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Analytical evaluation of a new Microdisk™ technology-based multiplex HPV genotyping system – the QPLEX™ HPV genotyping kit

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Abstract

Background: Recently, the QPLEX™ human papillomavirus (HPV) genotyping kit (QuantaMatrix, Seoul, Korea), a Microdisk™ technology-based multiplex system, was developed to detect 32 HPV genotypes. We evaluated the analytical performance of this kit by conducting a comparison study, precision evaluation and interference testing.
Methods: A total of 1594 cervical swab specimens were used to compare the QPLEX™ HPV genotyping kit with other commercially available kits (GeneFinder HPV Liquid Bead MicroArray Genotype polymerase chain reaction [PCR] kit, Infopia, Seoul, Korea; PANArray™ HPV Genotyping Chip, PANAGENE, Daejeon, Korea). For the determination of precision, we evaluated four types of precision profiles: repeatability, lot-to-lot variability, operator-to-operator variability and site-to-site variability. In addition, interference tests were performed with various interferents.
Results: The results of the QPLEX™ HPV genotyping kit showed almost perfect agreement with the other commercially available HPV genotyping assays. The combined precision was acceptable. In addition, there was no tested interferent that affected the results of the QPLEX™ HPV genotyping kit.
Conclusions: The QPLEX™ HPV genotyping kit showed acceptable analytical performance in our study. This assay could be a suitable option for HPV genotyping in routine and follow-up tests.

Keywords: cervical cancer; evaluation; genotyping; human papillomavirus (HPV).

Brief summary: The QPLEX™ human papillomavirus (HPV) genotyping kit is one of the most recently introduced HPV genotyping assays. The QPLEX™ HPV genotyping kit showed acceptable performance for clinical laboratories.

Introduction

Cervical cancer is a common cancer in women: 528,000 new patients were diagnosed and 266,000 women died of cervical cancer worldwide in 2012 [1]. It is well known that most cervical cancers are caused by the human papillomavirus (HPV) [2]. Among the various HPV genotypes, some HPVs are classified as high-risk for their oncogenic effect, and the detection of these HPVs is very important for the prevention and control of cervical cancer [3, 4]. Therefore, molecular HPV testing is recommended for women over the age of 30 years in many gynecological guidelines [1, 5, 6].

Adopting multiplex technologies, HPV assays have been developed to accurately specify not only the risk group of HPV, but also HPV genotypes in cervical swab specimens [7, 8]. Due to this advance in HPV testing,

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diagnosis and follow-up tests of specific HPV genotypes have become routine in the clinical field. The increasing needs of HPV genotyping have prompted manufacturers to develop HPV genotyping assays with novel multiplexing principles, and various HPV genotyping assays have been introduced in the market recently [9].

The QPLEX™ HPV genotyping kit (QuantaMatrix, Seoul, Korea) is one of the most recently introduced HPV genotyping assays and is based on a post-polymerase chain reaction (PCR) characterization system designed to detect 32 different HPV genotypes simultaneously. With novel multiplexing technology, it is expected that the QPLEX™ HPV genotyping kit will increase the diagnostic yield of HPV infection. The aim of this study was to evaluate the analytical performance of the QPLEX™ HPV genotyping kit and to perform a comparison study with other commercially available HPV genotyping kits, precision evaluation and interference testing.

Materials and methods

QPLEX™ HPV genotyping kit

The QPLEX™ HPV genotyping kit (QuantaMatrix, Seoul, Korea) is a Microdisk™ technology-based multiplex system for detecting 32 HPV genotypes. The Microdisk™ adopts a graphic coding system that can distinguish 1000 codes in one microwell according to a specific graphic appearance on the surface of the beads. The QPLEX™ HPV genotyping kit contains beads that are coupled with 32 HPV capture probes that bind to HPV-specific nucleic acids.

