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# **Technical Report on the Global HPV LabNet DNA Genotyping Proficiency Panel 2019**

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## Abbreviations

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

# Global HPV DNA genotyping proficiency panel 2019

*Distributed in December 2019*

## 1. Introduction

Accurate and internationally comparable HPV DNA detection and typing methodology is an essential component both for research and evaluation of HPV vaccines and in effective implementation and monitoring of HPV vaccination programmes. The WHO started a WHO Global HPV Laboratory Network (LabNet) in 2006 to support the world-wide development and implementation of HPV vaccines through improved laboratory standardization and quality assurance of HPV testing and typing methods used for research and evaluation of 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 was development, preparation and validation of proficiency panels (PP) to qualify methods and laboratories.

Call for participation in this proficiency study was sent to all laboratories that had participated in the HPV LabNet proficiency panels in previous years (Annex 1, 2).

## 2. Aims

The aims of this panel were:

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 International HPV Reference Center by the respective proprietors with written approval for use in this PP. All samples were purified plasmids diluted in TE buffer (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, 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:** 2019 HPV PP composition by randomised sample ID

HPV types	HPV genome equivalents (GE) or international unit (IU) (for HPV 16, 18) per 5 µl	Randomised Panel ID
16	50	9
16	5	6
18	50	29
18	5	21
6	500	36
6	50	14
11	500	19
11	50	23
31	500	5
31	50	27
33	500	34
33	50	17
35	500	37
35	50	10
39	500	25
39	50	38
45	500	1
45	50	30
51	500	41
51	50	15
52	500	33
52	50	3
56	500	31
56	50	8
58	500	22
58	50	40
59	500	11
59	50	18
68a	500	26
68a	50	4
68b	500	39
68b	50	12
6, 18, 39, 56	500	24
6, 18, 39, 56	50	16
11, 16, 52, 68a	500	32
11, 16, 52, 68a	50	2
31, 45, 58, 68b	500	20
31, 45, 58, 68b	50	35
33, 35, 51, 59	500	7
33, 35, 51, 59	50	28
TE buffer with 10 ng/µl human placenta DNA	0	13
HPV 16 positive SiHa cells	2500	B
HPV 16 positive SiHa cells	25	C
HPV- negative C33A cells	0	A

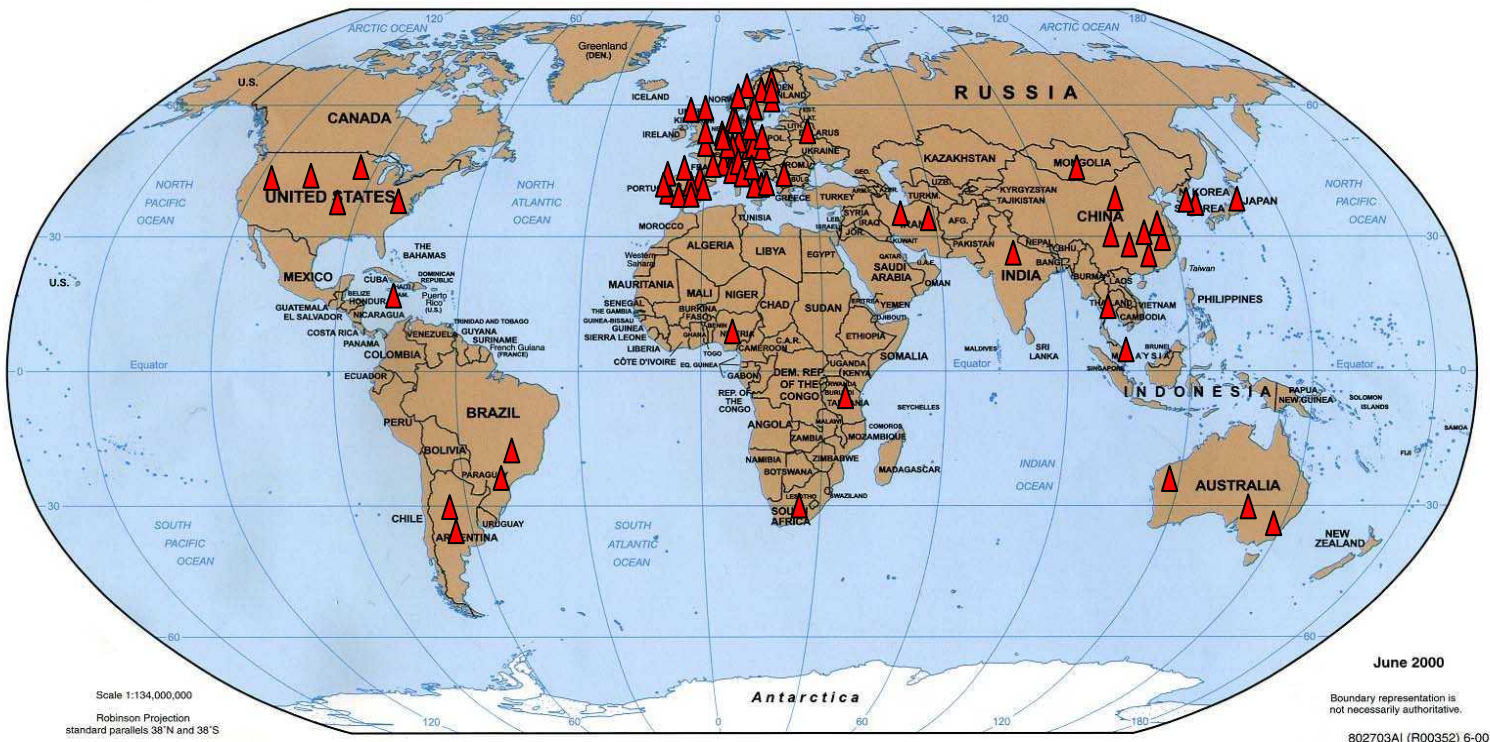
### 3.2 Validation of the PP

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, 66, 67, 68a, 68b, 69, 70, 73, 74, 81, 82, 86, 87, 89, 90 and 91.

### 3.3 Distribution of the PP

After pre-test validation by the International HPV Reference center in Sweden, the PP was compiled in September 2019 and distributed to 82 laboratories throughout six WHO regions in November 2019, following the call for participation and requests received from the laboratories. The fee for participation was for commercial entities 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. As in previous years the HPV reference laboratory in Sweden prepared the materials for the 2019 HPV DNA PP, but subcontracted the administration and distribution of the PP to Equalis (External quality assessment of laboratory medicine in Sweden; <https://www.equalis.se/en/> a public, non-profit company that administrates the external quality assessment for public health care laboratories in Sweden) 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.

The number (n) of laboratories submitting results per WHO Region is shown in Figure 1. These are EMRO (n = 2), EURO (n = 46), SEARO (n = 2), WPRO (n = 15), AFRO (n = 4) and PAHO (n = 9). One hundred ten datasets from 78 laboratories were obtained. Fifty-five laboratories submitted a data set from one assay, seventeen laboratories submitted data sets from 2 different assays, three laboratories submitted data sets from 3 assays and two laboratories submitted data sets from 4 different assays and one laboratory submitted data sets from 5 different assays.



**Figure 1: Global distribution of laboratories that submitted results for the 2019 HPV DNA PP.**

### 3.4 Data analysis

Results analysed in this report include all results returned prior to the 17<sup>th</sup> of February 2019. Data was compiled by Equalis and transferred to the HPV reference laboratory in Sweden for analyses. Each data set was designated a number from 1 to 110. The data were analysed by region of the laboratory, by assay used and by HPV type.

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 5 µl and 500 genome equivalents (GE) in 5 µl of the other HPV types, in both single and multiple infection. For proficiency, it was required that no false positive type was detected.

## 4. Results

Seventy eight of 82 participating laboratories submitted 110 data sets. Thirteen 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 /6800 test, Abbot Realtime PCR,

HybriBio 13 HR, HybriBio 14 HR, High risk HPV Screen, Harmonia HPV, and one in-house EIA). These data sets are only analyzed 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 laboratory was analyzed and a feedback letter was sent to all participating laboratories that had paid the fee in March 2020.

