



Sexual and reproductive health and rights
today and tomorrow

*ICRH celebrates 20 years of SRHR research,
training and advocacy*

4 & 5 December 2014, Het Pand, Gent (Belgium)

VALGENT (validation of HPV genotyping assays) implications for the introduction of primary HPV screening

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Introduction

- **Introduction of HPV screening and vaccination requires good HPV assays for:**
 - **Accurate detection of progressive infections (clinical accuracy)**
 - **Monitoring of HPV vaccination effects (analytical accuracy)**
- **Framework for assessment of analytical accuracy: WHO reference laboratory**
- **Framework for clinical performance: VALGENT**

VALGENT protocol

- **Archived cervical cell material**
 - 1000 continuous routine samples
 - Enriched by 300 pathological samples:
 - 100 ASC-US
 - 100 LSIL
 - 100 HSIL
 - Outcome monitored using usual follow-up procedures
 - Expected:
 - ≥ 90 women with CIN2+ (sensitivity)
 - ~900 women with 2 consecutive negative cyto results (specificity)
- **Testing with 4+7 HPV genotyping tests (Antwerp, Edinburgh)**
- **Data: ID, age, original cytology, follow-up cytology & histology, HPV test results**

Outcomes

- **Sensitivity for CIN2+, specificity (using double cyto-negative women)**
- **Relative sensi/speci vs GP5+6+**
- **Type concordance**
- **Definition of optimal cutoffs for test positivity**
- **Translation tables for HPV surveys (test+ matrices)**
- **Network of accuracy data obtained from different panels of samples (Antwerp, Edinburgh, ...)**
- **Ranked list of HPV tests ~ test performance**
- **Clinical validation of genotyping tests (~Meijer, 2009)**

Population from which samples are collected

- Methods used to find CIN2+ and non-diseased matters
- Antwerp: screening with very sensitive test & cytology interpreted with knowledge of HPV status => can generate more CIN2+ (a) than in cytology-based screening [Edinburg] (b) (Benoy, 2011 CEBP)
 - Situation a: advantage for sensitive tests
 - Situation b: advantage for specific tests

