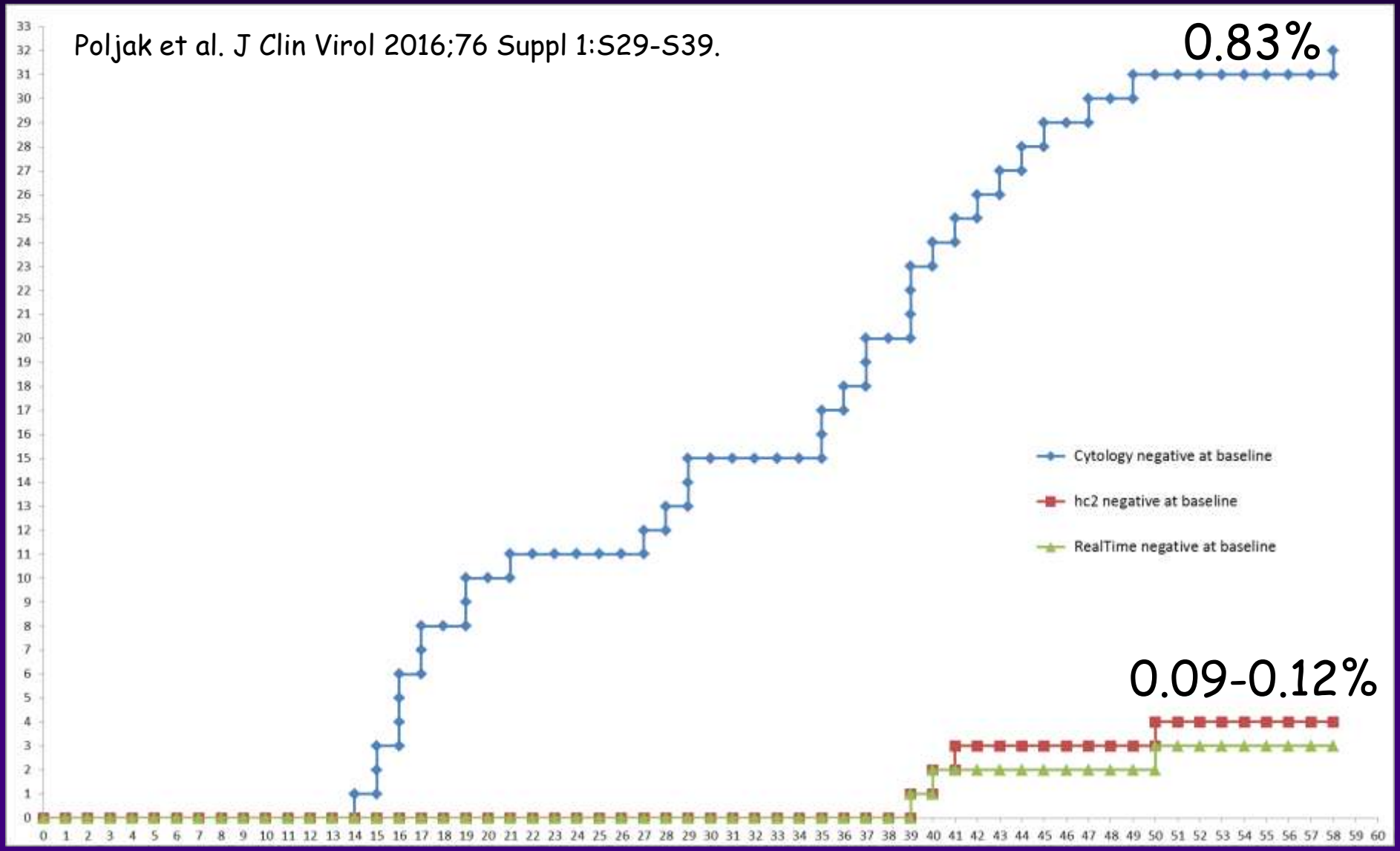
Second screening round

3,920 women that had 36-to 48-month follow-up result of hrHPV DNA and/or cytology after a baseline screening round

(Dec 2012-Dec 2014)

Time trends of incidence CIN2+ cases according to different baseline characteristic in women 20-65 years; Slovenia 2010-2014

country with cytology sensitivity of 66.2 % (95% CI, 53.6-76.9) for CIN2+ and sensitivity of 80.6 % (95% CI, 61.9-91.9) for CIN3+



Time trends of incidence CIN2+ cases according to different baseline characteristic in women 30-65 years; Slovenia 2010-2014