In our study, the DNAs of all the specimens were extracted with a MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Molecular Diagnostics, CA, USA) using a MagNA Pure 96 Instrument (Roche Molecular Diagnostics), and PCR reactions were performed using a SimpliAmp™ Thermal Cycler (Applied Biosystems, Foster city, CA, USA) according to the manufacturer's specifications. Then, the PCR products were denatured using Lambda Exonuclease at 37 °C for 30 min, followed by inactivation of the Lambda Nuclease at 70 °C for 10 min. Denatured PCR products were diluted in 45 µL of hybridization buffer and added to coupled beads in a 96-well plate. Hybridization was performed at 37 °C for 30 min with gentle shaking. These beads were then washed 3 times in 100 µL washing buffer, incubated at 25 °C with the streptavidin-R-phycoerythrin conjugate (Prozyme) solution for 10 min, and washed 3 times with 100 µL of washing buffer. Next, a QMAP™ image analyzer (QuantaMatrix) captured the microwells with bright field images and fluorescence images for data analysis. An image-processing algorithm decoded all the captured beads in the bright field image, and reported the HPV genotyping results using quantification of the fluorescence intensity using fluorescence imaging (Figure 1). The cut-off for a positive result was a mean fluorescence intensity (MFI) greater than 1000.

The QPLEX™ HPV genotyping kit can detect a total of 32 HPV genotypes including 19 high-risk HPV genotypes (16, 18, 26, 31, 32, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69, 70 and 73), and 13 low-risk HPV genotypes (6, 11, 34, 40, 42, 43, 44, 53, 54, 55, 61, 62 and 81). However, due to the

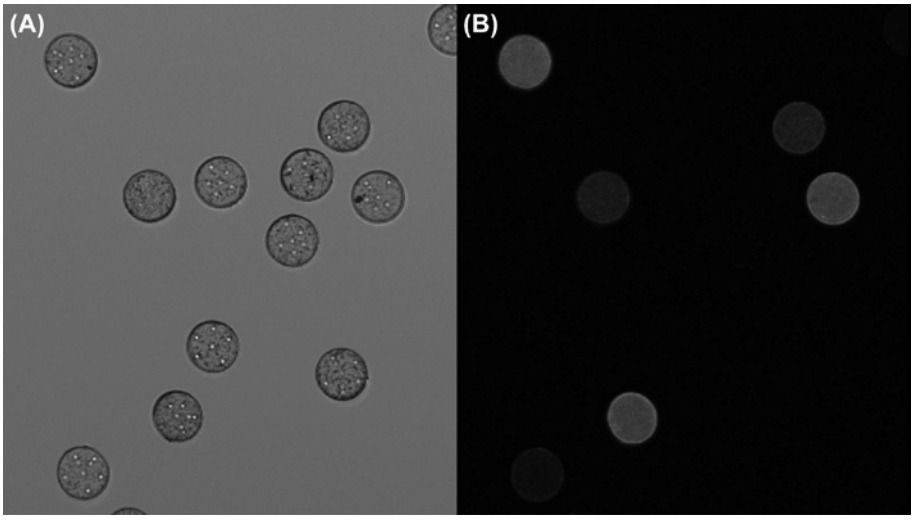

Figure 1: Bright-field (A) and fluorescence (B) images from the QMAP™ image analyzer.
The beads of the Microdisk™ are carboxyl functionalized magnetic disks that can be distinguished with a graphic code on the surface. Image processing algorithm decodes all captured MicroDisk™, and quantifies fluorescence intensity.

difference in categorization of carcinogenicity between literatures or manufacturer's claims [4, 9, 10], we classified HPV genotypes according to the carcinogen classification of the International Agency for Research on Cancer [10, 11] as follows: group 1 (carcinogenic to humans), group 2A (probably carcinogenic to humans), group 2B (possibly carcinogenic to humans), group 3 (not classifiable as to its carcinogenicity to humans) and unclassified group.

Samples

A total of 1594 cervical swab samples were obtained to evaluate the performance of the QPLEX™ HPV Genotyping kit. A total of 452 HPV-negative samples were enrolled, and at least 37 positive specimens for each HPV genotype were selected for comparative evaluation. These samples were residual samples requested for HPV genotyping between April 2017 and June 2017. Specimens were collected using the Digene Cervical Sampler (Qiagen, Hilden, Germany) and were stored at –20 °C. The specimens were anonymized and only the test results were available. All samples were genotyped using the GeneFinder HPV Liquid Bead MicroArray Genotype PCR kit (Infopia, Seoul, Korea), or PANArray™ HPV Genotyping Chip (PANAGENE, Daejeon, Korea), which were performed as routine cervical cancer screening assays during the study period. These two assays had been evaluated previously, and are widely used in various clinical laboratories as a HPV screening assay [12, 13]. Ethical approval was granted by the Chung-Ang University Hospital Ethics Committee C2016082 (1825).

