Comparative study of squamous intraepithelial lesion detection and unsatisfactory rates between liquid-based cytology and conventional smears from a split sample in cervical cancer screening: A Japanese experience

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Summary

In this study, we analyzed the detection rates of squamous intraepithelial lesions (SILs) using BD SurePath™ liquid-based cytology (LBC) and conventional cytology with Cervex-Brush® performed for cervical cancer screening. The split-sample procedure involved direct sampling and spreading with Cervex-Brush®, followed by the collection of the brush tip in a BD SurePath™ vial and BD SurePath™ specimen preparation. SIL detection rates were investigated in two groups: conventional cytology and LBC performed using the split-sample procedure. Split samples were collected from 2,025 women. A SIL was detected in 63 women (3.1%) by conventional cytology [33 cases, low-grade (LSIL); 30 cases, high-grade (HSIL)] and 69 women (3.4%) by BD SurePath™ LBC (37, LSIL; 32, HSIL). The unsatisfactory rate was significantly higher in the conventional cytology than in the BD SurePath™ LBC (p < 0.001). The unsatisfactory rate for BD SurePath™ LBC was 0%. The LBC platform is a standardized LBC system with improved HSIL detection rates and a lower unsatisfactory rate, and is very useful in cervical cancer screening conducted during health check-ups.

Key words
cervical cancer screening, Cervex-Brush®, BD SurePath™, liquid-based cytology, squamous intraepithelial lesion

I Introduction

The Japanese health check-up system refers to a voluntary health examination1) designed to confirm patient well-being and identify risk factors for diseases such as uterine cervical cancer. The detection, early treatment, and follow-up of precancerous lesions lowers cancer morbidity and mortality rates,2) and recipients with no abnormal test findings feel more secure. High-precision screening procedures are therefore needed.

The Bethesda System 2001 (TBS)3) is the gold standard for cervical cytology in the United States and many other countries. European nations, Australia, and New Zealand use a modified version of TBS.4) The most desirable feature of TBS is that specimens with insufficient squamous cellularity or obscured by inflammation or blood are classified as unsatisfactory.3)-4) The Japan Association of Obstetricians and Gynecologists endorsed TBS in May 2009,5) and the Japan Society of Ningen Dock followed suit in April 2012.5) Under TBS, unsatisfactory specimens should be avoided. Liquid-based cytology (LBC) platforms are necessary for this purpose.

The greatest advantage of LBC is that excessive smearing, drying, and improper fixation are minimal from sampling to smearing, because the sample cells are collected directly into the collection fluid.6)-17) BD SurePath™ (Nippon Becton Dickinson Company, Ltd.) is an
LBC system that minimizes unsatisfactory specimens through a pretreatment process for the elimination of artifacts unnecessary for diagnostic examinations. AlSharif and colleagues reported a 0.1% (360 in 232,022) incidence of unsatisfactory results for the BD SurePath™ LBC platform. Unsatisfactory test results were attributable to scant cellularity in 95.68% of the patients tested, who tended to be postmenopausal or had hysterectomy. The College of American Pathologists reported unsatisfactory rates of 0.3% for the BD SurePath™ LBC and 1.1% for the ThinPrep® LBC (Hologic, Inc.).

The detection of highly clinically significant high-grade lesions is a suitable benchmark for evaluating LBC. In an analysis of 3,036 patients with a high-grade squamous intraepithelial lesion (HSIL), the BD SurePath™ LBC outperformed conventional cytology by 38.2%. To provide data for improving the precision of cervical cytology, HSIL detection rate was investigated by conventional cytology and BD SurePath™ LBC performed using split-sample procedures. Unsatisfactory rates in the BD SurePath™ were also determined.

II Materials and Methods

1. Subjects
   The results of cervical cytology performed at a health check-up office were analyzed. One gynecologist used colposcopy to collect all samples. Split sampling was performed from September 2011 to December 2012 (16 months). Cells were collected only from test recipients who gave their informed consent.

