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Human Papillomavirus (HPV) Genotyping Assay Suitable for Monitoring the Impact of the 9-Valent HPV Vaccine

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In Japan, a bivalent human papillomavirus (HPV) vaccine against carcinogenic HPV16/18 was licensed in 2009, and a quadrivalent vaccines against HPV16/18 and non-carcinogenic HPV6/11 was licensed in 2011. Recently, the next-generation 9-valent vaccine targeting HPV6/11/16/18/31/33/45/52/58 has been approved. Accurate HPV genotyping is essential for HPV vaccine research and surveillance. The Roche Linear Array (LA) has long been a standard assay for HPV genotyping, but its recent product discontinuation notice has urged us to introduce an alternative assay with comparable performance. In the present study, an in-house HPV genotyping assay that employs PCR with PGMY09/11 primers and reverse blotting hybridization (PGMY-CHUV) was compared with LA to assess genotype-specific agreement. A total of 100 cervical precancer specimens were subjected to both PGMY-CHUV and LA. For detection of genotypes included in the 9-valent vaccine, PGMY-CHUV completely agreed with LA for detection of HPV6, HPV11, HPV16, HPV18, HPV33 and HPV45, and showed near-complete agreement for HPV31 and HPV58 (98% and 99%, respectively). Moreover, PGMY-CHUV detected a significantly higher prevalence of HPV52 than LA (22% vs. 14%, P = 0.008 by McNemar's exact test), with 92.0% overall agreement, 63.6% positive agreement and a kappa value of 0.73. Most (87.5%) of HPV52 discordant cases involved mixed infections with HPV35 or HPV58. In conclusion, while the two assays present equivalent data for assessing the effectiveness of the bivalent and quadrivalent vaccines, PGMY-CHUV is more suitable for evaluating the impact of the current 9-valent vaccine because of its superior detection of HPV52 in co-infection cases.

Keywords: cervical cancer; cervical intraepithelial neoplasia; genotyping; human papillomavirus; vaccine
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Introduction

Cervical cancer is caused by persistent infections with carcinogenic genotypes of human papillomavirus (HPV). Prophylactic HPV vaccines against HPV16/18 (bivalent HPV16/18 and quadrivalent HPV6/11/16/18 vaccines) have

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the potential to prevent approximately 70% of cervical cancers (de Sanjose et al. 2010; Serrano et al. 2012; Onuki et al. 2009). The next-generation 9-valent vaccine further extends coverage to HPV31, 33, 45, 52 and 58, and is estimated to provide around 90% protection of cervical cancers worldwide (de Sanjose et al. 2010; Serrano et al. 2012). Since prophylactic immunity elicited by HPV vaccines is basically viral genotype-specific (FUTURE II Study Group 2007; Paavonen et al. 2007), accurate HPV genotyping is essential for vaccine research and surveillance.

To our knowledge, the MINT study I was the largest nation-wide study monitoring HPV vaccination impact and HPV genotype-specific disease incidence in Japan (UMIN Clinical Trails Registry: UMIN000008891) (Matsumoto et al. 2014, 2017, 2019; Onuki et al. 2020). In that study, we recruited 7,709 Japanese women aged 16-39 years who were newly diagnosed with invasive cervical cancer (ICC), cervical intraepithelial neoplasia grades 1-3 (CIN1-3) or adenocarcinoma *in situ* (AIS) at 21 participating institutions from 2012 to 2017. HPV genotyping was conducted for 5,045 women using the commercially available Linear Array (LA) assay (Roche Molecular Systems, Pleasanton, CA, USA). The MINT study I assessed the population-level impact of the current HPV16/18 vaccines (Matsumoto et al. 2017, 2019) and attributed the contribution of individual HPV genotypes to cervical cancer and precancer in Japan (Onuki et al. 2020). To further monitor the long-term impact of HPV vaccination in Japan, we have recently initiated the MINT study II using nearly the same study design in April 2019 (UMIN Clinical Trails Registry: UMIN000038883).

