Comparison of ThinPrep and SurePath Liquid-Based Cytology and Subsequent Human Papillomavirus DNA Testing in China

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BACKGROUND: Liquid-based cytology (LBC) has been compared with conventional cytology in numerous studies. In the current study of 2 LBC systems, the accuracy, rates of unsatisfactory cytology, and sufficiency of residual LBC specimens for Hybrid Capture 2 (HC2) HPV DNA testing were compared.

METHODS: Eligible women ages 30 to 49 years were recruited for this cross-sectional population-based study in rural China. Women were assessed by visual inspection with acetic acid (VIA), LBC, and high-risk HPV HC2 DNA assay. Cervical specimens were preserved according to SurePath or ThinPrep protocols. LBC results were manually read. HC2 testing was performed on specimens with sufficient residual volume. Colposcopies and biopsies were performed on women who were VIA positive at the time of initial screening. Women with abnormal LBC or HC2 test results were called back for colposcopies and 4-quadrant cervical biopsies.

RESULTS: Of 2005 eligible women, 972 were tested by SurePath and 1033 by ThinPrep. Compared with SurePath samples, ThinPrep samples had higher rates of unsatisfactory cytology (0.2% for SurePath and 1.5% for ThinPrep) and insufficient residual volume for HC2 (0.0% for SurePath and 18.2% for ThinPrep). SurePath samples yielded higher sensitivities and similar specificities for LBC and HC2 testing of residual specimens, but these differences were not determined to be significant by area-under-the-curve analysis (LBC performance: 0.89 for SurePath and 0.85 for ThinPrep; HC2 performance: 0.91 for SurePath and 0.89 for ThinPrep). CONCLUSIONS: Both methods yielded similar validity in detecting significant cervical lesions. However, SurePath samples yielded higher rates of satisfactory LBC slides and sufficient residual volume for HC2. Cancer (Cancer Cytopathol) 2011;000:000–000. © 2011 American Cancer Society.

KEY WORDS: cytology, human papillomavirus (HPV), DNA testing, screening, cervical cancer.
Cervical cancer rates have fallen in most of the developed world as a result of screening and treatment programs since the introduction of the Papanicolaou (Pap) test in the 1950s. Considerable attention has been focused on specific shortcomings of the Pap test despite its population-based successes. Errors in sampling, preparation, and screening account for the majority of false-negative Pap results. The development of new sampling devices, slide preparation technologies, automated screening devices, and molecular methods such as high-risk human papillomavirus (HPV) detection are a response to some of these limitations.

One of these new methods is liquid-based cervical cytology (LBC), which produces thin-layer smears. Numerous studies have compared LBC with conventional cytology. Some have shown the relative benefits of LBC, including increased sensitivity and the ability to perform HPV DNA testing on the same specimen; others have reported decreased specificity and lower cost-effectiveness. ThinPrep and SurePath (formerly AutoCyte Prep) are 2 commonly used LBC systems. Each method follows a detailed manufacturer’s protocol for sample preservation and slide preparation. To our knowledge, only a few studies and a questionnaire survey have compared the accuracy and rates of unsatisfactory cytology of ThinPrep and SurePath.

Our project was embedded in the START (Screening Technologies to Advance Rapid Testing) project for cervical cancer prevention, a 5-year project (2003-2007) to develop simple, accurate, and inexpensive biochemical tests for use in low-resource settings. In our first year of screening women for the START project, we compared ThinPrep and SurePath LBC methods by rates of unsatisfactory cytology, insufficient residual specimen for HPV DNA testing, and accuracy of LBC and HPV DNA testing compared with cervical histology.