## 4.1 Results by assays used

### 4.1.1 Commercial assays

A total of 88 data sets were obtained using commercially available tests. The most commonly used assays were the Anyplex II HPV 28 (Seegene) that was used in 20 laboratories, HPV Direct Flow-Chip (Master Diagnostica) that was used in 7 laboratories and Linear Array (Roche) HPV genotyping assay, that was used in 6 laboratories (Table 2).

### 4.1.2 In-house assays

Twenty-two 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

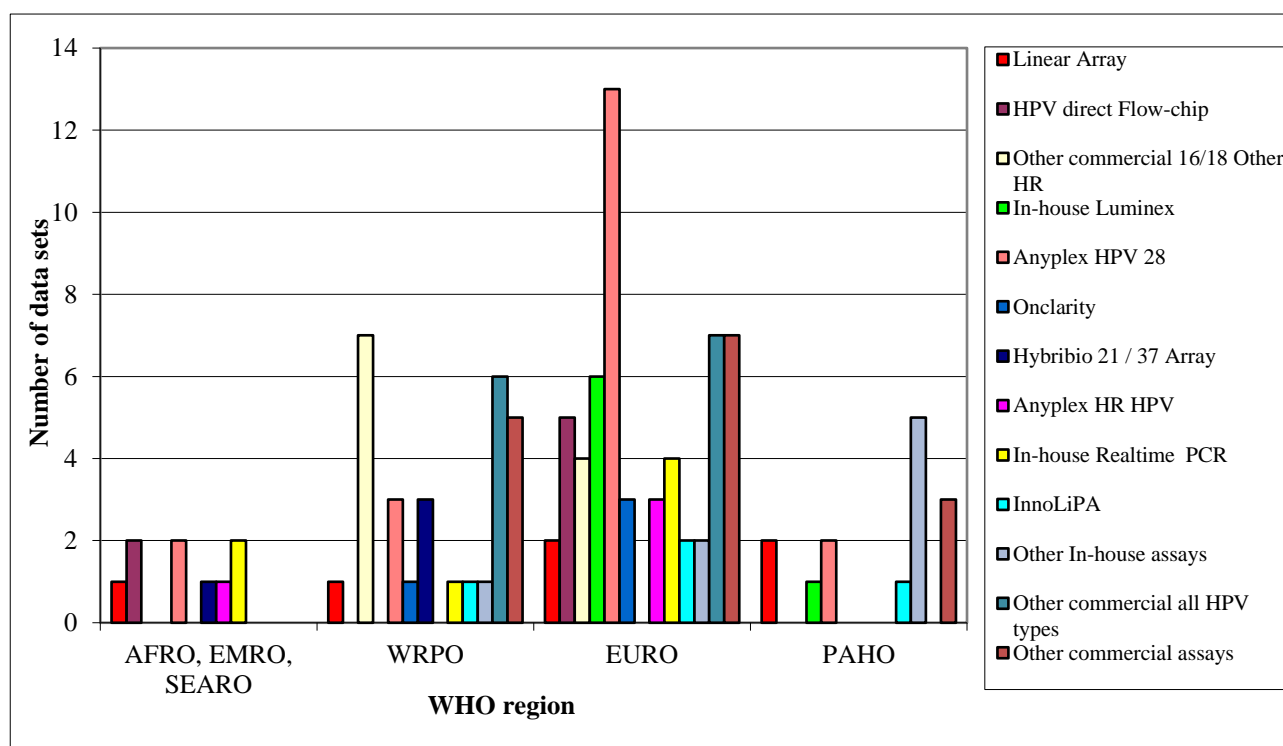
The different assays used for testing and typing of HPV as well as the number of submitted data sets and different part of the HPV genome targeted by each assay is shown in Table 2. The distribution of different assays in different WHO regions is shown in Figure 2.

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

HPV assay type	Number of data sets	HPV region targeted (primers)
<b>All assays</b>	<b>110</b>	<b>L1/L2/E1/E2/E4/E5/E6/E7</b>
Anyplex II HPV 28 (Seegene)	20	L1
In-house PCR Luminex	7	L1 / E7
In-house realtime PCR	7	L1/L2/E1/E4/E6/E7
HPV Direct Flow-chip (Master Diagnostica)	7	L1 (GP)
Linear Array (Roche)	6	L1 (PGMY)
InnoLiPA Extra (Fujirebio)	4	L1 (SPF10)
Anyplex HR HPV (Seegene)	4	L1
Onclarity (BD)	4	E6 / E7
HybriBio 21 HPV / 37 Array (HybriBio)	4	L1 (MY09/11)
OncoPredict HPV-DNA (Hiantis)	3	E6 / E7
Cobas 4800 / 6800 (Roche)	3	L1
Abbott m2000 / Alinity M (Abbott)	3	L1

In-house blot	3	L1
HPV-23 Genotyping (Hybriobio)	3	L1/L2/E1/E2/E4/E6/E7
GenoFlow HPV array (DiagCor)	2	L1 (PGMY)
Diamex (Diamex)	2	L1
Ampliquality (AB Analitica)	2	L1
In-house PGMY-CHUV	2	L1 (PGMY)
HPV SPF10-LiPA25 (Labo-bio)	2	L1 (SPF10)
VisionArray HPV (ZytoVision)	2	L1
Hybriobio 13 (Hybriobio)	2	E6 / E7
Hybriobio 14 HR (Hybriobio)	2	E6 / E7
Other Commercial assays <sup>a)</sup>	13	L1/L2/E1/E6/E7
Other In-house assays <sup>b)</sup>	3	L1 / E6 / E7

- a) Other commercial assays include one laboratory using each of: EUROArray HPV test, Agena MassARRAY iPLEX, Venus HPV, Harmonia HPV, OmniPlex-HPV, AmpFire, Riatol qPCR kit, Chapter NGS, amplisens HPV, SACACE HPV screen, yd-diagnostics, aid-diagnostika, Cepheid Xpert
- b) Other In-house assays include one laboratory using each of: In-house RFLP, In-house EIA, In-house seq



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

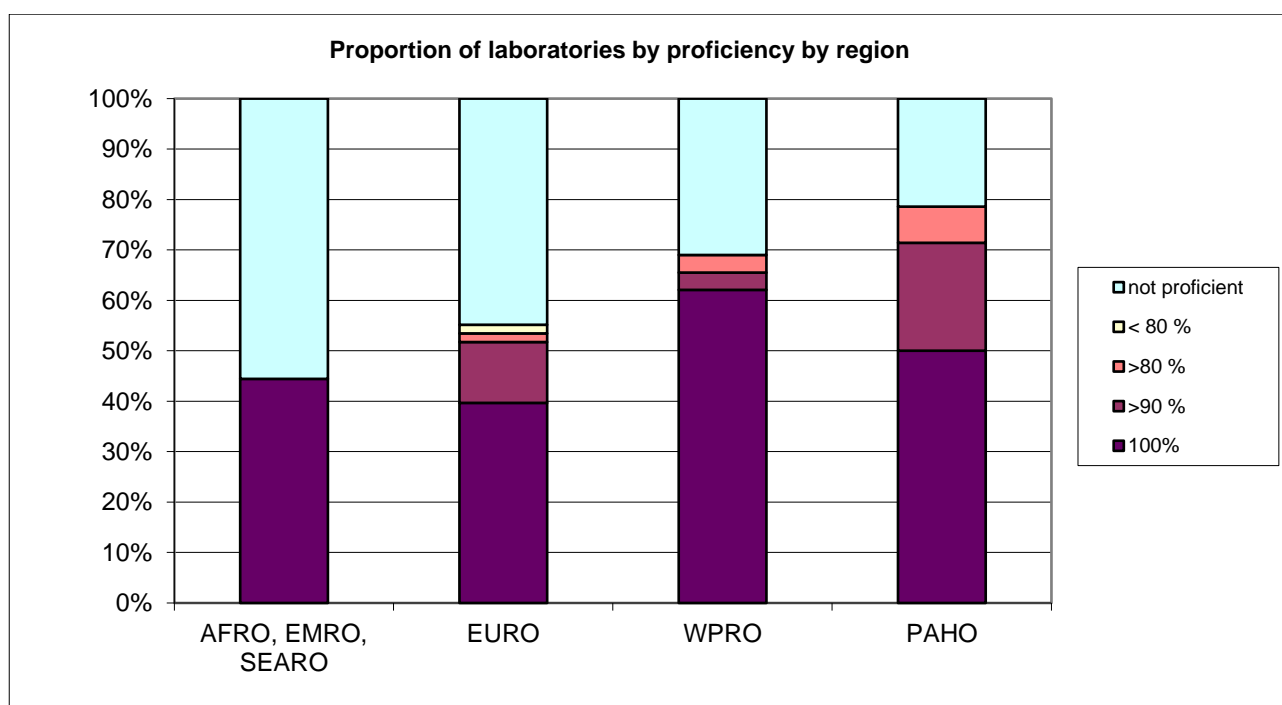
#### 4.2.2 Results achieved by participating laboratories