The LA assay, which uses PCR-primers (PGMY09/11) to amplify a subregion of the *L1* gene of mucosal HPVs, has been the gold standard for assessing new HPV genotyping assays for many years (Castle et al. 2008; Dalstein et al. 2009; Estrade et al. 2011; Demarco et al. 2018; Wagner et al. 2019). LA has also been extensively used in research on HPV epidemiology, cervical cancer screening and vaccine surveillance (e.g., ALTS trial [Wheeler et al. 2006], ARTISTIC trial [Kitchener et al. 2011], ATHENA study [Castle et al. 2011], VACCINE study [Garland et al. 2018] and MINT study I [Matsumoto et al. 2019]), but was discontinued in December 2019. In the MINT study II, we have selected an alternative HPV genotyping method based on PCR with the PGMY09/11 primers and reverse blotting hybridization with genotype-specific probes (hereafter designated as PGMY-CHUV).

PGMY-CHUV is a non-commercial assay originally described in the World Health Organization (WHO) HPV Laboratory Manual (Unger et al. 2009) and has been evaluated within the WHO HPV Laboratory Network (LabNet) by its member laboratories as a reliable typing method (Eklund et al. 2010, 2012). As a reference laboratory in the LabNet, we have used the PGMY-CHUV assay for HPV research for more than 10 years (Mori et al. 2011; Azuma et al. 2014; Hirose et al. 2019), and its performance has been consistently recognized as excellent in HPV DNA proficiency panel studies organized by the LabNet (Eklund et al. 2018). However, before starting the MINT study II, we needed to confirm whether HPV genotype-specific data from LA (MINT I) could be directly compared with those from our in-house PGMY-CHUV (MINT II), enabling analysis of year-on-year trends in HPV genotype prevalence. To address this issue, we statistically compared the HPV genotyping results obtained using LA and PGMY-CHUV with the same set of DNA samples.

Methods

Study design

We performed HPV genotyping in 100 Japanese women with CIN2 (n = 50) or CIN3 (n = 50) using the PGMY-CHUV and LA assays. Patients were recruited consecutively from Keio University Hospital in 2018. The mean ± standard deviation age was 35.7 ± 8.6 years (range: 19-67 years) for CIN2 patients and 37.6 ± 10.4 years (range: 24-65 years) for CIN3 patients. Cervical exfoliated cells were collected in ThinPrep PreservCyt solution (Hologic, Bedford, MA, USA) using a Cervex-Brush Combi (Rovers Medical Devices B.V., Oss, the Netherlands). The study protocol was approved by the Ethics Committees at Keio University Hospital and the National Institute of Infectious Diseases. Written informed consent was obtained from each patient.

DNA extraction and sample preparation

DNA extraction was performed at the National Institute of Infectious Diseases. Total DNA was extracted from 200-μL aliquots of cervical exfoliated cells using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Indianapolis, IN, USA) and a MagNA Pure LC 2.0 instrument (Roche). The PGMY-CHUV and LA assays were performed using a single DNA sample obtained from each specimen.

PGMY-CHUV assay

An aliquot (5 μL) of the purified DNA was PCR-amplified (total reaction volume 30 μL) with AmpliTaq Gold polymerase (Thermo Fisher Scientific, Waltham, MA, USA) and biotinylated PGMY09/11 primers to amplify the *L1* gene of mucosal HPVs. Biotinylated human leukocyte antigen (HLA) primers were used to amplify cellular HLA DNA. Positive (0.1 pg/mL of HPV16 full length genomic DNA in a plasmid) and negative controls (dH_2O) were used to assess the sensitivity of PCR and detect contaminating HPV DNA in reagents. The PCR products (10 μL) were analyzed on 1.5% agarose gels to assess HPV and HLA DNA amplification; amplification of HLA DNA served as an internal control to confirm template integrity. Reverse blotting hybridization was performed as described (Unger et al. 2009). Briefly, 15 μL of denatured PCR products were allowed to hybridize with oligonucleotide probes specific for 31 HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33,

34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 69, 70, 73, 82, 83, and 84) immobilized on a Biodyne C membrane (Pall corporation, Port Washington, NY, USA) using a Miniblotter MN45 (Immunetics, Cambridge, MA, USA). The hybridized DNA was detected using the horseradish peroxidase-conjugated streptavidin (GE Healthcare, Piscataway, NJ, USA) and the enhanced chemiluminescence detection reagent (GE Healthcare).

