Global Improvement in HPV genotyping services

Technical Report on the HPV LabNet 2011 HPV DNA Genotyping Proficiency Panel

Prepared by

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Abbreviations

AFRO	African Regional Office
EMRO	Eastern Mediterranean Regional Office
EQUALIS	External Quality Assurance in Laboratory Medicine In Sweden
EURO	European Regional Office
DKFZ	Deutsches Krebsforschungszentrum
GE	Genome Equivalent
GRL	Global Reference Laboratory
HPV	Human Papilloma Virus
HPV LabNet	HPV Laboratory Network
IU	International Unit
PAHO	Pan American Health Organisation
PP	Proficiency Panel
RRL	Regional Reference Laboratory
SEARO	South East Asian Regional Office
WHO	World Health Organization
WPRO	Western Pacific Regional Office

WHO HPV LabNet DNA proficiency panel 2011

Time for Distribution November - December 2011

1. Introduction

Accurate and internationally comparable HPV DNA detection and typing methodology is an essential component in the evaluation of HPV vaccines and in effective implementation and monitoring of HPV vaccination programmes. The WHO Global HPV LabNet is a WHO initiative established to support the world-wide implementation of HPV vaccines through improved laboratory standardization and quality assurance of HPV testing and typing methods used for evaluating HPV vaccines, for HPV surveillance and monitoring of HPV vaccination programmes (*http://www.who.int/biologicals/vaccines/hpv/en/index.html*). A major method for achieving progress towards this goal is development, preparation and validation of proficiency panels (PP) to qualify methods and laboratories.

Call for participation in this proficiency study was announced at the International Papilloma Virus Conference in Berlin 2011 and sent to all laboratories that had participated in the previous years WHO HPV LabNet proficiency panels (Annex 1, 2).

2. Aims

The aims of this panel are:

1. To assess the proficiency of HPV typing assays when routinely used in laboratories worldwide
2. To evaluate the sensitivity and type-specificity of HPV detection of the different HPV assays when routinely used in laboratories worldwide
3. Identify problems with any assays routinely used

3. Methods

3.1 Panel composition

Complete genomes of HPV cloned into plasmid vectors had been provided to the WHO HPV LabNet Global Reference Laboratory (GRL) at the University Hospital in Malmö Sweden by the respective proprietors with written approval for use in this WHO PP. All samples were purified plasmids diluted in TE buffert (10 mM TRIS-HCl, 0.1 mM EDTA, pH 8.0) with 10 ng/µl of human placental DNA (Sigma-Aldrich no 7011). The HPV types included were: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a (HPV 68 prototype) and 68b (ME 180 isolate).

Three additional samples, A, B and C were cell lines used as controls for the DNA extraction step in the testing. The composition of the panel is shown in Table 1.

Table 1: 2011 WHO HPV PP composition by randomised sample ID.

Randomised Panel ID	HPV types	HPV genome equivalents (GE) or international unit (IU) (for HPV 16, 18) per 5 µl
32	16	50
5	16	5
38	18	50
43	18	5
40	6	500
1	6	50
15	11	500
4	11	50
39	31	500
30	31	50
22	33	500
31	33	50
11	35	500
2	35	50
18	39	500
29	39	50
41	45	500
9	45	50
24	51	500
33	51	50
12	52	500
23	52	50
42	56	500
3	56	50
36	58	500
8	58	50
14	59	500
34	59	50
16	66	500
21	66	50
10	68a	500
27	68a	50
20	68b	500
13	68b	50
25	16, 45, 51, 33	500
19	16, 45, 51, 33	50
26	11, 18, 31, 52	500
6	11, 18, 31, 52	50
37	35, 39, 59, 66, 68b	500
17	35, 39, 59, 66, 68b	50
28	6, 56, 58, 68a	500
7	6, 56, 58, 68a	50
35	None	0
C	HPV 16; SiHa Cervical cancer cells	2500
A	HPV 16; SiHa Cervical cancer cells	25
B	HPV-negative C33A cells	0

3.2 Validation of the PP

The PP was pre-tested at GRL Sweden using a modified GP5+/6+ PCR followed by Luminex-based typing for HPV types 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68a, 68b, 69, 70, 73, 74, 81, 82, 86, 87, 89, 90, 91 and 114.

The PP was also pre-tested by one external laboratory before release, namely the German Cancer Research Center (DKFZ) in Heidelberg that used the BSGP5+/6+ PCR/MPG assay (a modification of GP5+/6+-PCR followed by Luminex-based typing) for HPV types 6, 7, 11, 13, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68b, 68a, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 97, 102, 106 and 114.

3.3 Distribution of the PP

After pre-test validation by GRL Sweden and DKFZ, the PP was compiled in October 2011 and distributed to 100 laboratories throughout five WHO regions in November and December 2011, following the call for participation and requests received from the laboratories. While participation had been free of charge previous years, this year there was a fee for laboratories to participate. The fee for commercial entities was 800 Euros, whereas academic and public health entities had a fee of 450 Euros. Participants from low and lower-middle income countries could apply for waiving of the fee.

The number (n) of laboratories included per WHO Region is shown in Figure 1. These are EMRO (n = 7), EURO (n = 51), SEARO (n = 5), WPRO (n = 15) and PAHO (n = 18). One hundred thirty four datasets with results were returned before the deadline from 96 laboratories. 71 laboratories submitted a data set from one assay only, 19 laboratories submitted data sets from 2 different assays, 2 laboratories submitted data sets from 3 assays, one laboratory submitted data sets from 4 different assays and 3 laboratories submitted data sets from 5 different assays.

As had also been the procedure for the 2010 PP, the GRL Sweden prepared the materials for the 2011 WHO HPV DNA PP, but subcontracted the administration and distribution of the PP to EQUALIS (External quality assurance of laboratory services in Sweden; *http://www.equalis.se/en/start.aspx* a public, non-profit company that administrates the external quality assurance for public health care laboratories in Sweden and that handled the logistics and distribution of the panel. This model has continued to work well and is a possible mode of operation of a sustainable long term activity with global distribution of an HPV DNA typing proficiency panel every year.

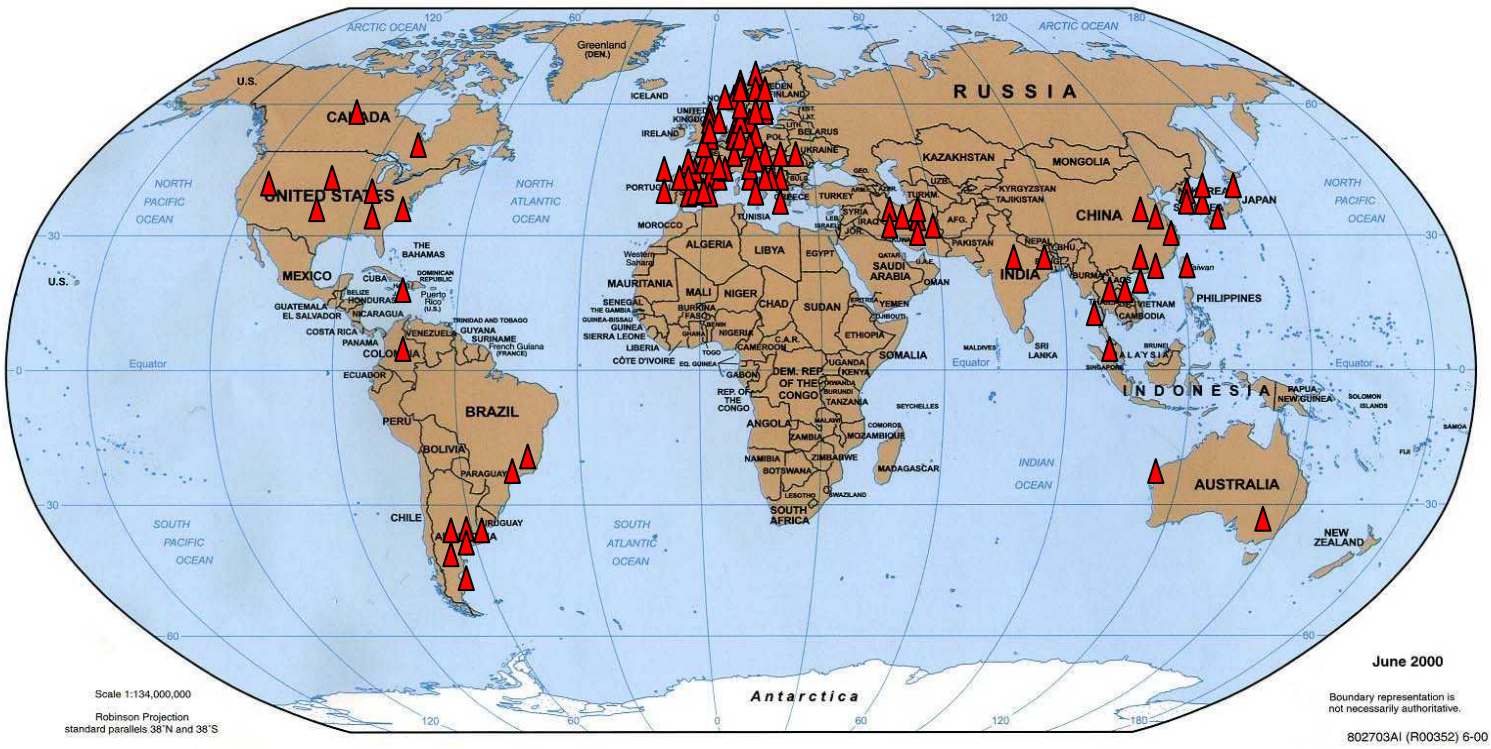

Figure 1: **Global distribution of laboratories that submitted results for the HPV DNA PP.**