MATERIALS AND METHODS

Subject Enrollment and Study Design
In November 2003, women from Xiangyuan County, Shanxi Province, a poor rural area of China, were enrolled and screened in a cross-sectional study. Four communes were selected by simple random cluster sampling. Eligible subjects were nonpregnant women between the ages of 30 and 49 years who had never been screened for cervical cancer, exhibited no signs of debilitating disease, and had no past medical history of cervical intraepithelial neoplasia, cervical cancer, or hysterectomy. Eligible women were invited to participate in our study and screening appointments were scheduled for those interested. Women who were menstruating on the day of their appointment were asked to return in 7 to 14 days. Trained local health care providers explained specimen collection and testing procedures to patients at their screening session. Providers then obtained informed consent after answering any questions the patients may have had. The Human Subjects Review Board of the Cancer Institute/Hospital of the Chinese Academy of Medical Sciences (CICAMS) in Beijing and PATH, Seattle, Washington, approved this study.

Screening Procedure and Specimen Collection
At the screening appointment, patients were greeted and interviewed as previously described. Each patient self-collected 3 nylon swab specimens. Patients were instructed to grasp a swab at midshaft, insert it to a depth of approximately 6 cm or until resistance was met, and rotate it 2 times before withdrawal. One nylon swab and 2 cervical brush (Cervical Sampler; formerly Digene now Qiagen, Gaithersburg, MD) specimens were then collected by physicians. Finally, 1 cervical specimen for cytology was collected by a physician using a broom-type sampling device (Cervex-Brush; Rovers Medical Devices B.V., Oss, The Netherlands). Physician-obtained cervical specimens from the first half of screening participants were placed in SurePath Preservative Fluid (TriPath Imaging, Burlington, NC); specimens from the second half of screening participants were placed in PreservCyt solution (ThinPrep; Hologic-Cytyc Company, Marlborough, Mass.). Patients were blinded to their LBC assignments. Visual inspection with 5% acetic acid (VIA) was performed on all patients, and VIA-positive patients underwent immediate digital colposcopy (Goldway, Shenzhen, China) with directed biopsy.

Cervical specimens in PreservCyt or SurePath Preservative Fluid were stored at room temperature and sent weekly to CICAMS (Beijing, China) to be processed in a centralized laboratory using slide systems for ThinPrep.
and SurePath, respectively. Residual samples from LBC specimen preparation were tested for high-risk oncogenic HPV subtypes using the Hybrid Capture 2 (HC2) test (formerly Digene now Qiagen) with mixed probes to detect 13 carcinogenic HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).\(^{18,19}\) LBC diagnosis of atypical squamous cells of uncertain significance (ASC-US) or higher was considered a positive test result. The cutpoint for HC2 results expressed by a ratio of relative light units to standard positive control (RLU/PC) was 1.0, with a RLU/PC ratio >1.0 considered positive and a ratio <1.0 considered negative. Women who were VIA negative but positive by LBC or HC2 were called back within 2 weeks for colposcopy with directed biopsy, 4-quadrant biopsies, and endocervical curettage (ECC).

**Histology and Cytology Classification**
Chinese and French pathologists were blinded to LBC preparation methods and clinical findings. Pathologists in China used the World Health Organization classification whereas a French pathologist used the Bethesda System to classify slides for histology and cytology.\(^ {20,21}\) Two gynecologic pathologists at CICAMS read slides independently and a third gynecologic pathologist served as a tie-breaker. A French pathologist then read all the histology and cytology slides deemed positive by majority consensus in China plus a 10% random sample of negative slides. There was ample opportunity for the discussion of diagnostic discrepancies among all pathologists. The final histological diagnosis for each slide was determined by the French pathologist. The final clinical diagnosis for each subject was based on the highest reading across all histology results including directed biopsy, 4-quadrant biopsies, and ECC. A subject was considered negative for cervical neoplasia when a biopsy was not indicated (and both cytology and HPV test results were negative), or when a biopsy was indicated but the histological diagnosis was negative.

