

Cytological Evaluation and REBA HPV-ID HPV Testing of Newly Developed Liquid-Based Cytology, EASYPREP: Comparison with SurePath

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Background: The objective of this study was to evaluate a newly-developed EASYPREP liquid-based cytology method in cervicovaginal specimens and compare it with SurePath. **Methods:** Cervicovaginal specimens were prospectively collected from 1,000 patients with EASYPREP and SurePath. The specimens were first collected by brushing for SurePath and second for EASYPREP. The specimens of both methods were diagnosed according to the Bethesda System. Additionally, we performed to REBA HPV-ID genotyping and sequencing analysis for human papillomavirus (HPV) on 249 specimens. **Results:** EASYPREP and SurePath showed even distribution of cells and were equal in cellularity and staining quality. The diagnostic agreement between the two methods was 96.5%. Based on the standard of SurePath, the sensitivity, specificity, positive predictive value, and negative predictive value of EASYPREP were 90.7%, 99.2%, 94.8%, and 98.5%, respectively. The positivity of REBA HPV-ID was 49.4% and 95.1% in normal and abnormal cytological samples, respectively. The result of REBA HPV-ID had high concordance with sequencing analysis. **Conclusions:** EASYPREP provided comparable results to SurePath in the diagnosis and staining quality of cytology examinations and in HPV testing with REBA HPV-ID. EASYPREP could be another LBC method choice for the cervicovaginal specimens. Additionally, REBA HPV-ID may be a useful method for HPV genotyping.

Key Words: Cytological techniques; Vaginal smears; EASYPREP; Human papillomavirus; REBA HPV-ID

Cervical cancer is a common malignancy in women, though the incidence has decreased as a result of screening programs using cervicovaginal cytology. A recent investigation revealed that the incidence and mortality of cervical cancer in the Republic of Korea were 15.5 and 4.0 per 100,000 people, respectively, in 2010.¹ In cervical cancer screening programs, obtaining high specimen adequacy and definite cytological morphology is necessary for diagnosis. Because human papillomavirus (HPV) infection is associated with cervical cancer, HPV testing

is also performed for cervical cancer screening.

Liquid-based cytology (LBC) has been widely used for cervical cancer screening. LBC provides a clear cytological specimen and limited field of the glass slide by an automated production system. The low incidence of inadequate specimens and high sensitivity are some advantages of LBC.²⁻⁵

The merit of LBC is that immunocytochemistry and molecular pathology for HPV can be performed using the same specimens.^{6,7} In Korea, LBC and HPV testing have become major

procedures for cervical cancer screening. ThinPrep and SurePath, both utilize the thin-layer cytology method and are widely used for cervical cancer screening.^{8,9}

In March 2012, a novel LBC method, designated EASYPREP (YD Diagnostics Corp., Seoul, Korea) was developed in Korea. EASYPREP is the world's first fully automated thin layer cell preparation processor using centrifugation.

This study was conducted to describe the cytological findings of EASYPREP and to assess the additional immunocytochemistry and HPV testing with REBA HPV-ID (YD Diagnostics Corp.) using the same cytology specimens and determine whether the EASYPREP procedure is suitable in primary cervical cancer screening programs when compared with another widely used standard LBC system, SurePath (BD Diagnostics, Burlington, NC, USA). Additionally, the performance of the REBA HPV-ID, which is based on a polymerase chain reaction (PCR)-reverse blot hybridization assay was evaluated by comparison with sequencing analysis.

MATERIALS AND METHODS

Subjects and specimen collection

The cervicovaginal cytology specimens were obtained from 1,000 patients with their informed consent at three hospitals between May 1 and August 31, 2012 after approval from the Institutional Review Board. The specimens were prospectively collected with the EASYPREP and SurePath methods at the same time for comparison. Each cervicovaginal sample was obtained using cervixbrush (SurePath) and cytobrush (EASYPREP). Initially, samples from the first brushing were placed into SurePath preservation solution and then samples from the second brushing were placed into EASYPREP preservation solution. The cytology specimens of both EASYPREP and SurePath methods were screened by three cytotechnologists and were subsequently diagnosed by two pathologists. For both the EASYPREP and SurePath methods, the cytological diagnosis was determined according to The Bethesda System with access to other relevant patient background information. The diagnoses of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and smear characteristics using the EASYPREP method were compared with the results of the SurePath method.