3.3 Data analysis

Results analysed in this report include all results returned prior to the 1st of March 2012. Data was compiled by EQUALIS and transferred to GRL Sweden for analyses of the results.

Each data set submitted was designated a number from 1 to 134. The data were analysed by region of the laboratory, by assay used and by HPV type, respectively.

From the data sets submitted, it was noted that participating laboratories used a range of commercial assays as well as in-house assays (Table 2). The proportion of correct HPV typing results, reported by the laboratory, was analyzed as data sets by laboratory and according to assay used.

A data set was considered proficient when it detected at least 50 international units (IU) of HPV 16 and HPV 18 in 5µl and 500 genome equivalents (GE) in 5µl of the other HPV types, in both single and multiple infection. For proficiency, it was also required that not more than one false positive type was detected. This corresponds to a specificity of 97%.

4. Results

Ninety-six of 100 participating laboratories submitted 134 data sets. Eleven data sets were generated using assays that either did not discriminate specific HPV types or reported results as HPV 16, 18 and "other" High Risk HPV types (Roche Cobas 4800 test, Hybribio 13 HR and

Hybribio 14 HR). These data sets and 7 data sets from type-specific HPV 16 / 18 / 52 / 68 PCR are only analysed for the specific types tested for individually.

Detection of 5 IU of HPV16 and HPV18 and 50 GE of the other HPV types was not required for proficiency - these samples are intended for training and for providing information on whether the test just barely met the requirements or whether it exceeded them.

Each data set submitted by each individual laboratory was analysed and a feedback letter was sent to the participating laboratory that had paid the fee in March 2012.

4.1 Results by assays used

4.1.1 Commercial assays

A total of 77 data sets were obtained using commercially available tests. The most commonly used assay was the Linear Array (Roche) HPV genotyping assay that was used in 18 laboratories. Other widely used assays were the Microarray test Papillocheck (Greiner) used by 9 laboratories and Inno-LiPA (Innogenetics) used by 8 laboratories (Table 2).

4.1.2 In-house assays

Fifty-seven of the data sets had been obtained using a variety of in-house assays (Table 2).

4.2 Results analysed by assay

4.2.1 Assay Details

The different assays used for testing and typing of HPV as well as the number of submitted data sets and different part of the HPV genome targeted by each assay is shown in Table 2. The distribution of different assays in different WHO regions is shown in Figure 2.

Table 2: Assays used for testing and typing of HPV.

HPV assay type	Number of data sets	HPV region targeted (primers)
All assays	**134**	**L1/L2/E1/E2/E4/E5/E6/E7/URR**
Linear Array (Roche)	18	L1 (PGMY)
In-house PCR Luminex	11	E6, E7, L1
Papillocheck (Greiner)	9	E1
InnoLiPA (Innogenetics)	8	L1 (SPF10)
In-house Lineblot	8	E6, E7, L1
In-house PGMY-CHUV	8	L1 (PGMY)
In-house real-time PCR	7	L1/E1/E4/E6/E7
In-house Type-specific PCR	7	L1/L2/E1/E5/E6/E7/URR
CLART HPV 2 / 3 (Genomica)	6	L1 (PGMY)
Hybribio 21 HPV GenoArray	5	L1 (PGMY)
Cobas 4800 (Roche)	4	L1
In-house PCR-EIA	4	L1/E6/E7
In-house PCR-RFLP	3	L1/E6/E7
In-house pyrosequencing	3	L1 (GP/PGMY)

Hybribio 14 HR (Hybribio)	3	E6/E7
PCR Luminex (Multimetrix)	2	L1 (GP)
In-house Microarray-chip	2	L1 (My/GPM)
PANArray	2	L1 (GP)
Digene HPV genotyping RH test (QIAGEN)	2	L1 (GP)
Hybribio 13 HR	2	E6/E7
HPV Direct Flow-chip (Master Diagnostica)	2	L1 (GP)
HPV SPF10-LiPA25 (DDL)	2	L1 (SPF 10)
LCD array (Chipron)	2	L1 (PGMY)
Other Commercial assays[a)]	10	L1/E1/E2/E6/E7
Other In-house assays [b)]	4	L1/E1/E7

a) Other commercial assays include one laboratory using each of; GenoFlow HPV array kit, High risk screen TM Sacace, AdvenSure HPV GenoBlot Assay, BMT HPV Genotyping 9G, F-HPV typing Molgentix, ProDect Chip HPV typing, PapType, f-HPV genomed-biotech, Type-specific PCR Analitica, SPF10 Hybridization (Delft lab)

b) Other In-house assays include one laboratory using each of; In-house PCR, In-house multiplex PCR, In-house hybridization-chemiluminescence, PCR-Sequencing My-GP, FAP

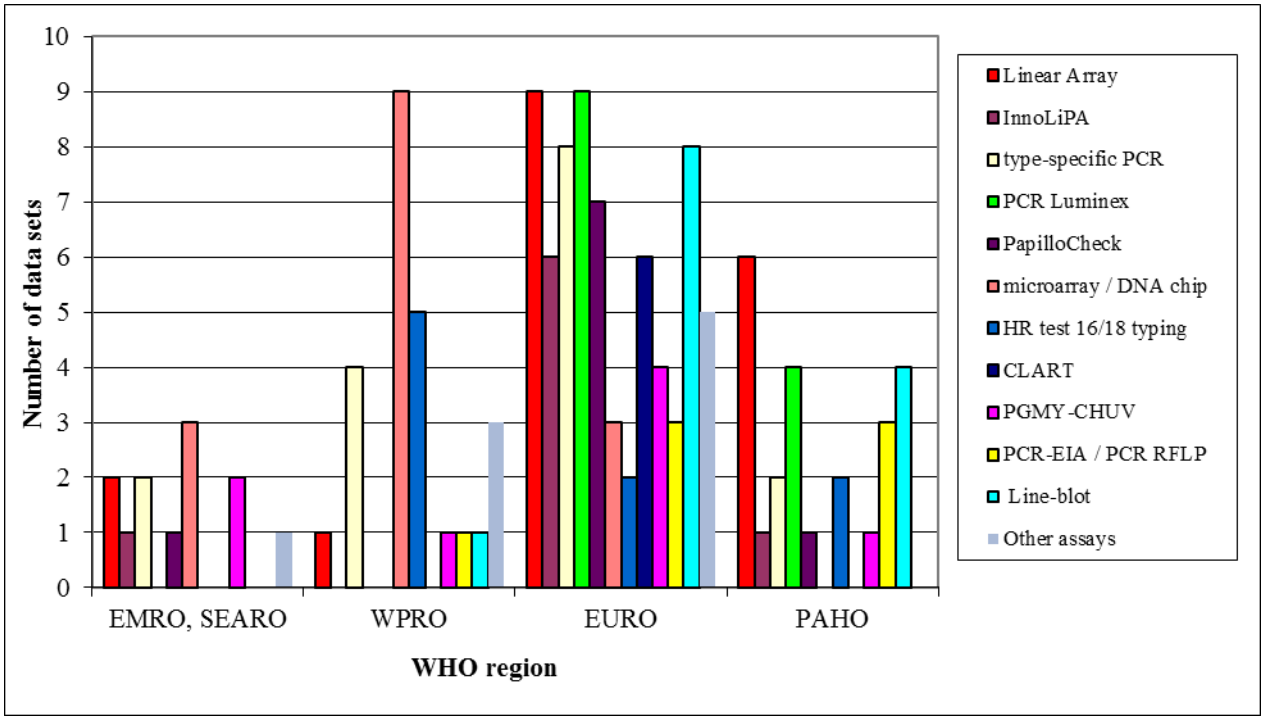

Figure 2: Type of assay in use for HPV DNA typing by WHO region, data for EMRO and SEARO region are combined.

