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Global Improvement in Genotyping of Human Papillomavirus DNA: the 2011 HPV LabNet International Proficiency Study

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Accurate and internationally comparable human papillomavirus (HPV) DNA genotyping is essential for HPV vaccine research and for HPV surveillance. The HPV Laboratory Network (LabNet) has designed international proficiency studies that can be issued regularly and in a reproducible manner. The 2011 HPV genotyping proficiency panel contained 43 coded samples composed of purified plasmids of 16 HPV types (HPV6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68a, and -68b) and 3 extraction controls. Tests that detected 50 IU of HPV16 and HPV18 and 500 genome equivalents for the other 14 HPV types in both single and multiple infections were considered proficient. Ninety-six laboratories worldwide submitted 134 data sets. Twenty-five different HPV genotyping assay methods were used, including the Linear Array, line blot/INNO-LiPA, Papillo-Check, and PCR Luminex assays. The major oncogenic HPV types, HPV16 and HPV18, were proficiently detected in 97.0% (113/116) and 87.0% (103/118) of the data sets, respectively. In 2011, 51 data sets (39%) were 100% proficient for the detection of at least one HPV type, and 37 data sets (28%) were proficient for all 16 HPV types; this was an improvement over the panel results from the 2008 and 2010 studies, when <25 data sets (23% and 19% for 2008 and 2010, respectively) were fully proficient. The improvement was also evident for the 54 laboratories that had also participated in the previous proficiency studies. In conclusion, a continuing global proficiency program has documented worldwide improvement in the comparability and reliability of HPV genotyping assay performances.

Cervical cancer is the third most common cancer among women worldwide, with an estimated 530,000 new cases diagnosed annually. Human papillomavirus (HPV) infection is linked to >99% of cervical cancers (1). The most important high-risk types (HPV16 and HPV18) account for about 70% of all invasive cervical cancers worldwide (2).

The introduction of HPV vaccines has highlighted the need for accurate and internationally comparable HPV DNA detection and genotyping methodologies. This is an essential component in both the development and evaluation of HPV vaccines, as well as in the effective implementation and monitoring of HPV vaccination programs. The genotyping assays used today differ in their analytical performances with regard to type-specific sensitivities and specificities. Several studies have compared different HPV typing assays to assess their performances in screening and epidemiology using clinical samples (3–5). However, evaluations of assay performance in different laboratories need to be performed in a standardized manner, such that different assay performance measures can be evaluated and the results can be compared over time against a known and accepted standard (4). The use of regularly issued global proficiency studies is an essential tool for establishing comparable and reliable laboratory services (6). International proficiency panels for quality assurance of laboratory testing are being widely distributed for a number of infectious agents. Recently, the World Health Organization (WHO) issued new reference genotype panels for both parvovirus B19 and hepatitis B virus (7, 8).

With the objective of facilitating the development and implementation of HPV vaccines by improving and standardizing the quality of HPV laboratory services, the WHO established a global HPV laboratory network (HPV LabNet) in 2005. The main activities within the HPV LabNet were the harmonization and standardization of the laboratory procedures used for HPV vaccine research and HPV vaccination program impact monitoring by the development of internationally comparable quality assurance methods, international standards, and reference reagents, as well as a laboratory manual for vaccinology (9–12). In 2008, recombinant HPV DNA plasmids were used to establish international standards (ISs) for HPV16 and HPV18 DNA, with an assigned potency in international units (IU) (13). That same year, WHO HPV LabNet conducted the first proficiency study that was open for participation from laboratories worldwide based on HPV DNA plasmids containing the genomes of 14 oncogenic and 2 benign HPV types (12, 14). In 2010, the proficiency study was repeated, demonstrating that it is possible to perform continuous global studies based on plasmid DNA with unitage traceable to ISs, and that such studies can provide an overview of the status of HPV detection and typing methodologies worldwide (6). The international HPV LabNet proficiency study described herein was designed for the genotyping needs in HPV vaccinology, and the proficiency criteria are not intended for clinical HPV screening purposes, as the requirements for analytical sensitivity may be different.

This report was based on a proficiency panel composed of the same HPV DNA plasmid material as in 2008 and 2010, with the amount of DNA titrated in amounts traceable to the IS. The use of

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the same panel material and proficiency criteria allowed for a standardized comparison of laboratory performance and reproducibility, as well as assay sensitivity and specificity over time. The sample preprocessing was evaluated with extraction controls of cervical cancer cell lines.

The panel was distributed to 100 laboratories worldwide and analyzed using a range of HPV DNA typing assays performed in a blinded manner. We report the results in terms of the ability of participating laboratories to correctly identify the HPV types, with the data grouped by the methods they used, and we assess the analytical sensitivities of these tests for the detection of the HPV types included in the study. We also compare the results of the panels from the years 2008, 2010, and 2011.

MATERIALS AND METHODS

Source of panel material. The complete genomes of HPV types cloned into plasmid vectors were provided by the respective proprietors with a written approval to be used in this proficiency panel: HPV6, -11, -16, -18, and -45 from Ethel-Michele de Villiers, HPV33, -39, -66, and -68a prototype from Gérard Orth, HPV68b from Elisabeth Schwarz, HPV51 from Saul Silverstein, HPV31, -35, and -56 from Attila Lörincz, HPV52 from Wayne Lancaster, and HPV58 and HPV59 from Toshihiko Matsukura. These agreements allowed for the distribution of the plasmids for performance in this WHO proficiency study only.

The HPV genomes were cloned into different cloning vectors as previously described (6). For 2011, two changes were made in the plasmids: HPV18 was recloned into a pGEM-5Zf vector in the L2 region, and the full-length HPV68a prototype, which was cloned into a pBluescript vector, was included, in comparison to previous panels in which a plasmid containing only the L1 gene of HPV68a was used.