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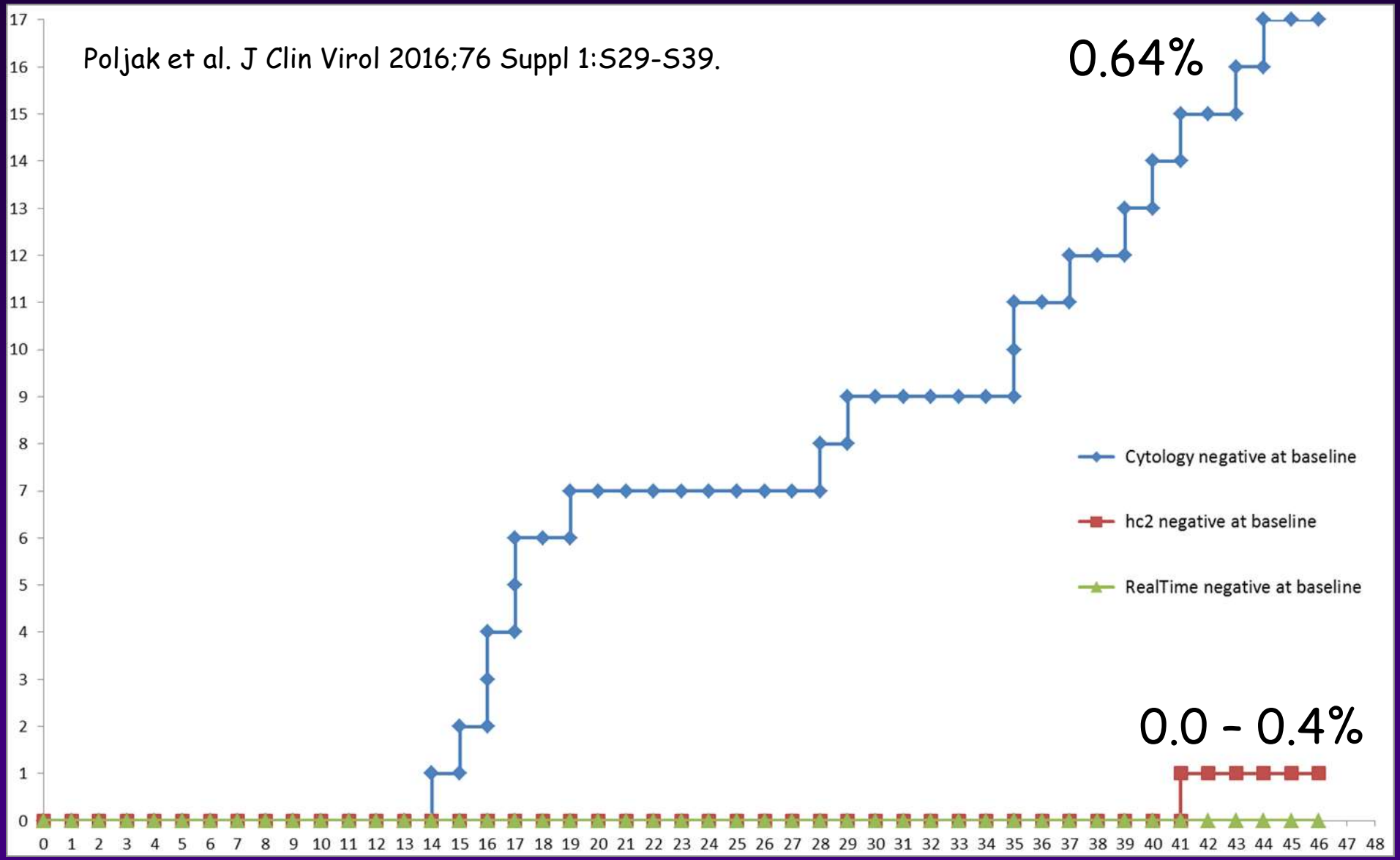


HPV !!!

HPV test ?

Detection of HPV infection

Direct detection (detection of current infection)

- light microscopy (koilocytes)
- electron microscopy (viral particles)
- detection of viral proteins
- detection of viral DNA
- detection of viral mRNA

Detection of past and/or current infection

- detection of anti-HPV antibodies (measurement of cumulative exposure)

Pre-vaccinaton seropositivity for HPV-6, HPV-11, HPV-16 and HPV-18 with 95% confidence intervals according to age among 3,259 women screened for cervical cancer in Slovenia (2009/2010)

HPV types tested: 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 68, 73

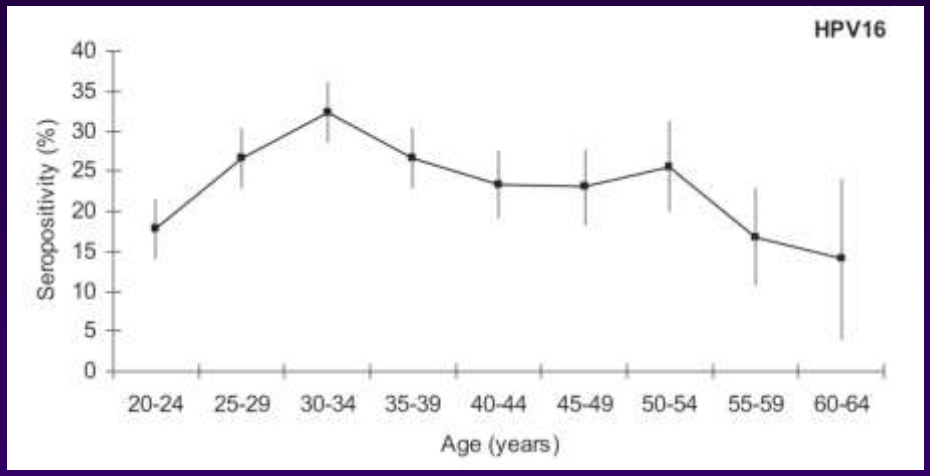
- seropositivity for any of the 15 HPV types = 65.7% (95% CI, 64.0-67.3)