Assays evaluated

● VALGENT 1

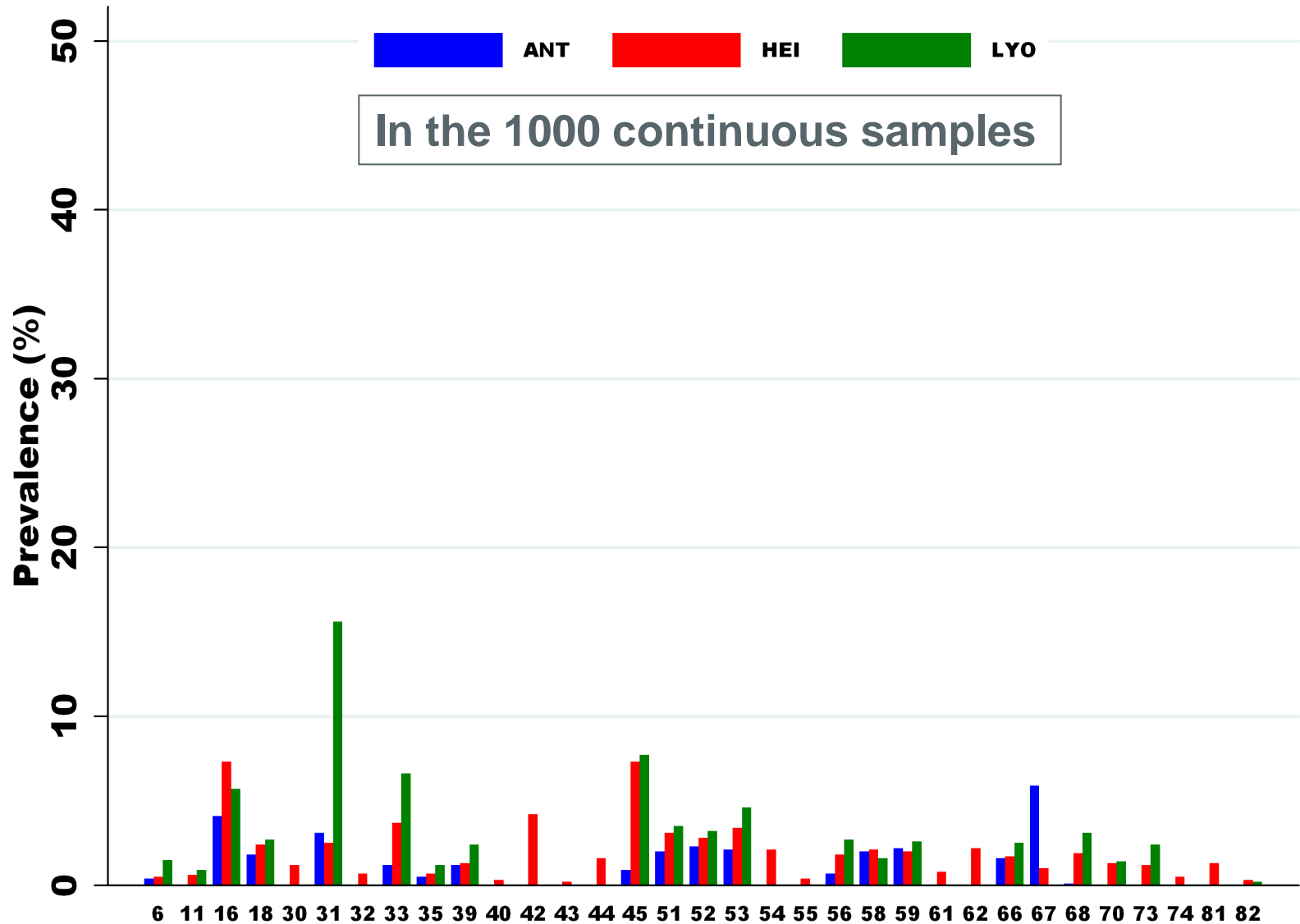
- qRTPCR (Antwerp)
- BSGP5+/6+-PCR/MPG assay (Heidelberg)
- Multiplex PCR/APEX assay (Lyon)
- GP5+/6+-EIA (Voorburg)