2. Methods
   The split-sample procedure involved direct spreading with the Cervex-Brush® followed by collection of the brush tip in a BD SurePath™ vial and specimen preparation by the BD SurePath™ LBC. LBC with the BD SurePath™ was performed by (1) removing the sample adhering to the brush with a vortex mixer, (2) uniformly mixing the sample and collecting 8.0 mL of the 10.0 mL suspension using the BD PrepMate™ automated mixer and dispenser (Nippon Becton Dickinson Company, Ltd.), and (3) removing blood, mucous, and other artifacts with a density reagent and centrifugation. Cell smears were prepared using the BD PrepStain™ system (Nippon Becton Dickinson Company, Ltd.), an automated slide processor. The specimens were fixed in 95% ethanol and Papanicolaou stain. All BD SurePath™ LBC specimens underwent a primary screening by a cytotechnologist and were then double-checked at 20x magnification by a senior cytotechnologist. The specimens were evaluated according to TBS.

The 2,025 women who underwent split-sample collection were classified into a newly tested group and a previously tested group. SIL detection rate was analyzed in each group. Specimen quality was reviewed for women with a BD SurePath™ LBC result of LSIL or HSIL and who were NILM at the previous comprehensive health examination. The results were used to demonstrate the efficiency of the BD SurePath™ LBC in these recipients of multiple tests. When women in the previously tested group had a BD SurePath™ result of SIL, the medical records over the past 5 years were reviewed. The sampling devices used at the most recent cervical cancer screening were classified as cotton swab, Cytobrush, Cytopick, or Cervex-Brush®, and re-evaluable specimens were reviewed. Biopsy was regarded as the gold standard. The results were cross-tabulated and analyzed. The chi-squared test with a significance level of 5% was used. Statistical analysis was performed with HALBAU7 (CMIC Co., Ltd.).

III Results

The split-sample group included 2,025 women with a mean age of 48.1 years (range, 21.0 to 89.0 years).

The low-grade SIL (LSIL) and HSIL detection rates in each group are shown in Table 1. A SIL was detected in 63 women (3.1%) by conventional cytology and 69 women (3.4%) by BD SurePath™ LBC. An HSIL was detected in 30 women (1.5%) by conventional cytology and 32 women (1.6%) by BD SurePath™ LBC. The detection rates of SIL by BD SurePath™ LBC and the conventional cytology were almost comparable, but that the rates of unsatisfactory results was significantly greater in the conventional cytology (5.2%) than in the BD SurePath™ LBC (0%) ($p < 0.001$). The unsatisfactory specimens showed excess overload of pus and blood, making more than 75% of the smeared area unobservable (Figure 1a, c). The BD SurePath™ could remove the blood cells by hemolysis and collect large amounts of squamous cells contained in a vial (Figure 1b, d). Of the 2,025 women in the BD SurePath™, 693 were newly tested and 1,332 were previously tested (Table 2). A SIL was detected by BD SurePath™ LBC in 30 (4.3%) of the newly tested cases and 39 (2.9%) of the previously tested
Table 1  Comparison of BD SurePath™ liquid-based cytology with split-sample procedures and conventional cytology with Cervex-Brush™

<table>
<thead>
<tr>
<th>Methods</th>
<th>Unsat (%)</th>
<th>NILM (%)</th>
<th>ASC-US (%)</th>
<th>ASC-H (%)</th>
<th>LSIL (%)</th>
<th>HSIL (%)</th>
<th>SCC (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional smear</td>
<td>105 (5.2)</td>
<td>1,830 (90.4)</td>
<td>23 (1.1)</td>
<td>3 (0.1)</td>
<td>33 (1.6)</td>
<td>30 (1.5)</td>
<td>1 (0)</td>
<td>2,025 (100)</td>
</tr>
<tr>
<td>BD SurePath™</td>
<td>0 (0)</td>
<td>1,926 (95.1)</td>
<td>25 (1.2)</td>
<td>4 (0.2)</td>
<td>37 (1.8)</td>
<td>32 (1.6)</td>
<td>1 (0)</td>
<td>2,025 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>105 (2.6)</td>
<td>3,756 (92.7)</td>
<td>48 (1.2)</td>
<td>7 (0.2)</td>
<td>70 (1.7)</td>
<td>62 (1.5)</td>
<td>2 (0.05)</td>
<td>4,050 (100)</td>
</tr>
</tbody>
</table>


Figure 1  Conventional preparation versus BD SurePath™ preparation (split sample)
An unsatisfactory sample directly prepared using Cervix-Brush™ due to excess blood (a: Papanicolaou stain, Loupe image; b: Papanicolaou stain, ×100).
A satisfactory sample prepared using BD SurePath™. Red blood cells are removed by hemolysis and the collected cells are smeared within the 13-mm-diameter circle. (c: Papanicolaou stain, Loupe image; d: Papanicolaou stain, ×100)

