

Shiraz E-Medical Journal
Vol. 11, No. 4, October 2010

<http://semj.sums.ac.ir/vol11/oct2010/88038.htm>

**Adequacy Criteria and Cytomorphological Changes in Liqui-Prep
TM versus Conventional Cervical Cytology.**

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Received for Publication: December 5, 2009, Accepted for Publication: February 2, 2010.

Abstract:

Background: The subject of adequacy in cervical smears has an essential role in the prevention of cervical cancer deaths. The predicament of establishing exactly what constitutes an adequate sample has received increasing attention in recent years. In this study, we tried to compare specimen adequacy and cytomorphological changes between conventional cervical and liquid based cytology " Liqui-PREP TM".

Material and methods: A total of 170 asymptomatic women who undergo annual screening, were examined in cytopathology and gynecology departments (Al-Zahra Hospital of Isfahan University, Iran) during August 2008 to December 2008. Among which 153 cases, 93 cases: pre-menopause and 60 cases: post menopause women were subsequently selected. The conventional smears (CS) and Liquid-Based slides (LBS) using Liqui-PREP TM technique taken from each patient were evaluated carefully by a professional cytopathologist. The number of endocervical and transitional zone's cells per slide, covering diagnostic squamous cells (%) and degenerated cells per high power microscopic field (HPF) were also counted. Other cytomorphological changes evaluated for postmenopausal women were included: number of eosinophilic pyknotic cells and spindled cells, basophilic globules (per HPF) and the presences of basophilic filaments (+/-). SPSS software was used for data analysis using indices of frequency percent, mean, and standard deviation for descriptive analysis.

Results: Mean of the patients' age was 42.28 which ranged from 20 to 67 years. The numbers of satisfactory cases with CS were 30 (31.9%) compared to 58 (62.4%) with the LBC ($P<0.0001$). Data for menopausal women included respectively: 11 (18.30%) and 39 (65%) and the $P<0.0001$. A significant difference was observed two methods in the number of degenerated cells and the amount of slide coverage by squamous cells in both age groups ($P=0.0001$) but bet the quantitative amount of endocervical cells was more in CS method rather than LBC method ($P=0.0001$). Other morphologic changes (variations) assessed in

menopause age group included: eosinophilic pyknotic cells, basophilic globules, spindled cells, and basophilic filaments which demonstrated a significant difference between two methods. The basophilic filaments were evaluated qualitatively as positive and negative. In CS 13 cases (21.7%) were negative and 47 ones (78.3%) were positive for filaments. All 13 negative cases in CS method were negative in LBC method also. But out of the 47 positive cases in LBC method, 13 cases (21.7%) were positive and 47 cases (78.7%) were negative (P value = 0.0001).

Conclusion: 1. Liquid-PREP TM is a straight-forward cytological procedure, relying on classic cell handling procedures. 2. Cellular material is encapsulated in a matrix material that assures quantitative, robust adherence to the slide. 3. The number of cells transferred to the slide is controlled by the cytologist. 4. Liquid-PREP TM is not expensive.

Keywords: Conventional cervical smear, Liquid based cytology, Liquid-PREP TM, Adequacy.

Introduction:

The subject of adequacy in cervical smears has an essential role in the prevention of cervical cancer deaths. The predicament of establishing exactly what constitutes an adequate sample has received increasing attention in recent years. Some guidelines have been proposed from various sources, including the British Society for Clinical Cytology and the Bethesda system.⁽¹⁾ However the most important thing for assessing smear quality according to these guidelines is that there should be adequate numbers of epithelial cells on the slide, with indicates that they are from the appropriate area of the cervix.⁽²⁾

Over the years, several methods for improving the cytological specimen have been proposed. Neugebauer et al.⁽³⁾ in 1981 described a sedimentation velocity separation method; and Naslund⁽⁴⁾ suggested a pulse wash method.