EASYPREP process

The specimen vials were processed in the automated smearing system following the manufacturer's instructions. All prep-

aration procedures for LBC were processed automatically in the EASYPREP after vortexing and then transferred onto EASYPREP. In the EASYPREP 5 mL of gradient density reagent and 5 mL of preserved specimen were dispensed successively into the centrifugal tube, centrifuged for 3 minutes at 1,000 rpm and the non-diagnostic debris in the supernatant (including mucus, red blood cells and excess inflammatory cells from the sample) were removed by aspiration. The sample was then centrifuged for 7 minutes at 2,000 rpm and the residual gradient density reagent removed by aspiration. The pelleted cells were resuspended with EASYPREP suspension buffer, mixed and transferred to an EASYPREP slide chamber mounted on the EASYPREP slide. The cells were sedimented by gravity and attached to the slide by electronic charge for 10-20 minutes. After cells were completely attached onto the slide, excessive smeared cells were removed by repeating the washing step 3-4 times. Staining and coverslipping of specimen slides were performed according to laboratory procedures, and slides were examined under a microscope by trained cytotechnologists and pathologists.

Reverse blot hybridization assay for HPV DNA using EASYPREP

EASYPREP cell samples were preserved at room temperature. Thereafter, 1 mL of specimen from the remnant cell suspension was used to extract HPV viral DNA within a week after preparing specimens for diagnosis.

REBA HPV-ID is a system for HPV genotyping that can identify the 18 high-risk groups (HPV genotypes 16, 26, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73), 1 probable high-risk group (HPV genotype 34), and the 13 low-risk groups (HPV genotypes 6, 11, 40, 42, 43, 44, 54, 70, 72, 81, 84, 87). DNA preparation was performed using the supplied DNA extraction solution according to the manufacturer's instructions. Specimens of 1 mL were centrifuged at 13,000 rpm and 4°C for 5 minutes and the supernatant was discarded. Sterile distilled water (1.2 mL) was used to adjust the pellet volume. After vortexing, the homogenate was centrifuged at 13,000 rpm and 4°C for 5 minutes and the supernatant was discarded. The resulting pellet was washed 2-3 times by homogenization as described above. The DNA extraction solution (50 µL; supplied by YD Diagnostics Corp.) was added to the pellet. The sample was immediately boiled at 100°C for 10 minutes on a heat block and centrifuged at 17,590×g for 3 minutes (25°C). The resulting supernatant was transferred to a new 1.5 mL tube. A sample 3-5 µL of the supernatant was taken to

be used as a template in the simultaneous PCR amplification of the HPV and β -globin gene. For HPV genotyping, PCR products were denatured by the addition of a denaturation solution after PCR amplification of the target region. The denatured DNA was added into each well in order to adjust the supplied membrane strip onto a provided blotting tray with hybridization solution and hybridization was processed at the appropriate temperature for 30 minutes. The hybridization solution was removed and the membrane was washed twice in a pre-heated washing solution. The alkaline-phosphatase enzyme reaction was performed for 30 minutes and chromogenic reaction for 10 minutes until the color was detected. The membrane was washed twice with distilled water and completely dried. The developed strips were placed onto the data sheet provided.

Immunocytochemistry

All EASYPREP slides for immunocytochemical analysis were processed with the EASYPREP processor according to the manufacturer's instructions. For immunocytochemical staining, slides were first fixed with spray fixative and then rinsed in 50% ethanol for 30 minutes. Antigen retrieval was first conducted for 20 minutes at 99°C by heating with the microwave processor. Slides remained in the Coplin jar with distilled water at room temperature for 30 minutes. After blocking endogenous peroxidase activity, the slides were incubated with primary antibody for 30 minutes. Immunostaining with p63 (1:200, Zeta, Sierra Madre, CA, USA) and Ki-67 (1:50, MIB-1, Dako, Glostrup, Denmark) was performed using the standard biotin streptavidin detection system (Dako, Carpinteria, CA, USA). 3,3-Diaminobenzidine was used as the chromogen and the slides were counter-stained with hematoxylin. All stains were performed on the Dako Autostainer.

Sequencing analysis

Sequencing analysis was performed for all specimens that were examined by REBA HPV-ID. For sequencing analysis, nested PCR was performed to amplify the target regions (GP5 and GP6).