The nucleic acid sequences for each of these HPV genomes have been reported previously and are available in GenBank with the following accession numbers: X00203 (HPV6), M14119 (HPV11), K02718 (HPV16), X05015 (HPV18), J04353 (HPV31), M12732 (HPV33), M74117 (HPV35), M62849 (HPV39), X74479 (HPV45), M62877 (HPV51), X74481 (HPV52), X74483 (HPV56), D90400 (HPV58), X77858 (HPV59), U31794 (HPV66), X67161 (HPV68a), and FR751039 (HPV68b).

Panel composition and production. The plasmids were prepared and characterized as previously described (6, 14). Purified plasmids containing cloned genomic DNAs for HPV6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68a, and -68b were prepared to make the 43 different panel samples by diluting the HPV recombinant DNA plasmid stock solution in Tris-EDTA (TE) buffer in the background of human placental DNA, as previously described (6). Table 1 summarizes the composition of the panel. The different amounts of plasmid (5 to 500 genomic equivalents [GE] or IU) were chosen to reflect the lower spectrum of the amount of virus that would typically be present in clinical samples (15). The plasmid concentration equivalent to 50 genome copies (IU) varied from 0.53 fg to 0.67 fg/5 μl because of a small variation in the length of the HPV genome and the use of different cloning vectors. The background concentration of human DNA was 50 ng/5 μl in all samples.

After the production of each of the 43 reference samples, the preparation was dispensed in 100-μl volumes in 1.5-ml siliconized vials. The vials were labeled as WHO HPV DNA 2011 and were randomly assigned numbers from 1 through 43. The panels were stored at −20°C before shipment to the participating laboratories. The participants were instructed to perform HPV typing according to their standard methods using their standard sample input volume.

Two different cell lines were used as controls for the extraction process at the participating laboratories. The HPV-negative epithelial cell line C33A, derived from human cervical carcinoma, and the HPV16-positive epithelial cell line SiHa, derived from squamous cell carcinoma, were purchased from the American Type Culture Collection and cultured in Dulbecco's modified Eagle medium (catalog no. 11960; Gibco). The cells were diluted in PreservCyt (catalog no. 0234004; Cytyc) to concentrations of 5 and 500 SiHa cells/μl in a background of 5,000 C33A cells/μl, and one sample contained the background C33A cells only. Two hundred microliters of each preparation was dispensed in 1.5-ml vials and labeled as WHO HPV DNA A, B, or C.

TABLE 1 HPV DNA proficiency panel composition and HPV testing results

HPV or cell type(s)	HPV (IU/genome equivalents per 5 μl)	% correct data sets[a] (no. correct/no. total) (false-positive results)
16	50	97.4 (113/116)
16	5	68.0 (85/125)
18	50	87.3 (103/118)
18	5	50.0 (58/116)
6	500	96.1 (99/103)
6	50	78.8 (82/104)
11	500	100 (104/104)
11	50	87.5 (91/104)
31	500	89.7 (96/107)
31	50	67.6 (71/105)
33	500	95.4 (104/109)
33	50	78.3 (87/111)
35	500	93.3 (98/105)
35	50	70.8 (80/113)
39	500	88.0 (95/108)
39	50	66.4 (73/110)
45	500	93.6 (102/109)
45	50	72.7 (80/110)
51	500	92.5 (99/107)
51	50	73.8 (79/107)
52	500	92.7 (102/110)
52	50	75.7 (84/111)
56	500	89.6 (95/106)
56	50	68.8 (75/109)
58	500	94.5 (104/110)
58	50	77.0 (84/109)
59	500	86.8 (92/106)
59	50	61.7 (66/107)
66	500	92.2 (94/102)
66	50	72.7 (729)
68a	500	62.1 (35/58)
68a	50	42.2 (27/64)
68b	500	84.2 (85/101)
68b	50	64.9 (637)
6, 56, 58, 68a	500	65.0 (65/100)[b,c]
6, 56, 58, 68a	50	42.1 (45/107)[b,c]
11, 18, 31, 52	500	77.3 (92/119)[b]
11, 18, 31, 52	50	58.3 (70/120)[b]
16, 33, 45, 51	500	79.0 (94/119)[b]
16, 33, 45, 51	50	73.9 (85/115)[b]
35, 39, 59, 66, 68b	500	61.0 (64/105)[b]
35, 39, 59, 66, 68b	50	41.8 (46/110)[b]
None	0	98.5 (130/132)
HPV16-positive	25	87.8 (108/123) (5)
HPV-negative	0	95.3 (121/127) (6)
HPV16-positive	2,500	91.1 (112/123) (4)

[a] Data sets that detected the correct type as claimed; no false-positive types were detected.
[b] Including the data set generated by type-specific HPV16, HPV, HPV52, and HPV68 PCRs.
[c] The data sets known not to detect the HPV68a plasmids in this panel are considered to be correct when the other HPV types in the sample were detected.

Before distribution of the WHO HPV DNA proficiency panel, the samples were tested (in a blinded fashion) by the WHO HPV LabNet Global Reference Laboratory (GRL) in Sweden and one other laboratory, the German Cancer Research Center (DKFZ) in Heidelberg, Germany (Michael Pawlita), using the technologies previously described (6). The appropriate negative and positive controls were used to monitor the performances of the methods. DNA samples from the extraction controls A, B, and C were extracted using the QIAamp DNA mini and blood kit (Qiagen), according to the manufacturer's instructions.