The liquid-based cytology (LBC) technique was introduced in the mid-1990s to increase the sensitivity and specificity of cervical cancer screening and for reduction in the number of unsatisfactory and "satisfactory-but-limited-by" reports seen with conventional cytology (CC).⁽⁵⁻⁸⁾

There are many articles in the world literature suggesting that LBC is more accurate than conventional screening. But LBC is expensive in terms of equipment, capital costs, maintenance, consumables, training, technical preparation time, transportation and disposal of liquid media.⁽⁹⁾ Liquid-based cervical cytologic specimens are processed according to manufacturers' directions for slide preparation. Papanicolaous stain is used for both types of specimens. Whether the stain is a progressive or regressive method, there should be clear visualization of the nuclear configuration and chromatin. In addition, the cytoplasm should be transparent and allow for distinction of cellular maturation.⁽²⁾

This study compared adequacy and cytomorphological criteria in conventional and liquid based cervical cytology according to premenopausal and menopausal status.

Material and Methods:

This study was carried out in cytopathology and gynecology departments of Al-Zahra Hospital of Isfahan University, Iran during August 2008 to December 2008. For this purpose 170 asymptomatic

women who undergo annual screening, were examined. Based on the age group, the patients were subdivided into two premenopausal and menopausal groups. Patients were considered asymptomatic if they didn't have symptoms such as vaginal itching, burning, dryness, abnormal discharge and bleeding. Patients with a previous history of hysterectomy, chemotherapy, radiation therapy, hormonal therapy and abnormal gross findings were excluded from the data analysis. Finally 153 smears were selected for evaluation. In this way, the sampling method was convenient and non-randomized. I) Sample collection:

Samples were obtained from the uterine cervix via Rovers Cervex-Brush. After conventional smearing on slides, the remaining material was rinsed and swirled vigorously in a vial containing Liqui-PREPTM preservative solution; the head of the brush is removed and placed into the vial. Then the labeled vial and conventional cytology slide were sent to cytology laboratory.

II) Sample processing:

Step 1: The labeled Liqui-PREPTM preservative vial specimens came to the laboratory with the head of the Rovers Cervex Brush in the preservative vial.

Step 2: Four milliliter Liqui-PREPTM cleaning solution was added into each of the centrifuge tubes.

Step 3: Each specimen vial was shaken by vortex and the specimen was carefully poured on top of the cleaning solution.

Step 4: The centrifuge tubes were centrifuged for 10 minutes at 1,000g.

Step 5: After centrifugation, the liquid was poured off and immediately the tube turned upright.

Step 6: Liqui-PREPTM Cellular Base was added into the centrifuge tube containing the cellular pellet.

Step 7: Each specimen was mixed using a vortex immediately prior to the application of the random-homogeneous, 50 micro liter sample onto the clean glass microscope slide.

Step 8: The processed slides were dried at room temperature.

Step 9: Both Conventional smears (CS) and Liquid-Based slides (LBS) were stained by Papanicolaou method.

III) Slides Evaluation:

The conventional smears (CS) and Liquid-Based slides (LBS) using Liqui-PREPTM technique taken from each patient were evaluated carefully by a professional cytopatologist. Smears were interpreted according to the Bethesda system. Adequacy for either method was determined by the presence adequate number of endocervical cells or transformation zone (TZ) component, adequate number of squamous cells and absence of obscuring factors such as inflammation, blood, thickness areas and poor-fixation.⁽¹⁰⁾ According to these criteria, adequacy for slides was classified in three groups:

1) Satisfactory, 2) Satisfactory but limited (SBL), and 3) Unsatisfactory.

The number of endocervical and transitional zone's cells per slide, covering diagnostic squamous cells (%) and degenerated cells per high power microscopic field (HPF) were also counted. Other cytomorphological changes evaluated for postmenopausal women were included: number of eosinophilic pyknotic cells and spindled cells, basophilic globules (per HPF) and the presences of basophilic filaments (+/-).

IV) Statistical analysis:

SPSS software was used for data analysis using indices of frequency percent, mean, and standard deviation for descriptive analysis. McNemar χ^2 was employed for investigative analysis, purposed for determining the association between qualitative indices used in two utilized methods. For comparing quantitative indices in two methods, regarding the lack of independence in samples or their lack of normal distribution (using Kolmogorov-smirnov) Wilcoxon test was used.