Table 2  2,025 women undergoing BD SurePath™ liquid-based cytology according to history of cervical cancer screening at health check-ups

<table>
<thead>
<tr>
<th></th>
<th>NILM (%)</th>
<th>ASC-US (%)</th>
<th>ASC-H (%)</th>
<th>LSIL (%)</th>
<th>HSIL (%)</th>
<th>SCC (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly</td>
<td>658 (94.9)</td>
<td>4 (0.6)</td>
<td>0 (0)</td>
<td>14 (2.0)</td>
<td>16 (2.3)</td>
<td>1 (0.1)</td>
<td>693 (100)</td>
</tr>
<tr>
<td>Re-examination</td>
<td>1,268 (95.2)</td>
<td>21 (1.6)</td>
<td>4 (0.3)</td>
<td>23 (1.7)</td>
<td>16 (1.2)</td>
<td>0 (0)</td>
<td>1,332 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>1,926 (95.1)</td>
<td>25 (1.2)</td>
<td>4 (0.2)</td>
<td>37 (1.8)</td>
<td>32 (1.6)</td>
<td>0 (0)</td>
<td>2,025 (100)</td>
</tr>
</tbody>
</table>

An HSIL was detected in 16 of the 693 (2.3%) newly tested cases and in 16 of the 1,332 (1.2%) previously tested cases by BD SurePath™. Of the 39 previously tested cases with a SIL detected, 21 of the 23 LSIL cases were NILM (Table 3), and 10 of the 16 HSIL cases were NILM in the previous test (Table 4). Thirty of these 39 cases underwent a test two or one year previously.

The cytology specimens from 31 of the 39 SIL cases with BD SurePath™ re-examination and who were NILM in the previous test (21 LSIL cases and 10 HSIL cases) were re-evaluated (Tables 3, 4). The results of the review of the previous cytology specimens of the 21 LSIL cases using BD SurePath™ were unsatisfactory (11 cases), NILM (7 cases), and atypical squamous cells of undetermined significance (ASC-US) (3 cases) (Table 4). Results of the review of the 10 HSIL cases using BD SurePath™ were unsatisfactory (5 cases), NILM (1 case), ASC-US (2 cases), and ASC cannot exclude HSIL (ASC-H) (2 cases) (Table 5). Retrospectively, 16 of the unsatisfactory results (51.6%) were due to very scant squamous epithelial cellularity. The rates of unsatisfactory test samples and sample repreparation using BD SurePath™ were both 0%.

Biopsy results for the 36 women examined in our hospital are shown in Table 5. Biopsy results for HSIL were cervical intraepithelial neoplasia (CIN) 1 (19 cases), CIN2 (6 cases), CIN3 (1 case), and microinvasive squamous cell carcinoma (1 case).

### IV Discussion

The rates of unsatisfactory test samples and sample repreparation in the BD SurePath™ were both 0%. Sixteen of the 32 cases with HSIL identified using BD SurePath™ were previously tested. None of the 10 women who were previously diagnosed to be NILM had undergone conventional cytology with a cotton swab. In the review, 5 showed unsatisfactory results, 1 was NILM, and 3 showed ASC-US/ASC-H.

A meta-analysis of unsatisfactory samples associated with LBC platforms found an unsatisfactory rate of 0.3% for BD SurePath™, which was significantly lower than the 1.3% rate for ThinPrep®.15 A follow-up histological examination of women who were NILM and showed unsatisfactory results indicated a significantly higher detection rate of high-grade lesions in the unsatisfactory samples.7) Avoiding unsatisfactory specimens is therefore paramount. A recent large-scale study showed that

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Table 3 Re-evaluation of cervical cytology specimens and sampling devices used in 21 cases with BD SurePath™ evaluation of LSIL found to have NILM at the last previous cytology

<table>
<thead>
<tr>
<th>Sampling devices (Conventional smears)</th>
<th>Unsatisfactory (Unsat)</th>
<th>NILM</th>
<th>ASC-US</th>
<th>ASC-H</th>
<th>ASC-H</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton swab</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Cytobrush</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cytobrush</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cervex-Brush®</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: ASC-US: Atypical squamous cells of undetermined significance, ASC-H: Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions, NILM: Negative for intraepithelial lesion or malignancy, SIL: squamous intraepithelial lesion, Unsat: Unsatisfactory.