Statistical analysis

Diagnosis between EASYPREP and SurePath methods were compared by the Pearson chi-square test. All p-values were 2-tailed; $p < 0.05$ were considered statistically significant. Analyses were conducted using SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Smear characteristics

SurePath and EASYPREP thin layer slides were very similar in their overall appearance in the 17 mm in diameter round observation field of EASYPREP and 11 mm observation field of SurePath. The gross areas of EASYPREP and SurePath were 213.7 mm² and 103.8 mm², respectively. EASYPREP was 2.1 times larger than SurePath. Both preparations displayed a uniform layer of cells across their respective cellular areas. Mucus and red cells were removed, and the number of leukocytes was reduced or distributed randomly throughout the preparation. The individual cells showed even staining intensity. Air-dried artifacts and obscuring and overlapping cellular material and debris were largely eliminated. The inflammatory cells did not overlap with the epithelial cells, which allowed for easier visualization of epithelial cells, diagnostically relevant cells and infectious organisms. The red blood cells were cytolized at the smear specimens. Lactobacilli were confirmed outside of the cells as well as in the cytoplasm of epithelial cells. Candida infections were detected in 0.8% of specimens. There were no significant differences between EASYPREP and SurePath in the quality of preservation, cellularity, infectious organisms and specimen adequacy. EASYPREP and SurePath methods provided evenly distributed thin layers of cells. Total smeared cells of EASYPREP were calculated to average 129,585. The total squamous cells of SurePath were 78,406 on average. The amount of smeared cells of EASYPREP was 1.7 times higher than SurePath. The cell density (643 cells) under the 200 \times magnification field of EASYPREP was 1.2 times lower than that of SurePath (800 cells). Cellularity and staining quality were the same in the EASYPREP and SurePath methods.

Diagnosis

Table 1 presents the results of cytological diagnosis performed on 1,000 cases using EASYPREP and SurePath methods. The mean age was 45 ± 2.5 years. The diagnosis results using the EASYPREP technique were negative for intraepithelial lesion or malignancy (NILM) in 86.6% of cases, atypical squamous cells of undetermined significance (ASC-US) in 4.3%, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) in 0.6%, low grade squamous intraepithelial lesion (LSIL) in 5.9% and high grade squamous intraepithelial lesion (HSIL) in 2.0% of cases. The squamous cell carcinoma (SCC) was detected in 0.4%. The atypical glandular cells of undetermined significance (AGUS) and adenocarcinoma were

detected in 0.1%, respectively. The cytological appearance for each diagnosis when using EASYPREP is presented in Fig. 1.

Table 1. Comparison of the diagnosis between SurePath and EASYPREP

	SurePath	EASYPREP
NILM	860 (86.0)	866 (86.6)
ASC-US	48 (4.8)	43 (4.3)
ASC-H	8 (0.8)	6 (0.6)
LSIL	59 (5.9)	59 (5.9)
HSIL	17 (1.7)	20 (2.0)
SCC	6 (0.6)	4 (0.4)
AGUS	1 (0.1)	1 (0.1)
AC	1 (0.1)	1 (0.1)
	1,000	1,000

Values are presented as number (%).

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma.

The proportion of diagnostic results did not differ significantly between the two methods ($p=0.087$). The negative rate (86.6%) was slightly higher in the EASYPREP method and the detection rate of abnormal cells (14.0%) was slightly higher in the SurePath method. Table 2 shows diagnostic agreement between the EASYPREP and SurePath methods. Based on the standard of the SurePath method, the sensitivity, specificity, PPV, and NPV of EASYPREP were 90.7%, 99.2%, 94.8%, and 98.5%, respectively. The concordance rate of diagnosis was 96.5% in total, 99.2% in NILM, 80.0% in abnormal diagnosis cases more than ASC-US and 92.9% in abnormal diagnosis cases more than LSIL. The concordance of diagnosis with the two methods is presented in Table 3. Although the numbers are small, the 2 methods agreed exactly in AGUS and adenocarcinoma (1 case, 0.1%, respectively). Among the 860 cases of NILM diagnosed using SurePath, five cases were reported as ASC-US, one case as LSIL and one case as HSIL when using EASYPREP. Among 48 cases interpreted as ASC-US using SurePath, nine were reported