This year the criteria for proficiency was increased to no false positive results allowed (in previous years, a maximum of one false positive had been allowed for proficiency). According to the criteria

described in 3.4, fifty-two (47 %) data sets out of the 110 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, 28 data set 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 and Table 3. 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 110 data-set.

**Table 3:** Proportion of data sets submitted by WHO region with  $\geq 90$  % proficient HPV typing results.

Region (data sets)	Proportion of laboratories with 100 % correct typing	Proportion of laboratories with $\geq 90$ % correct typing
EURO (58)	40 %	52 %
AFRO, EMRO, SEARO (9)	44 %	44 %
PAHO (14)	50 %	71 %
WPRO (29)	62 %	66 %

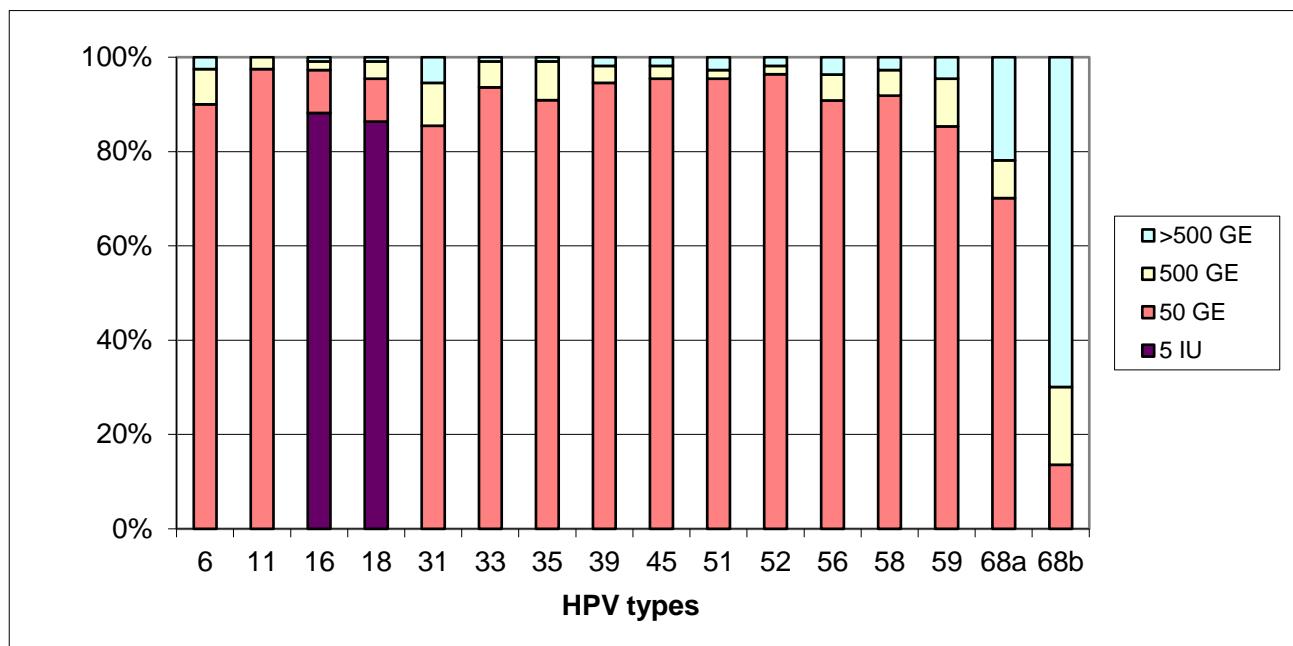
**Table 4:** Proficiency for detection of specific HPV types by assay used. (not HPV 68b)

HPV assay type	Number of data sets	No. of proficient data sets				
		100 % proficient	99-90 % proficient	89-80 % proficient	<80 % proficient	Not proficient
<b>All assays</b>	<b>110</b>	<b>52</b>	<b>9</b>	<b>4</b>	<b>2</b>	<b>43</b>
Anyplex II HPV 28 (Seegene)	20	12	2	0	0	6
In- house PCR Luminex	7	4	0	0	0	3
In-house realtime PCR	7	4	0	0	0	3
HPV Direct Flow-chip (Master Diagnostica)	7	1	0	0	0	6
Linear Array (Roche)	6	3	0	0	0	3
InnoLiPA Extra (Fujirebio)	4	1	2	0	0	1
Anyplex HR HPV (Seegene)	4	2	0	0	0	2
Onclarity (BD)	4	2	0	0	0	2
HybriBio 21 HPV / 37 Array (HybriBio)	4	4	0	0	0	0
OncoPredict HPV-DNA (Hiantis)	3	3	0	0	0	0
Cobas 4800 / 6800 (Roche)	3	0	0	0	0	3
Abbott m2000 / Alinity M (Abbott)	3	1	2	0	0	0
In-house blot	3	0	1	0	0	2
HPV-23 Genotyping (HybriBio)	3	2	0	0	0	1
GenoFlow HPV array (DiagCor)	2	2	0	0	0	0
Diamex (Diamex)	2	0	0	2	0	0
Ampliquality (AB Analitica)	2	0	0	0	0	2
In-house PGMY-CHUV	2	2	0	0	0	0
HPV SPF10-LiPA25 (Labo-bio)	2	0	0	0	0	2
VisionArray HPV (ZytoVision)	2	0	0	0	0	2
HybriBio 13 (HybriBio)	2	2	0	0	0	0
HybriBio 14 HR (HybriBio)	2	2	0	0	0	0
Other Commercial assays <sup>a)</sup>	13	4	2	2	1	4
Other In-house assays <sup>b)</sup>	3	1	0	0	1	1

- a) Other commercial assays include one laboratory using each of; EUROArray HPV test, Agena MassARRAY iPLEX, Venus HPV, Harmonia HPV, OmniPlex-HPV, AmpFire, Riatol qPCR kit, Chapter NGS, amplisens HPV, SACACE HPV screen, yd-diagnostics, aid-diagnostika, Cepheid Xpert
- b) Other In-house assays include one laboratory using each of; In-house RFLP, In-house EIA, In-house seq

### 4.2.3 HPV types detected

The sensitivity to detect each HPV type, as percent of laboratories detecting the different copy number (IU / GE) of the HPV types is shown in Figure 4. This data includes laboratories with multiple false positives. 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  $\mu$ l 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  $\mu$ l were classified as testing sample with 10-fold higher IU/GE content compared to that of 5  $\mu$ l input. Input with 10 or 15  $\mu$ l was classified as same IU/GE content as compared to input with 5  $\mu$ l. Three laboratories used 50  $\mu$ l input volume in Linear Array. This gives different number of possible data sets for different concentrations of different HPV types in Table 5 and 6.