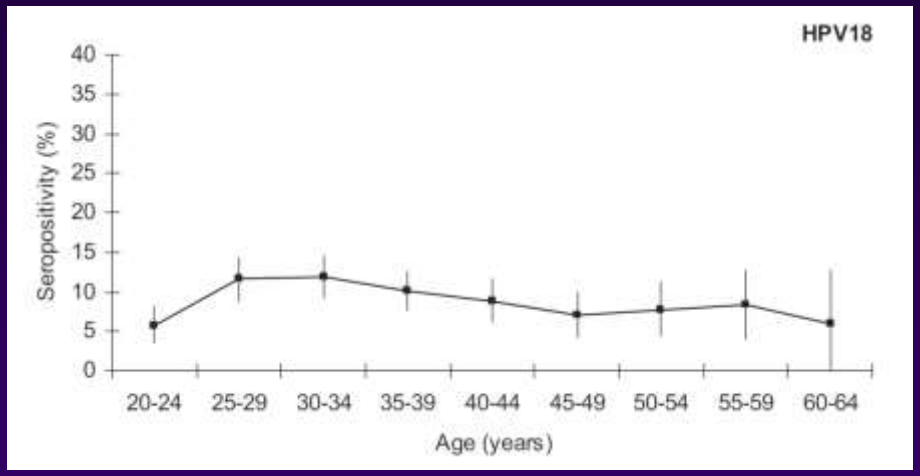
- seropositivity for any of the 11 hr-HPV types = 59.2% (95% CI, 57.5-60.9)

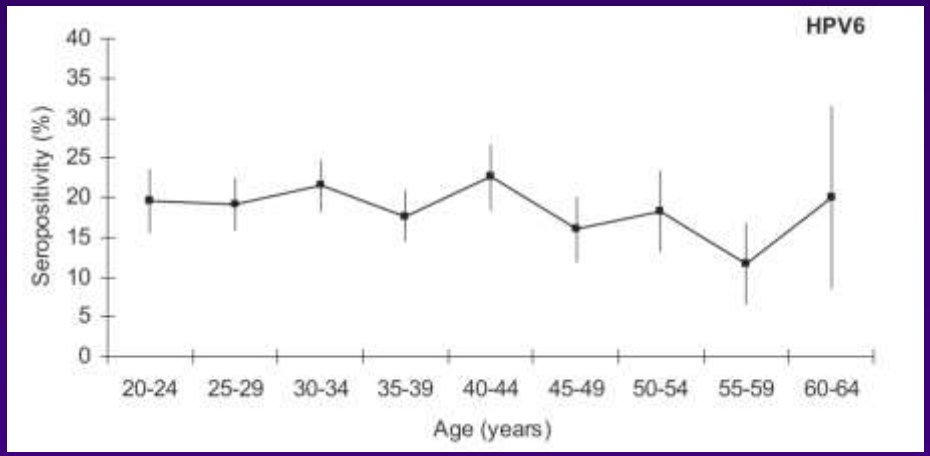
- seropositivity for any of the 4 lr-HPV types = 33.1% (95% CI, 31.5-34.7)

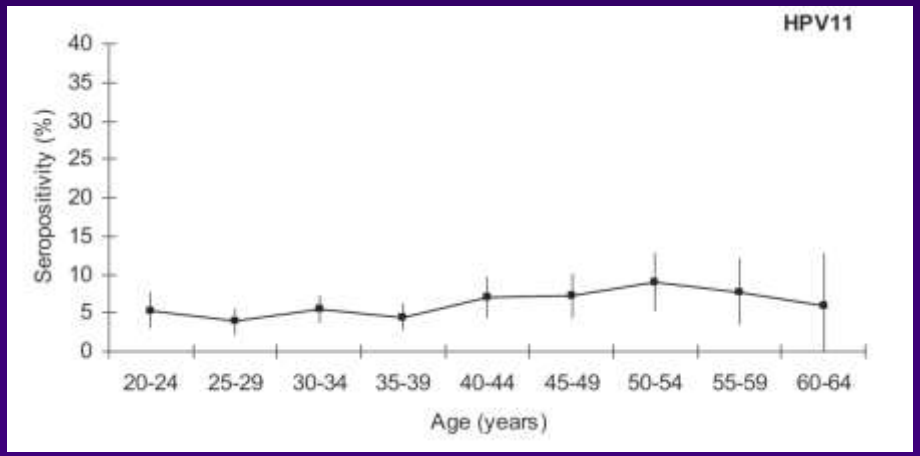
Vaccine 31 (2013) 4935–4939

Pre-vaccinaton seropositivity for HPV-6, HPV-11, HPV-16 and HPV-18 with 95% confidence intervals according to age among 3,259 women screened for cervical cancer in Slovenia (2009/2010)









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Detection of past and/or current infection

- detection of anti-HPV antibodies (measurement of cumulative exposure)

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-F106.

