Interobserver Reproducibility with LiquiPrep™ Liquid-based Cervical Cytology Screening in a Developing Country

Jongkolnee Settakorn, Samreung Rangdaeng*, Natchanun Preechapornkul, Sudaporn Nateewatana, Kampol Pongsiralai, Jatupol Srisomboon, Paul S Thorner

Abstract

Objective: A modified liquid-based techniques known as the “LiquiPrep™ (LP) system” requires neither expensive equipment nor complicated specimen preparation. The aim of this study was to assess the applicability of the LP for use in a developing country. Methods: Cervical cytology specimens were collected from 777 women, using the Cervex-Brush™. The brush was first smeared on a glass slide for conventional Papanicolaou (CP) stain, and then immersed in preservation fluid for LP preparation. Cytologic interpretations were classified into four categories: 1) no atypical cells, 2) atypical squamous epithelial cells (ASC), 3) definite epithelial cell abnormality, and 4) unsatisfactory specimen. Interobserver variability was tested using weighted kappa statistics. Results: An LP specimen cost $9 per case compared to $3 per case for a conventional Pap smear. The time to learn the technique was only a few days. Forty six (5.92%) specimens by LP were unsatisfactory. The overall agreement between cytopathologists was 96.7% (weighted κ=0.62), with 95.6% (weighted κ=0.44) for the cases enrolled earlier, increasing to 97.9% (weighted κ=0.78) for the cases enrolled later. Conclusions: In summary, after a short learning curve, interobserver reproducibility of LP smear was near perfect. This feature of the LP, together with the relatively low cost and simple protocol, makes it quite suitable for cervical cytology screening in developing countries. Moreover, with this technique, some of each sample can be reserved for additional studies such as HPV detection and subtyping.

Key Words: Liquid-based cytology - cervical cancer screening - LiquiPrep™ - Papanicolaou

Introduction

Liquid based cytology preparations (LBCP) are replacing conventional Papanicolaou (CP) smears for cervical screening cytology in the Western world (Doyle et al., 2006). Use of this technique has been shown to improve detection rates of cervical intraepithelial neoplasia when compared with conventional preparations (Cheung et al., 2003; Renshaw et al., 2004). Results correlate very well with biopsy diagnoses (Vassilakos et al., 2000). Interobserver reproducibility and satisfactory sample rates may be better than with CP smears (Chhieng et al., 2002; Cheung et al., 2003).

The commonly used automated LBCP techniques are ThinPrep™ test (Cytyc Corporation, Boxborough, MA, USA) and SurePath™ (TriPath Imaging, Burlington, NC, USA), plus there are some newer LBCP techniques, PapSpin™ (ThermoElectron, Pittsburgh, PA, USA) and DNACITOLIQ (Digene Brazil, Sao Paulo, Brazil) (Alves et al., 2004; Rosenthal et al., 2006). Liqui-Prep™ (LGM International, Fort Lauderdale, FL, USA) is a new manual LBCP technique. Instead of using expensive equipment combined with disposable filters, this chemical reagent-based technique encapsulates each cell in a transparent envelope. This method allows the cells to spread evenly on a slide thereby minimizing cell overlap. Liqui-Prep™ (LP) is claimed to provide a preparation comparable to other LBCP techniques, but is more cost effective for cervical cancer screening and non-gynecology specimens since it requires no special equipment.

Such a technique offers advantages for developing countries but its reliability in this setting has not been assessed. The purpose of this study was to assess this technique in cervical cytology, with respect to cost, ease of training, number of unsatisfactory specimens, interobserver variability, correlation between CP and LP smears and whether the latter are better for detecting high grade intraepithelial lesions. We report here the results of the first study in Thailand.

Materials and Methods

Participants

In this prospective study, cervical cytology specimens were collected from 777 women who attended a gynecology clinic at Chiang Mai University Hospital. All...

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patients gave informed consent. Pregnant women were excluded from the study. The participants were enrolled between October 2005 and June 2006. This study was approved by the Research Ethic Committee of Medical Faculty at Chiang Mai University.