**Table 5:** HPV GE or IU detected and typed per 5 µl in samples in both single and multiple HPV infections among the most commonly used commercial assays. Lowest detected GE / IU are indicated.

HPV type	HPV IU/GE	All Assays (%)	Anyplex II HPV 28	HPV Direct Flow-chip	Linear Array	InnoLiPA	HybriBio 21 / 37	Onclarity	Anyplex HR HPV	Onco - Predict
16	5	89 / 100 (89)	20 / 20	6 / 7	4 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
16	50	98 / 100 (98)		7 / 7	6 / 6					
16	500	99 / 100 (99)								
18	5	88 / 100 (88)	19 / 20	6 / 7	5 / 6	4 / 4	4 / 4	2 / 4	4 / 4	3 / 3
18	50	97 / 100 (97)	20 / 20	7 / 7	6 / 6			4 / 4		
18	500	98 / 100 (98)								
6	50	72 / 80 (90)	20 / 20	7 / 7	6 / 6	4 / 4	4 / 4	nt <sup>a)</sup>	nt <sup>a)</sup>	nt <sup>a)</sup>
6	500	79 / 80 (99)								
11	50	78 / 80 (98)	20 / 20	7 / 7	6 / 6	4 / 4	4 / 4	nt <sup>a)</sup>	nt <sup>a)</sup>	nt <sup>a)</sup>
11	500	80 / 80 (100)								
31	50	85 / 100 (85)	19 / 20	6 / 7	5 / 6	1 / 4	4 / 4	4 / 4	4 / 4	3 / 3
31	500	94 / 100 (94)	20 / 20	7 / 7	6 / 6					
33	50	93 / 100 (93)	20 / 20	5 / 7	4 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
33	500	99 / 100 (99)		6 / 7	6 / 6					
35	50	90 / 100 (90)	20 / 20	7 / 7	5 / 6	4 / 4	3 / 4	3 / 4	4 / 4	3 / 3
35	500	99 / 100 (99)			6 / 6		4 / 4	4 / 4		
39	50	94 / 100 (94)	20 / 20	6 / 7	6 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
39	500	98 / 100 (98)		7 / 7						
45	50	95 / 100 (95)	19 / 20	7 / 7	6 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
45	500	98 / 100 (98)	20 / 20							
51	50	94 / 99 (95)	20 / 20	7 / 7	5 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
51	500	96 / 99 (97)								
52	50	96 / 100 (96)	20 / 20	7 / 7	6 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
52	500	98 / 100 (98)								
56	50	89 / 99 (90)	18 / 20	6 / 7	4 / 6	4 / 4	3 / 4	4 / 4	4 / 4	3 / 3
56	500	95 / 99 (96)			6 / 6		4 / 4			
58	50	91 / 100 (91)	19 / 20	6 / 7	6 / 6	4 / 4	4 / 4	4 / 4	4 / 4	3 / 3
58	500	97 / 100 (97)	20 / 20	7 / 7						
59	50	83 / 99 (84)	19 / 20	6 / 7	5 / 6	4 / 4	3 / 4	4 / 4	4 / 4	3 / 3

59	500	94 / 99 (95)	20 / 20		6 / 6		4 / 4			
68a	50	56 / 77 (73)	20 / 20	7 / 7	nt <sup>a)</sup>	4 / 4	3 / 4	nt <sup>a)</sup>	4 / 4	nt <sup>a)</sup>
68a	500	63 / 77 (82)					4 / 4			
68b	50	12 / 92 (13)	1 / 20		1 / 6		2 / 4			nt <sup>a)</sup>
68b	500	26 / 92 (28)	4 / 20	3 / 7	2 / 6		3 / 4			

a) Nt: Not tested

**Table 6:** HPV GE or IU detected and typed per 5 µl in samples with in both single and multiple HPV infections among in-house assays and commercial assays used by less than 3 laboratories. Lowest detected GE / IU are indicated.

HPV type	HPV IU/GE	In-house Lumindex	In-house Realtime PCR	In-house Blot	HPV 23 Genotyping (HybriBio)	Other In-house <sup>a)</sup>	Other Commercial <sup>b)</sup>	Other Commercial <sup>c)</sup>
16	5	7 / 7	6 / 7	2 / 3	3 / 3	3 / 5	8 / 10	12 / 13
16	50			3 / 3		5 / 5	10 / 10	13 / 13
16	500							
18	5	6 / 7	5 / 7	1 / 3	3 / 3	4 / 4	10 / 10	12 / 13
18	50	7 / 7	6 / 7	3 / 3				
18	500		7 / 7					
6	50	6 / 6	4 / 5	2 / 3	3 / 3	4 / 5	8 / 10	6 / 7
6	500			3 / 3		5 / 5		7 / 7
11	50	6 / 6	5 / 5	3 / 3	3 / 3	5 / 5	10 / 10	7 / 7
11	500							
31	50	6 / 7	7 / 7	1 / 3	3 / 3	3 / 5	8 / 10	11 / 13
31	500	7 / 7		3 / 3			10 / 10	13 / 13
33	50	7 / 7	6 / 7	3 / 3	3 / 3	5 / 5	10 / 10	11 / 13
33	500		7 / 7					13 / 13
35	50	6 / 7	5 / 7	3 / 3	3 / 3	4 / 5	10 / 10	10 / 13
35	500	7 / 7	7 / 7					11 / 13
39	50	7 / 7	6 / 7	3 / 3	3 / 3	3 / 5	10 / 10	12 / 13
39	500					4 / 5		13 / 13
45	50	7 / 7	6 / 7	2 / 3	3 / 3	4 / 5	10 / 10	12 / 13
45	500			3 / 3				13 / 13
51	50	7 / 7	5 / 6	3 / 3	3 / 3	4 / 5	10 / 10	11 / 13
51	500					5 / 5		12 / 13

52	50	7 / 7	5 / 7	3 / 3	3 / 3	4 / 5	10 / 10	12 / 13
52	500					5 / 5		13 / 13
56	50	7 / 7	5 / 6	3 / 3	3 / 3	5 / 5	9 / 10	11 / 13
56	500		6 / 6				10 / 10	12 / 13
58	50	7 / 7	5 / 7	3 / 3	3 / 3	5 / 5	8 / 10	10 / 13
58	500						10 / 10	12 / 13
59	50	6 / 7	3 / 6	1 / 3	3 / 3	3 / 5	8 / 10	11 / 13
59	500	7 / 7	6 / 6	2 / 3			10 / 10	12 / 13
68a	50	5 / 6	1 / 5	1 / 3	2 / 3	3 / 5	2 / 10	5 / 12
68a	500				3 / 3	4 / 5	5 / 10	
68b	50	2 / 7			1 / 3			3 / 12
68b	500		1 / 5		3 / 3	2 / 5	1 / 10	

- a) Other In-house assays include two laboratories using; In-house PGMY-CHUV, and one laboratory using each of; In-house PCR EIA, In-house PCR-RFLP, In-house sequencing
- b) Other commercial assays include two laboratories using each of; HPV SPF10-LiPA25, GenoFlow HPV array, Diamex, Ampliquality, VisionArray HPV
- c) Other commercial assays include one laboratory using each of;; EUROArray HPV test, Agena MassARRAY iPLEX, VenusHPV, HarmoniaHPV, OmniPlex-HPV, AmpFire, Riatol qPCR kit, Chapter NGS, amplisens HPV, SACACE HPV screen, yd-diagnostics, aid-diagnostika, Cephid Xpert
- d) Nt: Not tested
- e) Cobas 4800 / 6800, Abbott, HybriBio 13 / 14 do not type, not included

### 4.3 Results by sample number

The numbers of laboratories typing 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, including test that type HPV 16 and HPV 18 and other HR, reported by sample number.