Organization of the study. The call for participation in this proficiency study was announced at the International Papilloma Virus Conference in Berlin, Germany, 2011, and sent to all laboratories that had participated in the 2010 WHO HPV LabNet proficiency panels. Laboratories that were or were planning to be involved in HPV surveillance and/or vaccine development were particularly welcome to participate in the study. The panels were prepared by the WHO HPV LabNet GRL in Sweden and were distributed by Equalis (the laboratory external quality assurance company of Sweden) at ambient temperature to 100 laboratories worldwide. The numbers of participating laboratories according to the WHO regions are as follows: American region, 18 laboratories; Eastern Mediterranean region, 7 laboratories; European region, 51 laboratories; South East Asia region, 5 laboratories; and Western Pacific region, 15 laboratories. The package also included a letter with instructions, a form for reporting the results of the panel testing, and technical information on the procedures to be performed. The participating laboratories were asked to submit the results of the tests performed to Equalis online within 4 weeks of receipt of the specimens. In registering for the proficiency study, each participating laboratory agreed to assign WHO the right to publish the data, and it was agreed that only coded results from all participating laboratories would be presented, grouped by methods they performed.

All the results submitted to Equalis were coded and analyzed anonymously by the GRL in Sweden. The generated data sets were assigned numbers from 1 through 134. The individual results of the proficiency study were disclosed only to the participating laboratory that generated the particular data set.

HPV technologies used by study participants. The different HPV typing methods that were used to generate results for the WHO LabNet proficiency study to detect HPV DNA (11, 16–34) are summarized in Table 2.

Data analysis. The criteria used for considering a data set to be proficient for HPV vaccinology were the following: (i) detection of ≥50 IU per 5 μl of HPV16 and HPV18, both in single and multiple HPV infections, (ii) detection of ≥500 GE per 5 μl of the other HPV types included, both in single and multiple infections, and (iii) one false-positive result, at most. These criteria were arrived at by a consensus opinion of international experts participating in an international WHO workshop in Geneva, Switzerland, in 2008 (10), and they were based on a consideration of which performance requirements should be required and were realistic. A higher requirement for HPV16 and HPV18 was considered essential because of the pivotal role of these HPV types in causing cervical cancer.

RESULTS

Validation of the HPV proficiency panel. The results from the initial panel validation at the GRL Sweden and at DKFZ included the qualitative characterization of HPV and human genomic DNAs. Both of these laboratories used Luminex-based assays with modified GP5+/6+ primers. No false-positive HPV types were detected in the samples in any of the reference laboratories. All HPV types were detected by both laboratories in the lowest concentration included in the panel, except for HPV18, -31, -35, -59, and -68a, which were detected by only one of these laboratories. The results from the reference laboratory evaluation revealed that the panel performed as expected, and the panel was then distributed to participating laboratories worldwide.

Panel distribution and response. Ninety-six of 100 participating laboratories, including the two laboratories that conducted the panel validation, submitted 134 data sets according to the timeline in Table 2. Eleven data sets were generated using assays (Roche Cobas 4800 test, Hybribio 13 HR, and Hybribio 14 HR) that either did not discriminate between specific HPV types or reported the results as HPV16, HPV18, and "other" high-risk HPV types. These data sets and 7 data sets from type-specific HPV16/-18/-52/-68 PCRs were only analyzed for the specific types tested. Some participating laboratories did not perform tests that typed all HPV types included in the proficiency panel. Therefore, the denominator for the number of test results included in the analyses varied for the different HPV types.

In 77 data sets, the results were obtained using commercially available tests. The most commonly used assay was the Linear Array (Roche), which was used to generate 18 data sets. Other widely used assays include the PapilloCheck (Greiner), INNO-LiPA (Innogenetics), CLART HPV 2 (Genomica), PGMY-CHUV, and other in-house line blot, in-house type-specific PCR, Luminex, and microarray-based assays (Table 2). According to the survey, the annual number of samples analyzed for HPV typing per laboratory varied from 250 to 200,000, with an average of approximately 7,000 samples tested yearly with the HPV genotyping assays.

Performance of HPV assays and participating laboratories. The participating laboratories were requested to perform testing using their standard protocols. Accordingly, the input volumes of the DNA panel varied from 1 μl to 50 μl between the laboratories. The data are presented by the lowest category of concentration (5, 50, or 500 GE or IU) that was proven to be detectable; e.g., a lab using a 2-μl input instead of 5-μl input that detects the 2 GE level is considered to be able to detect 5 GE level. HPV16 and HPV18 were included as single plasmids at the highest concentration of 10 IU/μl, which was correctly detected in 97% and 87% of the data sets, respectively. The samples containing single plasmids at a concentration of 100 GE/μl of HPV6, HPV11, and HPV33 were correctly identified, without false-positive types, in >95% of the data sets (Table 1). HPV68a was correctly identified in <80% of the data sets. In the samples containing multiple HPV types, between 61% and 79% of the data sets correctly identified the included types. The negative-control sample containing only human genomic DNA was correctly identified as negative in 130 of 132 data sets.

The proficiency of detecting HPV types by the type of assay is shown in Table 2. Fifty-four data sets were 100% proficient (detected ≥50 IU of HPV16 and HPV18 in 5 μl and 500 GE in 5 μl of the other HPV types tested, also when present together with other HPV types), without having more than one false-positive result. As the Linear Array assay used a large (50 μl) input volume in some laboratories, the Linear Array data sets did not test for the presence of amounts <50 IU of HPV16 and HPV18 in 5 μl and 500 genome equivalents in 5 μl of the other HPV types. Two commercial assays, the HPV Direct-Flow Chip (Master Diagnóstica) and the LCD Array (Chipron) both had 100% proficient results. More than two-thirds of the data sets generated by the Linear Array were 100% proficient. Several in-house assays based on general-primer PCR followed by hybridization (PGMY-CHUV) or Luminex were also 100% proficient.