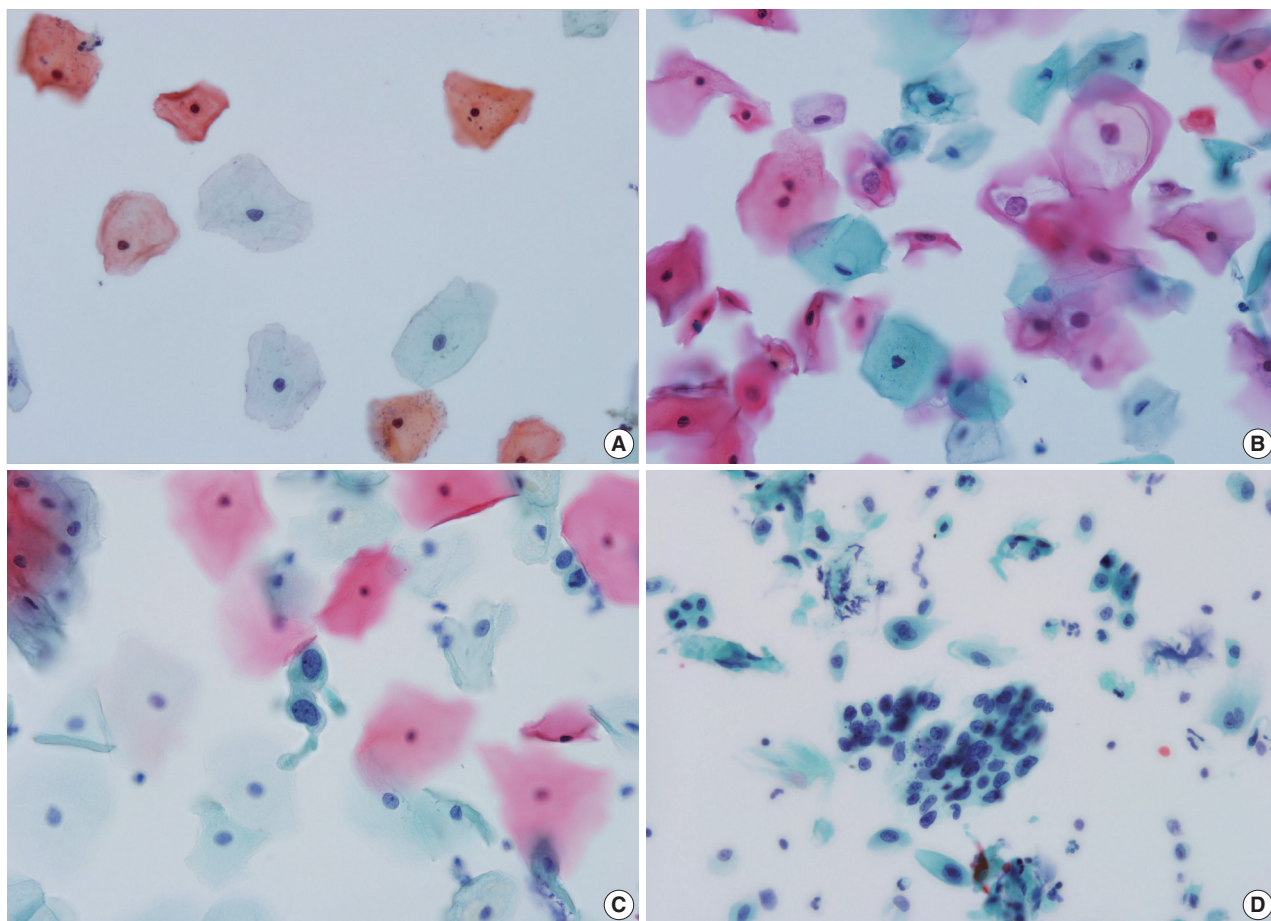


Fig. 1. Cytological appearance of EASYPREP. (A) Negative for intraepithelial lesion or malignancy. (B) Low grade squamous intraepithelial lesion. (C) High grade squamous intraepithelial lesion. (D) Squamous cell carcinoma.