Table 4 Re-evaluation of cervical cytology specimens and sampling devices used in 10 cases with BD SurePath™ evaluation of HSIL found to have NILM at the last previous cytology

<table>
<thead>
<tr>
<th>Sampling devices (Conventional smears)</th>
<th>Unsatisfactory (Unsat)</th>
<th>NILM</th>
<th>ASC-US</th>
<th>ASC-H</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton swab</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Cytobrush</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: ASC-US: Atypical squamous cells of undetermined significance, ASC-H: Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions, NILM: Negative for intraepithelial lesion or malignancy, SIL: squamous intraepithelial lesion, Unsat: Unsatisfactory.

### Table 5 Biopsy findings for BD SurePath™ evaluation of cervical intraepithelial neoplasm (%)

<table>
<thead>
<tr>
<th>BD SurePath™</th>
<th>Histologic diagnosis</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign (%)</td>
<td>CIN1 (%)</td>
</tr>
<tr>
<td>HSIL</td>
<td>2 (12.5)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>LSIL</td>
<td>7 (36.8)</td>
<td>16 (63.2)</td>
</tr>
<tr>
<td>SCC</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (25.0)</td>
<td>19 (52.8)</td>
</tr>
</tbody>
</table>

Abbreviations: CIN: Cervical intraepithelial neoplasm, HSIL: High-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, SCC: squamous cell carcinoma.
BD SurePath™ detected all grades of dysplasia significantly better in appropriate specimens with transformation zone components and that the HSIL detection rate was significantly lower when unsatisfactory specimens were used. Although TBS does not require transformation zone cells for adequate specimen evaluation, the collection of cells over a wide region from the endocervical gland to the portio vaginalis uteri facilitates HSIL detection.

Fremont-Smith et al. found that BD SurePath™ LBC provided an HSIL+ detection rate of 64.1% ($p < 0.00001$), which is higher than that of conventional cytology. Positivity rates for conventional cytology with the Cervex-Brush® and BD SurePath™ LBC did not differ significantly because split sampling was used. The significantly higher rate of HSIL detection by BD SurePath™ LBC than by cotton swabbing in this study may therefore suggest that the sampling device should be changed to the Cervex-Brush®. This issue caused by split sampling in this study suggests the need for a large-scale comparative study. Nevertheless, 100% of the BD SurePath™ specimens were properly prepared despite split sampling, which indicates that its performances are at least comparable to those of conventional cytology and that the BD SurePath™ is a precise detection system.

Thirty-nine (LSIL: 23 cases, 1.7%; HSIL: 16 cases, 1.2%) of the 69 cases with a SIL detected with BD SurePath™ were previously tested. Thirty-one of these were previously NILM. A review of cytology specimens showed that cotton swab sampling was used for 24 women (61.5%). Of these, 16 (51.6%) showed unsatisfactory results owing to insufficient squamous epithelial cellularity. This BD SurePath™ performance shows that adopting an LBC platform for women previously tested by a cotton swab technique will improve LSIL and HSIL detection and increase test sensitivity and specificity. Cotton swabbing is unsuitable for collecting cells because it collects fewer cells. On the basis of the findings of the present study, our center switched to the Cervex-Brush®. A randomized, controlled trial comparing Thin-Prep®, another LBC platform with a different sample preparation principle, with conventional cytology with the Cervex-Brush® found no significant difference in positivity rates but showed comparable SIL detection rates for the two methodologies. The repreparation rates and specific reasons for repreparation, however, are not stated.

A previously reported disadvantage is that the larger number of collected cells more commonly requires re-mounting owing to excessive smearing in conventional cytology. Conventional cytology with Cervex-Brush® showed higher SIL detection rates than cotton swab sampling, but a higher risk of screening errors owing to excessive smearing and cell detachment. The greatest disadvantage, however, was a longer observation time. BD SurePath™ uniformly deposited cells in a 13 mm circle, which facilitated SIL detection in this study.