Results:

Mean of the patients' age was 42.28 which ranged from 20 to 67 years. According to menopausal status, they were divided into two age groups: 93 cases were pre-menopause and 60 cases were post menopause. In premenopausal age group: The numbers of satisfactory cases with CS were 30 (31.9%) compared to 58 (62.4%) with the LBC ($P < 0.0001$). Data for menopausal women included respectively: 11 (18.30%) and 39 (65%) and the $P < 0.0001$. The comparison of smears adequacy between two methods for premenopausal and postmenopausal age groups is demonstrated in tables 1 and 2. (figure 1)

Morphological quantitative criteria assessed for both age groups included:

EC: Endocervical cell (per/ slide)

CD: Covering diagnostic squamous epithelial cells in slide surface (%)

DC: Degenerated cell (per /HPF)

A significant difference was observed two methods in the number of degenerated cells and the amount of slide coverage by squamous cells in both age groups ($P = 0.0001$) but the quantitative amount of endocervical cells was more in CS method rather than LBC method ($P = 0.0001$). (Tables 3, 4).

Other morphologic changes (variations) assessed in menopause age group included: eosinophilic pyknotic cells, basophilic globules, spindled cells, and basophilic filaments which demonstrated a significant difference between two methods. The basophilic filaments were evaluated qualitatively as positive and negative. In CS 13 cases (21.7%) were negative and 47 ones (78.3%) were positive for filaments. All 13 negative cases in CS method were negative in LBC method also. But out of the 47 positive cases in LBC method, 13 cases (21.7%) were positive and 47 cases (78.7%) were negative (P value = 0.0001) (figure 2-5)

Table 1. Comparison of smears adequacy between CS and LBC in pre-menopausal age group .n (%)

Methods			LBC		
			satisfactory	SBL	Unsatisfactory
CS	satisfactory	30(32.3)	26(28.0)	3(3.2)	1(1.1)
	SBL	40(43.0)	22(23.7)	17(18.3)	1(1.1)
	Unsatisfactory	23(24.7)	10(10.8)	8(8.6)	5(5.4)
Total		93(100.0)	58(62.4)	28(30.1)	7(7.5)

P value = 0.0001

Table 2. Comparison of smears adequacy between CS and LBC in menopausal age group .n (%)

Methods			LBC		
			satisfactory	SBL	Unsatisfactory
CS	satisfactory	11(18.3)	10(16.7)	1(1.7)	0(0.0)
	SBL	34(56.7)	21(35.0)	13(21.7)	0(0.0)
	Unsatisfactory	15(25.0)	8(13.3)	4(6.7)	3(5.0)
Total		60(100.0)	39(65.0)	18(30.0)	3(5.0)

P value = 0.0001**Table 3:** comparison of cytomorphological changes in CS and LBC in pre- menopausal groups. (Mean+ SD)

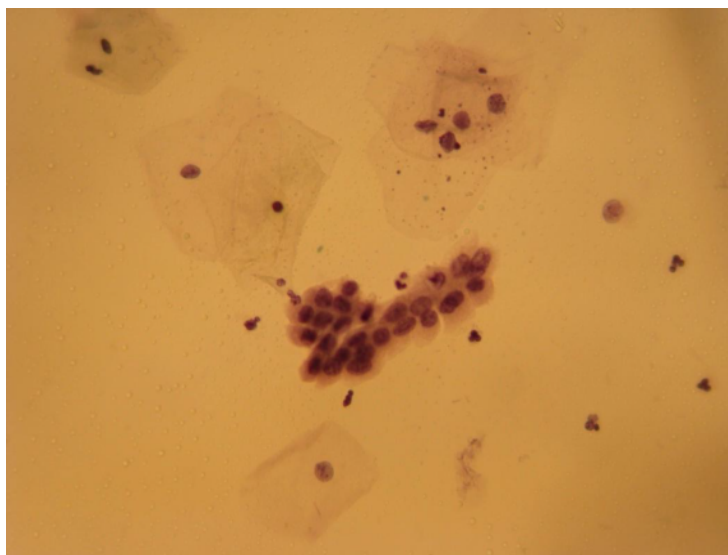
	Degenerated cells (Per/HPF)	Endocervical/TZ cells (Per/slide)	CDS* (%)
CS	4.53+ 4.44	22.17+ 36.49	21.80+ 15.18
LBC	0.36+ 0.72	8.11+ 13.53	39.25+ 20 12
P value	0.0001	0.0001	0.0001