4.2.2 Results achieved by participating laboratories

According to the criteria described in 3.3, fifty-one (39 %) data sets out of the 130 data sets that typed for **at least one HPV** type were 100 % proficient for the types claimed to be detected by the test. Of these, 7 data sets correctly identified the content of all samples, including the samples with copy number amounts that were lower than required for proficiency. Tests that did not type for all

the types in the panel could still be 100% proficient, as the denominator was the number of types claimed to be detected by the test (not the number of types included in the panel).

The proportion of laboratory proficiency including **all** datasets grouped by WHO region is shown in Figure 3. Table 3 includes datasets that type for **more than 2 HPV types**. The percent proficiency of detecting HPV types grouped by assay used for testing is shown in Table 4.

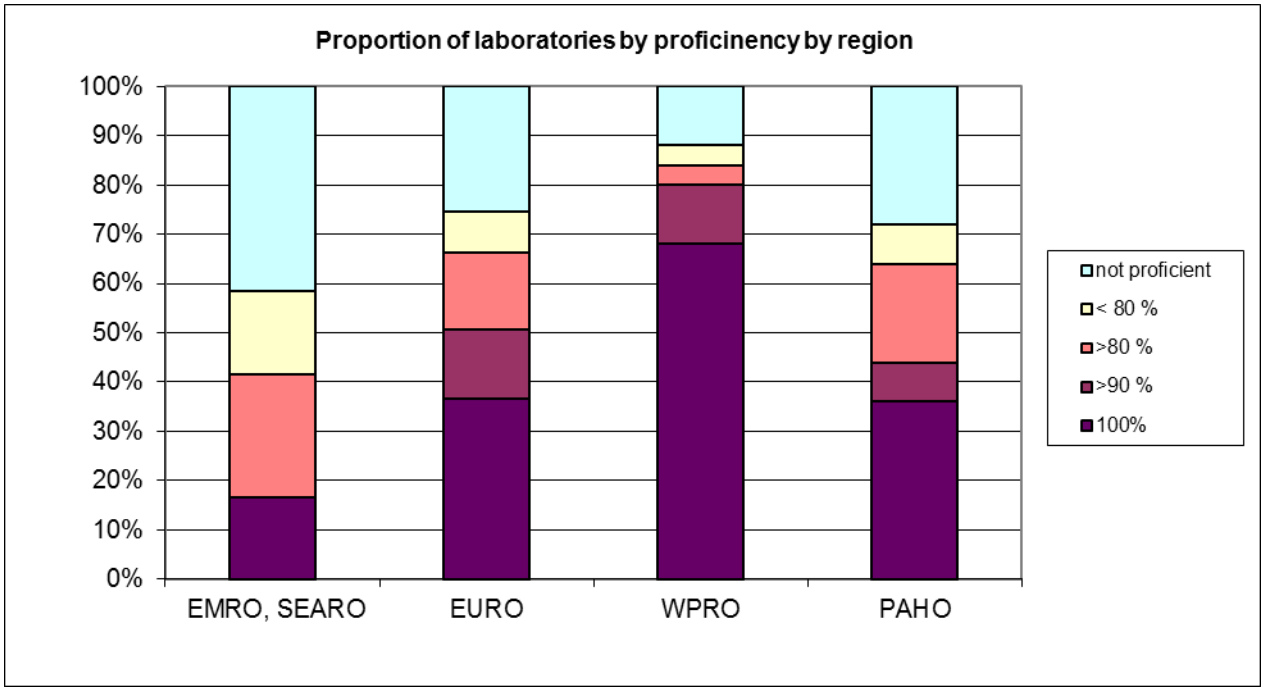

Figure 3: Proficiency for HPV DNA typing by WHO region. The figure includes 133 data-sets (all data sets, except one that only tested the extraction control).

Table 3: Proportion of data sets submitted by WHO region with ≥90% proficient HPV typing results when typing for **more than two HPV types**.

Region (data sets)	Proportion of laboratories with 100% correct typing	Proportion of laboratories with ≥90% correct typing
EURO (66)	33 %	48 %
EMRO, SEARO (12)	17 %	17 %
PAHO (22)	32 %	41 %
WPRO (17)	53 %	71 %

Table 4: Proficiency for detection of HPV types by assay used

HPV assay type	Number of data sets	No. of proficient data sets				
		100% proficient	99-90 % proficient	89-80 % proficient	<80 % proficient	Not proficient
All assays	**134**	**54**	**15**	**20**	**12**	**33**
Linear Array (Roche)	18	13	0	1	0	4
In- house PCR Luminex	11	5	3	1	0	2
Papillocheck (Greiner)	9	2	3	3	1	0
InnoLiPA (Innogenetics)	8	0	0	2	0	6
In-house Lineblot	8	1	1	4	0	2
In-house PGMY-CHUV	8	6	0	0	1	1
In-house realtime PCR	7	5	1	0	0	1
In-house Type-specific PCR	7	1	1	1	1	3
CLART HPV 2 / 3 (Genomica)	6	0	0	2	2	2
Hybribio 21 HPV GenoArray	5	3	0	2	0	0
Cobas 4800 (Roche)	4	2	0	1	1	0
In- house PCR-EIA	4	3	0	0	0	1
In-house PCR-RFLP	3	0	0	0	2	1
In-house pyrosequencing	3	0	0	1	1	1
Hybribio 14 HR (Hybribio)	3	3	0	0	0	0
PCR Luminex (Multimetrix)	2	0	1	1	0	0
In-house Microarray-chip	2	1	0	0	0	1
PANArray	2	0	2	0	0	0
Digene HPV genotyping RH test (QIAGEN)	2	0	0	1	0	1
Hybribio 13 HR	2	2	0	0	0	0
HPV Direct Flow-chip (Master Diagnostica)	2	2	0	0	0	0
HPV SPF10-LiPA25 (DDL)	2	0	1	0	0	1
LCD array (Chipron)	2	2	0	0	0	0
Other Commercial assays[a)]	10	3	2	0	1	4
Other In-house assays [b)]	4	0	0	0	2	2

a) Other commercial assays include one laboratory using each of; GenoFlow HPV array kit, High risk screen TM Sacace, AdvenSure HPV GenoBlot Assay, BMT HPV Genotyping 9G, F-HPV typing Molgentix, ProDect Chip HPV typing, PapType, f-HPV genomed-biotech, Type-specific PCR Analitica, SPF10 Hybridization (Delft lab)

b) Other In-house assays include one laboratory using each of; In-house PCR, In-house multiplex PCR, In-house hybridization-chemiluminescence, PCR-Sequencing My-GP, FAP

4.2.3 HPV types detected

The sensitivity to detect each HPV type included in the panel, as percent of laboratories detecting the different copy number (IU / GE) of the HPV types is shown in Figure 4. This data includes the laboratories with multiple false positives. Samples reported as High risk, Low risk or Other High risk are not included. In table 5 and 6 the lowest detected GE / IU grouped by assay used are shown.