HPV types	HPV genome equivalents per 5 µl	Percent correct data sets (N)
16	50	<b>99.1 (109 / 110)</b>
16	5	88.2 (97 / 110)
18	50	<b>93.6 (103 / 110)</b>
18	5	84.5 (93 / 110)
6	500	<b>96.2 (77 / 80)</b>
6	50	91.2 (73 / 80)
11	500	<b>98.8 (79 / 80)</b>
11	50	96.2 (77 / 80)
31	500	<b>64.5 (71 / 110)<sup>b</sup></b>
31	50	88.2 (97 / 110) <sup>b</sup>
33	500	<b>94.5 (104 / 110)</b>
33	50	94.5 (104 / 110)
35	500	<b>92.7 (102 / 110)</b>
35	50	90.0 (99 / 110)
39	500	<b>96.4 (106 / 110)</b>
39	50	93.6 (103 / 110)
45	500	<b>98.2 (108 / 110)</b>
45	50	96.4 (106 / 110)
51	500	<b>93.6 (102 / 109)</b>
51	50	94.5 (103 / 109)
52	500	<b>96.4 (106 / 110)</b>
52	50	96.4 (106 / 110)
56	500	<b>94.5 (103 / 109)</b>
56	50	91.7 (100 / 109)
58	500	<b>91.8 (101 / 110)</b>
58	50	89.1 (98 / 110)
59	500	<b>94.5 (103 / 109)</b>
59	50	90.1 (99 / 109)
68a	500	<b>81.8 (72 / 88)</b>
68a	50	72.7 (64 / 88)
68b	500	<b>37.2 (38 / 102)</b>
68b	50	28.4 (29 / 102)
6, 18, 39, 56	500	<b>85.4 (94 / 110)</b>
6, 18, 39, 56	50	87.3 (96 / 110)
11, 16, 52, 68a	500	<b>84.5 (93 / 110)<sup>a</sup></b>
11, 16, 52, 68a	50	80.0 (88 / 110) <sup>a</sup>
31, 45, 58, 68b	500	<b>45.4 (50 / 110)</b>
31, 45, 58, 68b	50	29.1 (32 / 110)
33, 35, 51, 59	500	<b>89.1 (98 / 110)</b>
33, 35, 51, 59	50	84.5 (93 / 110)
TE buffer with 10 ng/µl human placenta DNA	0	<b>93.7 (103 / 110)</b>
HPV 16 positive SiHa cells	2500	96.2 (102 / 106) (1 false positive)

HPV 16 positive SiHa cells	25	96.3 (105 / 109) (1 false positive)
HPV-negative C33A cells	0	98.2 (107 / 109) (2 false positive)

<sup>a</sup> 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.

<sup>b</sup>It is correct that more laboratories were able to detect the lower amounts of HPV31. Real-time quantitative PCR in several laboratories confirmed that the amounts stated are correct and that there was no mix-up of samples. Laboratories who detected HPV31 only in the lower amount were considered as having detected HPV31.

#### 4.4 Analysis of false positive results

To be considered as proficient for HPV testing no false positive result was accepted. The number of false positive samples by assay 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
<b>All assays</b>	110	67	19	6	9	9
Anyplex II HPV 28 (Seegene)	20	14	2	2	2	0
In- house PCR Luminex	7	4	0	0	1	2
In-house realtime PCR	7	4	1	0	0	2
HPV Direct Flow-chip (Master Diagnostica)	7	1	1	1	3	1
Linear Array (Roche)	6	3	2	0	0	1
InnoLiPA Extra (Fujirebio)	4	3	1	0	0	0
Anyplex HR HPV (Seegene)	4	2	2	0	0	0
Onclarity (BD)	4	2	1	1	0	0
HybriBio 21 HPV / 37 Array (HybriBio)	4	4	0	0	0	0
OncoPredict HPV-DNA (Hinatis)	3	3	0	0	0	0
Cobas 4800 / 6800 (Roche)	3	0	3	0	0	0
Abbott m2000 / Alinity M (Abbott)	3	3	0	0	0	0
In-house blot	3	1	0	0	2	0
HPV-23 Genotyping (HybriBio)	3	2	1	0	0	0
GenoFlow HPV array (DiagCor)	2	2	0	0	0	0
Diamex (Diamex)	2	2	0	0	0	0
Ampliquality (AB Analytica)	2	0	1	0	0	1
In-house PGMY-CHUV	2	2	0	0	0	0

HPV SPF10-LiPA25 (Labo-bio)	2	0	0	1	1	0
VisionArray HPV (ZytoVision)	2	0	2	0	0	0
HybriBio 13 (HybriBio)	2	2	0	0	0	0
HybriBio 14 HR (HybriBio)	2	2	0	0	0	0
Other Commercial assays <sup>a)</sup>	13	9	1	1	0	2
Other In-house assays <sup>b)</sup>	3	2	1	0	0	0

- a) Other commercial assays include one laboratory using each of; EUROArray HPV test, Agena MassARRAY iPLEX, VenusHPV, HarmoniaHPV, OmniPlex-HPV, AmpFire, Riatol qPCR kit, Chapter NGS, amplisens HPV, SACACE HPV screen, yd-diagnostics, aid-diagnostika, Cepheid Xpert
- b) Other In-house assays include one laboratory using each of; In-house RFLP, In-house EIA, In-house seq

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). Five laboratories detected HPV 31 in the sample that contained only human DNA. The assays used was Anyplex HPV 28 (used by in total 20 laboratories - 18 tested this sample as correctly negative), Onclarity and in-house Luminex.

#### **4.5 Comparison of results for laboratories that participated 2019 and in the years 2008, 2010, 2011, 2013, 2014 and 2017**

In total 54 laboratories that participated in 2019 had also participated in the HPV LabNet PPs from at least one previous year. Thirteen laboratories participated in all 7 PPs (2008, 2010, 2011, 2013, 2014, 2017 and 2019). Six laboratories analysed the PP in 6 years, 7 laboratories in 5 years, 6 laboratories in 4 years, 10 laboratories in 3 years and 12 laboratories analysed the panel in 2017 and 2019. 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 each year and compared with the results from all data sets submitted 2019 is shown in Table 9a and 9b, the sensitivity for individual HPV types in Table 10 and the specificity with number of false positive samples in Table 11a and 11b.

**Table 9a:** Proficiency of detecting HPV types by laboratories that participated in 2019 PP, using identical assays in 2008, 2010, 2011, 2013, 2014 and 2017 in comparison with all data sets submitted in 2019.

**Not proficient = One or more false positive results**

Proficiency	Laboratories that participated in 2008, 2010, 2011, 2013, 2014, 2017 and 2019 using identical assays							All datasets 2019 (%)
	2008 (%)	2010 (%)	2011 (%)	2013 (%)	2014 (%)	2017 (%)	2019 (%)	
<b>100 % proficient</b>	4 / 14 (29)	4 / 19 (21)	13 / 26 (50)	16 / 33 (48)	19 / 35 (54)	33 / 62 (53)	33 / 65 (51)	52 / 110 (47)
<b>99-90 % proficient</b>	1 / 14 (7.1)	1 / 19 (5.3)	1 / 26 (3.8)	2 / 33 (6.1)	2 / 35 (5.7)	4 / 62 (6.5)	3 / 65 (4.6)	11 / 110 (10)
<b>89-80 % proficient</b>	0 / 14 (0)	3 / 19 (16)	4 / 26 (15)	2 / 33 (6.1)	1 / 35 (2.9)	5 / 62 (8.1)	2 / 65 (3.1)	3 / 110 (2.7)
<b>&lt;80 % proficient</b>	1 / 14 (7.1)	1 / 19 (5.3)	1 / 26 (3.8)	0 / 33 (0)	0 / 35 (0)	2 / 62 (3.2)	1 / 65 (1.5)	1 / 110 (0.9)
<b>Not proficient</b>	8 / 14 (57)	10 / 19 (53)	7 / 26 (27)	13 / 33 (39)	13 / 35 (37)	18 / 62 (29)	26 / 65 (40)	43 / 110 (39)

**Table 9b:** Proficiency of detecting HPV types by laboratories that participated in 2019 PP, with data from 2008, 2010, 2011, 2013, 2014 and 2017 in comparison with all data sets submitted 2019.