* CDS: Covering diagnostic squamous epithelial cells

Table 4. Comparison of cytomorphological changes in CS and LBC in menopausal groups.(Mean + SD)

	Degenerated cells (Per/HPF)	Endocervical /TZ cells (Per/slide)	*CDS (%)	**EPC (Per/HPF)	Basophilic globules (Per/HPF)	spindled cells (Per/HPF)
CS	3.90+ 3.35	2.10+ 7.10	30.85+ 20.00	0.79+ 0.92	0.11+ 0.28	0.01+ 0.07
LBC	1.15 +1.45	3.58+ 3.65	40.35+ 16.18	0.62+ 0.62	0.01+ 0.12	0.34+ 0.66
P value	0.0001	0.001	0.0001	0 .085	0.010	0.0001

*EPC: Eosinophilic pyknotic cells, **CDS: Covering diagnostic squamous epithelial cells

Fig 1: Endocervical and squamous cells in LBC cervical smear of premenopausal woman (Papanicolaou stain, x400)**Fig 2:** Eosinophilic pyknotic cells in conventional cervical smear of postmenopausal woman (Papanicolaou stain , x400)

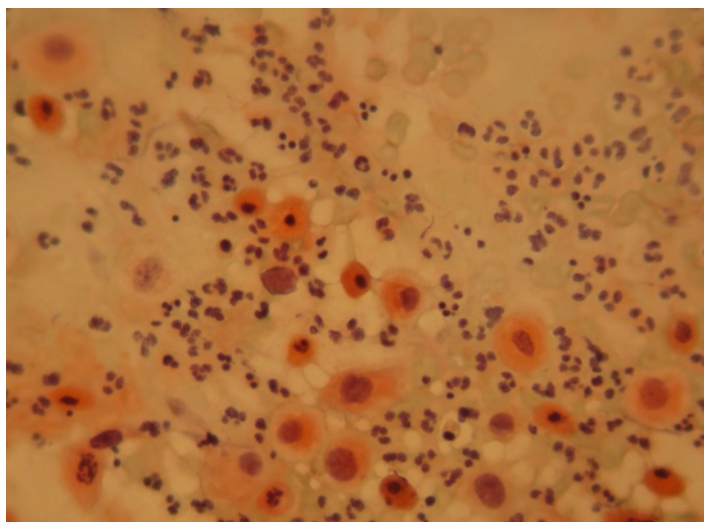


Fig 3: Spindled cells in conventional cervical smear of postmenopausal woman (Papanicolaou stain , x400)

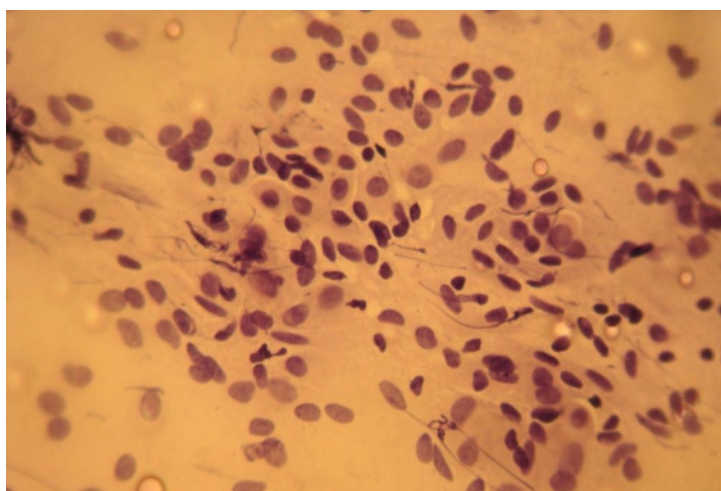


Fig 4: Basophilic filaments in conventional cervical smear of postmenopausal woman (Papanicolaou stain , x400)