Roche LA assay

The LA assay (Roche Molecular Systems, Pleasanton, CA, USA) was carried out according to the manufacturer's recommended protocol. Briefly, an aliquot (20 μL) of the purified DNA was used for PCR amplification with PGMY09/11 primers. The PCR products were subjected to reverse line blot hybridization for detection of 37 individual HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 56, 58, 59, 61, 62, 64, 66 to 73, 81 to 84, and 89). LA detects nine additional HPV genotypes not detected by PGMY-CHUV: HPV61, 62, 64, 67, 71, 72, 81, 82 (IS39) and 89 (CP6108). HPV genotyping using LA was performed at a clinical testing laboratory (SRL, Tokyo, Japan). Genotypes determined by PGMY-CHUV were recorded prior to the disclosure of LA results. The LA assays were performed by individuals who were blinded to the PGMY-CHUV results.

Statistical analyses

Our analysis focused on the 28 genotypes that PGMY-CHUV and LA can both detect. Using analyses of agreement (Kappa value, percent total agreement and percent positive agreement), PGMY-CHUV was compared with LA for detection of all 28 genotypes, detection of 14 carcinogenic genotypes (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (Walboomers et al. 1999) and detection of individual HPV genotypes. Kappa values were rated as follows: 0.0-0.20, poor agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, good agreement; and 0.81-1.00, very good agreement. The binomial test (McNemar's exact test) was used to determine whether detection differences between the two methods were statistically significant. The number of genotypes per sample was determined after combining PGMY-CHUV and LA results, assuming 100% probe specificity for each assay. Bowker's symmetry test was used to assess differences in the number of genotypes detected per sample. R version 3.6.3 (https://www.r-project.org) was used for all statistical analyses. Two-sided P values < 0.05 were considered statistically significant.

Results

Detection of 28 common HPV genotypes

The PGMY-CHUV and LA assays can detect 28 genotypes in common (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 69, 70, 73, 82, 83 and 84). Fig. 1 shows the results of HPV genotyping of 100 CIN2 and CIN3 cases using PGMY-CHUV and LA. Overall, the distribution of HPV genotypes was very consistent between the two methods; however, a lower number of HPV52 was detected by LA. The four most prevalent genotypes were HPV16 (50%), HPV52 (22%), HPV58 (22%) and HPV31 (19%) by PGMY-CHUV, and HPV16