LiquiPrep™ procedure

Collection: The specimen was collected using a broom like device known as Rover Cervex Brush™ (Rovers Medical Devices Bv, Oss, the Netherlands). The brush was first smeared on a glass side for a CP stain. Then, the head of the brush was removed and placed into the LP preservation fluid and submitted to the laboratory.

Cleaning and concentration: The vial containing the head of the brush was vigorously shaken using a vortex for 10 seconds. Then the contents of each vial were poured into a 15 ml centrifuge tube. For specimens containing blood or mucus, 4 ml of cleaning solution were added. The tubes were then centrifuged for 10 minutes at 1000 g.

Slide preparation: After centrifugation, the supernatant was discarded. An aliquot of cell base reagent was added in proportion to the size of the cell pellet according to the manufacturer’s instructions. The cell pellet was resuspended using a vortex for 10 seconds. Following this step, 50 µl of the suspension was pipetted onto an uncoated slide to form a 1.5 cm diameter circle. The slide was then air-dried and Pap-stained.

Data and statistics

The participants were questioned with regards to age, reproduction, and menstruation history. The CP smears were evaluated in a routine fashion by two cytopathologists (JS and SR). Discrepant cases were reevaluated to arrive at a consensus diagnosis. Both pathologists then independently examined the LP slides, blinded to the diagnosis made on the CP smears. The cytologic interpretation was classified into four categories: 1) no epithelial cell abnormality (NEA), 2) atypical squamous epithelial cell (ASC), 3) definite epithelial cell abnormality (low grade squamous intrapithelial lesion—LSIL, high grade squamous intraepithelial lesion-HSIL, or squamous cell carcinoma), and 4) unsatisfactory specimen. Interobserver variability was tested using weighted kappa statistics. Specifically, the weights were 1.00 for data cells on the diagonal (i.e., exact agreement), 0.5 for cells adjacent to the diagonal, and 0 for cells 2 units from the diagonal. Percentage of cases with diagnostic agreement between two cytopathologists was reported. Disagreement was arranged in major and minor groups. Major disagreement referred to a discrepancy between categories 1 and 3. Minor disagreement referred to a discrepancy between categories 1 and 2; or categories 2 and 3. For testing the learning curve effect, subgroup analysis was done by dividing the participants into two groups; i.e., a first group (case numbers 1 to 388, enrolled between October 2005 and February 2006) and a second group (case numbers 389 to 777, enrolled between February 2006 and June 2006).

Results

The participants’ age ranged from 17 to 80 years with mean age of 42.2 (+11.3) years. The characteristics of the patients are shown in Table 1. Forty six (5.9%) LP smears were unsatisfactory and were not evaluated further. By comparison, only one CP smear was felt to be inadequate. The reproductive status of the participants with unsatisfactory specimen was significantly different from those with satisfactory smears. There was a higher proportion of post partum (37.0%) and post menopausal (39.1%) women in the unsatisfactory group (p<0.001).

Table 1. Characteristics of the Patient Study Group for LiquiPrep™ smears

<table>
<thead>
<tr>
<th>Classification</th>
<th>Satisfactory (n=731)</th>
<th>Unsatisfactory (n=46)</th>
<th>Total (n=777)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean+SD)</td>
<td>42.10 +11.10</td>
<td>42.96 +14.64</td>
<td>42.15+11.33</td>
<td>0.70</td>
</tr>
<tr>
<td>Number of children (range/median)</td>
<td>0-2 / 2</td>
<td>0-7 / 2</td>
<td>0-9 / 2</td>
<td>0.80</td>
</tr>
<tr>
<td>Reproductive status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>394 (53.90%)</td>
<td>8 (17.39%)</td>
<td>402 (51.74%)</td>
<td></td>
</tr>
<tr>
<td>Postpartum</td>
<td>108 (14.77%)</td>
<td>17 (36.96%)</td>
<td>125 (16.09%)</td>
<td></td>
</tr>
<tr>
<td>Perimenopausal</td>
<td>77 (10.53%)</td>
<td>3 (6.52%)</td>
<td>80 (10.30%)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>152 (20.97%)</td>
<td>18 (39.13%)</td>
<td>170 (21.88%)</td>
<td></td>
</tr>
<tr>
<td>Menstruation history</td>
<td></td>
<td></td>
<td></td>
<td>0.32*</td>
</tr>
<tr>
<td>Normal</td>
<td>649 (88.78%)</td>
<td>43 (93.48%)</td>
<td>692 (89.06%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>82 (11.22%)</td>
<td>3 (6.52%)</td>
<td>85 (10.94%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison of Cervical Cytology Diagnoses Using LiquiPrep™