**Not proficient = false positive result**

Proficiency	All test by laboratories that participated in 2008, 2010, 2011, 2013, 2014, 2017 and 2019							All datasets 2019 (%)
	2008 (%)	2010 (%)	2011 (%)	2013 (%)	2014 (%)	2017 (%)	2019 (%)	
<b>100 % proficient</b>	7 / 21 (33)	8 / 33 (24)	20 / 39 (51)	21 / 44 (48)	26 / 46 (57)	37 / 71 (52)	39 / 78 (50)	52 / 110 (47)
<b>99-90 % proficient</b>	1 / 21 (4.8)	1 / 33 (3.0)	2 / 39 (5.1)	4 / 44 (9.1)	2 / 46 (4.3)	5 / 71 (7.0)	4 / 78 (5.1)	11 / 110 (10)
<b>89-80 % proficient</b>	0 / 21 (0)	5 / 33 (15)	4 / 39 (10)	3 / 44 (6.8)	3 / 46 (6.6)	5 / 71 (7.0)	2 / 78 (2.6)	3 / 110 (2.7)
<b>&lt;80 % proficient</b>	1 / 21 (4.8)	2 / 33 (6.1)	2 / 39 (5.1)	0 / 44 (0)	1 / 46 (2.2)	2 / 71 (2.8)	1 / 78 (1.3)	1 / 110 (0.9)
<b>Not proficient</b>	12 / 21 (57)	17 / 33 (52)	11 / 39 (28)	16 / 44 (36)	14 / 46 (30)	22 / 71 (31)	32 / 78 (41)	43 / 110 (39)

**Table 10:** HPV GE or IU detected and typed per 5 µl in both single and multiple infections by laboratories participating in 2010, 2011, 2013, 2014, 2017 and 2019 in comparison with all datasets 2019. Table includes samples with detection of false positive HPV types. Lowest detected GE/IU is indicated.

HPV type	HPV IU /GE	All test by laboratories participating both in 2010, 2011, 2013, 2014, 2017 and 2019						All datasets 2019 (%)
		2010 (%)	2011 (%)	2013 (%)	2014 (%)	2017 (%)	2019 (%)	
16	5	27 / 33 (82)	28 / 37 (76)	35 / 44 (80)	37 / 46 (80)	54 / 71 (76)	70 / 78 (90)	89 / 100 (89)
16	50	31 / 33 (94)	37 / 37 (100)	39 / 44 (89)	42 / 46 (91)	62 / 71 (87)	76 / 78 (97)	98 / 100 (98)
16	500			44 / 44 (100)	46 / 46 (100)	70 / 71 (99)	78 / 78 (100)	99 / 100 (99)
18	5	25 / 32 (78)	22 / 37 (59)	36 / 44 (82)	35 / 45 (78)	48 / 71 (68)	66 / 78 (84)	88 / 100 (88)
18	50	29 / 32 (91)	34 / 37 (92)	39 / 44 (89)	39 / 45 (87)	61 / 71 (86)	75 / 78 (96)	97 / 100 (97)
18	500		36 / 37 (97)	44 / 44 (100)	44 / 45 (98)	70 / 71 (99)	77 / 78 (99)	98 / 100 (98)
6	50	20 / 30 (67)	26 / 33 (79)	28 / 39 (72)	32 / 41 (78)	43 / 54 (80)	55 / 59 (93)	72 / 80 (90)
6	500	27 / 30 (90)	31 / 33 (94)	35 / 39 (90)	40 / 41 (98)	52 / 54 (96)	59 / 59 (100)	79 / 80 (99)
11	50	28 / 30 (93)	28 / 33 (85)	32 / 39 (82)	35 / 41 (85)	47 / 54 (87)	58 / 59 (98)	78 / 80 (98)
11	500		32 / 33 (97)	39 / 39 (100)	40 / 41 (98)	53 / 54 (98)	59 / 59 (100)	80 / 80 (100)
31	50	22 / 32 (69)	25 / 37 (68)	29 / 44 (66)	32 / 46 (70)	49 / 71 (69)	67 / 78 (86)	85 / 100 (85)
31	500	26 / 32 (81)	34 / 37 (92)	38 / 44 (86)	42 / 46 (91)	64 / 71 (90)	74 / 78 (95)	94 / 100 (94)
33	50	27 / 32 (84)	29 / 37 (78)	37 / 44 (84)	41 / 46 (89)	61 / 71 (86)	74 / 78 (95)	93 / 100 (93)
33	500	28 / 32 (88)	35 / 37 (95)	44 / 44 (100)	46 / 46 (100)	69 / 71 (97)	78 / 78 (100)	99 / 100 (99)
35	50	29 / 32 (91)	30 / 37 (81)	31 / 42 (74)	39 / 45 (87)	61 / 70 (87)	70 / 78 (90)	90 / 100 (90)
35	500	30 / 32 (94)	34 / 37 (92)	42 / 42 (100)	45 / 45 (100)	67 / 70 (96)	77 / 78 (99)	99 / 100 (99)
39	50	20 / 32 (62)	24 / 37 (65)	32 / 43 (74)	34 / 45 (76)	60 / 70 (86)	74 / 78 (95)	94 / 100 (94)
39	500	25 / 32 (78)	33 / 37 (89)	41 / 43 (95)	43 / 45 (96)	69 / 70 (99)	77 / 78 (99)	98 / 100 (98)
45	50	28 / 32 (87)	29 / 36 (81)	36 / 44 (82)	38 / 46 (83)	63 / 71 (89)	75 / 78 (96)	95 / 100 (95)
45	500	29 / 32 (91)	34 / 36 (94)	42 / 44 (95)	45 / 46 (98)	70 / 71 (99)	77 / 78 (99)	98 / 100 (98)
51	50	27 / 32 (84)	31 / 36 (86)	34 / 43 (79)	39 / 45 (87)	59 / 71 (83)	74 / 78 (95)	94 / 99 (95)
51	500	28 / 32 (88)	36 / 36 (100)	41 / 43 (95)	44 / 45 (98)	66 / 71 (93)	76 / 78 (97)	96 / 99 (97)
52	50	28 / 32 (88)	30 / 38 (79)	35 / 44 (80)	40 / 46 (87)	63 / 71 (89)	76 / 78 (97)	96 / 100 (96)
52	500	29 / 32 (91)	37 / 38 (97)	42 / 44 (95)	45 / 46 (98)	71 / 71 (100)	78 / 78 (100)	98 / 100 (98)
56	50	24 / 32 (75)	28 / 37 (76)	33 / 43 (77)	38 / 45 (84)	52 / 70 (74)	71 / 78 (91)	89 / 99 (90)
56	500	27 / 32 (84)	36 / 37 (97)	41 / 43 (95)	44 / 45 (98)	65 / 70 (93)	76 / 78 (97)	95 / 99 (96)
58	50	22 / 32 (69)	27 / 37 (73)	32 / 43 (74)	40 / 45 (89)	56 / 70 (80)	76 / 78 (97)	91 / 100 (91)
58	500	27 / 32 (84)	35 / 37 (95)	39 / 43 (91)	44 / 45 (98)	68 / 70 (97)	78 / 78 (100)	97 / 100 (97)
59	50	16 / 32 (50)	25 / 36 (69)	29 / 42 (69)	30 / 45 (67)	54 / 70 (77)	65 / 78 (83)	83 / 99 (84)
59	500	20 / 32 (62)	29 / 36 (81)	39 / 42 (93)	42 / 45 (93)	67 / 70 (96)	74 / 78 (95)	94 / 99 (95)
68a	50	1 / 13 <sup>a)</sup> (7.7)	7 / 21 (33)	10 / 26 (38)	16 / 28 (57)	34 / 55 (62)	46 / 72 (64)	56 / 77 (73)
68a	500	4 / 13 (31)	10 / 21 (48)	14 / 26 (54)	19 / 28 (68)	35 / 55 (64)	50 / 72 (69)	63 / 77 (82)
68b	50	21 / 29 (72)	25 / 35 (71)	27 / 40 (68)	32 / 43 (74)	54 / 68 (79)	10 / 75 (13)	12 / 92 (13)
68b	500	24 / 29 (83)	31 / 35 (89)	38 / 40 (95)	39 / 43 (91)	61 / 68 (90)	24 / 75 (32)	26 / 92 (28)

a) 68a cannot be detected by PGMV based primers except version 2 of PGMV-CHUV, the plasmid used contains the L1 fragment only.

