RESEARCH COMMUNICATION

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

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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 side 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

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

	Satisfactory for evaluation (n=731)	Unsatisfactory for evaluation (n=46)	Total (n=777)	p value
Age (mean+SD)	42.10 +11.10	42.96 +14.64	42.15+11.33	0.70[t]
Number of children (range/median)	0-2 / 2	0-7 / 2	0-9 / 2	0.80[w]
Reproductive status				<0.001[c]
Normal	394 (53.90%)	8 (17.39%)	402 (51.74%)	
Postpartum	108 (14.77%)	17 (36.96%)	125 (16.09%)	
Perimenopausal	77 (10.53%)	3 (6.52%)	80 (10.30%)	
Postmenopausal	152 (20.97%)	18 (39.13%)	170 (21.88%)	
Menstruation history				0.32[c]
Normal	649 (88.78%)	43 (93.48%)	692 (89.06%)	
Abnormal	82 (11.22%)	3 (6.52%)	85 (10.94%)	

[t]=t-test, [w]=Wilcoxon rank-sum test, [c]=Chi-square test

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 2. Comparison of Cervical Cytology Diagnoses Using LiquiPrep™

		Cytopathologist #2			Total
	Classification	1	2	3	
Cytopathologist #1	1	659	17	2	678
	2	21	15	0	36
	3	2	2	13	17
Total		682	34	15	731

Table 3. Percentage of Agreement and Weighted Kappa between Both Pathologists

	Agreement	Kappa (95%CI)
Cases enrolled earlier	95.59%	0.44 (0.36-0.53)
Cases enrolled later	97.90%	0.78 (0.69-0.86)
Overall	96.72%	0.62 (0.56-0.68)

Table 4. Summary of Disagreement Cases

Disagreement between	Level of disagreement	Number of cases (%)
NEA and LSIL	Major	1 (2.27)
NEA and HSIL	Major	3 (6.82)
NEA and ASCUS	Minor	27 (61.36)
NEA and ASC-H	Minor	11 (25)
LSIL and HSIL	Minor	1 (2.27)
ASC-H and SCC	Minor	1 (2.27)
Total		44 (100.00)

(Table 1).

Findings for LP smears with the two pathologists are summarized in Table 2. The overall agreement is shown in Table 3). Agreement in the later group of specimens was significantly better than in the first group ($p<0.001$). Details for the 44 disagreements in diagnosis are covered in Table 4.

By CP smear, 674 cases were assigned to category 1, 39 cases to category 2, and 18 cases to category 3, (Table 5) 13/18 in category 3 showing HSIL. Weighted agreement between LP and CP smears was 94.19% (weighted κ=0.37, 95%CI=0.31-0.44) for the first cytopathologist, and 94.60% (weighted κ=0.40, 95%CI=0.33-0.46) for the second cytopathologist.

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 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 (Rosenthat 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

Classification		Conventional			Total	Classification		Conventional			Total
		1	2	3				1	2	3	
#1 LP	1	638	36	4	678	#2 LP	1	644	34	4	682
	2	34	1	1	36		2	29	2	3	34
	3	2	2	13	17		3	1	3	11	15
	Total	674	39	18	731		Total	674	39	18	731

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

(Stoler & Schiffman, 2001) In our study, subgroup analysis demonstrated much better diagnostic agreement on the later group of the specimens (weighted κ=0.78, 95%CI=0.69-0.86) compared to the earlier (weighted κ=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 κ=0.37, 95%CI=0.31-0.44 and weighted κ=0.40, 95%CI=0.33-0.46) was slightly lower than their agreement on LP smears (weighted κ=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 κ 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 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.

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