Evaluation protocol

Among 1594 cervical swab samples, 48 specimens were excluded from the experiment because the quantity of extracted DNA was too small (less than 1 ng/μL). A total of 1546 samples were genotyped using the QPLEX™ HPV genotyping kit. For comparison with commercially available kits (GeneFinder HPV Liquid Bead MicroArray Genotype PCR kit and PANArray™ Genotyping Chip), the overall agreement, percent positive agreement (agreement between positive samples), percent negative agreement (agreement between negative samples) and Cohen's kappa coefficient were calculated for each of the HPV genotypes.

To confirm the genotyping results which showed discrepancies between kits, we carried out the type-specific PCR with direct sequencing analysis using the previously reported method [9, 14]. All type-specific primers were designed to detect the L1 gene region for PCR and sequencing analyses. A total of 395 sequences were analyzed among the 137 specimens, and compared with the GenBank Basic Local Alignment Search Tool (BLAST) database.

Precision analysis was carried out on HPV DNA standard materials (Human Papilloma Virus L1 DNA, Korea Centers for Disease Control & Prevention, Chungcheongbuk-do, Korea), which consisted of 41 vials of each HPV genotype for the evaluation of *in vitro* diagnostic medical devices. These standards were stored at –70 °C, and open vials were stored at 4 °C for 1 day. HPV DNA samples were prepared in two different DNA concentrations (1.0×10^2 copies/run and 1.0×10^3 copies/run). To account for variable factors present in the clinical laboratory, we evaluated four types of precision profiles according to the Clinical and Laboratory Standards Institute (CLSI) document EP05-A3 [15]: repeatability, lot-to-lot variability, operator-to-operator variability and site-to-site variability. All precision profiles were calculated from the MFI from the QPLEX™ HPV genotyping kit. Each sample was tested with the following protocols: two replicate measurements per run, two runs per day for 20 days ($2\times2\times2$, repeatability); three lots per run, four runs per day for 20 days ($3\times4\times5$, lot-to-lot variability); three operators per run, two runs per day for 3 days, ($3\times2\times3$, operator-to-operator variability); two laboratories per run and two runs per day for 3 days ($2\times2\times3$, site-to-site variability). For quantifying the site-to-site variability, additional tests were conducted in the U_2Bio Laboratory (Seoul, Korea). Next, to give a more definitive estimate of precision, three or four types of precision profiles for each HPV genotype were pooled for calculating the combined precision.

To evaluate the cross-reaction and interference of bacteria, fungi, parasites, viruses and other interfering substances, we spiked the HPV-negative and -positive specimens with various microorganisms, DNA of viruses and interferents (Table 1). Then, we tested these specimens 3 times using the QPLEX™ HPV genotyping kit.

Statistics

To evaluate the agreement of genotyping results, the overall agreement, percent positive agreement, percent negative agreement and Cohen's kappa coefficient were calculated using Microsoft Office Excel 2010 (Microsoft Co., Redmond, WA, USA) and R version 3.4.3 (http://

Table 1: List of interfering substances and test concentrations.

Interfering substances	Concentrations
Gram-negative bacteria	
Escherichia coli ATCC 25922	1.8×10^5 copies
Pseudomonas aeruginosa ATCC 27853	1.4×10^5 copies
Klebsiella pneumoniae ATCC 700603	1.8×10^5 copies
Chlamydia trachomatis ATCC VR-879D	7.9×10^8 copies
Neisseria gonorrhoeae ATCC 53420D-5	4.2×10^5 copies
Ureaplasma parvum[a]	3.1×10^8 copies
Gram-positive bacteria	
Staphylococcus aureus ATCC 29213	1.2×10^7 copies
Enterococcus faecalis ATCC 29212	3.2×10^5 copies
Streptococcus pneumoniae ATCC 49619	4.2×10^5 copies
Other bacteria	
Gardnerella vaginalis KCTC 5096	1.0×10^7 copies
Mycoplasma genitalium[b]	6.2×10^8 copies
Mycoplasma hominis[a]	3.1×10^8 copies
Ureaplasma urealyticum[a]	3.1×10^8 copies
Fungus	
Candida albicans[c]	6.2×10^8 copies
Parasite	
Trichomonas vaginalis ATCC 30001D	9.3×10^8 copies
Viruses	
HSV Type 1 ATCC VR-260	6.1×10^6 copies
HSV Type 2 ATCC VR-540	6.1×10^6 copies
HPV 57[d]	1.0×10^4 copies
HPV 67[d]	1.0×10^4 copies
HPV 72[d]	1.0×10^4 copies
HPV 84[d]	1.0×10^4 copies
Interferents	
Antifriction	2%
Feminine cleanser	2%
Antifungal agent	2%
Blood	2%