**Statistical Analysis**
The sensitivity, specificity, positive predictive value, negative predictive value, unsatisfactory rate of LBC testing of SurePath and ThinPrep specimens, and HC2 test results of residual LBC samples were evaluated. A final histologic diagnosis of high-grade disease (CIN2+) served as the positive cutpoint for sensitivity and specificity calculations. Differences in accuracy between ThinPrep and SurePath preparations were calculated and evaluated by the area under the receiver operating characteristic (ROC) curves.\(^ {22}\) Area under ROC curves between the 2 LBC systems and preservative solutions were compared by the \(U\) value, in which \(U\) is defined as:

\[
U = \frac{|A_1 - A_2|}{\sqrt{SE_1^2 + SE_2^2}}
\]

in which \(A_1\) refers to the observed area and \(SE_1\) refers to the estimated standard error of the ROC area associated with diagnostic method 1; \(A_2\) and \(SE_2\) refer to the corresponding quantities for diagnostic method 2.\(^ {23}\) ThinPrep and SurePath rates of unsatisfactory LBC and rates of insufficient residual volume for HC2 were compared by the chi-square test. All \(P\) values were 2 tailed; \(P\) values <.05 were considered statistically significant. Analyses were conducted in SPSS for Windows (version 13.0; SPSS Inc, Chicago, Ill.).

**RESULTS**
From November 1 to November 29, 2003, a total of 2594 women were invited for screening. Of the 2033 women (78.4%) who agreed to enroll, 28 were excluded for the following reasons: 10 were aged younger than the target age range, 7 were unable to attend callback colposcopy, 6 were menstruating on the day of screening and were subsequently lost to follow-up, 2 were found to be pregnant at the time of clinical examination, 2 refused to be called back after initial screening, and 1 had been screened for cervical cancer previously (Fig. 1). Of the 2005 eligible women who completed our screening process, the first 972 were allocated to SurePath and the remaining 1033 to ThinPrep (Fig. 1). A total of 94.8% of SurePath specimens were collected from women in 2 communes, and 94.1% of ThinPrep specimens were collected from another 2 communes. Sociodemographic data and reproductive histories between patients who provided SurePath specimens and those who provided ThinPrep specimens were similar (Table 1). The prevalence of CIN2+, ASC-US, and atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion or severe
lesions (ASC-H+) in both groups was not significantly different (CIN2+: 3.7% of SurePath specimens and 4.0% of ThinPrep specimens [chi-square = 0.10; \( P = .757 \)]; ASC-US: 10.5% of SurePath specimens and 9.1% of ThinPrep specimens [chi-square = 1.10; \( P = .295 \)]; and ASC-H+: 5.7% of SurePath specimens and 6.9% of ThinPrep specimens [chi-square = 1.26; \( P = .264 \)]. No statistically significant differences were observed in the prevalence of carcinogenic types of HPV DNA detected by HC2 (HC2: 17.5% of SurePath specimens and 19.2% of ThinPrep specimens [chi-square = 0.86; \( P = .355 \)]) (Table 1).

There was a statistically significant difference between the 0.2% (2/972) of SurePath specimens and the 1.5% (15/1033) of ThinPrep specimens determined to be unsatisfactory for cytologic evaluation due to scant cellularity (chi-square = 9.25; \( P = .002 \)) (Table 2). A significant difference was also noted between the rates of insufficient volume for HC2 testing of residual specimens, which were 0.0% (0/972) for SurePath specimens and 18.2% (188/1033) for ThinPrep specimens (chi-square = 195.20; \( P < .0001 \)) (Table 2).

DISCUSSION
The SurePath system resulted in a significantly lower rate of unsatisfactory slides for cytology and a lower rate of inadequate residual volume for HC2 when compared with the ThinPrep system as part of a screening study in Shanxi Province, China, an area with high rates of cervical cancer mortality. However, no differences were observed with regard to the sensitivity and specificity of LBC or HC2 HPV testing on residual specimens of SurePath or ThinPrep samples.