To be considered proficient in this study, no more than one false-positive sample per data set was acceptable. The number of

TABLE 2 Proficiency of detecting HPV types tested for by assay type[a]

HPV assay type (manufacturer)	No. of data sets	HPV region(s) targeted (primers)	No. of data sets with a proficiency (%) of:				
			100	90–99	80–89	<80	Not proficient
Linear Array (Roche)[b]	18	L1 (PGMY)	13	0	1	1	4
In-house PCR Luminex[c]	11	E6, E7, L1	5	3	1	0	2
PapilloCheck (Greiner)	9	E1	2	3	3	1	0
INNO-LiPA (Innogenetics)	8	L1 (SPF10)	0	0	2	0	6
In-house line blot[d]	8	E6, E7, L1	1	1	4	0	2
In-house PGMY-CHUV[b]	8	L1 (PGMY)	6	0	0	1	1
In-house real-time PCR[e]	7	L1/E1/E4/E6/E7	5	1	0	0	1
In-house type-specific PCR[f]	7	L1/L2/E1/E5/E6/E7/URR	1	1	1	1	3
CLART HPV 2/3 (Genomica)[b]	6	L1 (PGMY)	0	0	2	2	2
Hybribio 21 HPV GenoArray	5	L1 (PGMY)	3	0	2	0	0
Cobas 4800 (Roche)[g]	4	L1	2	0	1	1	0
In-house PCR-EIA[h]	4	L1/E6/E7	3	0	0	0	1
In-house PCR-RFLP[i]	3	L1/E6/E7	0	0	0	2	1
In-house pyrosequencing[j]	3	L1 (GP/PGMY)	0	0	1	1	1
Hybribio 14 HR[g]	3	E6/E7	3	0	0	0	0
Luminex PCR (Multimetrix)	2	L1 (GP)	0	1	1	0	0
In-house microarray chip[b]	2	L1 (My/GPM)	1	0	0	0	1
PANArray	2	L1 (GP)	0	2	0	0	0
Digene HPV genotyping RH test (Qiagen)[k]	2	L1 (GP)	0	0	1	0	1
Hybribio 13 HR[l]	2	E6/E7	2	0	0	0	0
HPV Direct-Flow chip (Master Diagnóstica)	2	L1 (GP)	2	0	0	0	0
SPF(10)-LiPA25 version 1 (Labo Biomedical Products)	2	L1 (SPF10)	0	1	0	0	1
LCD array (Chipron)[b]	2	L1 (PGMY)	2	0	0	0	0
Other commercial assays[m]	10	L1/E1/E2/E6/E7	3	2	0	1	4
Other in-house assays[n]	4	L1/E1/E7	0	0	0	2	2
All assays	134	L1/L2/E1/E2/E4/E5/E6/E7/URR	54	15	20	12	33

[a] Unless otherwise stated, all assays test for 17 HPV types.
[b] These assays, using PGMY-based primers, do not test for HPV68a.
[c] Four assays do not test for HPV68a and one of these does not test for HPV6 and HPV11.
[d] One assay types 9 different HPV types and one assay types 8 different HPV types.
[e] One assay does not type HPV6 and HPV11, two assays do not type HPV66 and HPV68, one assay types HPV16 and HPV18 only, one assay types HPV52, and one assay types HPV68.
[f] Two assays do not type HPV66, one assay does not type HPV66 and HPV68, and one assay types HPV16 and HPV18 only.
[g] These assays type HPV16 and HPV18, and the other 12 high-risk types are reported as "other high risk."
[h] One assay does not type HPV68a, one does not type HPV45, -51, -59, and -68a, and one assay types HPV16 and HPV18 only. PCR-EIA, PCR enzyme immunoassay.
[i] This assay, using PGMY-based primers, does not test for HPV68a; in addition, one assay does not type HPV51. PCR-RFLP, PCR restriction fragment length polymorphism.
[j] This assay, using PGMY-based primers, does not test for HPV68a, one assay types 12 different HPV types, and one assay types 8 different HPV types.
[k] This assay does not test for HPV6 or HPV11.
[l] This assay tests for 13 high-risk types, which are reported as positive or negative.
[m] Other commercial assays include one laboratory using each of GenoFlow HPV array kit, Sacace high-risk screen real-TM, AdvenSure HPV GenoBlot assay, BMT HPV Genotyping 9G, Molgentix F-HPV typing assay, ProDect CHIP HPV typing assay, PapType assay, genomed-biotech f-HPV typing assay, Analitica type-specific PCR, and SPF(10)-LiPA25 version 1 (Labo Biomedical Products).
[n] 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, and FAP.

false-positive HPV types detected per data set is shown in Table 3. Eighty-four of the 134 data sets did not have any false-positive results, whereas 17 data sets reported >3 false-positive results. Among these, 3 data sets reported false-positive HPV types in ≥20 samples. The data sets generated by the commercial tests INNO-LiPA, CLART, and Linear Array reported more than one false-positive sample in 75%, 33%, and 22% of the data sets, respectively. The 9 data sets generated by PapilloCheck, as well as several in-house and commercial assays that were performed by only a few laboratories, reported no false-positive results at all.

The lowest GE or IU of each HPV type included in the panel that was detected in both single and multiple infections by different assays is shown in Tables 4 and 5. HPV6, -11, -16, -18, and -33 were the types detected at the lowest concentration by >75% of the data sets. Only one data set did not detect the highest concentration of HPV16. In contrast, for HPV31, HPV59, and HPV39, there were 28, 24, and 22 data sets, respectively, that did not detect these viruses at the highest concentration (Table 4).