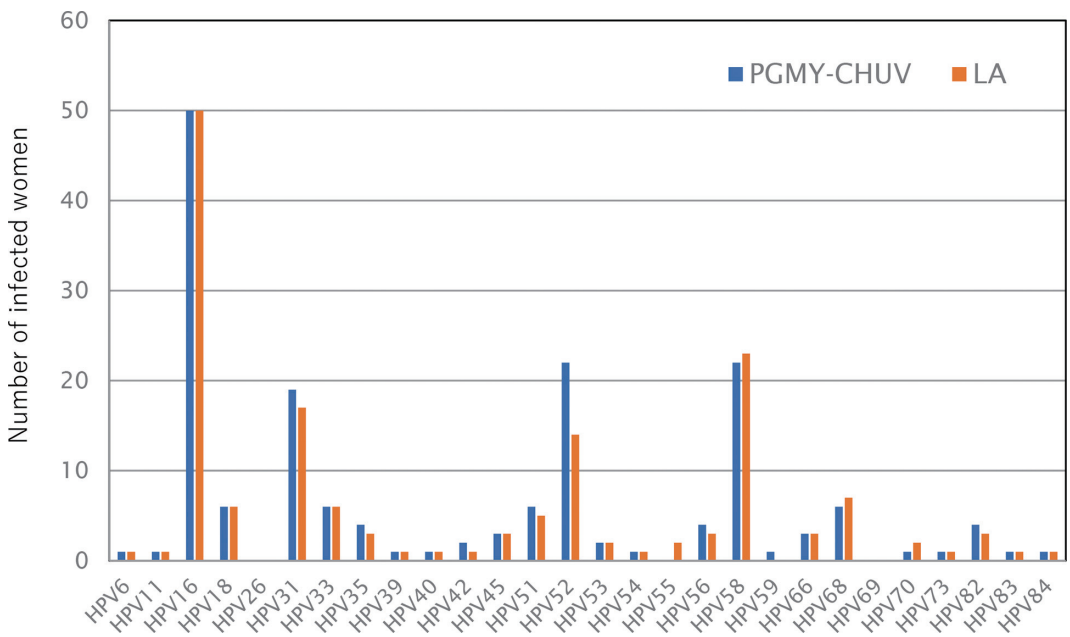

Fig. 1. HPV genotyping results of 100 CIN2/3 cases using PGMY-CHUV and Linear Array.
Only the 28 HPV genotypes detectable using both methods were considered in the analysis. The numbers of individual HPV genotypes detected in 100 women with cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) are shown separately for PGMY-CHUV (■) and Linear Array (LA) (■).

(50%), HPV58 (23%), HPV31 (17%) and HPV52 (14%) by LA.

The two methods agreed on all samples in terms of detection of any of the 28 genotypes: 99 cases were positive, and one case was negative according to both methods (Table 1). PGMY-CHUV detected 169 unique HPV infections (59 single and 40 multiple infections), while LA detected 158 infections (62 single and 37 multiple infections) (Table 2). Although PGMY-CHUV was more likely to detect multiple infections than LA, this difference was not statistically significant (P = 0.93 by Bowker's symmetry test). Kappa statistics showed good agreement in the number of HPV infections detected by the two methods (Kappa = 0.70, 95% confidence interval [95% CI] 0.59-0.82).

Detection of carcinogenic HPVs

Fourteen genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) were regarded as carcinogenic (Walboomers et al. 1999). There was complete agreement between PGMY-CHUV and LA in terms of detection of one or more carcinogenic HPV genotypes by the two methods: 96 cases were positive and four cases were negative according to both methods (Table 1). The two methods also detected a similar prevalence of multiple infection by

Table 1. Comparison of human papillomavirus genotypes detected by PGMY-CHUV and Linear Array.