Several possible reasons may account for differences in SurePath and ThinPrep performance. First, their slide processing mechanisms are different. The ThinPrep fixative liquid, PreservCyt, is methanol based. The ThinPrep process involves dispersion and cell collection by vacuum filtration followed by slide transfer using air pressure for adherence. SurePath preservative fluid is ethanol based; cells are centrifuged and resuspended in a sucrose density gradient followed by slide transfer using gravity for adherence (a manual imprint system is used to prepare slides in advance for SurePath). Blood, mucus, and vaginal discharge can adhere to the filter during ThinPrep processing, which could mimic an increase in epithelial cell density. This may increase the occurrence of unsatisfactory LBC by inducing a mechanical sensor in the ThinPrep automated slide processing system to prematurely stop cell collection, which would ultimately reduce the number of cells transferred to a slide. Two other comparison studies between both LBC systems have shown similar results. By simulating sample preparation conditions of excessive blood or mucus, these studies showed that SurePath’s cell enrichment process was able to handle...
significantly greater amounts of blood or mucus than ThinPrep’s membrane filtration process. This applies directly to women with a high incidence of cervicitis and fragile cervixes, which result in bloody specimens. This issue has been reported in a previous study conducted in the same county as the current study. Second, distinctive sampling devices could also factor into rate differences. ThinPrep specimens were collected with Cervex-Brushes. Each brush was rinsed in a vial of PreservCyt solution by pressing it into the bottom of the vial 10 times, forcing the bristles apart, and vigorously swirling the brush in the solution to release any remaining material prior to disposal. A fraction of each ThinPrep specimen may be lost depending on how thoroughly a brush is rinsed. The quality of cytology results may have also been affected by variations in the technical skills of practitioners. Cytology samples for SurePath specimens were collected with a different broom-like device with a detachable head. The entire head of the device was removed from its handle and placed into a vial of SurePath preservative fluid, which may have helped to preserve a greater proportion of each specimen.

This study was embedded in a well-designed project (START) and conducted according to strict protocol. Screening test observers were blinded to the results of Table 1. Characteristics of Participants

<table>
<thead>
<tr>
<th>Factor</th>
<th>SurePath, Mean ± SD or No. (%) (n = 972)</th>
<th>ThinPrep, Mean ± SD or No. (%) (n = 1033)</th>
<th>Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>38.3 ± 5.6</td>
<td>37.6 ± 5.5</td>
<td>t = 3.06</td>
<td>.002</td>
</tr>
<tr>
<td>Mean y of education</td>
<td>7.0 ± 2.3</td>
<td>7.3 ± 2.3</td>
<td>t = −2.84</td>
<td>.005</td>
</tr>
<tr>
<td>Mean age of first sexual experience, y</td>
<td>20.8 ± 2.1</td>
<td>20.7 ± 2.1</td>
<td>t = 0.47</td>
<td>.641</td>
</tr>
<tr>
<td>Mean no. of pregnancies</td>
<td>2.8 ± 1.2</td>
<td>2.8 ± 1.2</td>
<td>t = −0.40</td>
<td>.686</td>
</tr>
<tr>
<td>Mean no. of live births</td>
<td>2.1 ± 0.8</td>
<td>2.1 ± 0.7</td>
<td>t = 1.00</td>
<td>.317</td>
</tr>
<tr>
<td>Married</td>
<td>959 (98.7)</td>
<td>1028 (99.5)</td>
<td>Chi-square = 4.10</td>
<td>.043</td>
</tr>
<tr>
<td>Farmer</td>
<td>910 (93.6)</td>
<td>941 (91.1)</td>
<td>Chi-square = 4.51</td>
<td>.034</td>
</tr>
<tr>
<td>Never smoked</td>
<td>934 (96.1)</td>
<td>993 (96.1)</td>
<td>Chi-square = 0.002</td>
<td>.966</td>
</tr>
<tr>
<td>HR-HPV positive*</td>
<td>170 (17.5)</td>
<td>162 (19.2)</td>
<td>Chi-square = 0.86</td>
<td>.355</td>
</tr>
</tbody>
</table>