<table>
<thead>
<tr>
<th>Classification</th>
<th>Cytopathologist #1</th>
<th>Cytopathologist #2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cytopathologist #1</td>
<td>659</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>682</td>
<td>34</td>
<td>15</td>
</tr>
</tbody>
</table>
Discussion

The Pap smear is generally accepted as the most successful screening test for cancer detection. It can take 8 to 10 years from an initial HPV infection to a diagnosis of HSIL. The natural history of cervical cancer enables the detection of most lesions at an early stage, even after 1 or 2 missed opportunities or underinterpreted Pap smears. (Rosenthal et al., 2006) Moreover, the standard Pap smear is an inexpensive test.

The introduction of automated LBCP testing resulted in a need for expensive new processing devices as well as training of laboratory personnel and changes in laboratory space allocation. The cost per test at least doubled as a consequence. In addition, cytopathologist and cytotechnologists encountered new cytomorphologic criteria depending on the preparation. To deal with this, a few days for training the technologist. Required days of training, followed by weeks to months on the learning curve. Nevertheless, the decrease in the number of indeterminate results (ASC), the increase in the detection of neoplasia (Bishop et al., 1998; Limaye et al., 2003; Tench, 2000), and the ability to perform HPV testing on the residual material (Bolick et al., 2003; Levi et al., 2003) convinced many laboratories to convert to the LBPC type of testing.

The LP method counters many of these difficulties, since it makes use of the already available processor producing specimens with familiar cytologic features. We therefore carried out a study to determine whether use of this technique was advantageous for a developing country such as Thailand. There are no published studies comparing this technique to the conventional Pap smear and ours is the first such study to be carried out in Thailand.

We found that using the LP method, the background on slides was cleaner, cell preservation was better and there was no problem with air-drying artifact when compared to CP smears. The area for examination on the slide was also decreased, which meant a reduction in screening time and reduced fatigue in screeners. The latter should lead to reduced false negative results.

Cost comparison placed the LP higher than the conventional smear, but less expensive than the automated LBCP procedures. In Thailand, the laboratory cost for CP, LP, and ThinPrep smears were $3, $9, and $15 US, respectively. Moreover, in our experience, it required only a few days for training the technologist.

In our study, there was only one (0.13%) unsatisfactory CP smear. The unsatisfactory rate (5.9%) of LP cervical cytology specimens is higher than the value of <3% reported for previous LBCP studies. (Doyle et al., 2006; Rosenthal et al., 2006; Williams, 2006) This may reflect the fact that the CP was prepared first and the residual specimen used for the liquid based smear. Thus, the true rate of unsatisfactory smears is very likely lower than 5.9%. Nevertheless, our study design using split samples offers the advantage that the same patients are examined by both techniques, whereas most studies comparing CP and liquid-based cytology methods compare different groups of patients. Our choice of study design is supported by a recent study that also employed a split-sample design to compare conventional smears to a different liquid-based cytology technique and found comparable detection of epithelial abnormalities by both techniques (Rosenthal et al., 2006).

The interobserver reproducibility from the present study (weighted κ=0.62, 95%CI=0.56-0.68) was in keeping with the published literature including the result from the ALTS trial (weighted κ=0.59, 95%CI=0.57-0.61).

Table 5. Comparison of Cervical Cytology Diagnoses using LiquiPrep™ and Conventional Method

<table>
<thead>
<tr>
<th>Classification</th>
<th>Conventional</th>
<th>Total</th>
<th>Classification</th>
<th>Conventional</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 LP</td>
<td>1 638 36 4 678</td>
<td>#2 LP</td>
<td>1 644 34 4 682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 34 1 36</td>
<td>2 29 2 3 34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 2 2 13 17</td>
<td>3 1 3 11 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 674 39 18 731</td>
<td>Total 674 39 18 731</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#1, the first cytopathologist #2, the second cytopathologist

in the detection of an additional 2,240 HSIL cases with
an improved approach to cervical screening.