Genotype	PGMY-CHUV Positive	Linear Array Positive	Comparison between PGMY-CHUV and Linear Array				% Agreement	% Positive Agreement	Kappa (95% CI)
			PGMY-CHUV − Linear Array −	PGMY-CHUV + Linear Array −	PGMY-CHUV − Linear Array +	PGMY-CHUV + Linear Array +			
Grouped detection									
28 HPVs	99	99	1	0	0	99	100%	100%	1.00 (1.00-1.00)
14 carcinogenic HPVs	96	96	4	0	0	96	100%	100%	1.00 (1.00-1.00)
HPV 6/11	2	2	98	0	0	2	100%	100%	1.00 (1.00-1.00)
HPV 16/18	53	53	47	0	0	53	100%	100%	1.00 (1.00-1.00)
HPV 31/33/45/52/58	58	56	42	2	0	56	96.6%	95.9%	0.96 (0.90-1.00)
Individual detection									
HPV 6	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 11	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 16	50	50	50	0	0	50	100%	100%	1.00 (1.00-1.00)
HPV 18	6	6	94	0	0	6	100%	100%	1.00 (1.00-1.00)
HPV 26	0	0	100	0	0	0	NA	NA	NA
HPV 31	19	17	81	2	0	17	98.0%	89.5%	0.93 (0.84-1.00)
HPV 33	6	6	94	0	0	6	100%	100%	1.00 (1.00-1.00)
HPV 35	4	3	96	1	0	3	99.0%	75.0%	0.85 (0.57-1.00)
HPV 39	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 40	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 42	2	1	98	1	0	1	99.0%	50.0%	0.66 (0.04-1.00)
HPV 45	3	3	97	0	0	3	100%	100%	1.00 (1.00-1.00)
HPV 51	6	5	94	1	0	5	99.0%	83.3%	0.90 (0.72-1.00)
HPV 52	**22**	**14**	**78**	**8**	**0**	**14**	**92.0%**	**63.6%**	**0.73 (0.56-0.90)**
HPV 53	2	2	98	0	0	2	100%	100%	1.00 (1.00-1.00)
HPV 54	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 55	0	2	98	0	2	0	98.0%	0.0%	0.00 (0.00-0.00)
HPV 56	4	3	96	1	0	3	99.0%	75.0%	0.85 (0.57-1.00)
HPV 58	22	23	77	0	1	22	99.0%	95.7%	0.97 (0.92-1.00)
HPV 59	1	0	99	1	0	0	99.0%	0.0%	0.00 (0.00-0.00)
HPV 66	3	3	97	0	0	3	100%	100%	1.00 (1.00-1.00)
HPV 68	6	7	92	1	2	5	97.0%	62.5%	0.75 (0.49-1.00)
HPV 69	0	0	100	0	0	0	NA	NA	NA
HPV 70	1	2	98	0	1	1	99.0%	50.0%	0.66 (0.04-1.00)
HPV 73	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 82	4	3	96	1	0	3	99.0%	75.0%	0.85 (0.57-1.00)
HPV 83	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)
HPV 84	1	1	99	0	0	1	100%	100%	1.00 (1.00-1.00)

Only the 28 HPV genotypes detectable using both methods were considered in the analysis.
The 14 carcinogenic HPVs included HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (Walboomers et al. 1999). HPV52 was more often detected using PGMY-CHUV (P = 0.008 by McNemar's exact test).
HPV, human papillomavirus; 95% CI, 95% confidence interval; NA, not applicable.

carcinogenic HPVs (data not shown).

Inter-method agreement for individual HPV genotypes

The two methods showed very good agreement for detection of individual HPV genotypes, except for HPV52. HPV26 and HPV69 were not detected by either PGMY-CHUV or LA and therefore could not be evaluated. PGMY-CHUV completely agreed with LA for detection of HPV6, HPV11, HPV16, HPV18, HPV33, HPV39, HPV40, HPV53, HPV54 HPV66, HPV73, HPV83 and HPV84, although the numbers of positive cases were small. Overall agreement was also very high (range: 97-100%) for detection of HPV31, HPV35, HPV42 HPV51, HPV55, HPV56, HPV58, HPV59, HPV68, HPV70 and HPV82.

PGMY-CHUV detected a significantly higher prevalence of HPV52 than LA (22% vs. 14%, P = 0.008 by McNemar's exact test), with 92.0% overall agreement, 63.6% positive agreement and a kappa value of 0.73 (95% CI 0.56-0.90). As shown in Table 3, all discordant cases were HPV52-positive by PGMY-CHUV, HPV52-negative by LA and involved multiple infections. Of eight cases with discordant HPV52 results, seven cases (87.5%) were co-infected with HPV35 or HPV58. Of note, LA cannot exclude HPV52 when the sample is positive for HPV33, HPV35 and/or HPV58. The two assays showed complete concordance for HPV52 single infection.

Discussion

The WHO HPV LabNet recommends the PGMY-CHUV assay as a reliable HPV genotyping method for

Table 2. Comparison of the number of human papillomavirus genotypes detected using PGMY-CHUV and Linear Array.