● VALGENT 2

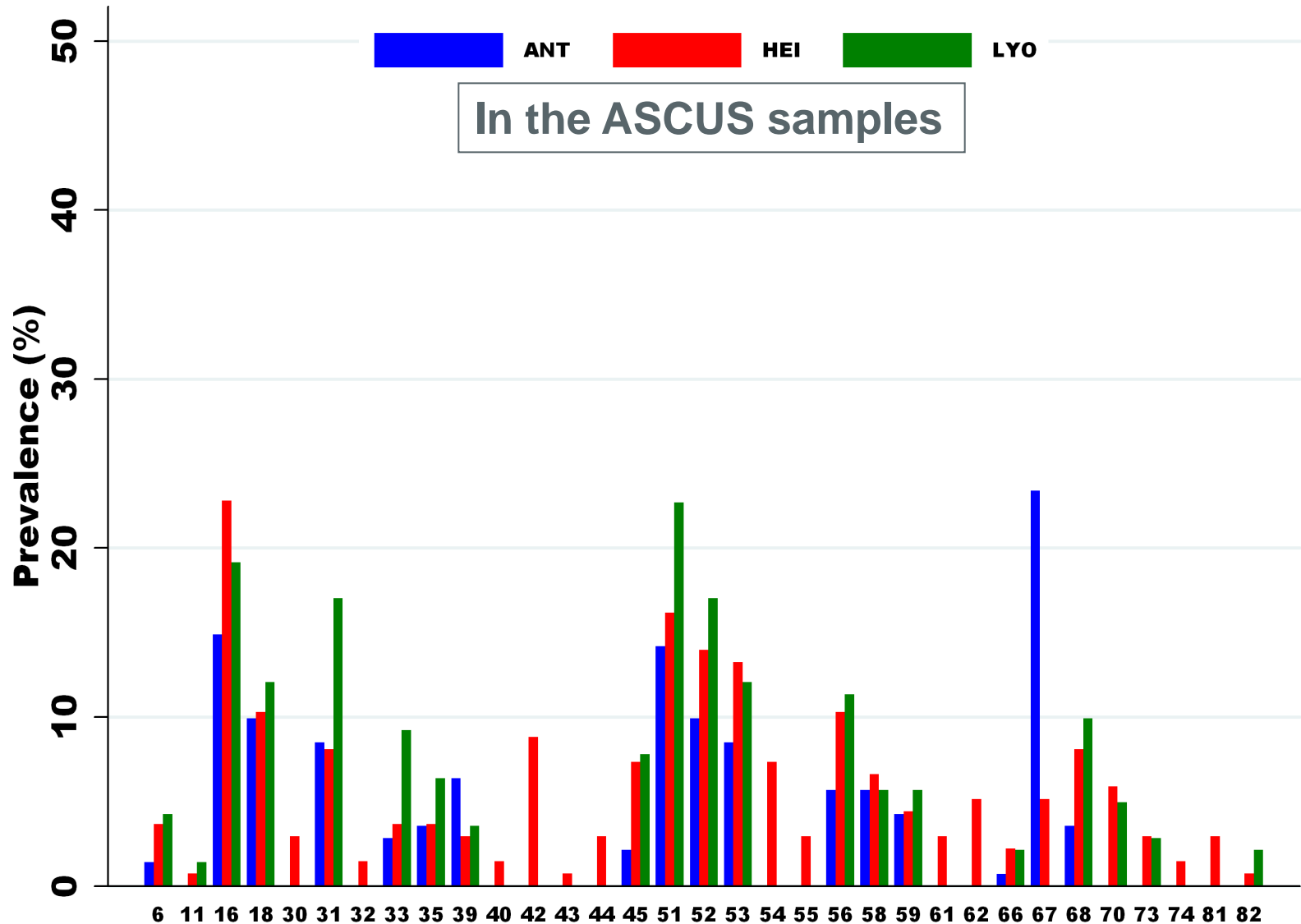
- The HPV Onclarity™ (BD) (Edinburgh)
- Cepheid HPV test (Edinburgh)
- PapilloCheck® (Paris)
- GP5+/6+-EIA (Voorburg)
- GP5+/6+-Luminex (Voorburg)
- qRTPCR (Antwerp)
- BSGP5+/6+-PCR/MPG assay (Heidelberg)

