

Direct-to-Vial Comparison of a New Liquid-Based Cytology System, Liqui-PREP™ Versus the Conventional Pap Smear

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The objective of the study was to assess the clinical utility of a liquid-based cytology system on cervicovaginal screening in a clinical commercial laboratory.

Twenty-six thousand, one hundred and seventy eight cervicovaginal specimens were prepared by the Liqui-PREP™ (LGM International Inc., Ft. Lauderdale, FL) with a direct-to-vial comparison to 218,548 cases of the conventional direct smear from August 2005 through December 2005. Biopsy data was obtained to confirm the sensitivity for each method.

Liqui-PREP™ showed a marked increase in HSIL+ detection compared to the conventional smear (P = 0.001). The rate of LSIL and AGC detection was higher with Liqui-PREP™ (P = 0.001 for both). The percentage of ASCUS specimens was higher than with conventional smear due to cleaner slides and easier detection of suspicious cells. The WNL rate was lower for Liqui-PREP™ (P = 0.001) consistent with increased HSIL+ and ASCUS. The unsatisfactory rate was lower for Liqui-PREP™ (P = 0.017). The histological predictive value of Liqui-PREP™ was slightly higher than the conventional smear (94.1% versus 89.9%).

The Liqui-PREP™ system similar to other reported LBC technologies shows an increased detection of squamous intraepithelial lesions, and gives higher-quality slides for interpretation than the conventional smear. Histological results confirm that this increase in cytological findings are clinically significant. LGM's new LBC technology is a more sensitive screening tool when compared with the conventional smear. Diagn. Cytopathol. 2007;35:488–492. © 2007 Wiley-Liss, Inc.

Key Words: Papanicolaou smear; cervical cancer; mass screening; liquid-based cytology; Liqui-PREP

Conventional cervical cytology screening (Pap smear) involves the microscopic examination of cell samples taken from the ectocervix and endocervix, smeared onto glass microscope slides, fixed, and stained by the Papanicolaou method.¹ The Pap smear has been utilized for cervical screening for >50 years and has reduced the mortality rate from invasive cervical carcinoma by 50–70%.^{2–5} In spite of this success, the Pap smear has a false negative rate reported as high as 55%.^{6–14} Errors due to poor sampling and nonrepresentative and partial transfer of the collected sample to the slides may account for as much as 62% of the false negative cervical smears.¹⁵

Sampling has been greatly improved by a new generation of collection devices that dependably remove large and representative cervical samples from the ectocervix and endocervix.^{16,17}

The transfer of the collected material to the conventional Pap smear is carried out in the clinic and is subject to inherent inconsistencies in the quality of fixation, thickness of the smear, amount of obscuring blood, mucus, inflammation, and diagnostic homogeneity of the final preparation.^{18,19}

In recent years, there have been many and varied approaches to improving the quality of cervical cytology.^{20,21} Most of these efforts involve the transfer of the collected sample into a vial of liquid preservative before making the slide preparation. This allows mucus and blood to be reduced and the sample to be thoroughly randomized before fixing a controlled and representative aliquot onto a microscope slide. The procedures are collectively known as liquid-based cytology. The ThinPrep® Pap Test (Cytec Corporation, Marlborough, MA) and SurePath® (TriPath Imaging, Burlington, NC) are repre-

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sentative of this technology and have been reported to improve the quality of cervical preparations.^{22,23}

The Liqui-PREP™ Preparation system (LGM International, Fort Lauderdale, FL) is a new liquid-based method of cytology specimen preparation. The purpose of this study is to evaluate Liqui-PREP™ (LP) and compare it to the conventional Pap smear (CS) for the detection of cervicovaginal abnormalities in the same population during the same time period.

Materials and Methods

One hundred and seventy-six (176) gynecologists from 117 different outpatient medical practices (one general hospital and the residual private GYN clinics) in Korea participated in this study. 26,178 cases of LP (LGM International, Fort Lauderdale, FL) and 218,548 cases of CS obtained from the referring physicians (August 2005 through December 2005) were processed and reviewed at the EONE Reference Laboratory, Seoul Korea. Each sample was either processed by the LP liquid-based or the CS procedure. None of the samples were processed by both techniques. The patient population can be characterized as a low-risk "typical screening population"²⁴ representing the full spectrum of ages and reproductive histories. The average age and standard deviation (SD) of the LP group were 38.78 and 10.13 yr, respectively, and those of the conventional Pap smear group were 43.29 and 11.87 yr, respectively.