[a]In-house synthetic DNA. [b]Amplirun *Mycoplasma genitalium* DNA control, Vircell, Granada, Spain. [c]Amplirun *Candida* DNA control, Vircell. [d]Human papilloma virus L1 DNA, Korea Centers for Disease Control & Prevention, Chungcheongbuk-do, Korea.
HPV, human papilloma virus; HSV, herpes simplex virus.

www.R-project.org/) [16]. Cohen's kappa coefficient (k) was assessed according to the following criteria: 0.81–1.00 for almost perfect agreement, 0.61–0.80 for substantial agreement, 0.41–0.60 for moderate agreement, 0.21–0.40 for fair agreement, 0.00–0.20 for slight agreement and <0.00 for poor agreement [17]. The comparisons between the results of QPLEX™ HPV genotyping kit and direct sequencing analysis were presented with the 2×2 contingency table, and the absolute agreements with 95% confidence intervals were calculated according to the CLSI guideline EP12-A2 [16]. The precision profiles were analyzed using R version 3.4.3 in accordance with the CLSI guideline EP05-A3 [15]. The MFI results of the cross-reaction and interference evaluations were compared by the T-test using R version 3.4.3, and differences with a p-value of less than 0.05 were considered statistically significant.

Results

The results of the comparative analysis for each HPV genotype are shown in Table 2. The overall agreement, percent positive agreement and percent negative agreement ranged from 98.7% to 100.0%, 92.5% to 100.0% and 98.7% to 100.0%, respectively. Cohen's kappa coefficient for the 32 HPV genotypes was between 0.79 and 1.00, and most of the HPV genotypes showed almost perfect agreement. Only HPV 52 showed substantial agreement (k = 0.79) when compared to the other commercial HPV genotyping kits.

A total of 137 samples showed discrepant genotyping results between QPLEX™ HPV genotyping kit and other commercially available kits. Among them, 132 samples were mixed infected with at least two HPV genotypes, and the last five samples were confirmed as a single genotype infection or negative result in the QPLEX™ HPV genotyping kit. Considering the mixed infection, we analyzed 395 HPV sequences, and the comparison results between the QPLEX™ HPV genotyping kit and direct sequencing analysis are summarized in Table 3. The absolute agreements of each HPV genotype were between 60% and 100%, and 91.1% (360/395) of results from the QPLEX™ HPV genotyping kit were concordant with sequencing analysis. Otherwise, there were relatively low agreements (under 80%) in the detection of HPV 31, 44, 55, 59 and 81.

The repeatability, lot-to-lot variability, operator-to-operator variability and site-to-site variability of the QPLEX™ HPV genotyping kit are summarized in Supplementary Tables 1–4. The combined precisions are shown in Figure 2. In the two different concentrations, the pooled precision of MFI was within 20% for HPV genotype detection. For every HPV genotype, there were no changes from a positive result to a negative result in the genotyping results. However, the combined precision of MFI unexpectedly exceeded 20% in some HPV genotypes: HPV 6, 26 and 69 for 1.0×10^2 copies/run, and HPV 52, 59 and 44 for 1.0×10^3 copies/run.

In the cross-reaction and interference evaluations, there was a slight change in MFI; however, no statistically significant changes were evident in the MFI results (data not shown). In addition, there were no cases where a negative result changed to a positive or a positive result changed to a negative result. We can conclude that none

Table 2: Overall agreement, percent positive agreement, percent negative agreement and Cohen's kappa coefficient (k-value) between the QPLEX™ HPV genotyping kit and commercially available HPV genotyping kits.