VALGENT 1

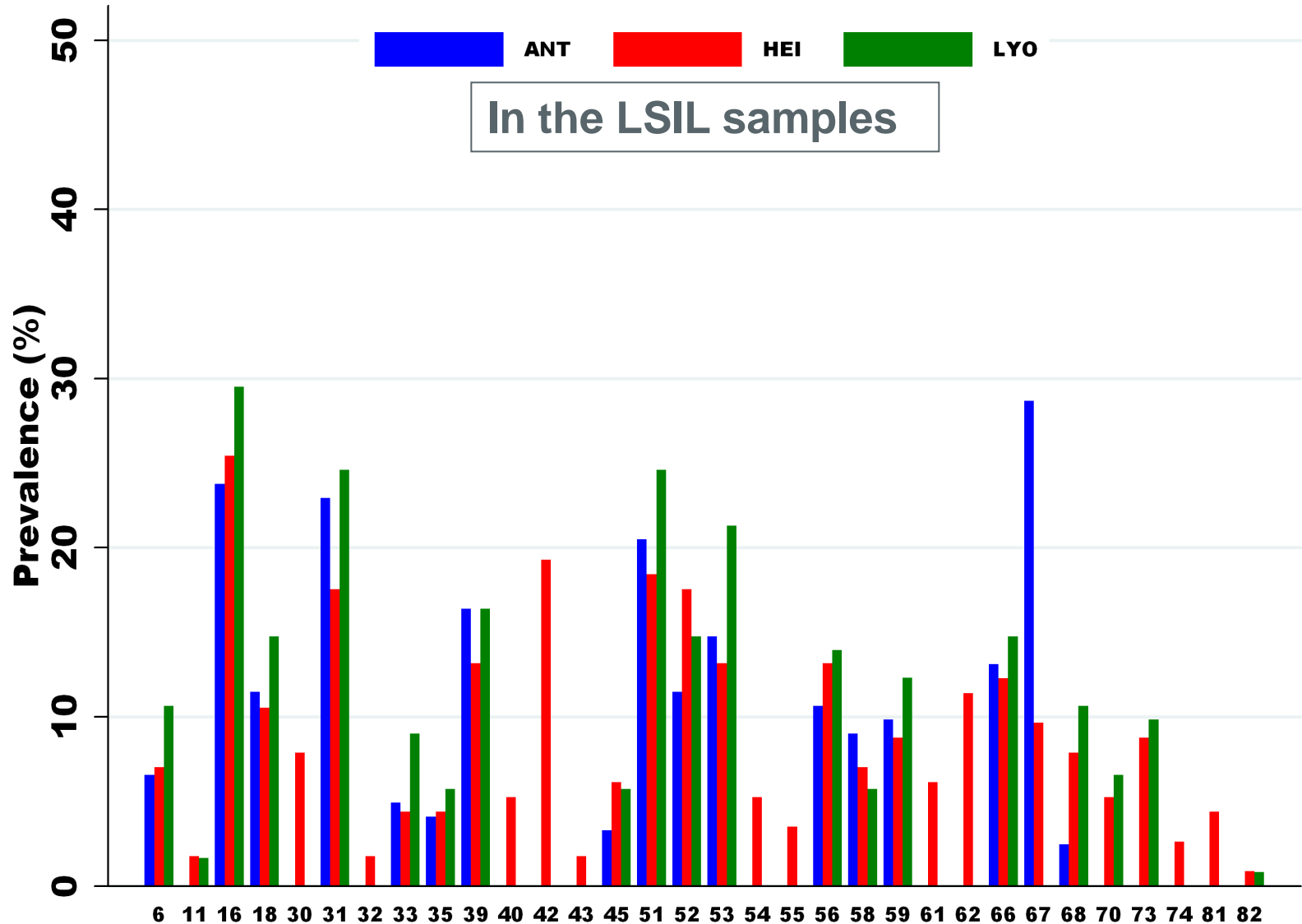
PREVALENCE OF HPV TYPES



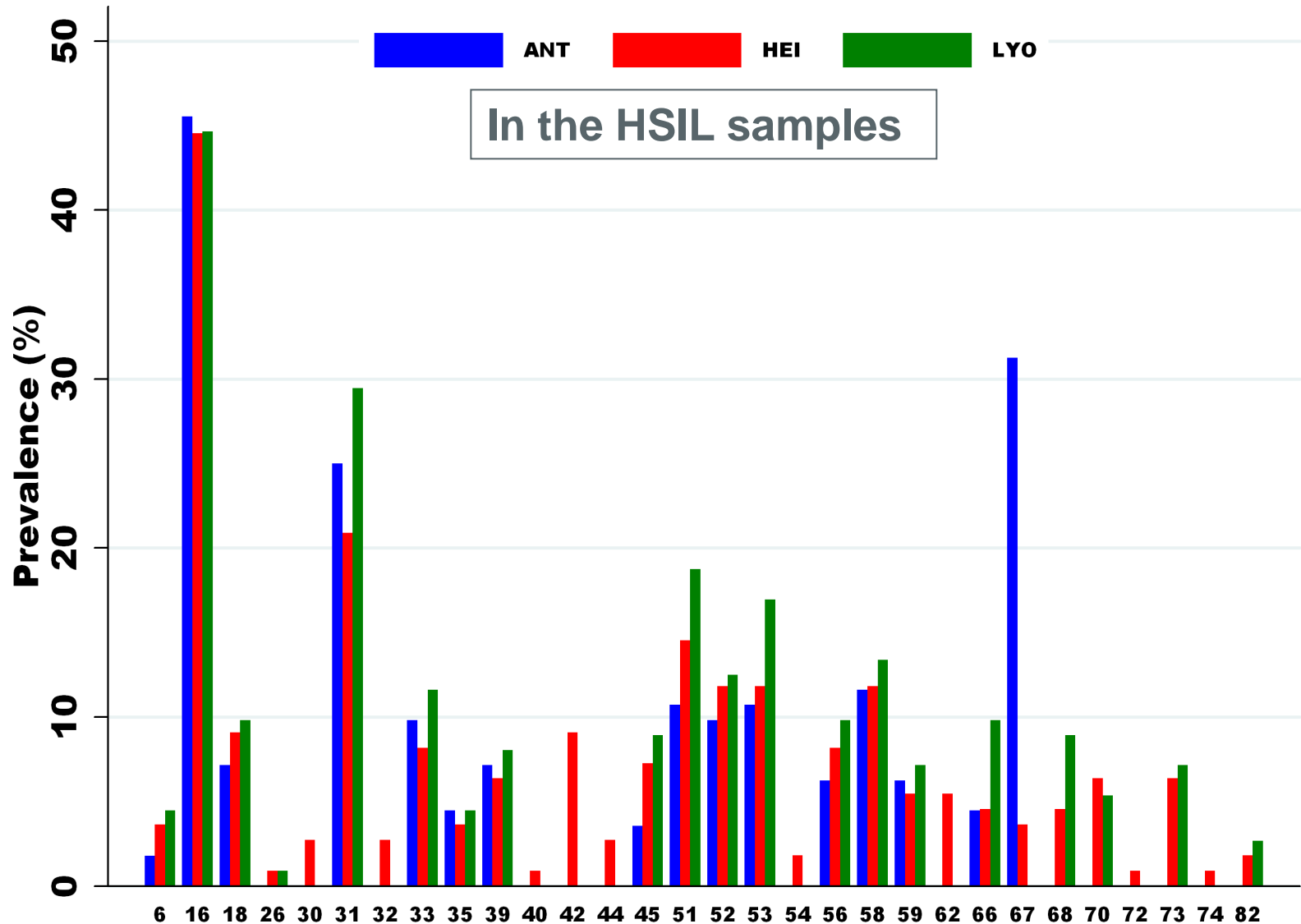