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.

- not 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
  - abstracts from main HPV-related conferences
  - internal files

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

Test vs. test variant

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

HPV TS 16 PCR-DEIA (Labo Bio-medical Products, Ev Rijswijk, Netherlands)

HPV TS 18 PCR-DEIA

HPV TS 31 PCR-DEIA

HPV TS 45 PCR-DEIA

2010 70 commercial HPV assays on the market

2012 125 commercial HPV assays (and 84 variants) on the market

2015 193 commercial HPV assays (and 127 variants) on the market

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

Surprising finding: extensive intra-manufacturer dynamics

manufacturers are constantly changing the design and names of their tests, resulting in delayed and non-updated data presented on vendors' webpages

data presented on the manufacturers' webpages not necessarily reliable

repeated contacts with responsible person(s) in diagnostic companies (mainly those not regularly present at major conferences) needed

main challenge in building HPV tests database is finding a reliable, longstanding person in each company to address questions and obtain reliable data

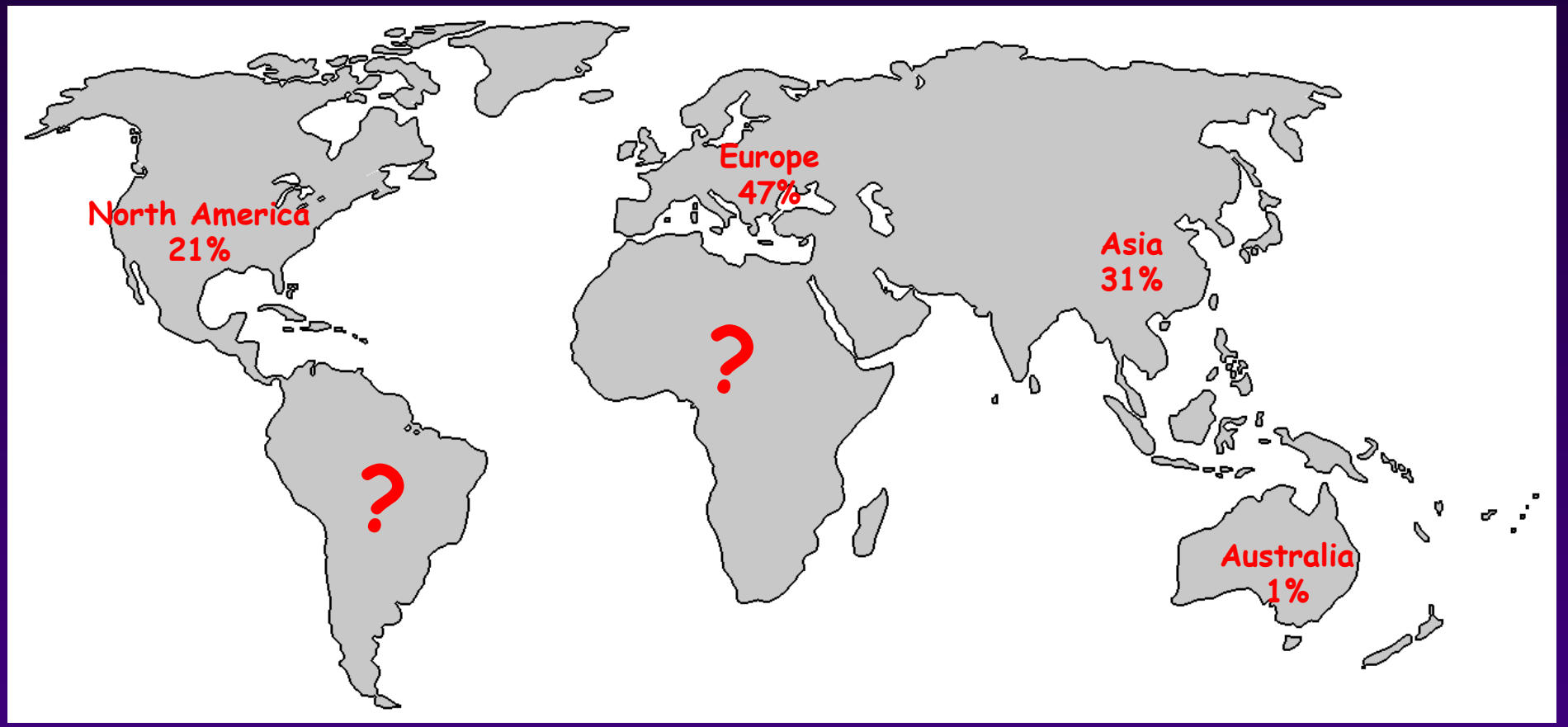
Problems with published data

some tests with published evaluation(s) never appear on the market

inaccurate names of the HPV tests often used in peer-reviewed publications

authors should consistently use accurate names of HPV tests in their publications !!!