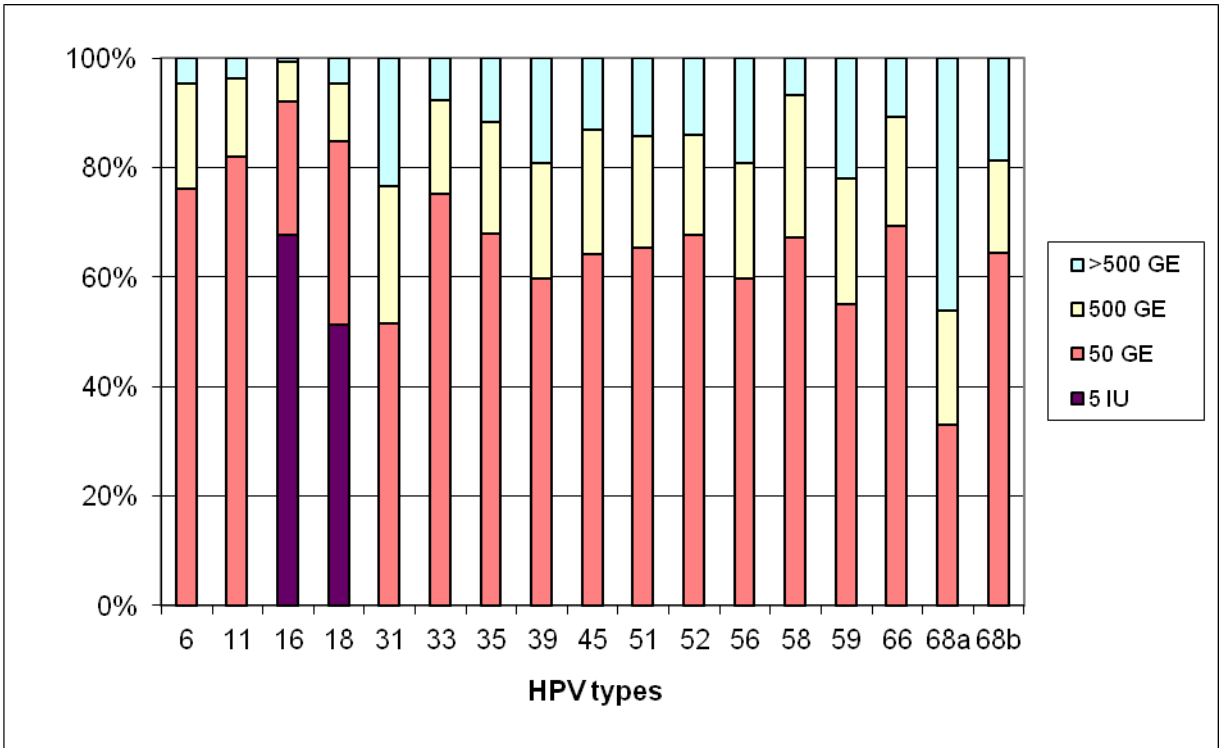

Figure 4: HPV Genome Equivalents (GE) or International Units (IU) detected per 5 µl in both single and multiple infections. Please note that only HPV16 and 18 were diluted to 5 IU (not all data sets analyze all HPV types).

Assays with input volume of 50 µl were classified as testing sample with 10-fold higher IU/GE content compared to that of 5 µl in put. Input with 10 or 15 µl was classified as same IU/GE content as compared to input with 5 µl. Thirteen laboratories used 50 µl input volume in Linear Array or Real-time PCR.

Table 5: HPV GE or IU detected per 5 µl in both single and multiple infections in commercially available assays used to test the HPV PP, lowest detected GE / IU indicated.

HPV type	HPV IU /GE	Linear Array	Papillocheck	InnoLiPA	CLART (HPV 2/3)	Hybribio 21	Luminex (Multimetrix)	Digene genotyping RH	HPV Direct Flow-chip	HPV SPF10-LiPA	LCD array (chipron)	PANArray	Other Commercial [a)]
16	5	6 / 18	7 / 9	6 / 8	5 / 6	5 / 5	2 / 2	2 / 2	2 / 2	1 / 2	2 / 2	2 / 2	5 / 9
16	50	18 / 18	8 / 9	8 / 8	6 / 6					2 / 2			7 / 9
16	500		9 / 9										9 / 9
18	5	3 / 18		6 / 8	3 / 5	4 / 5	2 / 2	2 / 2	2 / 2	1 / 2	2 / 2	2 / 2	6 / 9
18	50	18 / 18	2 / 9	7 / 8	5 / 5	5 / 5				2 / 2			9 / 9
18	500		7 / 9	8 / 8									
6	50	6 / 18	9 / 9	7 / 8	5 / 5	4 / 5	2 / 2		2 / 2	1 / 2	2 / 2	2 / 2	5 / 7
6	500	18 / 18		8 / 8		5 / 5		nt		2 / 2			
11	50	5 / 18	7 / 9	8 / 8	5 / 5	5 / 5	2 / 2		2 / 2	2 / 2	2 / 2	2 / 2	6 / 7
11	500	18 / 18	9 / 9					nt					7 / 7
31	50	4 / 18	2 / 9	7 / 8	5 / 5	4 / 5		2 / 2	2 / 2	1 / 2	2 / 2		4 / 9
31	500	17 / 18	6 / 9	8 / 8		5 / 5	1 / 2			2 / 2			5 / 9
33	50	6 / 18	5 / 9	8 / 8	6 / 6	5 / 5	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	5 / 9
33	500	18 / 18	8 / 9										8 / 9
35	50	6 / 18	2 / 9	8 / 8	5 / 5	3 / 5	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	6 / 8
35	500	18 / 18	6 / 9			4 / 5							8 / 8
39	50	6 / 18	9 / 9	7 / 8		4 / 5	2 / 2		2 / 2	2 / 2	2 / 2	2 / 2	5 / 8
39	500	18 / 18				5 / 5		1 / 2					6 / 8
45	50	6 / 18	5 / 9	6 / 8		4 / 5	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	6 / 9
45	500	18 / 18	9 / 9	7 / 8	2 / 5	5 / 5							8 / 9
51	50	6 / 18	8 / 9	8 / 8	5 / 5	3 / 5	2 / 2	1 / 2	2 / 2	2 / 2	2 / 2	2 / 2	6 / 8
51	500	18 / 18	9 / 9					2 / 2					
52	50	5 / 18	5 / 9	7 / 8	5 / 6	5 / 5	2 / 2		2 / 2	2 / 2	2 / 2	2 / 2	6 / 8
52	500	18 / 18	8 / 9	8 / 8				1 / 2					
56	50	2 / 18	9 / 9	8 / 8		3 / 5	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	7 / 8
56	500	17 / 18			2 / 5								
58	50	6 / 18	4 / 9	2 / 8	6 / 6	5 / 5	2 / 2	2 / 2	2 / 2	1 / 2	2 / 2	2 / 2	6 / 8
58	500	18 / 18	8 / 9	5 / 8						2 / 2			7 / 8
59	50	6 / 18	7 / 9		5 / 5	4 / 5	2 / 2	1 / 2	2 / 2	1 / 2	2 / 2	2 / 2	6 / 8
59	500	18 / 18	9 / 9	2 / 8		5 / 5		2 / 2		2 / 2			7 / 8
66	50	6 / 18	7 / 9	7 / 8	4 / 5	4 / 5	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	2 / 2	3 / 4
66	500	17 / 18	9 / 9	8 / 8	5 / 5	5 / 5							

68a	50		7 / 9			3 / 5			2 / 2			2 / 2	1 / 7
68a	500	nt [b)]	8 / 9		nt						nt		2 / 7
68b	50	6 / 18	8 / 9	7 / 8		4 / 5	2 / 2		2 / 2	2 / 2	2 / 2	2 / 2	4 / 7
68b	500	18 / 18	9 / 9	8 / 8	2 / 5	5 / 5							

a) Other commercial assays include one laboratory using each of; GenoFlow HPV array kit, High risk screen TM Sacace, AdvenSure HPV GenoBlot Assay, BMT HPV Genotyping 9G, F-HPV typing Molgentix, ProDect Chip HPV typing, PapType, f-HPV genomed-biotech, Type-specific PCR Analitica

b) Nt: Not tested

Table 6: HPV GE or IU detected per 5 µl in both single and multiple infections in in-house assays used to test the HPV PP, lowest detected GE / IU indicated.