HPV genotype	Results	Routine test results		Overall agreement, % (range)[a]		Positive agreement, % (range)[a]		Negative agreement, % (range)[a]		k-Value[a]	
		Positive	Negative								
Group 1											
16	Positive	121	0	99.9	(99.5–100)	100	(96.9–100)	99.9	(99.5–100)	0.99	(0.98–1)
	Negative	2	1423								
18	Positive	118	0	99.9	(99.5–100)	100	(96.9–100)	99.9	(99.5–100)	0.99	(0.98–1)
	Negative	2	1426								
31	Positive	39	0	99.5	(99.1–99.8)	100	(91.0–100)	99.5	(99.0–99.8)	0.92	(0.92–0.98)
	Negative	7	1500								
33	Positive	41	0	99.9	(99.6–100)	100	(91.4–100)	99.9	(99.6–100)	0.99	(0.96–1)
	Negative	1	1504								
35	Positive	41	0	99.9	(99.6–100)	100	(91.4–100)	99.9	(99.6–100)	0.99	(0.96–1)
	Negative	1	1504								
39	Positive	37	3	99.8	(99.4–99.9)	92.5	(80.1–97.4)	99.9	(99.5–100)	0.94	(0.88–0.99)
	Negative	2	1504								
45	Positive	38	0	99.8	(99.4–99.9)	100	(90.8–100)	99.8	(99.4–99.9)	0.96	(0.92–1)
	Negative	3	1505								
51	Positive	39	1	99.8	(99.4–99.9)	97.5	(87.1–99.6)	99.9	(99.5–100)	0.96	(0.92–1)
	Negative	2	1504								
52	Positive	40	0	98.7	(98.0–99.2)	100	(91.2–100)	98.7	(98.0–99.1)	0.79	(0.71–0.88)
	Negative	20	1486								
56	Positive	39	1	99.7	(99.2–99.9)	97.5	(87.1–99.6)	99.7	(99.3–99.9)	0.94	(0.88–0.99)
	Negative	4	1502								
58	Positive	41	0	99.9	(99.5–100)	100	(91.4–100)	99.9	(99.5–100)	0.98	(0.94–1)
	Negative	2	1503								
59	Positive	41	0	99.0	(98.4–99.4)	100	(91.4–100)	99.0	(98.4–99.4)	0.84	(0.76–0.92)
	Negative	15	1490								
Group 2A											
68	Positive	39	1	99.7	(99.3–99.9)	97.5	(87.1–99.6)	99.8	(99.4–99.9)	0.95	(0.90–1)
	Negative	3	1503								
Group 2B											
26	Positive	40	0	99.9	(99.6–100)	100	(91.2–99.9)	99.9	(99.6–100)	0.99	(0.96–1)
	Negative	1	1505								
34	Positive	38	1	99.9	(99.6–100)	97.4	(86.8–99.5)	100	(99.7–100)	0.99	(0.96–1)
	Negative	0	1507								
53	Positive	36	2	99.5	(99.1–99.8)	94.7	(82.7–98.5)	99.7	(99.2–99.9)	0.91	(0.84–0.98)
	Negative	5	1503								
66	Positive	40	1	99.5	(99.0–99.7)	97.6	(87.4–99.6)	99.5	(99.0–99.8)	0.91	(0.84–0.97)
	Negative	7	1498								
70	Positive	41	0	99.5	(99.1–99.8)	100	(91.4–100)	99.5	(99.0–99.8)	0.92	(0.86–0.98)
	Negative	7	1498								

Table 2 (continued)

HPV genotype	Results	Routine test results		Overall agreement, % (range)[a]		Positive agreement, % (range)[a]		Negative agreement, % (range)[a]		k-Value[a]	
		Positive	Negative								
73	Positive	41	0	99.8	(99.4–99.9)	100	(91.4–100)	99.8	(99.4–99.9)	0.96	(0.92–1)
	Negative	3	1502								
Group 3											
6	Positive	40	1	99.7	(99.2–99.9)	97.6	(87.4–99.6)	99.7	(99.3–99.9)	0.94	(0.89–0.99)
	Negative	4	1501								
11	Positive	41	0	100	(99.8–100)	100	(91.4–100)	100	(99.7–100)	1	(1–1)
	Negative	0	1505								
Unclassified group											
32	Positive	38	0	100	(99.8–100)	100	(90.8–100)	100	(99.7–100)	0.99	(0.96–1)
	Negative	0	1508								
40	Positive	39	1	99.9	(99.6–100)	97.5	(87.1–99.6)	100	(99.7–100)	0.99	(0.96–1)
	Negative	0	1506								
42	Positive	38	3	99.7	(99.3–99.9)	92.7	(80.6–97.5)	99.9	(99.6–100)	0.95	(0.90–1)
	Negative	1	1504								
43	Positive	40	0	99.7	(99.2–99.9)	100	(91.2–100)	99.7	(99.2–99.9)	0.94	(0.89–0.99)
	Negative	5	1501								
44	Positive	41	0	99.3	(98.7–99.6)	100	(91.4–100)	99.3	(98.7–99.6)	0.88	(0.81–0.95)
	Negative	11	1494								
54	Positive	38	1	99.9	(99.6–100)	97.4	(86.8–99.5)	100	(99.7–100)	0.99	(0.96–1)
	Negative	0	1507								
55	Positive	41	0	99.2	(98.6–99.5)	100	(91.4–100)	99.1	(98.5–99.5)	0.86	(0.78–0.93)
	Negative	13	1492								
61	Positive	37	0	99.4	(98.8–99.6)	100	(90.6–100)	99.3	(98.8–99.6)	0.88	(0.80–0.95)
	Negative	10	1499								
62	Positive	38	0	99.1	(98.5–99.5)	100	(90.8–100)	99.1	(98.4–99.4)	0.84	(0.76–0.92)
	Negative	14	1494								
69	Positive	40	0	100	(99.8–100)	100	(91.2–100)	100	(99.7–100)	1	(1–1)
	Negative	0	1506								
81	Positive	41	1	98.8	(98.2–99.3)	97.6	(87.7–99.6)	98.9	(98.2–99.3)	0.81	(0.73–0.90)
	Negative	17	1487								