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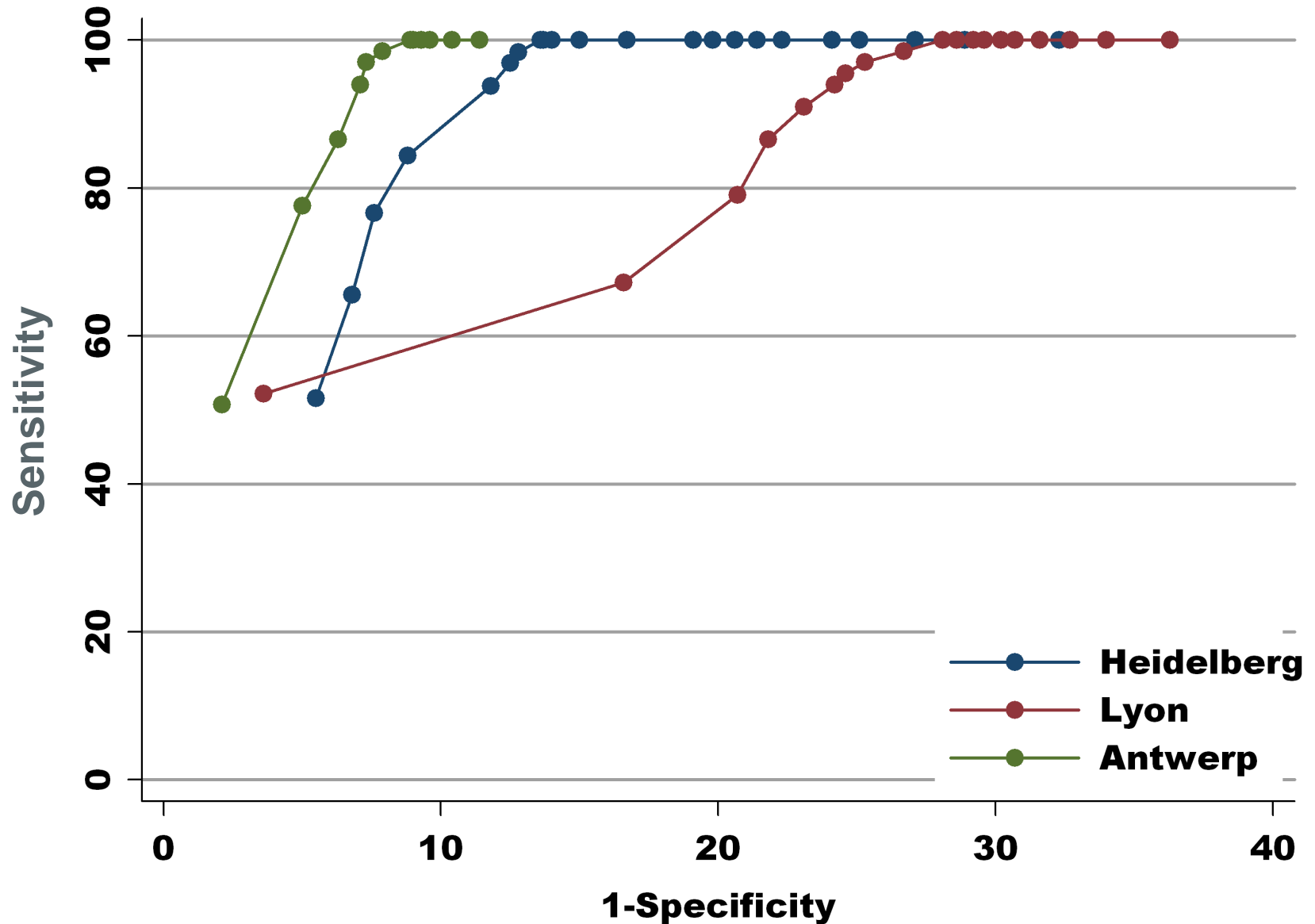