Three additional samples (A, B, and C) were used to evaluate the DNA extraction step before HPV testing and typing. Two of the samples contained cells from the cervical cancer cell line SiHa mixed with the HPV-negative cancer cell line C33A in different amounts. A sample with only C33A cells served as a negative control. In the sample containing 2,500 cells/5 μl of the cervical cancer cell line SiHa, HPV16 was correctly identified in 91% of the data sets. Four data sets reported false-positive HPV types in this sam-

TABLE 3 False-positive HPV types detected by assay type[a]

HPV assay type (manufacturer)	No. of data sets	HPV region(s) targeted (primers)	No. of data sets with indicated no. false-positive samples				
			0 samples	1 sample	2 samples	3 samples	>3 samples
Linear Array (Roche)[b]	18	L1 (PGMY)	12	2	2	0	2
In-house Luminex PCR[c]	11	E6, E7, L1	7	2	2	0	0
PapilloCheck (Greiner)	9	E1	9	0	0	0	0
INNO-LiPA (Innogenetics)	8	L1 (SPF10)	1	1	1	1	4
In-house line blot[d]	8	E6, E7, L1	4	2	1	0	1
In-house PGMY-CHUV[b]	8	L1 (PGMY)	5	2	0	0	1
In-house real-time PCR[e]	7	L1/E1/E4/E6/E7	6	0	1	0	0
In-house type-specific PCR[f]	7	L1/L2/E1/E5/E6/E7/URR	3	1	2	1	0
CLART HPV 2/3 (Genomica)[b]	6	L1 (PGMY)	3	1	1	0	1
Hybribio 21 HPV GenoArray	5	L1 (PGMY)	4	1	0	0	0
Cobas 4800 (Roche)[g]	4	L1	4	0	0	0	0
In-house PCR-EIA[h]	4	L1/E6/E7	3	0	0	0	1
In-house PCR-RFLP[i]	3	L1/E6/E7	2	0	1	0	0
In-house pyrosequencing[j]	3	L1 (GP/PGMY)	2	0	0	0	1
Hybribio 14 HR[k]	3	E6/E7	3	0	0	0	0
PCR Luminex (Multimetrix)	2	L1 (GP)	1	1	0	0	0
In-house microarray chip[b]	2	L1 (My/GPM)	1	0	0	1	0
PANArray	2	L1 (GP)	2	0	0	0	0
Digene HPV genotyping RH test (Qiagen)[k]	2	L1 (GP)	1	0	0	0	1
Hybribio 13 HR[l]	2	E6/E7	2	0	0	0	0
HPV Direct-Flow chip (Master Diagnóstica)	2	L1 (GP)	2	0	0	0	0
SPF(10)-LiPA25 version 1 (Labo Biomedical Products)	2	L1 (SPF10)	1	0	0	0	1
LCD array (Chipron)[b]	2	L1 (PGMY)	0	2	0	0	0
Other commercial assays[m]	10	L1/E1/E2/E6/E7	4	2	1	0	3
Other in-house assays[n]	4	L1/E1/E7	2	0	1	0	1
All assays	134	L1/L2/E1/E2/E4/E5/E6/E7/URR	84	17	13	3	17

[a] Unless otherwise stated, all assays test for 17 HPV types.
[b] These assays, using PGMY-based primers, do not test for HPV68a.
[c] Four assays do not test for HPV68a, and one of these does not test for HPV6 and HPV11.
[d] One assay types 9 different HPV types, and one assay types 8 different HPV types.
[e] One assay does not type HPV6 and HPV11, two assays do not type HPV66 and HPV68, one assay types HPV16 and HPV18 only, one assay types HPV52, and one assay types HPV68.
[f] Two assays do not type HPV 66, one assay does not type HPV66 and HPV68, and one assay types HPV16 and HPV18 only.
[g] These assays type HPV16 and HPV18, and the other 12 high-risk types are reported as "other high risk."
[h] One assay does not type HPV68a, one does not type HPV45, -51, -59, and -68a, and one assay types HPV16 and HPV18 only.
[i] This assay, using PGMY-based primers, does not test for HPV68a; in addition, one assay does not type HPV51.
[j] This assay, using PGMY-based primers, does not test for HPV68a, one assay types 12 different HPV types, and one assay types 8 different HPV types.
[k] This assay does not test for HPV6 or HPV11.
[l] This assay tests for 13 high-risk types, which are reported as positive or negative.
[m] Other commercial assays include one laboratory using each of GenoFlow HPV array kit, Sacace high-risk screen real-TM, AdvenSure HPV GenoBlot assay, BMT HPV Genotyping 9G, Molgentix F-HPV typing assay, ProDect CHIP HPV typing assay, PapType assay, genomed-biotech f-HPV typing assay, Analitica type-specific PCR, and the SPF(10)-LiPA25 version 1 (Labo Biomedical Products).
[n] 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, and FAP.

ple. The negative control containing only C33A cells was correctly reported as negative by 95% of the laboratories (Table 1).

Comparison of results for laboratories that participated both in 2011 and in 2008/2010. Fifty-four laboratories that participated in 2011 also participated in the HPV LabNet proficiency study during at least one previous year. Thirty laboratories participated in all 3 proficiency studies (2008, 2010, and 2011). Twenty-four laboratories analyzed the proficiency panel in 2010 and 2011. Some of the laboratories used the same assay during all study years, whereas some laboratories changed at least one of the assays used. The percent proficiency data, for all years and compared with the results from all data sets submitted for 2011, are shown in Table 6.