[a]Data are shown with 95% confidence intervals. HPV, human papilloma virus.

Table 3: Absolute agreements comparing the QPLEX™ HPV genotyping kit with direct sequencing analysis.

HPV genotypes	TP	TN	FP	FN	Absolute agreement with 95% CI, % (range)	
Group 1						
16	24	0	0	0	100	(80–100)
18	29	0	0	0	100	(83–100)
31	7	0	3	0	70	(0–100)
33	3	0	0	0	100	(17–100)
35	4	0	1	0	80	(0–100)
39	9	1	0	1	91	(31–100)
45	8	1	0	0	100	(56–100)
51	7	1	0	0	100	(52–100)
52	18	0	4	0	82	(13–100)
56	6	1	0	0	100	(48–100)
58	11	0	0	0	100	(62–100)
59	11	0	6	0	65	(0–100)
Group 2A						
68	11	1	1	0	92	(39–100)
Group 2B						
26	7	0	0	0	100	(48–100)
34	10	1	0	0	100	(62–100)
53	14	3	0	0	100	(73–100)
66	9	1	1	0	91	(31–100)
70	11	0	1	0	92	(35–100)
73	11	0	0	0	100	(62–100)
Group 3						
6	14	1	0	0	100	(70–100)
11	5	0	0	0	100	(36–100)
Unclassified group						
32	5	0	0	0	100	(36–100)
40	8	0	0	0	100	(52–100)
42	11	1	0	0	100	(64–100)
43	13	0	0	0	100	(66–100)
44	6	0	4	0	60	(0–100)
54	6	0	0	0	100	(42–100)
55	11	0	3	0	79	(0–100)
61	13	0	1	0	93	(42–100)
62	26	0	3	0	90	(41–100)
69	4	0	0	0	100	(28–100)
81	16	0	6	0	73	(0–100)

CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

of the material tested in our study had any interfering effect on the QPLEX™ HPV genotyping kit.

Discussion

Although prophylactic HPV vaccination shows efficacy in preventing cervical neoplasm [18], cervical cancer is still one of the most common gynecologic cancer diagnoses and causes of death worldwide [1, 19, 20]. Therefore, HPV genotyping test is an important molecular diagnostic test in the clinical laboratory, and should be accurate and precise for routine testing and monitoring [21].

In this study, we evaluated one of the new HPV genotyping assays, the QPLEX™ HPV genotyping kit based on the Microdisk™ technology. The Microdisk™ system can support various molecular bioassays as well as immunoassays by multiplex assay in a single well. The Microdisk™ is made of an ultraviolet (UV) curable polymer mixed with magnetic nanoparticles. Due to the unique material composition, the shape of the particles is freely controlled by photo-patterning and they can be massively handled or separated by a magnetic field. The QMAP™ image analyzing system would be comparable to all specifications