Manufacturers' distribution by continent according to the number of different HPV tests on the market in September 2015



- 110/193 (57%) of HPV tests with at least one publication
- dramatical improvement from 2012 (25% vs. 57%)

BUT

- only 69/193 (35.7%) of HPV tests with published performance evaluation (analytical and/or clinical)
- 41/193 HPV tests only cross-sectional descriptive studies - 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)

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

- the number of commercial HPV tests will continue to increase in the near future, due to promising marketing opportunities

- "traditional" molecular diagnostic microbiology testing areas are very mature, expected annual growth rates app. 5%

- growth rates of HPV tests expected to remain as high as 20% through 2020

- the total global cervical cancer diagnostic test market valued at USD 5.9 billion in 2013 and is expected to reach USD 8.9 billion in 2020

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 which define the purpose of the HPV test

(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 completely from transient HPV infections

based on prediction of cervical cancer and NOT the presence of virus

Pathogenic role of the eight probably/possibly carcinogenic HPV types 26, 53, 66, 67, 68, 70, 73 and 82 in cervical cancer

Gordana Halec,[1] Laia Alemany,[2,3] Belen Lloveras,[2,4] Markus Schmitt,[1] Maria Alejo,[5] Franz X Bosch,[6] Sara Tous,[2] Jo Ellen Klaustermeier,[2] Nuria Guimerà,[2,7] Niels Grabe,[8,9] Bernd Lahrmann,[8,10] Lutz Gissmann,[1,11] Wim Quint,[7] Francesc X Bosch,[2] Silvia de Sanjose,[2,3] and Michael Pawlita[1] on behalf of the Retrospective International Survey and HPV Time Trends Study Group†

J Pathol 2014;234:441-51.

97% of cervical cancers caused by 12 HR-HPVs:
HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59

eight HPV types rarely, but consistently identified as single HPV types in about 3% of cervical cancers

55 CxCa tissues harbouring single pHR-HPV infections (2–13 cases per type) and 266 tissues harbouring single HR-HPV (7–40 cases per type) from a worldwide cross-sectional study

in 55 CxCa tissues:
E6 mRNA expression 100%; high p16INK4a 98%; low pRb 96%; low CyD1 93%; and low p53 84%

individual frequencies of five markers compared to HPV16 as a reference did not differ significantly

eight HPV types, when present as a single infection in CxCa, are biologically active and affect the same cellular pathways as any of the fully recognized carcinogenic HR-HPV types

although this evidence is crucial for HPV-type carcinogenicity classification, per se it is not sufficient for inclusion of these HPV types into population-wide primary and secondary prevention programmes

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 !!!!

CIN 2+

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 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

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

Regulatory approvals

US Food and Drug Administration (FDA) approval

Co-testing (every 5 years, >=30 years)

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

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

cobas 4800 HPV Test (Roche)

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

HPV testing only (every 3 years, >=30 years)

cobas 4800 HPV Test (Roche)

several HPV tests on the market have been marked with the European Conformity mark for In Vitro Diagnostics (CE-IVD) compliant for diagnostic use in Europe

CE-IVD marking process is mainly technical and is substantially less demanding in comparison with the US Food and Drug Administration (FDA) or the Australian Therapeutic Goods Administration evaluation procedure

althought CE-IVD marking is necessary for an HPV test to be legally placed on the European market, CE-IVD marking per se does not particularly impress the great majority of laboratory directors or issuers of public tenders when deciding/requesting which HPV test to use

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 (PREDICTORS 3)

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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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[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

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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%.

Clinical validation of Anyplex™ II HPV HR Detection according to the guidelines for HPV test requirements for cervical cancer screening

A.T. Hesselink[a], R. Sahli[b], J. Berkhof[c], P.J.F. Snijders[d], M.L. van der Salm[a], D. Agard[d], M.C.G. Bleeker[d], D.A.M. Heideman[d,*]

[a] Self-screen B.V., Amsterdam, The Netherlands
[b] Institute of Microbiology, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland
[c] Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands
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ABSTRACT

Background: Anyplex™ II HPV HR Detection (Seegene, Seoul, Korea) is a multiplex real-time PCR using tagging oligonucleotide cleavage and extension (TOCE) technology for simultaneous detection and genotyping of 14 high-risk (HR) HPV types, including HPV16 and HPV18.
Objectives: To evaluate whether the clinical performance and reproducibility of Anyplex™ II HPV HR Detection meet the international consensus guidelines for HPV test requirements for cervical cancer screening [1].
Study design: The clinical performance of Anyplex™ II HPV HR Detection for detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) was determined relative to that of the reference assay, i.e., HR HPV GP5+/6+-PCR-EIA, by analysis of a total of 879 cervical liquid based cytology (LBC) specimens from a screening population, of which 60 were from women with CIN2+. The intra-laboratory reproducibility and inter-laboratory agreement were determined on 509 LBC samples, of which 172 were positive by the reference assay.
Results: Anyplex™ II HPV HR Detection showed a clinical sensitivity for CIN2+ of 98.3% (59/60; 95% CI: 89.1–99.8) and a clinical specificity for CIN2+ of 93.6% (764/816; 95% CI: 89.8–96.1). The clinical sensitivity and specificity were non-inferior to those of HR HPV GP5+/6+-PCR-EIA (non-inferiority score test: $P = 0.005$ and $P = 0.023$, respectively). Both intra-laboratory reproducibility (96.8%; 95% CI: 95.3–98.1; kappa value of 0.93) and inter-laboratory agreement (96.0%; 95% CI: 94.3–97.4; kappa value of 0.91) were high.
Conclusions: Anyplex™ II HPV HR Detection performs clinically non-inferior to HR HPV GP5+/6+-PCR-EIA. Anyplex™ II HPV HR Detection complies with international consensus validation metrics for HPV DNA tests for cervical cancer screening [1].