HPV type	HPV IU /GE	All Assays (%)	Luminex	Lineblot	PGMY-CHUV	Realtime PCR	Type-specific PCR	PCR-RFLP	PCR sequencing	Microarray-chip	Other In-house[a)]
16	5	86 / 126 (68)	9 / 11	5 / 8	6 / 8	3 / 5	5 / 7	1/ 3	3 / 4	2 / 2	2 / 4
16	50	117 / 125 (94)	11 / 11	8 / 8	7 / 8	5 / 5	6 / 7		4 / 4		
16	500	124 / 125 (99)			8 / 8		7 / 7	3 / 3			3 / 4
18	5	70 / 124 (56)	7 / 11	6 / 8	3 / 8	3 / 5	2 / 7	1 / 3	4 / 4	2 / 2	
18	50	107 / 124 (86)	10 / 11	8 / 8	7 / 8	5 / 5	6 / 7				
18	500	119 / 124 (96)	11 / 11				7 / 7				2 / 4
6	50	80 / 103 (78)	8 / 10	5 / 7	8 / 8	3 / 3	5 / 5	1 / 3	2 / 2	2 / 2	1 / 2
6	500	99 / 103 (96)	9 / 10	7 / 7				2 / 3			2 / 2
11	50	87 / 104 (84)	10 / 10	7 / 7	8 / 8	3 / 3	5/ 5	3 / 3	2 / 3	2 / 2	1 / 2
11	500	103 / 104 (99)									
31	50	55 / 113 (49)	3 / 11	5 / 8	5 / 8	3 / 4	1 / 6		1 / 3	2 / 2	1 / 4
31	500	85 / 113 (75)	9 / 11	6 / 8	6 / 8	4 / 4	3 / 6	1 / 3	1 / 3		
33	50	88 / 116 (76)	10 / 11	7 / 8	7 / 8	3 / 4	6 / 6	2 / 3	4 / 4	2 / 2	1 / 4
33	500	108 / 116 (93)	11 / 11	8 / 8				3 / 3			
35	50	76 / 113 (67)	8 / 11	6 / 7	7 / 8	3 / 4	5 / 6		2 / 4	1 / 2	1 / 4
35	500	101 / 113 (89)	11 / 11						4 / 4	2 / 2	2 / 4
39	50	65 / 113 (57)	8 / 11	3 / 6	6 / 8	3 / 4	4 / 6			1 / 2	
39	500	91 / 113 (80)	10 / 11	6 / 6	7 / 8		6 / 6		1 / 3	2 / 2	
45	50	73 / 114 (64)	10 / 11	7 / 8	7 / 8	3 / 4	4 / 6		1 / 4	2 / 2	
45	500	99 / 114 (87)	11 / 11			4 / 4	5 / 6				1 / 3
51	50	77 / 110 (70)	8 / 11	5 / 6	6 / 8	3 / 4	5 / 6		1 / 3	2 / 2	
51	500	94 / 110 (85)	10 / 11			4 / 4					
52	50	77 / 115 (67)	10 / 11	4 / 7	6 / 8	4 / 5	5 / 6		2 / 3	2 / 2	1 / 4
52	500	99 / 115 (86)	11 / 11	7 / 7							
56	50	70 / 114 (61)	11 / 11	5 / 6	5 / 8	2 / 4	3 / 6		1 / 4	1 / 2	1 / 4
56	500	93 / 114 (82)		6 / 6	7 / 8	3 / 4	4 / 6			2 / 2	

58	50	79 / 115 (69)	9 / 11	6 / 8	7 / 8	3 / 4	5 / 6	2 / 3	4 / 4	2 / 2	1 / 4
58	500	107 / 115 (93)	11 / 11	8 / 8		4 / 4	6 / 6	3 / 3			2 / 4
59	50	60 / 111 (54)	6 / 11	1 / 6	7 / 8	3 / 4	3 / 5			2 / 2	
59	500	87 / 111 (78)	8 / 11	3 / 6	8 / 8	4 / 4	3 / 5		1 / 4		1 / 3
66	50	70 / 102 (69)	8 / 11	5 / 6	6 / 8		3 / 4		2 / 2	2 / 2	1 / 4
66	500	93 / 103 (90)	10 / 11		7 / 8	1 / 2	4 / 4	2 / 3			
68a	50	22 / 63 (35)	1 / 7	1 / 6		2 / 3	2 / 4			1 / 1	
68a	500	30 / 63 (48)	5 / 7	2 / 6	nt[b)]		3 / 4	nt			
68b	50	65 / 109 (60)	7 / 11	3 / 6	7 / 8	2 / 3	3 / 4		1 / 2	2 / 2	1 / 4
68b	500	86 / 109 (79)	10 / 11	4 / 6			4 / 4				

a) Other In-house assays include one laboratory using each of; In-house PCR EIA, In-house PCR, In-house multiplex PCR, In-house hybridization-chemiluminescence

b) Nt: Not tested

4.3 Results by sample number

The numbers of laboratories testing for and reporting correct HPV type, with **no false positive** HPV type detected are shown in table 7. The number of datasets without false positive results differs for each sample.

Table 7: Percentage of laboratories reporting correct HPV type as claimed and with **no false** positive HPV type detected, reported by sample number.

Randomised Panel ID	HPV types	HPV genome equivalents per 5 µl	Percent correct data sets (N)
32	16	50	**97.4 (113 / 116)**
5	16	5	68.0 (85 / 125)
38	18	50	**87.3 (103 / 118)**
43	18	5	50.0 (58 / 116)
40	6	500	**96.1 (99 / 103)**
1	6	50	78.8 (82 / 104)
15	11	500	**100 (104 / 104)**
4	11	50	87.5 (91 / 104)
39	31	500	**89.7 (96 / 107)**
30	31	50	67.6 (71 / 105)
22	33	500	**95.4 (104 / 109)**
31	33	50	78.3 (87 / 111)
11	35	500	**93.3 (98 / 105)**
2	35	50	70.8 (80 / 113)
18	39	500	**88.0 (95 / 108)**
29	39	50	66.4 (73 / 110)
41	45	500	**93.6 (102 / 109)**
9	45	50	72.7 (80 / 110)
24	51	500	**92.5 (99 / 107)**
33	51	50	73.8 (79 / 107)
12	52	500	**92.7 (102 / 110)**
23	52	50	75.7 (84 / 111)
42	56	500	**89.6 (95 / 106)**
3	56	50	68.8 (75 / 109)
36	58	500	**94.5 (104 / 110)**
8	58	50	77.0 (84 / 109)
14	59	500	**86.8 (92 / 106)**
34	59	50	61.7 (66 / 107)
16	66	500	**92.2 (94 / 102)**

21	66	50	72.7 (72 / 99)
10	68a	500	**62.1 (35 / 58)**
27	68a	50	42.2 (27 / 64)
13	68b	500	**84.2 (85 / 101)**
20	68b	50	64.9 (63 / 97)
28	6, 56, 58, 68a	500	**65.0 (65 / 100)** [a, b]
7	6, 56, 58, 68a	50	42.1 (45 / 107) [a, b]
26	11, 18, 31, 52	500	**77.3 (92 / 119)** [a]
6	11, 18, 31, 52	50	58.3 (70 / 120) [a]
25	16, 33, 45, 51	500	**79.0 (94 / 119)** [a]
19	16, 33, 45, 51	50	73.9 (85 / 115) [a]
37	35, 39, 59, 66, 68b	500	**61.0 (64 / 105)**
17	35, 39, 59, 66, 68b	50	41.8 (46 / 110)
35	None	0	**98.5 (130 / 132)**
A	HPV 16 Cervical cancer	25	87.8 (108 / 123) (5 false positive)
B	HPV-negative cells	0	95.3 (121 /127) (6 false positive)
C	HPV 16 Cervical cancer	2500	91.1 (112 / 123) (4 false positive)

[a] Including data set generated by type specific HPV 16 / 18/ 52 / 68 PCR.

[b] Data sets known not to detect the HPV 68a plasmids in this panel are considered as correct when the other HPV types in the sample are detected.

4.4 Analysis of false positive results

To be considered as proficient for HPV testing in this study only one false positive result was accepted. The number of false positive samples by assay used is shown in Table 8.

Table 8: Number of false positive HPV types detected per data set reported by assay used.

HPV assay type	Number of data sets	No. of false positive samples per data set				
		0 samples	1 sample	2 samples	3 samples	> 3 samples
All assays	**134**	**84**	**17**	**13**	**3**	**17**
Linear Array (Roche)	18	12	2	2	0	2
In-house PCR Luminex	11	7	2	2	0	0
Papillocheck (Greiner)	9	9	0	0	0	0
InnoLiPA (Innogenetics)	8	1	1	1	1	4
In-house Lineblot	8	4	2	1	0	1
In-house PGMY-CHUV	8	5	2	0	0	1
In-house realtime PCR	7	6	0	1	0	0
In-house Type-specific PCR	7	3	1	2	1	0
CLART HPV 2 / 3 (Genomica)	6	3	1	1	0	1
Hybribio 21 HPV GenoArray	5	4	1	0	0	0
Cobas 4800 (Roche)	4	4	0	0	0	0
In- house PCR-EIA	4	3	0	0	0	1
In-house PCR-RFLP	3	2	0	1	0	0
In-house pyrosequencing	3	2	0	0	0	1

Hybribio 14 HR (Hybribio)	3	3	0	0	0	0
PCR Luminex (Multimetrix)	2	1	1	0	0	0
In-house Microarray-chip	2	1	0	0	1	0
PANArray	2	2	0	0	0	0
Digene HPV genotyping RH test (QIAGEN)	2	1	0	0	0	1
Hybribio 13 HR	2	2	0	0	0	0
HPV Direct Flow-chip (Master Diagnostica)	2	2	0	0	0	0
HPV SPF10-LiPA25 (DDL)	2	1	0	0	0	1
LCD array (Chipron)	2	0	2	0	0	0
Other Commercial assays[a)]	10	4	2	1	0	3
Other In-house assays [b)]	4	2	0	1	0	1

a) Other commercial assays include one laboratory using each of; GenoFlow HPV array kit, High risk screen TM Sacace, AdvenSure HPV GenoBlot Assay, BMT HPV Genotyping 9G, F-HPV typing Molgentix, ProDect Chip HPV typing, PapType, f-HPV genomed-biotech, Type-specific PCR Analitica, SPF10 Hybridization (Delft lab)

b) Other In-house assays include one laboratory using each of; In-house PCR, In-house multiplex PCR, In-house hybridization-chemiluminescence, PCR-Sequencing My-GP, FAP