Among the laboratories that used the same assay during all study years, 32% were proficient in 2008, 36% were proficient in 2010, and 42% were proficient in 2011. There was a definite trend toward fewer false-positive results in the laboratories through the years; e.g., 44% of the data sets submitted in 2008 detected no false-positive samples compared to 71% of the data sets in 2011 (Table 7). Twenty out of 26 laboratories that were not proficient in 2008 participated again in 2010, and nine out of these 20 laboratories had become proficient in 2010. Twenty-nine out of 45 laboratories that were not proficient in 2010 participated again in 2011, and 13 of these laboratories had become proficient in 2011. There were only 3 laboratories that participated in 2008, 2010, and 2011 and were continuously not proficient.

TABLE 4 HPV IU/GE detected per 5 μl in both single and multiple infections (commercial assays)

HPV type	HPV (IU/GE)	No. of data sets with indicated HPV IU/GE concn detected/total no. of data sets											
		Linear Array	PapilloCheck microarray	INNO-LiPA	CLART (HPV 2/3)	Hybribio 21	Luminex (Multimetrix)	Digene genotyping RH	HPV Direct-Flow chip	SPF(10)-LiPA25 (Labo Biomedical Products)	LCD array (Chipron)	PANArray	Other[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[b]		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
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	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, Sacace high-risk screen real-TM, AdvenSure HPV GenoBlot assay, BMT HPV Genotyping 9G, Molgentix F-HPV typing assay, ProDect CHIP HPV typing assay, PapType assay, genomed-biotech f-HPV typing assay, Analitica type-specific PCR, and the SPF(10)-LiPA25 version 1 (Labo Biomedical Products).

[b] NT, not tested.

DISCUSSION

We report that a reproducible, internationally comparable quality assurance methodology that is traceable to ISs has detected global improvement in the quality of HPV genotyping services. An accurate comparison of the methodologies used in laboratories worldwide requires a standardized methodology for an evaluation of laboratory performance. We have found that the repeated issuing of international proficiency panels containing known amounts of virus plasmids with unitage traceable to ISs can be used to globally monitor the development of the quality of HPV typing methodologies, and that laboratories participating continuously in such programs improve in the quality of their testing.

In the 2011 study, there was a charge for laboratories to participate, although participants from low- and lower-middle-income countries could apply to have their fees waived. Although the number of laboratories that participated was about the same as in previous years and there was little change in the global distribution of the laboratories, it cannot be excluded that laboratories from low-income countries may have chosen not to participate because of the participation fee. It is also possible that the improved performances observed in the 2011 proficiency study may reflect a bias, with preferential participation toward laboratories that were able to afford the fee and who may have come from high-income countries and/or had HPV genotyping as a central priority in their activities. However, the analysis that was restricted to laboratories that have participated multiple times also did find improvements in performance in 2011, indicating that global improvement in the performance of HPV genotyping has indeed occurred.

Overall, a majority of the HPV DNA typing methodologies used by laboratories participating in this study had a proficient performance according to the established criteria. However, some limitations were revealed.

As in the previous studies, there were systematic differences in the sensitivity to detect different HPV types; e.g., HPV16, HPV11, and HPV18 were still the types detected at the smallest amounts in most data sets (only 1, 1, and 5 data sets, respectively, did not detect these types at 500 IU/5 μl), whereas HPV31, HPV59, and HPV39 were not detected at 500 GE/5 μl by 28, 24, and 22 data sets, respectively. However, for the types with lower sensitivities, this was an improvement compared to in 2010 when, e.g., only

TABLE 5 HPV IU or GE detected per 5 μl with single and multiple infections (in-house assays)[a]

HPV type	HPV (IU/GE)	No. of data sets with indicated HPV IU/GE concn detected/total no. of data sets									
		All assays (%)	Luminex	Line blot	PGMY-CHUV	Real-time PCR	Type-specific PCR	PCR-RFLP	PCR sequencing	Microarray chip	Other in-house assay[b]
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[c]		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] Includes laboratories with multiple false positives. Detection with input volume of 50 μl classified as data for the next 10-fold dilution compared to input with 5 μl. Input with 10 or 15 μl was classified as the same dilution compared to input with 5 μl.
[b] Other in-house assays include one laboratory using each of in-house PCR EIA, in-house PCR, in-house multiplex PCR, and in-house hybridization-chemiluminescence.
[c] NT, not tested.

65% of the data sets detected HPV39 at 500 GE/5 μl. Many surveys of circulating HPV types might still systematically underestimate the prevalences of HPV31, HPV59, and HPV39 compared to those of HPV16 and HPV18.

A lower sensitivity in the samples with multiple HPV types has been seen in all our studies. In 2011, correct typing of the samples containing multiple HPV types at 500 GE/5 μl was reported in 61% to 79% of the data sets, compared to an average of 90% when only 1

TABLE 6 Proficiency of detecting HPV types by laboratories that participated in 2011 proficiency panel, with data from 2008 and 2010 in comparison with all data sets submitted in 2011

Proficiency (%)	No. of proficient assays/total no. of assays (%)						No. of proficient data sets/total no. of data sets from 2011 (%)
	Identical assays used in:			All tests run by laboratories that participated in:			
	2008	2010	2011	2008	2010	2011	
100	8/25 (32)	17/47 (36)	20/47 (42)	8/32 (25)	18/61 (29)	28/66 (42)	54/134 (40)
90–99	2/25 (8)	4/47 (8.5)	5/47 (11)	2/32 (6.2)	5/61 (8.2)	10/66 (15)	15/134 (11)
80–89	2/25 (8)	6/47 (13)	6/47 (13)	4/32 (12)	6/61 (9.8)	10/66 (15)	20/134 (15)
<80	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 7 Number of false-positive HPV types detected per data set reported by laboratories participating in 2008, 2010, and 2011 proficiency studies in comparison with all data sets submitted in 2011