Table 2. Diagnostic agreement between SurePath and EASYPREP

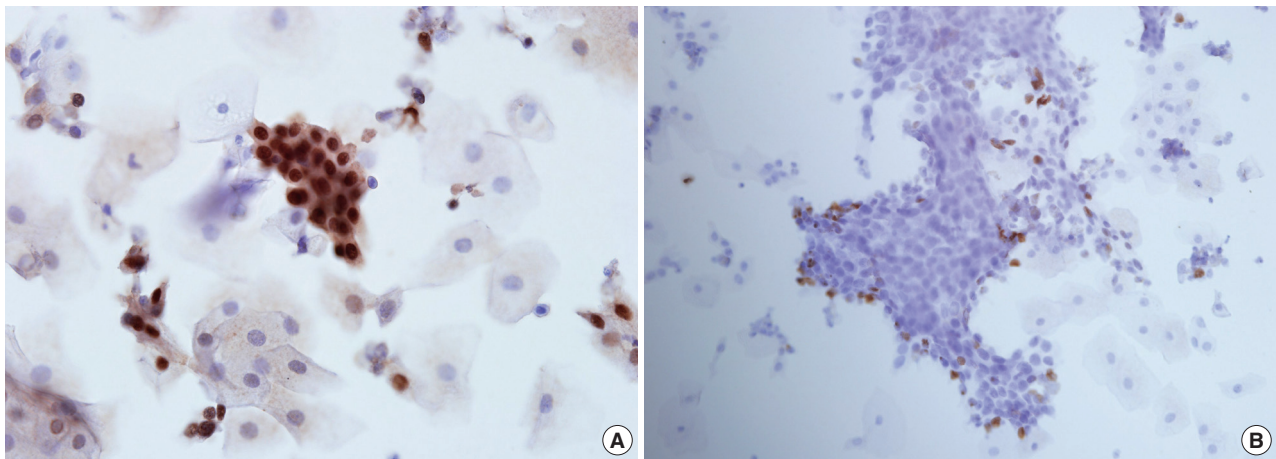
		EASYPREP								Total
		NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGUS	AC	
SurePath	NILM	853	5	0	1	1	0	0	0	860
	ASC-US	9	34	1	4	0	0	0	0	48
	ASC-H	2	0	4	0	2	0	0	0	8
	LSIL	1	4	0	53	1	0	0	0	59
	HSIL	0	0	1	1	15	0	0	0	17
	SCC	1	0	0	0	1	4	0	0	6
	AGUS	0	0	0	0	0	0	1	0	1
	AC	0	0	0	0	0	0	0	1	1
	Total	866	43	6	59	20	4	1	1	1,000

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma.

Table 3. Concordance of diagnosis with SurePath and EASYPREP

	NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGUS	AC	Total
SurePath	860	48	8	59	17	6	1	1	1,000
Concordant cases in EASYPREP	853	34	4	53	15	4	1	1	965
Concordance rate (%)	99.2	70.8	50.0	89.8	88.2	66.7	100	100	96.5

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma.

**Fig. 2.** Target cells demonstrate positive nuclear staining for immunocytochemistry of p63 (A) and Ki-67 (B) using EASYPREP.

as NILM, one as ACS-H and four as LSIL. Eight cases were interpreted as ASC-H when using SurePath, two of which were reported as NILM and two of which were reported as HSIL. A total of 59 cases were interpreted as LSIL when using SurePath, one of which was reported as NILM, four as ACS-US and one as HSIL. Among the 17 cases interpreted as HSIL when using SurePath, one was reported as ASC-H and one as LSIL. Six cases were interpreted as SCC when using SurePath, one of which was reported as NILM and one as HSIL. There was no inadequate specimen in either the EASYPREP or SurePath.

Immunohistochemistry

In all 5 different cases, dysplastic cells arranged in groups and reactive squamous cells demonstrated positive nuclear staining for p63 and Ki-67 (Fig. 2).

Reverse blot hybridization assay for HPV DNA genotyping using EASYPREP samples

HPV genotyping was performed on 249 specimens examined cytologically using the REBA HPV-ID. Tables 4 and 5 show the results of REBA-HPV DNA genotyping using EAS-

Table 4. The detection rate of HPV-positive cases by REBA HPV-ID for each cytological diagnosis

REBA HPV-ID	SurePath							Total	PPV (%)	NPV (%)
	NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGUS/AC			
HPV (+)	84	12	5	9	9	4	0	123	31.7 (39/123)	98.4 (124/126)
HPV (-)	124	0	1	0	1	0	0	126		
Total	208	12	6	9	10	4	0	249		
HPV (+) rate (%)	40.4	100.0	83.3	100.0	90.0	100.0	0.0	49.4		

HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma; PPV, positive predictive value; NPV, negative predictive value.