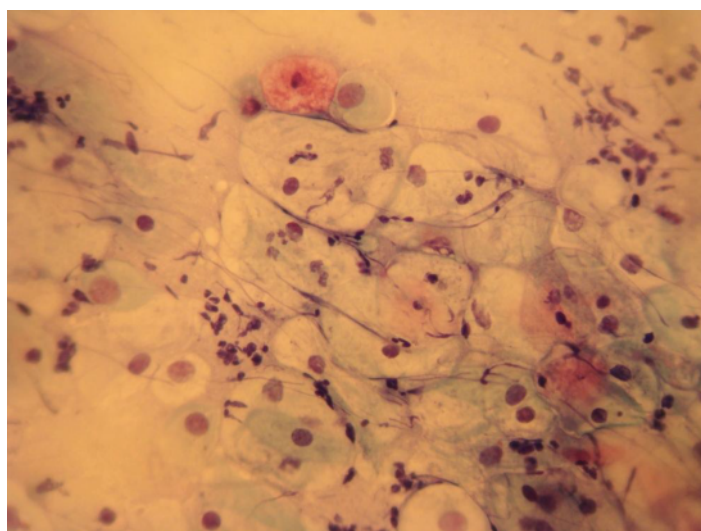
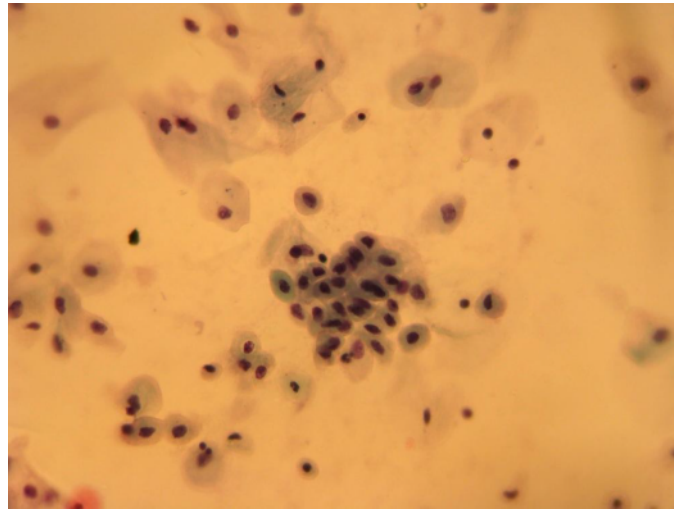


Fig 5: Atrophic parabasal cells in LBC of postmenopausal woman showing little degenerative changes (Pap-panicolaou stain , x400)



Discussion:

Cervical cancer is the only human malignancy for which effective prevention strategies exist, based on population-screening program by Pap test. These strategies have proven extremely successful, resulting in >80% reduction of cervical cancer incidence in countries where the strategies are properly implemented.⁽¹¹⁾

Screening for cervical cancer has been done using the conventional Pap smear test (CS) for more than 30 years. Despite the limited accuracy of the test, the incidence of cervical cancer has fallen substantially. Eventually, the LBC method was developed to increase accuracy.^(12 - 14)

It is of a particular importance to attain appropriate and adequate amount of smears in term of diagnostic cells because it affects the recognition of cervical pre-cancerous problems. Based on the guidelines presented by Bethesda System, smears considered to be satisfactory which meet the following criteria:

- Having at least two groups containing 5 endocervical or TZ cells.
- An adequate amount of squamous cells (at least 10%) cover the slide plane.
- Not having obscuring factors such as inflammation, blood, thick areas and cellular degeneration resulted from air-drying.⁽¹⁵⁾

The LBC technique involves rinsing all the material gathered on the sampling device into a preservative alcohol – based fluid, creating a cell suspension. This is the specimen sent to the laboratory rather than a glass slide pre-smeared with cellular material. All of the cells collected from the cervix should therefore be present in the cell suspension sent to the laboratory and the remaining cells well preserved for several weeks at room temperature in the preservative fluid. In the laboratory the cell suspension can be processed to remove excess blood and inflammatory exudates and a small representative aliquot of epithelial cells deposited in a thin layer on a glass slide. The slide preparations therefore should

contain a proportional representation of all epithelial cell types in the sample deposited within a small well-demarcated area on the glass slide. Slides from the cell suspension may be prepared manually or automatically.⁽¹⁶⁾ Most obscuring factors like mucus, blood and cell debris are diminished, as are preparation artifacts such as partial air drying, air bubbles in the mountant. With a heavy neutrophil polymorph exudate the preservative fluid tends to stick the cells together to form balls, thus not obscuring epithelial cells on the slide. Background material such as menstrual or inflammatory exudate, cytolysis and microorganisms can still be identified, although not obscuring the epithelial cells.