No. of false-positive samples	No. of assays with false-positive results/total no. of assays (%)						No. of assays with false-positive results/total no. of data sets from 2011 (%)
	Identical assays used in:			All tests run by laboratories that participated in:			
	2008	2010	2011	2008	2010	2011	
0	12/25 (48)	31/47 (66)	32/47 (68)	14/32 (44)	36/61 (59)	47/66 (71)	84/134 (63)
1	4/25 (16)	0/47 (0)	5/47 (11)	5/32 (16)	1/61 (1.6)	7/66 (11)	17/134 (13)
2	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	2/25 (8)	3/47 (6.4)	0/47 (0)	4/32 (12)	8/61 (13)	0/66 (0)	3/134 (2.2)
>3	4/25 (16)	7/47 (15)	6/47 (13)	4/32 (12)	10/61 (16)	8/66 (12)	17/134 (13)

HPV type was present in the sample. The underestimation of the prevalence of multiple infections introduces a systematic detection bias in epidemiological studies, with detectability being dependent on the determinants of HPV acquisition. Some high-risk HPV types will thus be more difficult to detect in patients in high-risk groups, because of their higher likelihood of multiple HPV infections.

The 2011 proficiency panel has overall been tested with no fewer than 25 different assays that target the L1, E6/E7, and E1 regions of the HPV genome. There were no noteworthy differences in proficiency depending on the region of the genome that was targeted by an assay. The proficiency panel contained only 2 entirely HPV-negative samples. The study was designed to evaluate HPV typing, and we considered that specificity should be measured primarily as the absence of detection of a specific HPV type, including when other HPV types were present. Thus, for each HPV type evaluated, there are at least 38 negative samples included in the panel, and thus, 1 false-positive result equals >97% specificity. We searched the data sets for patterns of consistent false positivity for any specific sample in the panel. There was no single sample that had systematic false positivity for the same type in several laboratories, indicating that the problem with false positives is usually not related to a property of the assays itself (e.g., cross-reactivity) but rather to the laboratory conditions of use (e.g., contamination). In the previous proficiency panel distributed in 2010, systematic false positivity was found in the HPV58 plasmid, for which 15 data sets also detected HPV52 in at least one of the samples containing HPV58. In the analyses of the results for 2010, the conclusion was that this was probably not due to contamination of the panel but more likely attributable to the fact that HPV52 and HPV58 are related and may cross-react in some assays. HPV58 plasmid preparation was analyzed with an E6/E7 type-specific PCR for both HPV52 and HPV58 (34) on both undiluted and diluted plasmid stock; only HPV58 was detected. For the 2011 panel, we nevertheless decided to make a new preparation of the HPV58 plasmid. In the 2011 proficiency panel, six laboratories still detected HPV52 in at least one of the samples containing HPV58 plasmid (5 laboratories using the INNO-LiPA and one using the Linear Array assay). Both the Linear Array assay and the INNO-LiPA do not exclude the presence of some HPV types when specific HPVs are present.

All plasmids in the panel contained full-length genomes, including that of HPV68a. Since all assays based on PGMY primers (directed against gene L1) cannot detect HPV68a, we also added HPV68b to the panel. HPV68b can be detected by PGMY-based primers and other common primer systems. All data sets that used the PGMY primers were thought of as not having tested for HPV68a. However, among the 64 data sets that were analyzed for their detection of HPV68a, 55% (35/64) detected 500 GE of HPV68a. This was the lowest number of correct data sets among all the HPV types tested (35, 36). The HPV18 plasmid used was recloned in 2011 into a new vector in the L2 region, in order to enable detection with the Microarray kit using the primers directed to the E1 region.

Four of the 18 data sets generated by the most commonly used commercial assay, the Linear Array, were not proficient, reporting false-positive results in between 2 and up to 8 samples. HPV52 and HPV66 were detected as false positives in 6 out of the 17 false-positive results submitted in the 18 data sets using the Linear Array assay. These laboratories reported that HPV52 was present in samples that contained HPV35 and HPV58. The Linear Array assay cannot make a type-specific HPV52 call in the presence of HPV33, HPV35, and/or HPV58. The Linear Array assay was designed with an intentionally cross-reactive probe. Six of the 18 laboratories performed an additional HPV52-specific real-time in-house PCR on these samples, with the result that they all detected the lowest concentration of HPV52 with no false-positive results in the panel. The fact that false positives for HPV66 were reported in 7 samples containing HPV56 was similar to the results in 2008 and 2010, when HPV66 false positives were reported in some samples containing the HPV56 plasmid, but only when the Linear Array test was used. Compared with the 2010 proficiency panel, the number of false-positive results in the data sets obtained with the Linear Array assay in 2011 decreased from 31 to 17, a major improvement.

In 2010, all four laboratories that used the microarray-based assay PapilloCheck were 100% proficient. In 2013, 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 data sets detected 5 copies/5 μl of the recloned HPV18 plasmid, whereas HPV18 at 50 copies/5 μl was detected by 2 of the 9 laboratories and 7 out of 9 detected HPV18 at 500 copies/5 μl. None of the other assays used to test the panel had this rather low sensitivity for HPV18.

The commercial tests INNO-LiPA and CLART did not generate any 100%-proficient data sets. For the INNO-LiPA, 6 out of 8 data sets were not proficient because of 2 to 21 false-positive results. One laboratory using the INNO-LiPA reported no false positives, showing that it is possible to perform the INNO-LiPA with no false-positive results. However, it seems that stringent conditions with attention to the prevention of contamination and negative controls are particularly important when using this test.