We searched the data sets for patterns of consistent false positivity for any specific sample in the panel. The false positivities appeared to be essentially randomly distributed among the samples, indicating that the problem with false positives is usually not related to a property of the assays itself (e.g. cross-reactivity), but rather with the laboratory conditions of use (e.g. contamination). In the PP for 2010 a systematic false positivity was found in the HPV 58 plasmid where 15 data sets also detected HPV 52 in at least one of the HPV 58 containing samples. The analyses in the report for 2010 concluded that this was probably not due to a contamination of the panel, but more likely attributable to the fact that HPV52 and 58 are related and may cross-react in some assays. For the 2011 PP, we nevertheless decided to make a new preparation of the HPV 58 plasmid. In the 2011 PP, six laboratories still detect HPV 52 in at least one of the samples containing HPV 58 plasmid (5 laboratories using Inno-Lipa and one using Linear Array). Both Linear Array and InnoLiPA don't exclude the presence of some HPV types when specific HPVs are present. So the limit cannot be due only to the assay but also to the laboratory that couldn't ascertain the presence of some genotypes (for example by additional HPV type-specific PCR).

4.5 Comparison of results for laboratories that participated 2011 and in the year 2008 and 2010

In total 54 laboratories that participated in 2011 had also participated in the HPV LabNet PPs from at least one previous year. Thirty laboratories participated in all 3 PPs (both 2008, 2010 and 2011). Twenty-four laboratories analysed the PP 2010 and 2011. Comparisons of these results were made for each laboratory. Some of the laboratories used the same tests during all years, whereas some laboratories had changed at least one of the tests used. Percent proficiency, for all years and compared with the results from all data sets submitted 2011 is shown in Table 9, the sensitivity for individual HPV types in Table 10 and the specificity with number of false positive samples in Table 11.

Table 9: Proficiency of detecting HPV types by laboratories that participated in 2011 PP, with data from 2008 and 2010 in comparison with all data sets submitted 2011.

Proficiency	**Identical assays used**			**All test by laboratories that participated both 2008, 2010 and 2011**			**All datasets 2011**
	2008 (%)	**2010 (%)**	**2011 (%)**	**2008 (%)**	**2010 (%)**	**2011 (%)**	**2011 (%)**
100 % proficient	8 / 25 (32)	17 / 47 (36)	20 / 47 (42)	8 / 32 (25)	18 / 61 (29)	28 / 66 (42)	54 / 134 (40)
99-90 % proficient	2 / 25 (8)	4 / 47 (8.5)	5 / 47 (11)	2 / 32 (6.2)	5 / 61 (8.2)	10 / 66 (15)	15 / 134 (11)
89-80 % proficient	2 / 25 (8)	6 / 47 (13)	6 / 47 (13)	4 / 32 (12)	6 / 61 (9.8)	10 / 66 (15)	20 / 134 (15)
<80 % proficient	4 / 25 (16)	5 / 47 (11)	6 / 47 (13)	5 / 32 (16)	9 / 61 (15)	6 / 66 (9.1)	12 / 134 (8.9)
Not proficient	9 / 25 (36)	15 / 47 (32)	10 / 47 (21)	13 / 32 (41)	23 / 61 (38)	12 / 66 (18)	33 / 134 (25)

Table 10: HPV GE or IU detected per 5 µl in both single and multiple infections by laboratories participating 2008, 2010 and 2011. Including samples with detection of additional false positive HPV types.

HPV type	HPV IU /GE	Identical assays used			All test by laboratories participating both 2008, 2010 and 2011			All datasets 2011
		2008 (%)	2010 (%)	2011 (%)	2008 (%)	2010 (%)	2011 (%)	2011 (%)
16	5	20 / 25 (80)	41 / 47 (87)	37 / 47 (79)	24 / 32 (75)	53 / 61 (87)	47 / 64 (88)	86 / 125 (69)
16	50	24 / 25 (96)	46 / 47 (98)	46 / 47 (98)	28 / 32 (88)	59 / 61 (97)	62 / 64 (97)	117 / 125 (94)
16	500			47 / 47 (100)	31 / 32 (97)	60 / 61 (98)	64 / 64 (100)	124 / 125 (99)
18	5	15 / 24 (62)	32 / 43 (74)	24 / 47 (51)	18 / 31 (58)	41 / 57 (72)	38 / 64 (59)	70 / 124 (56)
18	50	23 / 24 (96)	40 / 43 (93)	41 / 47 (87)	27 / 31 (87)	54 / 57 (95)	56 / 64 (88)	107 / 124 (86)
18	500			46 / 47 (98)	29 / 31 (94)	55 / 57 (96)	63 / 64 (98)	119 / 124 (96)
6	50	22 / 25 (88)	29 / 42 (69)	32 / 42 (76)	27 / 32 (84)	36 / 55 (65)	46 / 56 (82)	80 / 103 (78)
6	500	23 / 25 (92)	37 / 42 (88)	40 / 42 (95)	29 / 32 (91)	47 / 55 (85)	53 / 56 (95)	99 / 103 (96)
11	50	23 / 25 (92)	38 / 42 (90)	35 / 42 (83)	29 / 32 (91)	46 / 55 (84)	47 / 56 (84)	87 / 104 (84)
11	500	24 / 25 (96)	40 / 42 (95)	41 / 42 (98)	30 / 32 (94)	52 / 55 (94)	54 / 56 (96)	103 / 104 (99)
31	50	14 / 25 (56)	29 / 46 (63)	26 / 46 (56)	18 / 32 (56)	37 / 59 (63)	37 / 63 (59)	55 / 113 (49)
31	500	21 / 25 (84)	36 / 46 (78)	40 / 46 (87)	25 / 32 (78)	45 / 59 (76)	55 / 63 (87)	85 / 113 (75)
33	50	20 / 25 (80)	36 / 46 (78)	36 / 46 (78)	26 / 32 (81)	46 / 59 (78)	51 / 63 (81)	88 / 116 (76)
33	500	22 / 25 (88)	39 / 46 (85)	43 / 46 (93)	29 / 32 (91)	51 / 59 (86)	59 / 63 (94)	108 / 116 (93)
35	50	21 / 25 (84)	34 / 46 (74)	35 / 46 (76)	26 / 32 (81)	45 / 59 (76)	48 / 63 (76)	76 / 113 (67)
35	500	24 / 25 (96)	43 / 46 (93)	42 / 46 (91)	29 / 32 (91)	57 / 59 (97)	58 / 63 (92)	101 / 113 (89)
39	50	11 / 14[a)] (79)	31 / 46 (67)	27 / 46 (59)	13 / 18[a)] (72)	33 / 59 (56)	37 / 62 (60)	65 /113 (57)
39	500		36 / 46 (78)	36 / 46 (78)	14 /18 (78)	43 / 59 (73)	49 / 62 (79)	91 /113 (80)
45	50	18 / 24 (75)	35 / 46 (76)	32 / 45 (71)	20 / 31 (65)	43 / 59 (73)	46 / 62 (74)	73 /114 (64)
45	500	19 / 24 (79)	41 / 46 (89)	40 / 45 (89)	24 / 31 (77)	52 / 59 (88)	56 / 62 (90)	99 /114 (87)
51	50	21 / 25 (84)	39 / 46 (85)	35 / 45 (78)	26 / 32 (81)	46 / 58 (79)	48 / 61 (79)	77 /110 (70)
51	500	23 / 25 (92)	42 / 46 (91)	41 / 45 (91)	28 / 32 (88)	51 / 58 (88)	56 / 61 (92)	94 /110 (85)
52	50	17 / 25 (68)	39 / 46 (85)	31 / 46 (67)	20 / 32 (62)	47 / 59 (80)	43 / 64 (67)	77 / 115 (67)
52	500	18 / 25 (72)	42 / 46 (91)	41 / 46 (89)	23 / 32 (72)	53 / 59 (90)	58 / 64 (91)	99 /115 (86)
56	50	17 / 25 (68)	33 / 46 (72)	35 / 46 (76)	20 / 32 (62)	41 / 59 (69)	45 / 62 (73)	70 /114 (61)
56	500	19 / 25 (76)	37 / 46 (80)	40 / 46 (87)	22 / 32 (69)	48 / 59 (81)	54 / 62 (87)	93 /114 (82)
58	50	21 / 25 (84)	35 / 46 (76)	33 / 46 (72)	27 / 32 (84)	41 / 59 (69)	47 / 63 (75)	79 / 115 (69)
58	500	23 / 25 (92)	41 / 46 (89)	42 / 46 (91)	30 / 32 (94)	51 / 59 (86)	58 / 63 (92)	107 / 115 (93)
59	50	17 / 25 (68)	30 / 46 (65)	26 / 45 (58)	21 / 32 (66)	34 / 59 (58)	37 / 62 (60)	60 /111 (44)
59	500	18 / 25 (72)	34 / 46 (74)	34 / 45 (76)	22 / 32 (69)	43 / 59 (73)	47 / 62 (76)	87 / 111 (68)
66	50	18 / 21 (86)	33 / 42 (79)	32 / 42 (76)	23 / 28 (82)	42 / 55 (76)	43 / 58 (74)	70 /102 (69)
66	500	20 / 21 (95)	37 / 42 (88)	40 / 42 (95)	25 / 28 (89)	47 / 55 (85)	55 / 58 (95)	93 /102 (91)