Cytopathology results**

<table>
<thead>
<tr>
<th>Specimen Preparation Method</th>
<th>Un satisfactory LBC</th>
<th>Un satisfactory HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SurePath</td>
<td>0.2 (2/972)</td>
<td>0 (0/972)</td>
</tr>
<tr>
<td>ThinPrep</td>
<td>1.5 (15/1033)</td>
<td>18.2 (188/1033)</td>
</tr>
<tr>
<td>Total</td>
<td>0.8 (17/2005)</td>
<td>9.4 (188/2005)</td>
</tr>
</tbody>
</table>

Abbreviations: ASC-H, atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; SD, standard deviation.

* The HR-HPV prevalence was 19.2% (162 of 845 patients), excluding 188 participants with no Hybrid Capture 2 results due to insufficient specimen volume.

** Consensus was based on pathologists from China and France.

ASC-H included ASC-H, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, and squamous cell carcinoma.

Table 2. Unsatisfactory Rates of LBC and HC2 Testing by 2 LBC Systems

<table>
<thead>
<tr>
<th>Specimen Preparation Method</th>
<th>Unsatisfactory LBC</th>
<th>Unsatisfactory HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SurePath</td>
<td>0.2 (2/972)</td>
<td>0 (0/972)</td>
</tr>
<tr>
<td>ThinPrep</td>
<td>1.5 (15/1033)</td>
<td>18.2 (188/1033)</td>
</tr>
<tr>
<td>Total</td>
<td>0.8 (17/2005)</td>
<td>9.4 (188/2005)</td>
</tr>
</tbody>
</table>

Abbreviations: 95% CI, 95% confidence interval; HC2, Hybrid Capture 2; LBC, liquid-based cytology; NA, not applicable; OR, odds ratio.

* Chi-square = 9.25 (P = .002).

** Chi-square = 195.20 (P < .0001).
all other tests to ensure independent interpretation of each test. Women who were diagnosed as negative by colposcopy but positive by LBC or HC2 were recalled for 4-quadrant cervical biopsies and ECC to maximize ascertainment of disease and to reduce verification bias. Cytological and histological diagnoses were based on the consensus of pathologists in China and France.

One limitation of the current study was the selection of participants by simple random cluster sampling, which could have led to a reduced ability to generalize our findings to other women in the region. As opposed to our sequential design, a more powerful one could have been achieved by randomly allocating women to one preservative solution or the other. Although operational difficulties prevented us from implementing this design, patients in both sample groups appeared similar despite this potential weakness. Great care was taken to ensure uniformity between the clinical, laboratory, and pathology procedures and the personnel performing them. Another limitation of the current study was the decision to have patients self-collect 3 specimens for research purposes before physicians collected samples for cervical cytology, which did not precisely follow the manufacturer’s recommended protocol. This may have had an adverse impact on the adequacy of both types of specimens, potentially resulting in findings that are not applicable to clinical settings.

A few studies have compared the performances of ThinPrep and SurePath in a population. A study in the United Kingdom concluded that ThinPrep and SurePath had equivalent performances using a variety of predictive values. The study excluded HC2-tested samples that might have been at increased risk for high-grade cervical lesions and their cytology specimens were diagnosed according to UK National Health Service Cancer Screening Program classification guidelines. A 4-year retrospective study in the United States included rates of insufficient volume for HC2 testing in their analysis of reflex HC2 testing of women with abnormal ThinPrep and SurePath results. In concurrence with our study, they concluded that ThinPrep specimens had statistically significant higher rates of insufficient volume for HC2 testing. Their retrospective study design differed from ours in several aspects: the screening population was larger, and the majority of the subjects were African American or Caucasian; the age range was much greater, but the median age was lower; and the accuracy of HC2 testing could not be calculated because women with negative HC2 results did not undergo biopsy.