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

Clin Microbiol Infect 2015;21:817-26

M. Arbyn[1], P. J. F. Snijders[2], C. J. L. M. Meijer[2], J. Berkhof[3], K. Cuschieri[4], B. J. Kocjan[5] and M. Poljak[5]

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UPDATE OF THE LIST OF HPV ASSAYS THAT FULFILL REQUIREMENTS FOR PRIMARY CERVICAL CANCER SCREENING

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submitted

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

		Evaluated assay		Comparator assay			Evaluated/ comparator assay		Non-inferiority test*		
		Absolute			Absolute		Relative				
Evaluated assay	Study	sensitivity	specificity	Comparator assay	sensitivity	specificity	sensitivity	specificity	p_{sens}	p_{spec}	Validation level‡
GP5+/6+ EIA	Meijer, 2009 [9]	98.7%	96.0%	HC2*	98.7%	94.1%	1.00	1.02	0.0037	<0.0001	⊕⊕⊕⊕
PapilloCheck	Hesselink, 2010 [19]	95.8%	96.7%	GP5+/6+ EIA	96.4%	97.7%	0.99	0.99	<0.0001	0.0072	⊕⊕
Abbott RT hrHPV test	Carozzi, 2011 [20]	96.4%	92.3%	HC2	97.6%	92.6%	0.99	1.00	0.0040	0.0087	⊕⊕⊕
	Poljak, 2011 [21]	100.0%	93.3%	HC2	97.4%	91.8%	1.03	1.02	0.0112	0.0000	
	Hesselink, 2013 [22]	95.6%	92.0%	GP5+/6+ EIA	98.5%	91.8%	0.97	1.00	0.0278	0.0003	
cobas 4800	Heideman, 2011 [23]	90.0%	94.6%	HC2	91.7%	94.4%	0.98	1.00	0.0216	0.0009	⊕⊕⊕
	Lloveras, 2013 [24]	98.3%	86.2%	HC2	98.3%	85.3%	1.00	1.01	0.0093	0.0012	
qPCR(*E6/E7*)	Depuydt, 2012 [25]	93.5%	95.6%	HC2	83.9%	94.4%	1.11	1.01	0.0001	<0.0001	⊕⊕
APTIMA	Heideman, 2013 [26]	95.5%	94.5%	GP5+/6+ EIA	100.0%	93.6%	0.96	1.01	0.0394	0.0002	x
Cervista	Boers, 2014 [27]	89.0%	91.2%	HC2	93.4%	88.8%	0.95	1.03	0.0043	<0.0001	⊕
	Alameda, 2015 [28]	98.4%	85.2%	HC2	100.0%	86.4%	0.98	0.99	0.0122	0.3170†	
BD Onclarity	Ejegod, 2014 [30]	92.9%	87.7%	HC2	94.2%	88.8%	0.99	0.99	0.0009	0.0216	⊕⊕
HPV-Risk assay	Hesselink, 2014 [29]	97.1%	94.3%	GP5+/6+ EIA	97.1%	94.1%	1.00	1.00	0.0056	0.0003	⊕⊕

* p values for non-inferiority of the evaluated assay compared to the comparator assay.
† We corrected an error in Alameda, 2015 [28] (due to switching of + and − columns and rows). The corrected data showed that the non-inferiority test was not significant for specificity.
‡Validation level for the test accuracy criterion as proposed by Meijer *et al* 2009 [9].
⊕⊕⊕⊕ validated in large randomized controlled trials with cancer incidence as an outcome; considered as standard comparator tests.
⊕⊕⊕: fully validated in multiple studies.
⊕⊕ fully validated in one study.
⊕ partially validated.
X not evaluated since not a hrHPV DNA assay.

Arbyn et al. Clin Microbiol Infect 2015;21:817-26.

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

EIA kit HPV GP GP5+/6+ HR

cobas 4800 HPV Test (Roche)

APTIMA HPV Assay (Hologic, Gen-Probe)

Cervista HPV HR Test (Hologic)

RealTime High Risk HPV test (Abbott)

PapilloCheck HPV-screening test (Greiner Bio-One)

Real-time quantitative PCR (qPCR) assay targeting the E6 and E7 genes (Belgian private lab)

HPV-Risk assay (Self-Screen)

BD Onclarity HPV Assay (Becton Dickinson)

LMNX genotyping kit HPV GP HR (Diassay) - previous digene HPV Genotyping LQ Test

Anyplex II HPV HR (Seegene)

Xpert HPV (Cepheid)

Requirements for HPV tests in primary cervical screening

Longitudinal data ?