HPV59 was detected in only one of the samples, with the highest concentration, in 2 out of the 8 data sets that used the INNO-LiPA.

CLART was used by 6 laboratories, out of which 2 were considered not proficient because of reporting 2 to 7 false-positive results. Only two of the data sets detected HPV39 in one of the panel samples with the highest concentration, while HPV45, HPV56, and HPV68 were proficiently detected by two laboratories.

The line blot assay PGMY-CHUV is described in the WHO HPV Laboratory Manual (12). The assay was developed within the WHO HPV LabNet in order to provide an inexpensive assay that would be independent of any specific commercial vendor (11). In 2008, the assay was transferred to all WHO HPV LabNet members as an effort to build up testing capacity and to evaluate assay transferability. In the 2008 HPV DNA proficiency study, only one laboratory out of 7 was 100% proficient using this assay. In 2011, 6 of 8 laboratories were 100% proficient, and only one laboratory had substantial problems with the assay, showing that experience in performing the assay is critical in generating qualified results.

As was also found in our previous study (14), the differences in performance were much larger between laboratories than between the different types of assays. Proficiency panel testing is thus particularly useful to stimulate a learning process to improve performance in laboratories.

Three samples were included in the panel to evaluate the DNA extraction step before HPV testing and typing. There were at least 21 different extraction procedures used by the laboratories. The most commonly used (in 23 data sets) was a MagNA Pure kit (Roche). We did not observe any obvious differences in performance between the different extraction methods. The 9 laboratories reporting false-positive results in the extraction samples all used different extraction methods. SiHa cells have about 1 copy of HPV16 per cell and 2,500 cells/5μl were correctly identified in 91% of the data sets. In the sample containing only the HPV-negative cell line, 6 data sets reported false-positive results, and in total, 15 false-positive results were reported in the 3 extraction samples. This suggests that for a noteworthy minority of laboratories, contamination during the DNA extraction step is an issue.

This was the third HPV DNA proficiency panel issued by HPV LabNet that was open for worldwide participation. There were 54 laboratories participating in 2011 that also participated in at least one of the previous years. Thirty laboratories participated in all three proficiency studies. Their continued participation in the study shows that many laboratories are interested in quality assurance of their assay methodologies and laboratory performance.

We see a trend toward increased sensitivity of the assays. It is important to note that the sensitivity and specificity of an assay also depend on the experience, quality assurance, and performance of the individual laboratory, as is the case when highly standardized commercial kits are used. An example is the Linear Array kit, for which some laboratories were 100% proficient, while others were nonproficient when using the same kit. Comparing the results of the laboratories that used the same assay in 2008, 2010, and 2011, there was an increase in proficient laboratories from 32% in 2008 to 42% in 2011. In the results from 2011, we can also see a clear trend of increased specificity. Among the laboratories that used the same assay each year, the proportion reporting no false-positive samples increased from 48% to 68%. The percentage of laboratories with >3 false-positive results has been rather stable over the years, around 12 to 16%, whereas the number of laboratories that have few or no false-positive results has improved.

There are additional steps and considerations in the laboratory detection process that are not evaluated by the present strategy, e.g., patient sampling, handling of the samples at the laboratory before extraction, PCR-inhibiting substances, and the natural variability of circulating virus strains. To assess these, the HPV LabNet instead performed quality control using a confirmatory testing scheme. The participating laboratories submitted a part of their clinical samples tested annually for retesting to a higher level reference laboratory (10). The HPV LabNet has recommended that as part of a quality assurance program, a laboratory can submit a part of the clinical samples tested to a national HPV reference laboratory for confirmatory testing. National HPV reference laboratories can then send those samples to a regional HPV reference laboratory for confirmatory testing, and that laboratory can send samples to one of the two global reference laboratories for testing. The alternative strategy, including clinical samples in the proficiency panel, was not chosen because of the need to have exactly reproducible panels with defined content that can be distributed to hundreds of laboratories over many years. The use of clinical samples in proficiency panels does not allow the same reproducibility over time.

It should be emphasized that the current proficiency panel was designed to evaluate the performance of the HPV testing and typing tests used in HPV vaccinology and HPV surveillance, but not for the evaluation of tests used in cervical cancer screening. In vaccinology, a high analytical sensitivity is needed, as failure to detect prevalent infections at entry may result in false vaccine "failures" in vaccination trials. In contrast, the HPV-associated diseases, such as high-grade cervical intraepithelial neoplasia (CIN), typically contain larger amounts of virus, and cervical screening programs using HPV testing do not have as high demands for their analytical sensitivities (37). Assays that are used for HPV primary screening and HPV testing for triage of cytology need to balance clinical sensitivity and specificity for the detection of high-grade CIN and cervical cancer. An international expert group has suggested the use of the FDA-approved Hybrid Capture 2 (HC2) as a reference assay and recommends that any assay used for HPV screening have a clinical sensitivity for cervical intraepithelial neoplasia (CIN)2+ of ≥90% of that of HC2 and a clinical specificity for CIN2+ of ≥98% of the clinical specificity of HC2 (37). We are planning to issue a separate proficiency panel specially designed for assays used for HPV screening. This panel will include different high-risk HPV types in a concentration that represents the clinical sensitivity and specificity described above.

In conclusion, we find that the use of global HPV DNA typing proficiency panels for validating different HPV DNA tests and laboratories promotes the comparability of data generated from different laboratories worldwide. Regularly issued global HPV DNA typing proficiency panels that allow for a comparison of global results over time will be required for the continuing work toward international standardization and quality improvement of HPV DNA typing results worldwide.

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