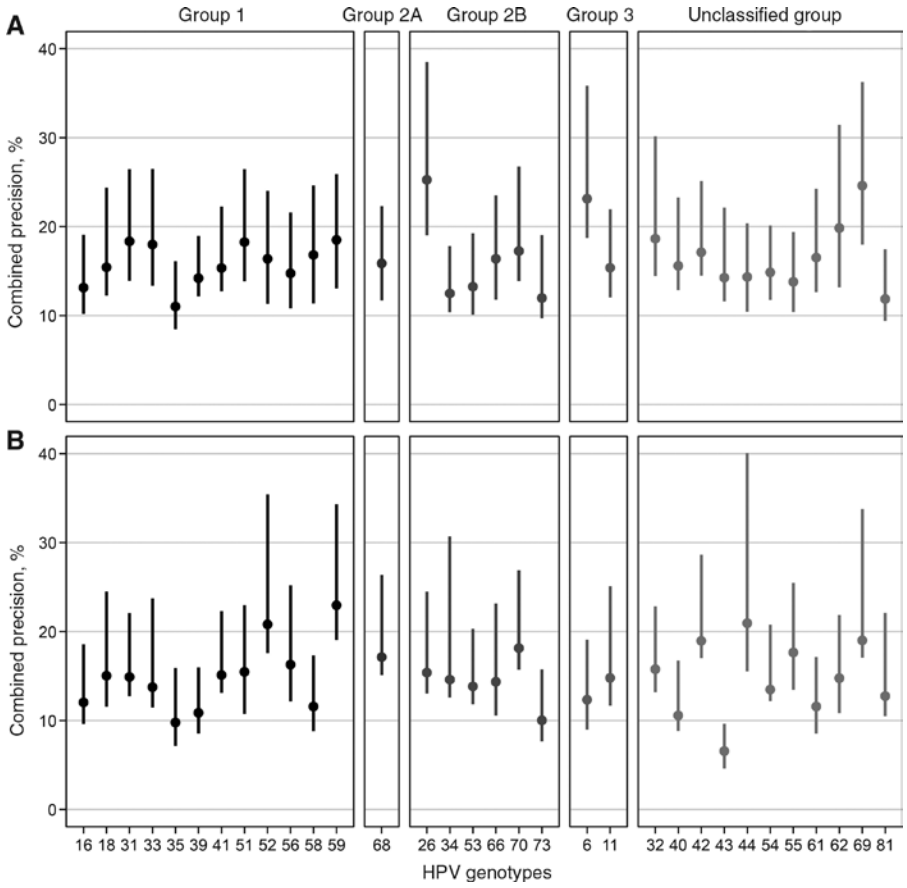

Figure 2: Combined precision (circles) with 95% confidence intervals (bold lines).
Two HPV DNA samples were used in the precision study; 1.0×10^2 copies/run (A) and 1.0×10^3 copies/run (B).

of the Luminex xMAP technology that uses fluorescent color coding. In addition, the Microdisk™ is expected to be more stable to light and temperature conditions than the Luminex beads because graphic codes are engraved on the beads.

Our data demonstrated that the results of the QPLEX™ HPV genotyping kit showed almost perfect agreement with commercially available HPV genotyping assays. Additionally, QPLEX™ HPV genotyping kit showed appropriate absolute agreements compared to direct sequencing analysis. However, there were unsatisfactory agreements for some of HPV genotypes, especially HPV 31, 44, 55, 59 and 81. We hypothesized that these problems would be caused by the similarities among the targeted sequences used in the QPLEX™ HPV genotyping kit.

In precision evaluation, we demonstrated that overall precision was acceptable. The QPLEX™ HPV genotyping kit showed repeatability, lot-to-lot variability, operator-to-operator variability, site-to-site variability and a combined precision within 20% of the MFI for detection of most of the HPV genotypes. However, we found that the precision profiles for some HPV genotypes were unexpectedly over 20% in the MFI results. Although no erroneous genotyping results, such as a change from a positive to a negative result or a negative to a positive result, were reported, due to large precision MFI profiles in some HPV genotypes, accurate HPV genotyping when DNA concentrations are close to the detection limit could be a problem. We have no clear explanation for such a large MFI difference in repeatability; however, we suggest that this may need to be improved by the manufacturer in the future.

Conclusions

In conclusion, we evaluated the recently developed Microdisk™ technology-based multiplex HPV genotyping system. The QPLEX™ HPV genotyping kit showed perfectly matched results compared to other commercially available HPV genotyping assays. In addition, this assay

showed acceptable performance for almost all HPV genotypes in the precision study, and no interferences were found by various interferents that could affect the test results. Therefore, we are convinced that the QPLEX™ HPV genotyping kit could be one of the best options for HPV genotyping in routine and follow-up protocols in the clinical field.

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