No. of HPV genotypes detected by PGMY-CHUV	No. of HPV genotypes detected by Linear Array							Total
	0	1	2	3	4	5	6	
0	**1 (1.0%)**	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)
1	0 (0.0%)	**57 (57.0%)**	2 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	59 (59.0%)
2	0 (0.0%)	5 (5.0%)	**17 (17.0%)**	0 (0.4%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	23 (23.0%)
3	0 (0.0%)	0 (0.0%)	3 (3.0%)	**6 (6.0%)**	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (9.0%)
4	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)	**2 (2.0%)**	0 (0.0%)	0 (0.0%)	5 (5.0%)
5	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	**0 (0.0%)**	0 (0.0%)	1 (1.0%)
6	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	1 (1.0%)	**0 (0.0%)**	2 (2.0%)
Total	1 (1.0%)	62 (62.0%)	22 (22.0%)	9 (9.0%)	5 (5.0%)	1 (1.0%)	0 (0.0%)	**100 (100.0%)**

Only the 28 HPV genotypes detectable using both methods were considered in the analysis. The kappa statistics showed good agreement between the two methods in terms of the number of detected HPV infections (kappa value 0.70, 95% confidence interval 0.59-0.82).
HPV, human papillomavirus.

Table 3. Discordant cases of HPV52 detection between PGMY-CHUV and Linear Array.

Case ID	Age	Diagnosis	HPV genotypes detected by PGMY-CHUV	HPV genotypes detected by Linear Array
#2299	43	CIN2	31, **35**, 52	31, **35**
#2339	29	CIN2	16, 52	16
#2382	33	CIN3	16, 31, 52, **58**	16, 31, **58**
#2402	27	CIN3	16, 52, 56, **58**, 68, 82	16, 56, **58**, 68
#2407	31	CIN2	16, 52, **58**	16, **58**
#2411	43	CIN3	52, **58**, 66, 68	**58**, 61, 66, 68
#2448	36	CIN2	16, 52	16, **58**
#2496	28	CIN3	16, 52, **58**, 68	16, **58**, 68

Only the 28 HPV genotypes detectable using both assays were considered in the analysis. HPV35 and HPV58 are indicated in bold.
HPV, human papillomavirus; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3.

HPV vaccine surveillance (Unger et al. 2009), while the Roche LA assay has been used in numerous studies of HPV epidemiology, screening and vaccination (Wheeler et al. 2006; Kitchener et al. 2011; Castle et al. 2011; Markowitz et al. 2013; Garland et al. 2018; Matsumoto et al. 2019; Onuki et al. 2020). PGMY-CHUV can detect 31 individual HPV genotypes at a much lower cost (Estrade et al. 2011), but requires proficient skills of laboratory work and strict quality control for the reagents and membranes. This might be the reason why PGMY-CHUV has not been widely employed in previous HPV studies. Although both methods are based on PCR with PGMY09/11 primers followed by reverse blotting hybridization, few direct comparisons of these two methods have been conducted. In this study, the overall agreement between PGMY-CHUV and LA was very high (> 97%) for detection of individual HPV genotypes common to both methods, except for HPV52. Our findings agree with those of a previous study reporting good concordance between the two methods and an advantage of PGMY-CHUV over LA in HPV52 detection (Estrade et al. 2011).

PGMY-CHUV detected a significantly higher prevalence of HPV52 infection than LA: eight discordant cases were all HPV52-positive by PGMY-CHUV but HPV52-negative by LA. As described in its product insert, the Roche LA assay cannot directly detect HPV52 because of intellectual property restrictions. Specimens testing negative for HPV33, HPV35 and HPV58 individually, but positive for the HPVmix (a combined probe for HPV33/35/52/58), are assumed to be HPV52 positive. Accordingly, LA cannot exclude HPV52 positivity in co-infected cases with HPV33, HPV35 and/or HPV58. In the present study, six (75.0%) out of eight HPV52 discordant cases showed multiple infections of HPV52 and HPV58 (n = 5) or HPV35 (n = 1) by PGMY-CHUV, which strongly suggests that LA missed HPV52 infections in these co-infection cases. In contrast, the two methods showed complete agreement for detection of HPV52 single infections and near-complete agreement for detection of HPV35 and HPV58. Although several vaccine studies used LA in tandem with HPV52-specific PCR to avoid misclassification of HPV52 status in patients with multiple infections (Markowitz et al. 2013; Garland et al. 2018), PGMY-CHUV does not require a complementary method.