Table 5. Type-specific HPV prevalence of REBA HPV-ID

REBA HPV-ID	HPV genotype	SurePath							Total	Prevalence (%) (n = 249)	
		NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGUS/AC			
High-risk	16	6	1	4	1	6	4	0	22	8.8	
	18	13	3	1	0	0	0	0	17	6.8	
	26	0	0	0	0	0	0	0	0	0.0	
	31	1	0	0	0	0	0	0	1	0.4	
	33	0	0	0	1	3	0	0	4	1.6	
	35	1	0	0	1	0	0	0	2	0.8	
	39	4	0	0	3	0	0	0	7	2.8	
	45	9	0	0	0	0	0	0	9	3.6	
	51	0	0	0	0	0	0	0	0	0.0	
	52	6	4	0	1	0	0	0	11	4.4	
	53	6	1	0	3	0	0	0	10	4.0	
	56	4	0	0	1	0	0	0	5	2.0	
	58	2	1	0	1	1	0	0	5	2.0	
	66	11	2	0	1	0	0	0	14	5.6	
	59/68	4	3	0	1	0	0	0	8	3.2	
Probable high-risk	69	3	0	0	0	0	0	0	3	1.2	
	73	0	1	0	0	0	0	0	1	0.4	
	34	0	0	0	0	0	0	0	0	0.0	
	Low-risk	6	4	0	0	0	0	0	0	4	1.6
		11	1	0	0	0	0	0	0	1	0.4
		32	12	1	0	0	1	0	0	14	5.6
		40	3	0	0	0	0	0	0	3	1.2
42		3	0	0	1	0	0	0	4	1.6	
43		1	0	0	0	0	0	0	1	0.4	
44		3	0	0	0	0	0	0	3	1.2	
54		13	0	0	0	0	0	0	13	5.2	
70		2	0	0	0	0	0	0	2	0.8	
72		2	0	0	0	0	0	0	2	0.8	
81/87	1	1	0	1	0	0	0	3	1.2		
84	1	0	0	0	0	0	0	1	0.4		
Other		15	2	1	0	0	0	0	18	7.2	
Total		131	20	6	16	11	4	0	261		

HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma.

YPREP samples. Out of 249 cases, 123 (49.4%) cases were HPV positive and 126 (50.6%) cases were HPV negative. The positivity rate of the REBA HPV-ID for 41 abnormal cytological samples was 95.1%. The positivity rate of the REBA HPV-ID was 100.0% for SCC, 90.0% for HSIL, 100.0% for LSIL, 83.3% for ASC-H, and 100.0% for ASC-US. Based on the stan-

dard of REBA HPV-ID genotyping using SurePath samples, the sensitivity, specificity, PPV, and NPV of REBA HPV-ID genotyping using EASYPREP samples were 95.1%, 59.6%, 31.7%, and 98.4%, respectively. The 123 positive cases consisted of 84 cases of NILM, 12 of ASC-US, 5 of ASC-H, 9 of LSIL, 9 of HSIL, and 4 of SCC. The REBA HPV-ID positivity

Table 6. The detection rate of HPV-positive cases by sequencing analysis for each cytological diagnosis

Sequencing analysis	SurePath							Total	PPV (%)	NPV (%)
	NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGUS/AC			
HPV (+)	77	12	5	9	9	4	0	116	33.6 (39/116)	98.5 (131/133)
HPV (-)	131	0	1	0	1	0	0	133		
Total	208	12	6	9	10	4	0	249		
HPV (+) rate (%)	37.0	100.0	83.3	100.0	90.0	100.0	0.0	46.6		

HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cell of undetermined significance; ASC-H, atypical squamous cell cannot exclude high grade lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGUS, atypical glandular cells of undetermined significance; AC, adenocarcinoma; PPV, positive predictive value; NPV, negative predictive value.

Table 7. Comparison results of REBA HPV-ID and sequencing analysis (except for 22 mixed infection specimens in sequencing analysis)

	Reference standard (sequencing analysis)				
	HPV (+)	HPV (-)	Total	PPV	NPV
REBA HPV-ID HPV (+)	94	7	101	93.1%	-
HPV (-)	0	126	126	-	100.0%
Total	94	133	227	-	-
	Sensitivity	Specificity	-	-	-
	100.0%	94.7%			

HPV, human papillomavirus; PPV, positive predictive value; NPV, negative predictive value.

rate with normal cytological samples was 40.4% (84/208). Forty-nine (58.3%) cases had a previous clinical history including LSIL, HSIL and invasive cancer among the 84 normal cytology samples with HPV positivity. Among the patients who were HPV positive (123 cases), 44.7% (55/123) had single type infections and 55.3% had multiple HPV types (data not shown). HPV-16 was the most prevalent genotype observed in 22 cases (8.8%). The five most common HPV genotypes detected using REBA HPV-ID were HPV 16, 52, 18, 33, and 53 which were found in specimens that had an abnormal cytological diagnosis. The five most prevalent HPV genotypes detected using the REBA HPV-ID in the specimens with normal cytology were HPV 18, 54, 32, 66, and 45 (Table 5).