The conventional slides were prepared from samples collected using a locally supplied cervical brush designed to brush and collect both cervical and endocervical specimens simultaneously (MoA Medical, Seoul, Korea), fixed with 95% ethanol at the collection site and processed in the EONE Reference Laboratory. Cellular material for LP was collected using the Cervex Brush™ (Rover's, Oss, The Netherlands). According to the manufacturer's instructions, the head of the Cervex Brush™ was detached and dropped into LP preservative fluid and forwarded to the EONE laboratory.

For preparation, the liquid-based samples were mixed and centrifuged through a carbohydrate based density cleaning solution at optimal centrifugal force and time (1,000 g for 10 minutes) a modification of the method to a single centrifugation reported by Otto et al.²⁵⁻²⁷ The supernatants were decanted and the cellular pellets were uniformly mixed into a cellular encapsulating^{28,29} (Cell Base) reagent. Cell density was controlled by estimating the ratio of cell pellet to Cell Base added. Fifty microliter aliquots of the homogeneous suspensions were placed onto clean microscope slides and spread into 17 ± 4 mm circles. The slides were allowed to dry before staining.

LP and CS were stained using the regressive Harris PAP stain. The slides were classified according to the Be-

thesda System for reporting cervical cytology (Figs. C1-C4).³⁰

All slides, CS and LP, were screened by a team of three cytotechnologists. All slides classified as other than "within normal limits" were reviewed by one or more members of a group of three cytopathologists. Prior to the introduction of LP at EONE Reference Laboratory, cytotechnologists received extensive (one month) training to gain experience with the recognition of a range of normal and abnormal cytologic morphologies on LP cytology preparations. This instruction was carried out under the direction of knowledgeable cytopathologists. Cellular morphology in LP is quite similar to that of CS contrary to our experience and reports with the ThinPrep System.³¹ Differences in the proportions of positive diagnoses were analyzed statistically using a calculated Z statistic and derived *P*-value.³²

Results

LP resulted in a marked increased detection of HSIL+ in line with leading commercially available liquid cytology systems over the conventional PAP smear. Unsatisfactory rates on both systems were low with LP being three times lower. The gynecologists were very careful and skillful during the collection of cervical specimens (Table I).

The percentage of ASCUS specimens was higher with the LP than with the CS. The LP slides are easier to find small numbers of abnormal cells due to clearer morphology of the individual cells. Therefore, the rate of ASCUS was increased, which was welcomed by gynecologists concerned about receiving false negative results.

The number of WNL diagnoses was as expected for liquid based cytology systems. It was, however, lower for LP than for the CS, because of higher detection of HSIL+ and ASCUS.

Biopsy results were obtained by EONE from local hospitals and clinics in order to compare histology and cytology diagnoses for LP and CS. Biopsy results were obtained on 461 CS cases and 119 LP cases having a diagnosis of either ASC-H, LSIL, HSIL, HSIL+ or AGC. In this study, biopsy was performed only on patients with a positive cytology diagnosis (Table II).

Discussion

Comparison of preparation systems for cervical screening can be challenging due to bias. We considered a split sample study, but lack of an easy method to accurately divide the sample makes this type of study impractical. The direct to vial comparison we used and the number of patients in the study were selected to minimize bias. The patient population for both arms of the study is considered "random screening" and samples were taken during