68a	50	5 / 11[b] (45)	4 / 20 (20)	7 / 23 (30)	5 / 13[b] (38)	6 / 29 (21)	11 / 34 (32)	22 / 63 (35)
68a	500	6 / 11 (54)	6 / 20 (30)	10 / 23 (43)	6 / 13 (46)	10 / 29 (34)	17 / 34 (50)	30 /63 (48)
68b	50		28 / 43 (65)	28 / 43 (65)		32 / 56 (57)	40 / 60 (67)	65 /109 (60)
68b	500	nt[c]	32 / 43 (74)	36 / 43 (84)	nt	40 / 56 (71)	50 / 60 (83)	86 /109 (79)

a) 2008 years panel used a HPV 39 plasmid where HPV 39 was cloned into the vector in L1, this plasmid could not be detected by PGMY based primers.
b) 68a cannot be detected by PGMY based primers, the plasmid used contains the L1 fragment only.
c) Nt: not tested

Table 11: Number of false positive HPV types detected per data set reported by laboratories participating in both 2008, 2010 and 2011 years proficiency studies in comparison with all data sets submitted 2011.

No of false positive samples	**Identical assays used**			**All test by laboratories participating both 2008, 2010 and 2011**			**All datasets 2011**
	2008 (%)	**2010 (%)**	**2011 (%)**	**2008 (%)**	**2010 (%)**	**2011 (%)**	**2011 (%)**
0 samples	12 / 25 (48)	31 / 47 (66)	32 / 47 (68)	14 / 32 (44)	36 / 61 (59)	47 / 66 (71)	84 / 134 (63)
1 sample	4 / 25 (16)	0 / 47 (0)	5 / 47 (11)	5 / 32 (16)	1 / 61 (1.6)	7 / 66 (11)	17 / 134 (13)
2 samples	3 / 25 (12)	6 / 47 (13)	4 / 47 (8.5)	5 / 32 (16)	6 / 61 (9.8)	4 / 66 (6.1)	13 / 134 (9.7)
3 samples	2 / 25 (8)	3 / 47 (6.4)	0 / 47 (0)	4 / 32 (12)	8 / 61 (13)	0 / 66 (0)	3 / 134 (2.2)
>3 samples	4 / 25 (16)	7 / 47 (15)	6 / 47 (13)	4 / 32 (12)	10 / 61 (16)	8 / 66 (12)	17 / 134 (13)

5. Discussion

The 2011 PP was distributed to 100 laboratories worldwide and 134 datasets were returned for analysis from 96 laboratories. Participating laboratories involved public health laboratories, research laboratories, diagnostic test manufacturers and vaccine companies. This year there was a charge for laboratories to participate, although participants from low and lower middle-income countries could have their fees waived. Although the number of laboratories that participated was about the same as previous years and although there was little change in the global distribution of laboratories, it can however not be excluded that laboratories from low income countries may have chosen not to participate because of the fee. It is also possible that the improved performance observed in the 2011 proficiency study may reflect a bias with a preferential participation of laboratories who can afford the fee and who may preferentially come from high income countries and/or have HPV genotyping as a central priority for their activities. However, the analysis that was restricted to laboratories that have participated multiple times did also find the improvement in performance in the 2011, indicating that there has indeed occurred a global improvement in the performance of HPV genotyping.

A proficiency of 100% for detection of at least 50 IU of HPV 16 and HPV 18 in 5µl and 500 GE in 5µl of the other HPV types tested for without having more than one false positive type detected was achieved in 40% of the datasets (54 data sets from 45 laboratories). However, the PP is designed for the genotyping needs in HPV vaccinology and the proficiency criteria are not intended for clinical HPV screening purposes, where the requirements for analytical sensitivity are different. Not all assays tested for every HPV types included in the panel. In the analyses for proficiency only the HPV types tested for were included. E.g., if an assay did not include HPV 66, laboratories using such an assay were considered as not tested for HPV 66.

A total of 77 data sets had been obtained by the use of commercial tests. The most commonly used assay was Linear Array (Roche), that was used to generate 18 data sets. Other widely used assays were the Microarray test Papillocheck (Greiner) and Inno-LiPA (Innogenetics). Fifty-seven of the data sets had been obtained using a variety of in-house assays. Eleven data sets were generated using assays unable to perform complete HPV typing. Eight of these datasets identified HPV 16 and 18, and gave the other types as High-risk HPV e.g. Cobas 4800 (Roche) and Hybribio 14 (Hybribio). The results from these datasets have been considered as proficient when the correct types have been identified and no false positive results have been reported.

Two commercially available assays were 100 % proficient for HPV typing in all submitted datasets. The HPV Direct flow-chip (Master Diagnostic) used by 2 European laboratories and LCD Array (Chipron) used by 2 laboratories in the Eastern Mediterranean Region. Among the commercial tests used by more than 2 laboratories, Linear Array had the highest proportion of 100 % proficient results, with 13 out of 18 laboratories being 100% proficient for HPV typing. Several data sets generated by in-house assays based on real time PCR, the PGMY-CHUV assay or by PCR followed by Luminex-based typing were 100 % proficient. The 33 data sets classified as not proficient all detected more than one false positive HPV type.

All plasmids in the panel contained full-length genomes, including HPV 68a. Since all PGMY-based assays (directed against L1) cannot detect HPV68a we also added HPV 68b in the panel. HPV68b can be detected by PGMY-based primers and other common primer systems. All data sets that used the PGMY primers were considered as not tested for HPV 68a in this study. However, among the 64 data sets that could be analysed for detection of HPV 68a, 55 % (35/64) detected 500 GE of HPV 68a. This was the lowest number of correct data sets among all HPV types tested. The used HPV 18 had this year been re-cloned into a new vector in the L2 region, to enable detection with the Microarray kit using primers directed to the E1 region.

Four data sets generated by the use of Linear Array were not proficient, reporting between 2 and up to 8 false positive results. HPV 52 and 66 were detected as false positive in 6 out of the 17 false positive results submitted in the 18 data sets using Linear Array. These laboratories reported that HPV 52 was present in samples that contained HPV 35 and 58. Linear Array cannot make a type-specific HPV 52 call in the presence of HPV 33, HPV 35 and / or HPV 58. The Linear Array assay was designed with an intentionally cross-reactive probe. Several laboratories perform an additional HPV 52 specific PCR on these samples. The false HPV66 positives were reported in samples containing HPV 56. Compared with the 2010 PP, the number of false positive results in the data-sets obtained with Linear Array decreased from 31 to 17 of in total 134 data-sets - a major improvement.

In 2010, all four laboratories that used the microarray-based assay Papillocheck were 100 % proficient. This year only 2 out of 9 laboratories using this assay were 100 % proficient, indicating that the results depend not only on the test but on the overall performance of the laboratory. None of the datasets could detect 5 copies / 5 µl of the re-cloned HPV 18 plasmid, whereas 50 copies HPV18/ 5 µl could be detected by 2 of the 9 laboratories and 7 out of 9 could detect 500 copies of

HPV18 / 5 μl. None of the other assays used to test the PP had this rather low sensitivity for HPV18.