In our study, LBC improved diagnostic yield compared to paired, same specimen handled by the CS. Satisfactory smears in LBC method in both age groups were significantly more than the amount CS method. Moreover, cellular obscuring and cellular degeneration were significantly lower in LBC method compared to CS method. Some studies have reported higher unsatisfactory test result rates by LBC than by CC^(12,14,17) but other studies have reported the reverse.⁽¹⁸⁻¹⁹⁾ Hodgson et al.⁽²⁰⁾ reported a 46% increase in unsatisfactory results with LBC after they began to use this method. The reasons for such results were large amounts of blood or inflammation (15.7% with LBC and 80.2% with CC), thick smears, scant cellularity (82% with LBC and 9.9% with CC), cytolysis, air-drying artifacts, clumped cells or too few cells because of lubricants, and the specialty of the person obtaining the smear.⁽²⁰⁾ Williams⁽²¹⁾ reported that the rate of unsatisfactory

smears fell from 13.6% to 1.9% once he switched from CC to LBC, and that colposcopic referrals for repeated unsatisfactory smears fell from almost 25% to 0.5%. Cheung et al.⁽¹⁸⁾ reported that with ThinPrep Pap tests the rate of unsatisfactory test results was reduced from 0.48% to 0.32%; Doyle et al.⁽²²⁾ reported that productivity improved and the number of unsatisfactory cases decreased following their switching to LBC; and Nanda et al.⁽²³⁾ obtained lower rates of satisfactory-but-limited-by results by LBC than by CC (6.5% vs. 27.9%) but significantly higher rates of unsatisfactory results (2.2% vs 0.8%). In the latter study, all unsatisfactory LBC results were due to scant cellularity (cervical atrophy in older women). In the study by Davey et al.⁽¹⁷⁾, using LBC neither reduced the proportion of unsatisfactory slides, nor improved the overall performance.⁽¹⁷⁾

In our study, despite the presence of endocervical cells in the majority of slides provided through LBC method, mean of the number of these cells in CS method was higher than LBC method. This finding may be due to the fact that samples containing endocervical cells are not randomized in CS method while cellular suspension is provided randomizedly in preservative liquid in LBC method.

Morphological variations related to menopause that sometimes lead confusing diagnoses, in LBC method were significantly lower than CS method; Because the squamous epithelium in many postmenopausal women is composed of immature parabasal type cells. Such atrophic epithelia are especially subject to injury and inflammation and often show a spectrum of changes that must

be recognized as benign and not mistaken for a significant lesion. The sheets of immature cells seen in atrophy may appear crowded, and the nuclei may be elongated and hyperchromatic (spindled cells), mimicking the architectural features of squamous cell carcinoma. Cellular degeneration with dense eosinophilic or orangeophilic cytoplasm and dark, pyknotic nuclei is seen in some cases (eosinophilic pyknotic cells). Air-drying, a common artifact in atrophic specimens, causes artificial nuclear crushing "basophilic filaments" and dark blue, rounded masses known as "basophilic globules" which are thought to represent either condensed mucus or degenerated bare nuclei.⁽²⁴⁾ Apparently, because of the rapid cellular fixation that takes place in LBC method, degenerative changes (variations) related to menopause which are usually observed in CS method are reduced in LBC method.

Conclusion:

1. Liquid-PREP TM is a straight-forward cytological procedure, relying on classic cell handling procedures.
2. Cellular material is encapsulated in a matrix material that assures quantitative, robust adherence to the slide.
3. The number of cells transferred to the slide is controlled by the cytologist.
4. Liquid-PREP TM is not expensive.

Acknowledgements:

Authors wish to express their gratitude to all women who participated in this study. We would also like to thank Mr. Chehrei

and Hasanzade for their sincere help in statistical analysis.

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