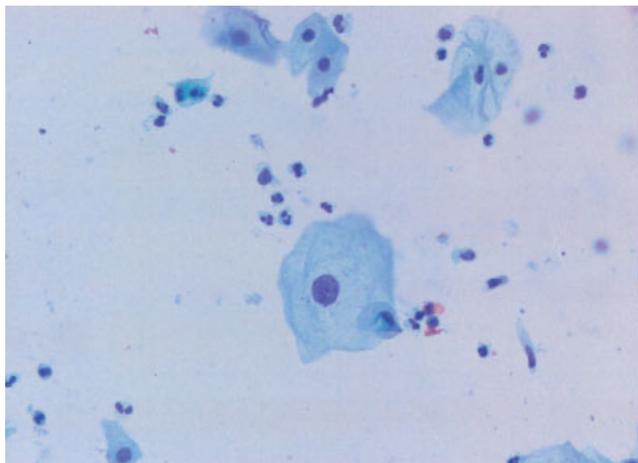


Fig. C-1

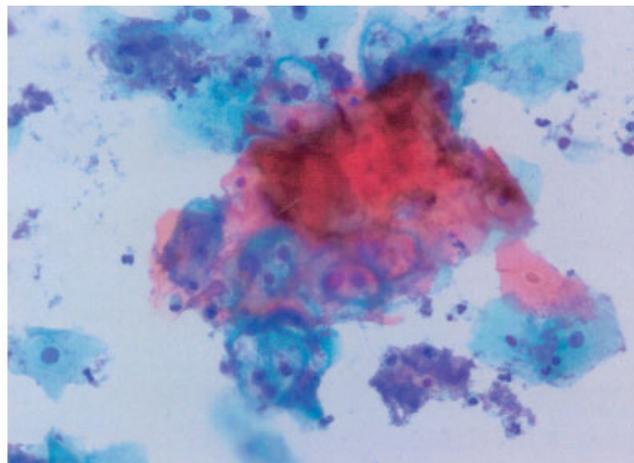


Fig. C-2

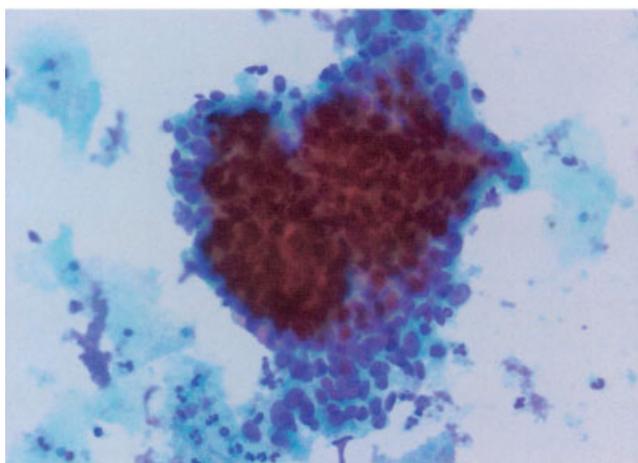


Fig. C-3

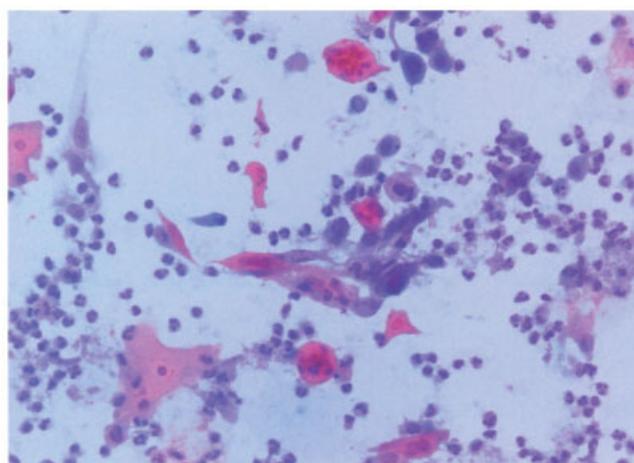


Fig. C-4

Figs. C-1–C-4. Fig. C-1. Cervicovaginal smear processed with the LP method diagnosed as ASC-US; Papanicolaou stain, $\times 400$. Fig. C-2. Cervicovaginal smear processed with the LP method diagnosed as LSIL; Papanicolaou stain, $\times 400$. Fig. C-3. Cervicovaginal smear processed with the LP method diagnosed as HSIL; Papanicolaou stain, $\times 400$. Fig. C-4. Cervicovaginal smear processed with the LP method diagnosed as SCC; Papanicolaou stain, $\times 400$.

the same calendar time. One possible bias can be attributed to different types of collection devices. The gynecologists participating in this study used the sapula-endocervical brush for collecting CS smears and a cervical broom for LB samples. The second possible bias is that the client is asked to pay additional money, though modest, to have the LP test performed, since national coverage is for CS only. This would indicate a potential bias towards a wealthier client opting for the LBC technology. A third possible bias could be that the average age of LP clients tended to be younger, 38.78 with a SD of 10.13 years versus the mean age for the CS, 43.29, SD of 11.87 years. (Student's *t*-test, *P*-value < 0.01) The data indicates the LP population may have a slight bias to a higher economic class and younger population of women compared to the CS.

These data confirm the increased detection of precancerous lesions when using the LP cytology preparation system. In our direct-to-vial study, LP showed a two fold improvement in the detection of LSIL and two fold improvement in the detection of HSIL+ compared to a similar patient population tested with the CS. The overwhelming diagnostic improvement of LP in our study, especially with respect to increased HSIL+ detection, should significantly and positively impact the success of cervical screening in Korea. The percent increase in the detection of LSIL and HSIL+ by LP are also similar to the results of direct-to-vial studies reported for other liquid-based methods.^{33–35} There were too few cases of carcinoma in our study to establish statistical significance, but these data should emerge as the number of patients we test with LP increases.