(at least 36+ months)

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

EIA kit HPV GP GP5+/6+ HR

cobas 4800 HPV Test (Roche)

RealTime High Risk HPV test (Abbott)

APTIMA HPV Assay (Hologic, Gen-Probe)

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 (PREDICTORS 3)

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 according to local guidelines will identify 70–130 CIN2+ cases and about 800-1,200 subjects without CIN, allowing computation of cinical sensitivity and specificity

VALGENT: A protocol for clinical validation of human papillomavirus assays

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ABSTRACT

Background: Testing for high-risk HPV is more effective in primary cervical cancer screening than the cytological examination of a Pap smear. Separate genotyping may be useful for triage in both HPV-based and cytology-based screening. Only clinically validated tests should be used in clinical practice.
Objectives: VALGENT is a study framework for test comparison and validation of HPV assays in general and HPV genotyping tests in particular according to clinically relevant outcomes and for clinical applications endorsed by scientific evidence.
Study design: VALGENT involves the collation of fresh or archived cervical cell specimen from women attending routine screening supplemented with cytologically abnormal samples. Multiple aliquots of residual material are sent from a central laboratory to participating laboratories for testing with novel HPV assays with limited, extended or full genotyping capacity. Outcomes are derived from screening and pathology registries. Each VALGENT panel includes an assay already validated for screening. A series of accuracy and concordance statistics were generated.
Results: Currently, two VALGENT study rounds, originated from laboratories in Antwerp (Belgium) and Edinburgh (Scotland), were completed. Two new assays (G5+/6+ PCR-LMNX and Xpert HPV) were validated for screening by showing similar accuracy for cervical precancer as the standard comparator test. For two other tests (BD Onclarity, PapilloCheck) validation was confirmed. Inter-test agreement was high although certain type-specific discordances were observed which warrant further analysis.
Conclusion: VALGENT extends current guidelines for high-risk HPV test validation in cervical cancer screening and has produced a large study resource for test comparison. More robust procedures of sample selection and handling and integration with the global WHO reference laboratory network focusing on analytical accuracy, may result in the generation of an international standard and a formalized system for clinical validation of HPV assays and quality control in HPV-based screening.

VALGENT 1

5 HPV assays - samples derived from a Belgian biobank

VALGENT 2

6 HPV assays - samples derived from Scottish HPV archive

VALGENT 3

13 HPV assays – samples derived from Slovenian national cohort

VALGENT 4

Danish samples

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

GP5+/6+ PCR-based EIA kit HPV GP HR (Diassay, Rijswijk, the Netherlands) for simultaneous identification of 14 hrHPV types, and LMNX Genotyping Kit GP HR (Diassay) for full genotyping of 18 types (DDL Diagnostic Laboratory, Rijswijk, The Netherland)

cobas 4800 HPV Test (Roche)

RealTime High Risk HPV test (Abbott)

HPV-Risk assay (Self-Screen BV)

Multiplex RT-qPCR, a real-time quantitative PCR targeting E6 or E7 genes of 17 HPV types (AML laboratory, Antwerp, Belgium)

Anyplex II HPV HR Detection (Seegene)

14 High-risk HPV with 16/18 Genotyping Real-time PCR kit (Hybribio)

Linear Array HPV Genotyping Test (Roche)

INNO-LiPA HPV Genotyping Extra II (Fujirebio)

EUROArray HPV (EUROIMMUN AG)

Anyplex II HPV28 Detection (Seegene)

21 HPV GenoArray Diagnostic Kit (Hybribio)

VALGENT-3
13 tests !

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Clinical and analytical performance of the PapilloCheck HPV-Screening assay using the VALGENT framework

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ABSTRACT

Background: The benefit of HPV testing for cervical cancer screening and disease management has been shown in many recent studies and is part of several new evidence-based guidelines. Assessment of emerging HPV tests in this context is essential, using well-annotated samples, such as those generated via the Validation of Genotyping Tests-HPV (VALGENT) framework.
Objective: Our aim was to assess the PapilloCheck HPV assay in terms of absolute and relative accuracy for primary cervical cancer screening, using a standard comparator test (GP5+/6+EIA)already validated in randomised trials.
Study design: Type-specific HPV prevalence was stratified by age and cytology grade and compared with the luminex typing assay incorporating a GP5+6+ PCR (GP5+/6+ LMNX Assay). Clinical outcomes were compared with GP5+/6+EIA.
Results: Prevalence of hrHPV types (high-risk HPV) increased with severity of cytology. The concordance between PapilloCheck and the GP5+/6+ LMNX Assay was excellent when assessed at the qualitative hrHPV presence/absence level also at the type-specific level in the whole population and in women over 30 years of age. Absolute clinical sensitivity and specificity of the PapilloCheck was high and ranged between 95.5% and 98.2% for sensitivity and between 82.7% and 91.6% for specificity, depending on the outcome and population.
Conclusion: The sensitivity and specificity of this assay for the outcomes of CIN2+ were similar to those of the standard comparator assay, GP5+/6+ EIA.