**Table 11a:** Number of false positive HPV types detected per data set reported by laboratories using identical assays in 2008, 2010, 2011, 2013, 2014, 2017 and 2019 years proficiency studies in comparison with all data sets submitted 2019.

No of false positive samples	Laboratories that participated in 2008, 2010, 2011, 2013, 2014, 2017 and 2019 using identical assays							All datasets 2019 (%)
	2008 (%)	2010 (%)	2011 (%)	2013 (%)	2014 (%)	2017 (%)	2019 (%)	
<b>0 samples</b>	6 / 14 (43)	9 / 19 (47)	20 / 26 (77)	20 / 33 (61)	22 / 35 (63)	46 / 64 (72)	41 / 69 (59)	67 / 110 (61)
<b>1 sample</b>	4 / 14 (29)	1 / 19 (5.2)	0 / 26 (0)	7 / 33 (21)	4 / 35 (11)	9 / 64 (14)	12 / 69 (17)	19 / 110 (17)
<b>2 samples</b>	2 / 14 (14)	4 / 19 (21)	3 / 26 (11)	0 / 33 (0)	2 / 35 (5.7)	2 / 64 (3.1)	4 / 69 (5.8)	6 / 110 (5.4)
<b>3 samples</b>	0 / 14 (0)	1 / 19 (5.2)	0 / 26 (0)	2 / 33 (6.1)	3 / 35 (8.6)	0 / 64 (0)	6 / 69 (8.7)	9 / 110 (8.2)
<b>&gt;3 samples</b>	2 / 14 (14)	4 / 19 (21)	3 / 26 (11)	4 / 33 (12)	4 / 35 (11)	7 / 64 (11)	6 / 69 (8.7)	9 / 110 (8.2)

**Table 11b:** Number of false positive HPV types detected per data set reported by laboratories participating in 2008, 2010, 2011, 2013, 2014, 2017 and 2019 years proficiency studies in comparison with all data sets submitted 2019.

No of false positive samples	All test by laboratories that participated in 2008, 2010, 2011, 2013, 2014, 2017 and 2019							All datasets 2019 (%)
	2008 (%)	2010 (%)	2011 (%)	2013 (%)	2014 (%)	2017 (%)	2019 (%)	
<b>0 samples</b>	9 / 21 (43)	16 / 33 (48)	28 / 39 (72)	28 / 44 (64)	32 / 46 (70)	49 / 71 (69)	46 / 78 (59)	67 / 110 (61)
<b>1 sample</b>	5 / 21 (24)	3 / 33 (9.1)	5 / 39 (13)	9 / 44 (20)	4 / 46 (8.7)	12 / 71 (17)	13 / 78 (17)	19 / 110 (17)
<b>2 samples</b>	3 / 21 (14)	6 / 33 (18)	3 / 39 (7.7)	1 / 44 (2.3)	3 / 46 (6.5)	2 / 71 (2.8)	4 / 78 (5.1)	6 / 110 (5.4)
<b>3 samples</b>	1 / 21 (4.8)	4 / 33 (12)	0 / 39 (0)	2 / 44 (4.5)	3 / 46 (6.5)	0 / 71 (0)	9 / 78 (12)	9 / 110 (8.2)
<b>&gt;3 samples</b>	3 / 21 (14)	4 / 33 (12)	3 / 39 (7.7)	4 / 44 (9.1)	4 / 46 (8.7)	8 / 71 (11)	6 / 78 (7.7)	9 / 110 (8.2)

## 5. Discussion

The 2019 PP was distributed to 82 laboratories worldwide and 110 datasets were returned from 78 laboratories. The promising result of a global improvement in testing quality that was seen in previous PP is maintained. The proportion of data sets that could correctly type all samples included in the panel increased from 15 % (20 / 136 datasets) in 2013, to 25 % (28 / 110 datasets) in 2019. Participating laboratories involved public health laboratories, research laboratories and diagnostic test manufacturers. There was a charge for laboratories to participate, although participants from low and lower middle-income countries could have their fees waived. It cannot be excluded that some laboratories from low income countries may have chosen not to participate because of the fee. It is thus possible that the improved performance observed in the 2019 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 2019, indicating that there has indeed occurred a global improvement in the performance of HPV genotyping.

There is an overall improvement in sensitivity of the assays used. HPV 16, 18, 33 and 35 could be detected in 500 GE / 5 µl in all but 1 dataset respectively.

A proficiency of 100 % for detection of at least 50 IU of HPV 16 and HPV 18 in 5 µl and 500 GE in 5 µl of the other HPV types tested for without having any false positive type detected was achieved in 47 % of the datasets (52 data sets from 42 laboratories). Not all assays tested for every HPV type 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 6, laboratories using such an assay were considered as not testing for HPV 6.

A total of 88 data sets had been obtained using commercial tests. The most commonly used assays were the Anyplex II HPV 28 (Seegene) that was used in 20 laboratories, HPV Direct Flow-chip (Master Diagnostica) that was used in 7 laboratories and Linear Array (Roche) HPV genotyping assay, that was used in 6 laboratories. Twenty-two of the data sets had been obtained using a variety of in-house assays. The proportion of commercial assays has increase from 57 % in 2011 to 80 % in 2019. Twelve data sets were generated using assays unable to perform complete HPV typing. Ten of these datasets identified HPV 16 and 18 and gave the other types as High-risk HPV: Cobas 4800 (Roche), HybriBio 14 (HybriBio), Abbot Realtime PCR, and one in-house EIA. 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.

Four commercial assays were 100 % proficient for HPV typing in all datasets: GenoFlow HPV array (Diagcor), OncoPredict (Hiantis), HybriBio 21, and HybriBio 13/14 (HybriBio). The HybriBio 21 were used by three laboratories in the Western Pacific Region and one in Africa, OncoPredict were used by three European laboratories, GenoFlow HPV array were used by two laboratories in the Western Pacific Region, HybriBio 13/14 were used by two laboratories in the Western Pacific Region. 50 % of the data sets generated by in-house assays were 100 % proficient, among these the PGMY-CHUV assay and in-house real-time PCR assays. The 43 data sets classified as not proficient all detected one or more false positive HPV types. There was no difference in reporting false positive results among commercial assays (39 %), and in-house assays (41 %).

Anyplex II HPV 28 was used to generate 20 data sets out of which 12 were 100 % proficient. Six datasets were not proficient reporting 1 to 3 false positive results. These results are an example of the common finding that the laboratory performing the test has a big impact on the performance of the test itself.

All plasmids in the panel contained full-length genomes, except HPV 68a that only contained L1. Since all PGMY-based assays (directed against L1) cannot detect HPV68a, except version 2 of PGMY-CHUV assay, we also added HPV 68b in the panel. HPV68b can be detected by PGMY-based primers and other common primer systems. All data sets generated using assays targeting other parts of HPV 68a or that used the PGMY primers were considered as not testing for HPV 68a in this study. Among the 88 data sets that could be analysed for detection of HPV 68a, 82 % (72/88) detected 500 GE of HPV 68a.

There were 37 % (38/102) data sets that could detect 500 GE of HPV 68b. This is considerably fewer than in 2017 when 97 % (130 / 134) data sets were positive. This is most probably because of a problem with instability of the plasmid in the proficiency panel and not depending on any of the assays.