Table I. Comparison of Cytology Results by TBS 2001 Reporting

| | WNL | ASC-US | ASC-H | AGC | LSIL | HSIL | Malignant | HSIL+ | Unsat. |
|-----------------|----------------|-------------|------------|-----------|-------------|-----------|-----------|-----------|------------|
| Conventional | 210,497 (96.3) | 6,092 (2.8) | 190 (0.09) | 63 (0.03) | 1,062 (0.5) | 472 (0.2) | 54 (0.02) | 526 (0.2) | 118 (0.05) |
| Liqui-PREP | 23,913 (91.4) | 1,710 (6.5) | 63 (0.24) | 24 (0.09) | 370 (1.4) | 93 (0.4) | 0 (0.0) | 93 (0.4) | 5 (0.02) |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.017 |
| Liqui-PREP (%Δ) | -5 | +134 | +177 | +217 | +187 | +64 | | +47 | -65 |

Values in parenthesis indicate percentages.

WNL, within normal limits; AGC, atypical glandular cells; HSIL+, HSIL or malignant lesion; Unsat., unsatisfactory for cytological evaluation.

Table II. Biopsy Data

| | Total biopsies | LSIL | HSIL+ | AGC |
|------------------------|----------------|------|-------|------|
| Conventional Pap smear | | | | |
| Total | 461 | 343 | 109 | 9 |
| Concordant Result | 414 | 306 | 102 | 6 |
| % Agreement | 89.8 | 89.2 | 93.6 | 66.7 |
| Liqui-PREP | | | | |
| Total | 119 | 93 | 22 | 4 |
| Concordant Result | 112 | 87 | 21 | 3 |
| % Agreement | 94.1 | 93.5 | 95.5 | 75.0 |

Two Liqui-Prep biopsy results were discarded due to inadequate specimen collection.

HSIL+, HSIL or malignant lesion; AGC, atypical glandular cells.

The LP results also showed an increase in the ASC-US rate. With the LP method, 6.8% of the cases were reported as ASCUS compared to 2.9% for the CS. Part of this increase probably relates to the conservative nature of our staff and their cautious interpretation of the new preparation but more importantly, the cellular morphology is clearer and small numbers of abnormal cells can be easily viewed versus the CS. In spite of this increase, the ASC-US rate found in our laboratory for both CS and LP is in the range found in the United States.^{36,37} In addition, the increased ASC-US rate for LP should be taken in the context of the overall detection of ASC-US and SIL by the two cervical preparation methods. Calculating ASC-US:LSIL ratios helped determine whether increased detection of precancerous lesions by LP is coming with a proportional increase in ASC-US. In our study, the ASC-US:LSIL ratio for the LP was 19% lower than for the CS, indicating a potentially more specific diagnosis with LP.

The LP tested patient population also showed an increase in the rate of AGC diagnoses. We feel that this was due to improved preservation, more cells available for testing, and the presentation of the cells by the LP system. EONE is a reference laboratory that carries out very few histologic evaluations. We were however able to confirm biopsy results from a number of outside laboratories. The correlations of the biopsy to the cytology results reported in Table II indicate that the histologic predictive value of LP is slightly higher than CS histologic results, 94.1% versus 89.8% respectively overall. This higher predictive value was also seen for each cytology diagnosis,

LSIL, 89.2% CS versus 93.5%, HSIL+, 93.6% CS versus 95.5% LP, and for AGC, 66.7% CS versus 75.0% LP. The mean age for CS biopsy patients was 38.2 years, with a standard deviation of 10.3 years, while the mean age for the LP was 36.4 years, with a standard deviation of 36.4, (*t*-test, *P*-value = 0.09), showing random distribution even though these biopsy specimens represent only a portion of the cytology positives.

The biopsy data confirms the cytology data which indicates that LP provides a more sensitive detection and diagnosis than CS in cervical specimens. Finally, in spite of our laboratories low conventional smear unsatisfactory rate, LP improved the percentage of unsatisfactory slides by 66% (from 0.05% to 0.017%). The new LP liquid-based cytology preparation has proven itself to be more diagnostic than the CS and an economical alternative to the original and popularly used liquid-based methods.

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