AGREEMENT BETWEEN TESTS

HPV type	HEI/LYO		HEI/ANT		ANT/LYO	
	% agreement	Kappa	% agreement	Kappa	% agreement	Kappa
all types	97.2	0.66	97.5	0.67	96.6	0.63
6	98.9	0.74	99.5	0.80	98.5	0.61
11	99.5	0.73				
16	95.2	0.77	95.1	0.75	97.0	0.84
18	97.7	0.73	98.3	0.77	97.7	0.73
26	100.0	1.00				
31	88.3	0.42	98.2	0.84	89.1	0.48
33	94.7	0.52	97.6	0.62	94.7	0.43
35	98.8	0.68	99.7	0.88	98.8	0.68
39	98.3	0.73	99.7	0.77	97.8	0.68
45	92.4	0.42	93.5	0.18	93.5	0.21
51	96.4	0.72	97.8	0.79	96.8	0.74
52	98.1	0.82	98.2	0.80	98.0	0.78
53	96.6	0.72	97.8	0.76	96.4	0.68
56	97.0	0.66	98.2	0.72	97.1	0.61
58	98.1	0.68	99.1	0.86	97.9	0.66
59	97.9	0.69	97.7	0.62	97.6	0.66
66	98.0	0.67	99.2	0.82	98.2	0.71
67			90.6	0.23		
68	98.2	0.74	97.8	0.36	96.1	0.23
70	99.5	0.89				
73	98.3	0.69				
82	99.5	0.53				
14hr pos	80.83	0.62	85.57	0.69	78.28	0.57

 Very strong agreement
 Strong agreement
 Moderate agreement
 Weak agreement

ROC curve by cumulative series of HPV types included in the test



VALGENT 2

Accuracy of 4 tests

Assay	Sensitivity	Sensitivity	Specificity	
	CIN2+ (N=102)	CIN3+ (N=55)	<CIN1 (N=746)	Nb FP
GP-EIA	94.1%	98.2%	90.3%	72
GP-LMX	96.1%	98.2%	90.8%	68
Onclarity	96.1%	98.2%	89.1%	81
PapilloCheck	96.1%	98.2%	89.7%	77
Cepheid test	94.1%	98.2%	90.3%	72