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 (PREDICTORS 3)

PREDICTORS 3

Comparing the performance of six human papillomavirus tests in a screening population

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Br J Cancer 2013;108:908-13

Background: Several new assays have been developed for high-risk HPV testing of cervical samples; we compare six HPV tests in a screening population.

Methods: Residual material from liquid-based PreservCyt samples was assayed. Four tests (Hybrid Capture 2, Cobas, Abbott and Becton-Dickinson (BD)) measured HPV DNA while two used RNA (APTIMA and NorChip).

Results: Positivity rates ranged from 13.4 to 16.3% for the DNA-based tests with a significantly lower positivity rate for the Abbott assay. The Gen-Probe APTIMA assay was positive in 10.3% of women, which was significantly lower than all the DNA tests; the NorChip PreTect HPV-Proofer test was much lower at 5.2%. 40 CIN2+ cases were identified, of which 19 were CIN3+. All CIN3+ cases were HPV positive by all tests except for one, which was negative by the Abbott assay and five which were negative by the NorChip test.

Conclusion: All HPV tests except NorChip showed high sensitivity for high-grade lesions positive by cytology, suggesting co-testing is unnecessary when using HPV tests. Positivity rates in cytology-negative specimens were similar for the DNA-based tests, but lower for the APTIMA test suggesting this maintains the high sensitivity of DNA tests, but with better specificity.

- 193+ commercial HPV assays (and 127+ variants) on the market
- 2 + 11 HPV assays fulfil cross-sectional criteria for primary screening
- 2 + 3 HPV assays have at least 36+ months longitudinal data

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

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 and vaccine-related studies

Broad genotype coverage	NECESSARY
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

Continuing global improvement in HPV genotyping services

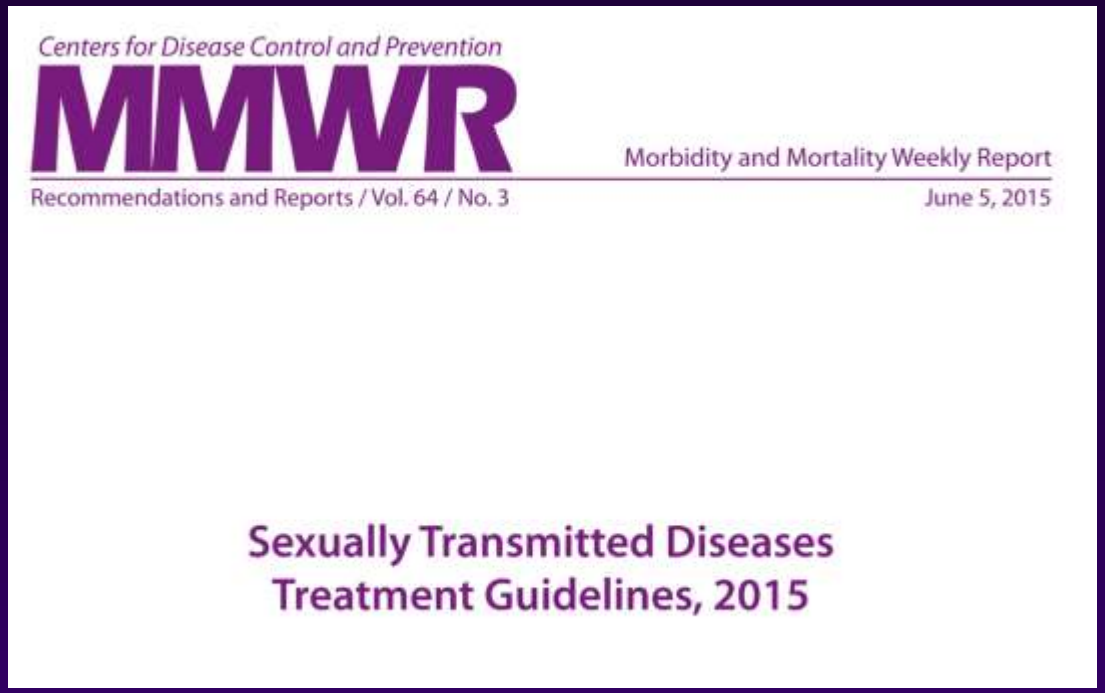
Technical Report on the Global HPV LabNet 2013 HPV DNA Genotyping Proficiency Panel

Prepared by

Carina Eklund[1], Keng-Ling Wallin[2], Ola Forslund[3] &

Joakim Dillner[1]

HPV testing for men ?



…"HPV test are available for women aged >30 years undergoing cervical cancer screening. These tests **should not be used for men**, …"

No HPV test is approved by FDA or appropriately clinically validated for detection of anogenital HPV infection in men.

HPV testing should not be used to screen partners of women with HPV!


HPV testing
for men ?

non-validated HPV tests should not be used in clinical management