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(Stoler & Schiffman, 2001) In our study, subgroup
analysis demonstrated much better diagnostic agreement
on the later group of the specimens (weighted $\kappa=0.78$,
95%CI=0.69-0.86) compared to the earlier (weighted
$\kappa=0.44$, 95%CI=0.36-0.53). We believe this difference
reflects the increase in pathologists’ expertise over time.
The most frequent disagreements in diagnoses were
between NEA and ASC of undetermined significant (ASC-
US) (n=27, 61.36%) and between NEA and ASC favor
HSIL. (ASC-H) (n=11, 25%). These findings are in
keeping with those of the other studies in which smears
in the categories of atypical squamous cells had poorer
interobserver agreement. (Crum et al., 1999)
The agreement between the CP smear and the LP
smears of two cytopathologists (weighted $\kappa=0.37$,
95%CI=0.31-0.44 and weighted $\kappa=0.40$, 95%CI=0.33-
0.46) was slightly lower than their agreement on LP
smears (weighted $\kappa=0.62$, 95%CI=0.56-0.68). Similary, the
ThinPrep and CP correlation study of Lerma E, et al that
also used split sample method in a low risk population
yielded the $\kappa$ of 0.55. (Lerma et al., 2007) We think this
may be influenced by the fact that the conventional
smears were prepared first; however, there were only slightly
fewer conventional smears in category 1 compared to LP,
(674 vs. 680) and slightly more in categories 2 (39 vs 35)
and 3 (18 vs 16). We found that the LP method and
conventional Pap smear were equally useful in detecting
HSIL smears. CP smear detected 13 HSIL smears, and
the LP method detected 13 HSIL smears by one
cytopathologist and 10 HSIL smears by the second
method.  CP smear detected 13 HSIL smears, and
3 (18 vs 16). We found that the LP method and
conventional Pap smear were equally useful in detecting
HSIL smears. CP smear detected 13 HSIL smears, and
the LP method detected 13 HSIL smears by one
cytopathologist and 10 HSIL smears by the second
cytopathologist. The number of cases in our study (i.e.,
13) is likely too small to detect any advantage of liquid-
based methods over CP smear with respect to detection
of HSIL cases. A recent analysis from Australia showed
that conversion from CP test to ThinPrep™ test resulted
in the detection of an additional 2,240 HSIL cases with
480 life-years gained and an estimated health care savings
of $5,536,000 per year (Neville & Quinn, 2005).

An HPV vaccine is now available in the market for
Thai women and, recently, the Ministry of Public Health
of Thailand has changed the guidelines for cervical cancer
screening to now include HPV testing. In this respect,
there is a distinct advantage of the LP system over CP
smears since this method of preparation (as with other
liquid-based methods) allows for part of the sample to be
retained for additional studies such as HPV detection and
subtyping. The remainder of the sample can be used for
immunohistochemistry, in situ hybridization and/or
molecular genetic methods, as needed. The conventional
smear does not lend itself readily to these ancillary
investigative techniques.

In summary, many features of the LP system make it
eminently suitable for cervical cytology screening in
developing countries, including ease of preparation, high
correlation between results obtained on CP smears, and
excellent interobserver reproducibility. While slightly
more expensive than the CP smear, we feel this increased
cost is offset by the advantages of the LP system such as
reduced screening time, reduced screener fatigue, and the
ease of performing additional studies on the same sample,
in particular HPV testing. HPV infection is an increasing
health problem in developing countries, and a system such
as LP would appear to provide an economical option for
an improved approach to cervical screening.

Conflict of Interest

LGM International, Inc (Fort Lauderdale, FL, USA)
is a manufacturer of LiquiPrep™. This firm provided the
kits, reagent, and technical support for the present study.
None of the authors of this article have any financial
benefit from conducting this study.

Acknowledgements

We would like to thank the LGM International, Inc
(Fort Lauderdale, FL, USA) for providing the kits and for
the technical support.

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practice with comparison of interpretive and probabilistic
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effectiveness analysis of ThinPrep in the Australian setting.