CIN2+: 6 cases missed by GP-EIA, 4 by all other tests

CIN3+: all tests missed the 1 case

Relative accuracy vs GP5+/6+ EIA

	Relative sensitivity	p McN	p non- inferiority
GP5+/6+ - Luminex	1.02 (0.99-1.05)	0.157	<0.001
Onclarity	1.02 (0.99-1.05)	0.157	<0.001
PapilloCheck	1.02 (0.99-1.05)	0.157	<0.001
Cepheid assay	1.00 (0.96-1.04)	1.000	0.017

Relative accuracy vs GP5+/6+ EIA

	Relative specificity	p McN	p non-inferiority
GP5+/6+ - Luminex	1.001 (0.994-1.009)	0.706	<0.001
Onclarity	0.986 (0.973-1.001)	0.061	0.186
PapilloCheck	0.993 (0.974-1.011)	0.435	0.097
Cepheid test	1.000 (0.980-1.020)	1.000	0.027

Reproducibility of hrHPV detection (14 types)

test 1	test 2	Agreement	Kappa	st err
GP5+/6+ Luminex	GP5+/6+ EIA	98.4%	0.94	0.0317
GP5+/6+ Luminex	Onclarity	96.3%	0.87	0.0316
GP5+/6+ Luminex	PapilloCheck	94.1%	0.80	0.0316
GP5+/6+ Luminex	qPCR~E6/E7	94.1%	0.81	0.0315
Onclarity	GP5+/6+ EIA	96.7%	0.89	0.0316
Onclarity	PapilloCheck	96.0%	0.87	0.0316
Onclarity	qPCR~E6/E7	94.3%	0.82	0.0316
PapilloCheck	GP5+/6+ EIA	93.9%	0.79	0.0316
qPCR~E6/E7	GP5+/6+ EIA	94.3%	0.81	0.0315
PapilloCheck	qPCR~E6/E7	91.8%	0.74	0.0316

Reproducibility of HPV16 genotyping

test 1	test 2	Agreement	Kappa	st err
GP5+/6+ Luminex	Onclarity	99.6%	0.93	0.0316
GP5+/6+ Luminex	PapilloCheck	99.5%	0.92	0.0316
GP5+/6+ Luminex	qPCR~E6/E7	98.5%	0.80	0.0310
Onclarity	PapilloCheck	99.7%	0.95	0.0316
Onclarity	qPCR~E6/E7	98.1%	0.74	0.0308
PapilloCheck	qPCR~E6/E7	98.0%	0.73	0.0307

Which tests clinically validated for 1ary screening?

- **HC2 & GP5+/6+ PCR: proven efficacy in 1ary screening (from RCTs)**
- **Other hrHPV DNA tests should demonstrate non-inferior sensitivity ($\geq 90\%$) and specificity ($\geq 98\%$) for CIN2+ and high reproducibility ($\geq 87\%$) using (archived) samples from a screening cohort (Meijer, Int J Cancer 2009)**