Sequencing analysis for HPV using EASYPREP samples

Sequencing analysis was performed to determine the accuracy and reliability of REBA HPV-ID with all specimens (249 cases) that were tested by REBA HPV-ID. The detection rate of HPV with sequencing analysis was 46.6% (116/249) (Table 6). The positivity rate of HPV with sequencing analysis for 41 abnormal cytological samples was 95.1%. The positivity rate of HPV with sequencing analysis was 100.0% for SCC, 90.0% for HSIL, 100.0% for LSIL, 83.3% for ASC-H, and 100.0% for ASC-US. HPV-16 was the most prevalent genotype observed in 18 (7.2%) cases. The HPV positivity rate with sequencing analysis with

normal cytological samples was 37.0% (77/208). Based on the standard of the SurePath method, the sensitivity, specificity, PPV, and NPV of sequencing analysis were 95.1%, 63.0%, 33.6%, and 98.5%, respectively. The 116 positive cases consisted of 77 cases of NILM, 12 of ASC-US, 5 of ASC-H, 9 of LSIL, 9 of HSIL, and 4 of SCC (Table 6). The REBA HPV-ID had high concordance with sequencing analysis as considered by the gold standard of molecular diagnostics. As shown in Table 7, the sensitivity, specificity, PPV, and NPV of REBA HPV-ID except for specimens of 22 mixed infections in the sequencing analysis were 100.0%, 94.7%, 93.1%, and 100.0%, respectively. Seven samples could not be interpreted by sequencing analysis because they were not amplified using HPV-specific primer pairs. The cytological diagnosis of these samples was NILM. Table 8 shows the type-specific HPV results of REBA HPV-ID and sequencing analysis.

DISCUSSION

In this study, we analyzed the cytological characteristics using the EASYPREP method, a newly developed LBC technique and performed HPV DNA genotyping using residual cell suspensions.

EASYPREP is the world's first fully automated thin layer cell preparation processor using centrifugation. The entire operation process of the system is controlled automatically and is displayed to the user by the monitor. The automated smearing processors generally use the filter-membrane method. However, the filtering method is problematic when the physical morphology of cells changes while adhering to the slide, and in the loss of cells not transferring to the slide. Instead of using a filter, EASYPREP uses the centrifugal sedimentation and specific gravity which partially remove non-diagnostic debris (including mucus and red blood cells) and excess inflammatory cells from the sample.

SurePath also utilizes centrifugation but is a semi-automated processor in which preparation process is performed manually

Table 8. The type-specific HPV result of REBA HPV-ID and sequencing analysis

MolecuTech REBA HPV-ID result	Sequencing analysis result																							Prevalence (n=227, %)										
	High-risk												Low-risk										Other HPV type (-)		To- tal									
16	18	26	31	33	35	39	45	51	52	53	56	58	66	59/ 68	69	73	34	6	11	32	40	42		43		44	54	70	72	84	81/ 87			
High-risk	16	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	18	7.2		
	18	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	6	2.4		
	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0		
	31	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.4		
	33	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1.6			
	35	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.4			
	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	0.8			
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	51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0			
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	56	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.8			
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	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	3	1.2			
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	2	0.8			
	42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0			
	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0			
	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0			
	54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	2	-	6	2.4			
	70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	0.4			
	72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	0.8			
	84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	2	0.8			
81/87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	0.8			
Other type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	3	18	7.2		
HPV(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	126	126	50.6			
Total	17	5	0	1	4	1	0	7	0	6	5	1	5	3	0	1	0	0	0	0	1	1	0	0	0	4	1	2	1	4	24	133	227	-

HPV, human papillomavirus.

and can cause differences in slide quality depending on the operators.¹⁰ Because EASYPREP is a fully automated walk-away processor, the operator does not have to be concerned about the slide quality after placing the consumables and specimens in the processor. Furthermore various types of processing software are installed in EASYPREP in accordance with the specimen characteristics allowing for objective cell preparation, which is an advantage for maintaining quality control. Moreover EASYPREP's sample loading capacity is 64 specimens. A characteristic of the EASYPREP method is the composition of the cytology-specific preservation vial, which can be directly used as a centrifuge tube. The surface of the glass slide is pretreated in order to have a positive charge for optimal cell adhesion.

In this study, the cells processed by the EASYPREP method

were evenly distributed in a monolayer without overlapping at the observation field. There were no significant differences in cellular distribution in the EASYPREP and SurePath methods. The gross areas of EASYPREP and SurePath were 213.7 mm² and 103.8 mm², respectively, EASYPREP was 2.1 times larger than SurePath. The total smeared cells of EASYPREP and SurePath were calculated as 129,585 and 78,406 on average, respectively. Because the total smeared cell count of EASYPREP was 1.6 times higher than SurePath, the possibility to detect abnormal cells could be high.¹¹ The cell density (643 cells) under the 200× magnification field of EASYPREP was 1.2 times lower than of SurePath (800 cells). Although there was no significant statistical difference, the cell density of the EASYPREP method was less than the SurePath method due to the larger circle of

smear area in the EASYPREP method.