In conventional cytology, only 10% of the cells sampled are available for screening. The BD SurePath™ platform, in contrast, features a cell collection rate of 100%, because the tip of Cervex-Brush® is directly collected in a dedicated vial. An analysis of sampling devices indicated that spatulas collect squamous epithelial cells, the cytobrush collects endocervical cells, and the Cervex-Brush® collects components of both. Thus, the Cervex-Brush® is the most efficient device for cervical cancer screening. Thirty of the 39 cases with a SIL detected using BD SurePath™ were tested within the past 2 years. Detecting SILs in the initial tests of such women who underwent ongoing testing will require a review of the sampling devices and a change to an LBC system. Conventional cytology smears are highly dependent on the sampling device used and the technique of the sampler. Thus, the reported unsatisfactory rates vary widely. The unsatisfactory rate for conventional smear by only cytobrush sampling is 10.5%.

Cytological specimens of 31 of the women who were NILM in a previous test but had a SIL detected using BD SurePath™ were reviewed. Sixteen were unsatisfactory owing to scant epithelial cellularity. Since definite SIL was not retrospectively detected, these unsatisfactory test samples are likely attributable to cell detachment or other errors in cell sampling, alcohol fixation, or Papanicolaou staining during slide smearing. Smear cells in BD SurePath™ specimens are firmly held by special chemical bonds that make cell detachment less likely. BD SurePath™ is a sample preparation system with no cell detachment. We learned that cotton swabbing is associated with a higher risk of unsatisfactory tests owing to errors during sampling or transfer errors during smearing. Collecting cells with the Cervex-Brush® and preparing appropriate specimens in BD SurePath™ LBC eliminated many unsatisfactory tests and significantly increased the HSIL detection rate in the present study.

Screening with LBC specimens is optimal for improving screening precision and detecting high-grade lesions.
in cervical cancer screening. Many factors, such as vaginal bleeding, endocervical polyps, and ≤ 3 months postpartum, significantly increase unsatisfactory rates. The standardized pretreatment steps of the BD SurePath™ platform eliminate these factors.

From this perspective, the BD SurePath™ platform would be a valuable addition. In combination, the Cervex-Brush® and BD SurePath™ LBC eliminate unsatisfactory tests and significantly increase the HSIL detection rate. Residual samples from LBC platforms can be used for high-risk HPV-DNA testing and other tests, generating additional data useful for preventing cancer and analysis at the molecular level.

V Conclusions

BD SurePath™ LBC is a standardized cervical cancer screening system. The performance of SurePath™ LBC was superior to that of conventional cytology.

A summary of this paper was presented at the 53rd Conference of the Japan Society of Ningen Dock (September 2012, Tokyo).

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References


There is no potential conflict of interest to disclose.
子宮頸がん健診における split-sample による液状化細胞診法と従来法の子宮頸部扁平上皮内病変検出率と検体不適正率に関する研究—日本での経験—

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要 旨

人間ドックにおける BD SurePath™ 法による液状化細胞診法と従来法の SIL 検出率と検体不適正率の分析を目的とした。Cervex-Brush®で採取し直接塗抹後、ブラシ先端を専用のバイアルに回収し Split-sample による従来法と BD SurePath™ 法での SIL 検出率、及び検体不適正率を比較した（χ² 検定、p < 0.05）。対象は Split-sample 2,025 例であった。SIL 検出数および検出率は、Split-sample による従来法 63 例（3.1%）のうち LSIL 33 例（1.6%），HSIL 30 例（1.5%），BD SurePath™ 法 SIL 69 例（3.4%）のうち LSIL 37 例（1.8%），HSIL 32 例（1.6%）であった。BD SurePath™ 法で SIL であった 69 例中 39 例（HSIL：16 例，LSIL：23 例）は受診歴があり、そのうち NILM であった 31 例中 24 例（61.5%）で前回細胞診では綿棒採取による従来法であった。31 例の標本の再評価では検体不適正（16 例），NILM（8 例），ASC-US（5 例），ASC-H（2 例）であった。BD SurePath™ 法では検体不適正が 0%であった。BD SurePath™ 法は、HSIL 検出率が向上し、検体不適正が改善された液状化細胞診法であり、子宮頸がん健診での有用性が高い。

キーワード：子宮頸がん健診、サーベックスブラシ®、BD シュアパス™ 法、液状化検体細胞診、子宮頸部扁平上皮内病変

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