Non inferior sensitivity & specificity

Evaluated assay	Study	Absolute		Reference assay	Absolute		Relative		Non inferiority test	
		sensitivity	specificity		sensitivity	specificity	sensitivity	specificity	p_sens	p_spec
GP5+/6+ PCR	Meijer, 2009	98.7%	96.0%	HC2	98.7%	94.1%	1.00	1.02	0.004	0.011
Abbott RT hrHPV test	Carozzi, 2011	96.4%	92.3%	HC2	97.6%	92.6%	0.99	1.00	0.004	0.009
	Poljak, 2011	100.0%	93.3%	HC2	97.4%	91.8%	1.03	1.02	0.011	0.000
	Hesselink, 2013	95.6%	92.0%	GP5+/6+ PCR	98.5%	91.8%	0.97	1.00	0.028	0.000
Cobas-4800	Heideman, 2011	90.0%	94.6%	HC2	91.7%	94.4%	0.98	1.00	0.022	0.001
	Lloveras, 2013	98.3%	86.2%	HC2	98.3%	85.3%	1.00	1.01	0.009	0.001
Papillocheck	Hesselink, 2010	95.8%	96.7%	GP5+/6+ PCR	96.4%	97.7%	0.99	0.99	0.004	0.007
qPCR(E6/E7)	Depuydt, 2012	93.6%	95.6%	HC2	83.9%	94.5%	1.12	1.01	0.000	0.000
qPCR(E6/E7)	Hesselink, 2014	97.1%	94.3%	GP5+/6+ PCR	97.1%	94.1%	1.00	1.00	0.004	0.007
Cervista	Boers, 2014	89.0%	91.2%	HC2	93.4%	88.8%	0.95	1.03	0.043	<0.001
APTIMA	Heideman, 2013	95.5%	94.5%	GP5+/6+ PCR	100.0%	93.6%	0.96	1.01	0.067	0.000
BD Onclarity	Ejegod, 2014	92.9%	87.7%	HC2	94.2%	88.8%	0.99	0.99	0.005	0.013

All these assays also show high intra- & interlaboratory reproducibility (data not shown)

Updated from: Arbyn, Vaccine 2012

Conclusions

- **Good reproducibility for HPV16 & hr panel**
- **GP5/6+ Luminex, Onclarity, PapilloCheck, Cepheid assay**
 - **Similar sensitivity as GP EIA**
 - **Similar specificity but p test of non inferiority not significant for Onclarity, PapilloCheck**
- **Further follow-up of HPV+ cyto-negative cases in next screening round could reveal incident CIN3+ not yet present at enrolment (FP->TP)**

Conclusions: VALGENT

- **2 tests can be added to list of (Meijer-) validated HPV assays**
- **VALGENT, extremely challenging forum for inter-test comparisons**
- **Largest database of this type worldwide, still growing**
- **No structural funding**
- **Contribution of manufacturers**

See also

Cancer Epidemiol Biom Prev 2013; 22 (3): 406-14.

Research Article

Cancer
Epidemiology,
Biomarkers
& Prevention

Viral Load of High-Risk Human Papillomaviruses as Reliable Clinical Predictor for the Presence of Cervical Lesions

Markus Schmitt¹, Christophe Depuydt², Ina Benoy², Johannes Bogers², Jerome Antoine³, Michael Pawlita¹, and Marc Arbyn³, on behalf of the VALGENT study group



IJC

International Journal of Cancer

Int. J. Cancer: 132, 2395–2403 (2013)

Prevalence and viral load of 51 genital human papillomavirus types and three subtypes

Markus Schmitt¹, Christophe Depuydt², Ina Benoy², Johannes Bogers², Jerome Antoine³, Marc Arbyn³ and Michael Pawlita¹ on behalf of the VALGENT Study Group



Multiple Human Papillomavirus Infections with High Viral Loads Are Associated with Cervical Lesions but Do Not Differentiate Grades of Cervical Abnormalities

2. Markus Schmitt,^a Christophe Depuydt,^b Ina Benoy,^b Johannes Bogers,^b Jerome Antoine,^c Marc Arbyn,^c Michael Pawlita,^a on behalf of the VALGENT Study Group

See also



Clinical Evaluation of a GP5+/6+-Based Luminex Assay Having Full High-Risk Human Papillomavirus Genotyping Capability and an Internal Control

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Future work

- **Comprehensive comparisons**
- **VALGENT 3 (Ljubljana) & 4 (Copenhagen)**
- **Network meta-analysis of all test comparisons**

Acknowledgements

- **European Commission: CoHear(*FP7*)**
- **Belgian Foundation Against Cancer**
- **BD**
- **Cepheid**
- **Bio-Greiner**
- **Laboratories of Antwerp, Heidelberg, Voorburg, Lyon, Pasteur-Paris, Edinburgh**