The proportion of diagnostic results using the EASYPREP method did not differ significantly from the SurePath method. The negative rate (86.6%) was slightly higher in the EASYPREP method than in SurePath (86.0%). The detection rate of abnormal cells (14.0%) was slightly higher in the SurePath method than EASYPREP method (13.4%). However, there were five cases of ASC-US, one case of LSIL and one case of HSIL when using EASYPREP among the 860 cases of NILM in SurePath. The patient diagnosed as LSIL in EASYPREP and as NILM in SurePath did not perform a follow up cervicovaginal cytology examination and biopsy. One case diagnosed as HSIL in EASYPREP and as NILM in SurePath was diagnosed as cervical intraepithelial neoplasia 2 by punch biopsy.

Additionally, there were 11 cases diagnosed as ASC-US or ASC-H, one case as LSIL and one case as SCC in SurePath among the 866 cases of NILM in EASYPREP. One case diagnosed as LSIL in SurePath and as NILM in EASYPREP had positive HPV testing results and NILM on five follow-up cervicovaginal cytology examinations without biopsy. One case diagnosed as SCC in SurePath and as NILM in EASYPREP was diagnosed as SCC by punch biopsy. The authors detected necrotic materials only without atypical squamous cells on review of the EASYPREP slide.

Based on the standard of the SurePath method, the sensitivity, specificity, PPV, and NPV of the EASYPREP method were 90.7%, 99.2%, 94.8%, and 98.5%, respectively. The concordance rate of diagnosis was 96.5% in total and 99.2% in NILM, 80.0% in abnormal diagnosis cases more than ASC-US and 92.9% in abnormal diagnosis cases more than LSIL. The disagreement of diagnosis between the two methods and the slightly low sensitivity of the EASYPREP method was possibly due to bias in specimen collection process in this study.

Several reports regarding LBC showed that sensitivity in detecting abnormal squamous and glandular lesion was better than conventional cytology procedures due to even distribution and staining quality and short observation time on the restricted field.²⁻⁵ Additionally, the number of unsatisfactory specimens was decreased. However, several reports showed similar performance characteristics between conventional cytology and LBC.¹²⁻¹⁴ Nevertheless, LBC has advantages for cervical smear readers with an easy microscopic examination due to short observation time and even distribution.¹⁵

Moreover, the same EASYPREP specimen was used to assess additional immunocytochemistry. The positive nuclear staining for Ki-67 and p63 was successfully detected in this study, show-

ing the EASYPREP specimen can be used for immunocytochemistry.

The merit of LBC is that the samples used for LBC can be used in HPV testing.⁷ In this study, HPV genotyping using EASYPREP samples was performed by REBA HPV-ID. The positivity rate of the REBA HPV-ID was 49.4% in total and 95.1% in abnormal cytology samples. Using REBA HPV-ID in the current study, the prevalence of HPV was found in patients with ASC-US (100.0%), ASC-H (83.3%), LSIL (100.0%), HSIL (90.0%), and SCC (100.0%). The REBA HPV-ID positivity rate with normal cytology samples was 40.4%. Forty-nine (58.3%) cases among the normal cytology samples with HPV positivity had previous clinical history including LSIL, HSIL, and invasive cancer. In this study, the REBA HPV-ID positive rate was lower than previously reported (49.4% vs 72.8%), but higher in abnormal cytology samples (95.1% vs 80.9%).¹⁶ Subsequent sequencing analysis was performed and confirmed that the specimens which were positive by REBA HPV-ID did contain HPV sequences. The detection rate (46.6%) of HPV with sequencing analysis was lower than REBA HPV-ID (49.4%). The REBA HPV-ID had high concordance with sequencing analysis as considered by the gold standard of molecular diagnostics. Based on the standard of sequencing analysis, the sensitivity, specificity, PPV, and NPV of REBA HPV-ID (except for 22 mixed infection specimens) in sequencing analysis were 100.0%, 94.7%, 93.1%, and 100.0%, respectively. The performance results of the REBA HPV-ID were more sensitive and specific than other HPV genotype testing in terms of detection rates for samples with abnormal and normal cytology.¹⁶

In conclusion, the EASYPREP method provided comparable results to the SurePath method in diagnosis and staining quality and the samples could be used for immunocytochemistry and HPV testing. The EASYPREP method is a feasible cytological procedure and could be another choice of LBC method for the cervicovaginal specimens. The REBA HPV-ID might be a very useful diagnostic method for HPV genotyping using EASYPREP residual samples.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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