In the previous study comparing the two methods, PGMY-CHUV detected significantly more HPV42 and HPV56 infections than LA (Estrade et al. 2011). However, our study did not find significant differences in detecting HPV42 and HPV56 between PGMY-CHUV and LA (both P = 0.99 by McNemar's exact test), probably due to limitations imposed by a lower prevalence of these genotypes in Japan. In this study, PGMY-CHUV and LA performed similarly for detecting individual HPV genotypes common to both methods, except for HPV52.

Based on LA genotyping results, the MINT study I reported a significant reduction in cervical precancer attributable to HPV16/18 among vaccinated patients from 2012 to 2017 (Matsumoto et al. 2019). To monitor long-term changes in the prevalence of HPV genotypes in Japan, the MINT study II is now in progress using PGMY-CHUV. The complete agreement between the two methods for detection of HPV16 and HPV18 suggested that HPV16/18-specific data from MINT studies I and II can be combined. For detection of individual genotypes included in a 9-valent vaccine, the overall agreement between the two methods was also very high (98-100%) for detection of HPV6, HPV11, HPV31, HPV33, HPV45 and HPV58. As mentioned above, however, LA cannot exclude HPV52 infections in cases of mixed infections with HPV33, HPV35 and/or HPV58. In general, multiple infections are more commonly associated with low-grade cervical abnormalities (Miura et al. 2006; Onuki et al. 2009, 2020), suggesting that HPV52 infections may be more frequently missed by LA in women with CIN1. In a recent report using LA data from the MINT study I, the genotype-specific risks of disease progression may have been slightly overestimated for HPV52 because these risks were calculated from prevalence ratios between CIN2-3/AIS/ICC and CIN1 (Onuki et al. 2020). Given that HPV52 prevalence is relatively high in patients with ICC (7.0-11.8%) and CIN2-3 (13.9-26.5%) in Japan (Miura et al. 2006; Onuki et al. 2009; Azuma et al. 2014; Sakamoto et al. 2018), accurate detection of HPV52 is important for vaccine surveillance. The 9-valent vaccine is reported to confer approximately 96% protection against HPV31/33/45/52/58 persistent infections and associated diseases (Joura et al. 2015), and has been approved for use in Japan in 2020. The MINT study II using PGMY-CHUV will provide more accurate data on HPV52 prevalence in the era of the 9-valent vaccine.

Our PGMY-CHUV assay has a drawback for HPV68 detection. HPV68 is divided into two subtypes, HPV68a and HPV68b, in which the L1 gene sequence differs by 7% (Longuet et al. 1996). A previous report demonstrated that the original PGMY09/11 primer set (PGv1) cannot amplify HPV68a, while the updated PGMY09/11 primer set (PGv2: equivalent to PGv1 with the additional RSMY09-L primer and HPV68a-specific probe) enables HPV68a detection (Estrade and Sahli 2014). In the present study, there was good agreement in HPV68 detection between PGMY-CHUV and LA (97.0% overall agreement and kappa value of 0.75) because both methods employed the same primer set PGv1. In the MINT study II, we are going to change the primer set from PGv1 to PGv2 to improve HPV68 detection. The PGv2-based PGMY-CHUV may provide more accurate data on HPV68 infections in the MINT study II because HPV68a was reported to be more prevalent than HPV68b in a Japanese population (Satoh et al. 2013).

In conclusion, PGMY-CHUV and LA showed excellent agreement for HPV genotype detectable using both methods in clinical specimens collected from CIN2-3 patients. Both assays were comparable for monitoring the impact of the bivalent and quadrivalent HPV16/18 vac-

cines. However, PGMY-CHUV is more suitable for monitoring the prophylactic effects of the 9-valent vaccine, because it can unambiguously identify HPV52 in the context of mixed infections.

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Conflict of Interest

The authors declare no conflict of interest.

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