In conclusion, compared with the ThinPrep method, the SurePath method yielded significantly lower rates of inadequate cytology and inadequate volumes for HC2 with comparable diagnostic accuracies. Researchers and clinicians should contemplate some of the factors we have discussed to optimize the potential of a specific method of LBC preparation for gynecologic specimens, with special consideration for the option of HPV DNA testing on

### Table 3. Accuracy of Screening Methods for Detecting CIN2+

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>No.</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>Area Under the ROC Curve (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBC (≥ASC-US)</td>
<td>SurePath</td>
<td>970</td>
<td>91.4 (77.6-97.0)</td>
<td>86.6 (84.3-88.7)</td>
<td>20.4 (14.8-27.4)</td>
<td>99.6 (98.9-99.9)</td>
</tr>
<tr>
<td></td>
<td>ThinPrep</td>
<td>1014</td>
<td>82.9 (68.7-91.5)</td>
<td>87.0 (84.7-88.9)</td>
<td>21.1 (15.5-28.1)</td>
<td>99.2 (98.3-99.6)</td>
</tr>
<tr>
<td>Statistic</td>
<td>Chi-square = 0.57</td>
<td>.452</td>
<td>Chi-square = 0.042</td>
<td>.838</td>
<td>Chi-square = 0.026</td>
<td>.871</td>
</tr>
<tr>
<td>HC2 (≥1.0 pg/mL)</td>
<td>SurePath</td>
<td>972</td>
<td>97.2 (85.8-99.5)</td>
<td>85.6 (83.2-87.7)</td>
<td>20.6 (15.2-27.3)</td>
<td>99.9 (99.3-100.0)</td>
</tr>
<tr>
<td></td>
<td>ThinPrep</td>
<td>841</td>
<td>94.1 (80.9-98.4)</td>
<td>84.4 (81.7-86.7)</td>
<td>20.3 (14.7-27.2)</td>
<td>99.7 (98.9-99.9)</td>
</tr>
<tr>
<td>Statistic</td>
<td>Chi-square = 0.003</td>
<td>.960</td>
<td>Chi-square = 0.48</td>
<td>.940</td>
<td>Chi-square = 0.006</td>
<td>.940</td>
</tr>
<tr>
<td>LBC + HC2 (either ≥ASC-US or ≥1.0 pg/mL)</td>
<td>SurePath</td>
<td>972</td>
<td>100.0 (88.5-100.0)</td>
<td>78.1 (75.3-80.6)</td>
<td>14.9 (11.0-20.0)</td>
<td>100.0 (99.4-100.0)</td>
</tr>
<tr>
<td></td>
<td>ThinPrep</td>
<td>864</td>
<td>95.1 (83.0-99.5)</td>
<td>74.7 (71.7-77.6)</td>
<td>15.8 (11.7-20.9)</td>
<td>99.7 (98.8-100.0)</td>
</tr>
<tr>
<td>Statistic</td>
<td>—</td>
<td>—</td>
<td>Chi-square = 2.77</td>
<td>—</td>
<td>Chi-square = 0.07</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; LBC, liquid-based cytology; NPV, negative predict value; PPV, positive predict value; ROC, receiver operating characteristic.

Four women with unsatisfactory biopsy results were excluded from analysis.

Derived using the Fisher exact test.
residual specimens. Although we did not discuss the cost and labor required for sample processing, the findings of the current study have important implications for improving methods of LBC slide preparation that are aimed at reducing the rates of unsatisfactory cytology, specifically by manufacturer and specimen collection. This is especially relevant for testing in rural populations with a high rate of cervicitis.

**FUNDING SUPPORT**

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**CONFLICT OF INTEREST DISCLOSURES**

The authors made no disclosures.

**REFERENCES**

Pap systems in the processing of mucus-rich specimens. *Cancer (Cancer Cytopathol)*. 2010;25:118:244-249.