The commercial tests InnoLiPA and CLART did not generate any 100 % proficient data sets. For InnoLiPA, 6 out of 8 data sets were not proficient because of 2 to 21 false positive results. One laboratory using InnoLiPA reported no false positives, showing that it is possible to perform InnoLipa with no false positive results. However, it seems that stringent conditions with attention to prevention of contamination and negative controls are particularly important when using this test. HPV 59 could only be detected in one of the samples, with highest concentration, in 2 out of the 8 data sets that used InnoLiPA.
CLART was used by 6 laboratories, out of which 2 were not proficient because of reporting 2 to 7 false positive results. Only two of the data-sets could detect HPV 39 in one of the panel samples with highest concentration; while HPV 45, 56, 68 could be proficiently detected by two laboratories.

Experience in performing assays is critical in generating qualified results, in addition to the assay per se. The in-house assay PGMY-CHUV had been transferred to all WHO HPV LabNet members in 2008 as an effort to build up testing capacity and evaluate assay transferability. In the 2008 HPV DNA PP only one laboratory out of 7 was 100 % proficient using this assay. This year, 6 of 8 laboratories were 100 % proficient and only one laboratory had substantial problems with this assay.

The majority of data sets submitted 2011 were generated using assays that were used by 4 or fewer laboratories. This makes it difficult to draw conclusions regarding the generalisability of the performance of the assays.

Three additional samples (A, B, C) in the PP were used to evaluate the DNA extraction step prior to the HPV testing and typing. Two of the samples contained different amounts of the cervical cancer cell line SiHa mixed with the HPV negative cancer cell line C33A and one sample with only C33A cells was served as negative control.
There were at least 21 different extraction procedures used by the laboratories. The most commonly used was a MagNaPure kit (Roche) that was used to generate 23 data-sets, followed by different extraction kits from Qiagen used to generate 21 data-sets. Other methods used were EZNA kit, phenol chloroform extraction, Papillocheck DNA extraction kit. One laboratory used an in-house method with proteinase K extraction. The HPV Direct flow-chip from Master Diagnostica are

performed without DNA extraction, the cell suspension are added directly to the PCR mix. We did not observe any obvious difference in performance between different extraction methods. The 9 laboratories reporting false positive results in sample A, B, C all used different extraction methods.
In sample C containing 2500 cells / 5 ul of the cervical cancer cell line SiHa, HPV 16 was correctly identified by 91% of the data sets. Four data sets reported false positive HPV types in this sample.
In sample A containing 25 SiHa cells / 5ul, HPV 16 was detected in 88% of the data sets with five false positive results reported. The negative control containing only C33A cells was correctly reported as negative by only 95% of the laboratories.
This is an improvement in sensitivity compared to the results in 2010 where the correct results ranged from 61 % for sample A to 83 % for sample C. The number of false positive results in the sample only containing C33A decreased from 12 / 126 to 6 / 127.

6. Conclusions and recommendations

This was the third WHO HPV DNA proficiency study that was open for participation to all laboratories worldwide. The panel gave the possibility to analyse the specificity and sensitivity for different HPV typing assays to correctly identify 14 high risk HPV types and 2 low risk HPV types- the HPV types that are the most important for HPV vaccine characterization as well as for HPV surveillance and monitoring.

The majority of participating laboratories were from the EURO, WPRO and PAHO regions, this year the AFRO region was not represented.

The 2011 WHO HPV DNA proficiency panel has shown that it is possible to perform global studies comparing the sensitivity and specificity of different HPV typing assays, as well as the performance of participating laboratories, in a consistent manner that allows comparison of results generated by different laboratories worldwide and over time.

Compared to the results of the 2008 and 2010 WHO HPV DNA PP, we can see overall improvements. E.g., comparison of laboratories that used the same assay during the 3 years, 32 % were proficient in 2008, as compared to 42 % in 2011.

We see a trend towards increased sensitivity of assays. This year we can also see a clear trend of increased specificity. Among the laboratories that used the same assay 2008 and 2011, the proportion reporting no false positive samples increased from 48% to 68%. The percentage of laboratories with more than 3 false positive results has been rather stable over the years (around 12 – 16%) whereas the laboratories with few or no false positive results has now improved.

We suggest that recommendations for HPV laboratory testing should continue to include a strong emphasis on the use of negative controls in the assays. Furthermore, we suggest that the requirements for proficiency in future WHO HPV DNA Proficiency Panels should at the outset announce that proficiency will require that there are no false positives at all.

As also detected in previous studies, HPV 16 and HPV 18 were the types detected at lowest IU in most data sets. Only 1 and 5 datasets, could not detect 500 IU / 5 µl of HPV16 and HPV18, respectively. In contrast, HPV 31, HPV 59 and HPV 39 could not be detected in 500 GE / 5 µl in both single and multiple infections by 28, 24 and 22 data sets respectively. The continued presence

of a differential analytic sensitivity for different HPV types suggests that many surveys of circulating HPV types may give biased results.

In summary, by repeated issuing of global HPV DNA typing proficiency panels for validating different HPV DNA tests and laboratories, we have demonstrated a global improvement in performance and comparability of HPV genotyping data generated from different laboratories worldwide.

Annex 1:

Call for participation:

The 2011 WHO HPV LabNet Proficiency Study of HPV DNA Typing

Accurate and internationally comparable HPV DNA detection and typing methodology is an essential component in the evaluation of HPV vaccines and in effective implementation and monitoring of HPV vaccination programs. The WHO Global HPV LabNet is a WHO initiative established to support the world-wide implementation of HPV vaccines through improved laboratory standardization and quality assurance of HPV testing and typing methods to promote international comparability of results. The major methods for achieving progress towards this goal are developing international biological standards as well as preparing and validating proficiency panels to qualify methods.

The WHO is now seeking international participation in an international WHO HPV DNA testing and typing proficiency study. Laboratories that are or will be involved in HPV surveillance and/or vaccine development are particularly welcome.
Participant laboratories will be asked to perform HPV typing using one or more of their usual assays on the 43 challenges in this panel. This challenge is intended to evaluate assays that type HPV and is not appropriate for assays that detect HPV in general or grouped as high risk/low risk.

The challenge material will be composed of purified whole genomic plasmids of **HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a and 68b** in a background of human cellular DNA. The samples will be prepared to include single types and mixtures at varying concentrations that are traceable to establish or candidate International Standards to evaluate the sensitivity and type-specificity of detection. Three samples with cell suspensions will also be provided to allow evaluation of DNA extraction methods. Laboratories that have more than one assay are encouraged to provide results on each assay they commonly use. The challenge samples will be shipped with instructions for how to store the specimens, volume to test and coded forms to return results and assay description.

Participants in the proficiency study may be subjected to a participation fee:

Fee for commercial entities: 800 Euros

Fee for academic entities: 450 Euros

Participants from low and lower-middle income countries (World Bank classification with GNI (gross national income) per capita: <3 975 USD) can apply for waiving of the fee.

The WHO HPV Global Reference Laboratory Sweden is organizing this study on behalf of WHO and the WHO HPV LabNet. The project is a collaboration with the Swedish external quality assurance provider EQUALIS, who is responsible for the management and distribution. Laboratories will be expected to return the results within **4 weeks** of specimen receipt. Data submitted will become the property of WHO, and may be analyzed for publication by the HPV LabNet either as an internal document or peer reviewed manuscript. All results will be handled in a coded anonymous fashion, with summaries grouped by method. WHO will ensure that the code linking data to originating laboratories will be kept confidential. Laboratories that provide data within the required time-frame will receive a copy of their own results and the summary data.

Any laboratory interested in participation should fill in the **Application Form** (downloadable). Requests should be sent to the address below and must be received no later than the **15th of October 2011**. Laboratories will be notified of their enrolment, the date and mode of shipment of the panel.

Contact persons:

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Annex 2:

Application for participating in The 2011 WHO HPV LabNet Proficiency Study of HPV DNA Typing

Fee for commercial entities: 800 Euros Fee for academic entities: 450 Euros Participants from low and lower middle-income countries (World Bank classification with GNI (gross national income) per capita: <3 975 USD) can apply for waiving of fee.			
Laboratory details			
Delivery address of samples			
Department /Laboratory			
Address			
City		Postal code:	
Province /State		Country:	
E-mail		Fax	
Phone			
Invoice address (if different from above)			
Department /Laboratory			
Address			
City		Postal code:	
Province /State		Country:	
Principal Investigator			
First Name			
Surname (Title)			
HPV DNA typing experience in your laboratory			
Methodology used (may be more than one)			
Annual number of HPV typing tests performed			
Brief description of involvement in HPV surveillance or HPV vaccine development			

Send this registration form, preferably by email or fax to:
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Annex 3:

List of participants for WHO HPV LabNet Proficiency Study of HPV DNA Typing, 2011

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