The PP is designed for the genotyping needs in HPV vaccine research and the proficiency criteria are not intended for clinical HPV screening purposes, where the requirements for analytical sensitivity may be different.

Several of the data sets submitted in 2019 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 these assays.

Three additional samples (A, B, C) in the PP were used to evaluate the DNA extraction step prior to 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 served as negative control.

There were at least 22 different extraction procedures used among the laboratories. The most commonly used was different extraction kits from Qiagen used to generate 30 data sets, followed by extraction kits using MagNa Pure LC (Roche) used to generate 10 datasets. Other utilised methods were BioMerieux kits, HybriBio extraction kit and STARMag from Seegene. The HPV Direct flow-chip from Master Diagnostica is performed without DNA extraction, the cell suspensions are added directly to the PCR mix. Three laboratories reported false positive results in sample A, B, C. We did not observe any obvious difference in performance between different extraction methods.

In sample B containing 2500 cells / 5 ul of the cervical cancer cell line SiHa, HPV 16 was correctly identified by 96 % of the data sets. Only one data set reported false positive HPV types in this sample. In sample C containing 25 SiHa cells / 5ul, HPV 16 was detected in 96 % of the data sets with one false positive result reported. The negative control containing only C33A cells was correctly reported as negative by 98 % of the laboratories.

## 6. Conclusions and recommendations

This was the seventh HPV LabNet HPV DNA proficiency study that was open for participation to all laboratories worldwide. The panel provided the possibility to analyse the specificity and sensitivity for different HPV typing assays to correctly identify 13 high risk HPV types and 2 low risk HPV types, the HPV types that are the most important for HPV vaccine research as well as for HPV surveillance and monitoring.

As a service to the laboratories using assays that could not detect the HPV68 prototype virus, but only the subtype HPV68b, we also included HPV68b in the proficiency panel starting in 2017, but not requiring that it should be detectable. It appears that this “extra” sample was this year not as adequate as in the PP of 2017 and we suggest that the results regarding the HPV68b subtype in 2019 should be disregarded.

Laboratories from all WHO regions participated in testing the 2019 PP. The majority of participating laboratories were from the EURO region.

The 2019 Global HPV LabNet HPV DNA proficiency panel further supports 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.

Comparing the results in 2008, 2010, 2011, 2013, 2014, 2017 and 2019 Global HPV DNA PPs, we can see overall improvements. E.g., comparison of laboratories that have participated during the 6 years: 29 % were 100 % proficient in 2010, as compared to 51 % in 2019.

We suggest that recommendations for HPV laboratory testing should continue to include a strong emphasis on the use of negative controls in the assays.

As also demonstrated in previous studies, HPV 16 and HPV 18 were the types detected at lowest IU in most data sets. Only 1 dataset could not detect 500 IU / 5 µl of HPV16 and 500 IU / 5 µl of HPV18. In contrast, HPV 59 and HPV 31 were not detected in 500 GE / 5 µl in both single and multiple infections by 5 and 6 data sets, respectively. This was still an improvement in sensitivity from 2013 when 26 datasets could not detect HPV 31 correctly. 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 2019 Global HPV LabNet Proficiency Study for HPV DNA Typing**

Accurate and internationally comparable HPV DNA detection and typing methodology is an essential component in research on HPV vaccines and in effective implementation and monitoring. A WHO initiative established a Global HPV LabNet to support the worldwide 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.

We are now seeking international participation in an international 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 44 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.

**Composition of sample material**

- 41 tubes containing purified whole genomic plasmids of **HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68a** and **68b** in a background of human cellular DNA. Each sample may contain either single types or a mixture of HPV types at varying concentrations.
- 3 samples containing cell suspensions.

**Participation fee**

Participation in the proficiency study is subject to a participation fee per panel consisting of 44 samples: 800 Euros for commercial entities and 450 Euros for academic entities. Laboratories that have outstanding payments from past Global HPV LabNet proficiency studies will need to clear their debts before their registration is accepted.

**Data submission**

The International HPV Reference Laboratory in Sweden is organizing this study in collaboration with the Swedish external quality assurance provider Equalis AB, who is responsible for management and distribution. Laboratories that have more than one assay are encouraged to provide results on each assay they commonly use. Data submitted will become the property of the organizers and may be analyzed for publication by the Global 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. 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.

Application forms for the 2019 study can be found at

<http://www.equalis.se/en/products-and-services/project/hpv-typing-proficiency-study/>

**Preliminary dates:**

19<sup>th</sup> of June 2019: Registration for participation opens.

30<sup>th</sup> of August 2019: Registration for participation closes.

October 2019: Dispatch of panels begins.

**Participation, management and practical issues**

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**Scientific issues**

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**Annex 2: Application for participating in  
The 2019 Global HPV LabNet DNA Typing Proficiency Study**

Fee for commercial entities: 1000 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.	
<b>Delivery address:</b>	
Lab ID (from previous participation):	
Department /Laboratory	
Address	
City	Postal code
Province /State	Country
E-mail	Fax
Phone	
<b>Invoice address (if different from above):</b>	
VAT number:	
Department /Laboratory	
Address	
City	Postal code
Province /State	Country
<b>Mode of payment (please check the preferred choice):</b>	
By Credit Card/PayPal:	
By Invoice:	
<b>Principal Investigator:</b>	
First Name	
Surname (Title)	
<b>HPV DNA typing experience in your laboratory</b>	
<b>Methodology used (may be more than one)</b>	
<b>Annual number of HPV typing tests performed</b>	
<b>Brief description of involvement in HPV surveillance or HPV vaccine development</b>	

Return registration form by email or fax to: [info@equalis.se](mailto:info@equalis.se). Fax: +46 18 69 31 46

**Annex 3:****Source of panel material.**

Complete genomes of HPV cloned into plasmid vectors had been provided to the Lund University by the respective proprietors with a written approval to be used in this proficiency panel: Dr Ethel-Michele de Villiers (HPV types 6, 11, 16, 18 and 45), Dr Gérard Orth (HPV types 33, 39 and 68a prototype), Dr Elisabeth Schwarz (HPV 68b), Dr Saul Silverstein (HPV type 51), Dr Attila Lőrincz (HPV types 31, 35 and 56), Dr Wayne Lancaster (HPV type 52) and Dr Toshihiko Matsukura (HPV types 58 and 59).

**The HPV genomes are cloned into different cloning vectors:** HPV 6 in pBR322 at position 4724 in the HPV genome, HPV 11 in pGEM4Z at position 4781, HPV 16 in pBR322 at position 6152, HPV 18 in pGEM-5Zf vector in the L2 region, HPV 31 in pT713 at position 3362, HPV 33 in pBR322 at position 2797, HPV 35 are cloned in two fragments 5012-956 and 956-5012 in pT713, HPV 39 in pGEM4z at position 1714, HPV 45 in pGEM4Z at position 75, HPV 51 in pGEM4z at position 4511, HPV 52 in pUC19 at position 7559, HPV 56 in pT713 at position 5521, HPV 58 in pGEM4Z at position 1158, HPV 59 in pUC9 at position 69, HPV 68a prototype in a bluescript vector, and HPV68b (ME180) of about 7 kb containing L1, L2, E1, E2, E4, E5, E6, E7 with an incomplete E2 gene in pCR4-TOPO.

The nucleic acid sequences for each of these HPV genomes have been reported previously and are available in Gene Bank with the following accession numbers; HPV 6 nr X00203; HPV 11 nr M14119; HPV 16 nr K02718; HPV 18 nr X05015; HPV 31 nr J04353; HPV 33 nr M12732; HPV 35 nr M74117; HPV 39 nr M62849; HPV 45 nr X74479; HPV 51 nr M62877; HPV 52 nr X74481; HPV 56 nr X74483; HPV 58 nr D90400; HPV 59 nr X77858; HPV 68a nr X67161 and HPV 68b nr FR751039.

**Annex 4:****List of participants in the HPV LabNet Proficiency Study  
of HPV DNA Typing, 2019**

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