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# LIQUID BASED CYTOLOGY- IS IT A GOOD ALTERNATIVE?

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## ABSTRACT

The objectives of the study were to evaluate Liquid Based Cytology (LBC) over conventional Pap smear with respect to adequacy of smear, preservation of morphological features, clarity of background, detection of infective organisms and dysplastic cells.

**Methodology**: the samples were collected at the OBG clinic using the cytobrush, and Pap staining and analysis was done in the Department of Pathology. The sample size was 114. Under speculum examination, the cytobrush was introduced into the cervical os and scraped. The material was smeared onto a slide to prepare a conventional Pap smear. The brush was then dropped into a vial containing the preservative. This sample was then subjected to processing which dispersed the sample to separate the representative cells and unwanted cell debris.

A smear was then prepared using the required cells. Both smears were stained by the routine Pap stain and examined under a microscope. Their findings were recorded and the differences were analyzed.

**Results**: There was not much difference in the sensitivity between the conventional Pap smear and LBC in detecting infective organisms. However dysplastic changes were detected in two smears using LBC whereas this was not possible using the conventional smear.

**Conclusion**: Using LBC it was possible to detect infective organisms even when their load was low. Since the cells are in a monolayer, and the smear is uniformly prepared, the quality of the smear is improved thereby decreasing the screening time and easier to read. Therefore LBC can be considered superior to conventional smear with respect to adequacy of smear, preservation of morphological features, clarity of background, detection of infective organisms like bacterial vaginosis, trichomonas vaginalis, Candida etc and dysplastic cells.

**Keywords**: LBC, Pap smear, bacterial vaginosis, trichomonas vaginalis, Candida

## INTRODUCTION

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. It is the third most common cancer among women worldwide and the most common cancer among women in India [1]. Most cervical cancers are squamous cell carcinomas (SCC), arising from the squamous epithelial cells that line the cervix. Adenocarcinoma, arising from glandular epithelial cells is the second most common type. Very rarely, cancer can arise in other types of cells in the cervix.

Cervical screening is a way of preventing cervical cancer from developing, and diagnosing the disease at an early pre-cancerous stage. Screening tests look for pre- cancerous changes in the cervix that could develop into cervical cancer. If the abnormal tissue or cells can be removed, then the disease can be prevented from developing and causing problems. The tests can also diagnose the disease by identifying

cancer cells that are already present. [10]

The Pap smear (Papanicalou test) is a conventional screening test used to detect potentially pre-cancerous and cancerous changes in the endocervical canal. In taking a Pap smear, a speculum is used to open the vaginal canal the cells from the ectocervix and endocervix are collected using a spatula, usually the Ayre's spatula. The collected material is smeared on a slide which is then placed in a container filled with fixative/preservative fluid (95% ethanol). The slide is stained using the Pap stain and examined under a microscope to detect dysplastic cells and infective organisms and mixed infections. The test may also detect infections in the endocervix and endometrium.

However this conventional test, though effective and is being widely used, has several disadvantages. Over 90% of the cells are discarded along with the spatula. Hence the cells may not be representative of the cervical mucosa. Also, the cells are not preserved adequately and maybe obscured by blood and mucous. This proves to be a hindrance while analyzing the slide and diagnosis may become difficult. There may be thicker and thinner areas of the smear and the cellular morphology may be obscured in the thicker areas. [7] Drying artifacts may be present.

Liquid Based Cytology (LBC) is a thin layer or monolayer preparation that has been introduced to overcome the disadvantages of the conventional Pap test in 1999.[2][4] In this technique, the smear sample is collected using a brush instead of a spatula. The head of the brush is removed and is put into a vial containing a methanol based preservative and is transported to the laboratory for processing where the samples are processed to separate the cervical cells and any debris (such as blood or mucus) is removed[9].Red blood cells are lysed in the transport medium. A thin layer of cervical epithelial cells are deposited in a diameter of 20mm.[11] This smear is stained with the Pap stain and the smeared slide is read under a microscope to detect dysplastic cells and infective organisms.

The smears prepared using LBC will have greater percentage of representative cells as the cells are separated from the brush during sampling. [3][6] Also, since it is a monolayer preparation, there will not be any overlapping of cells. Mucous and blood are removed during the processing and hence the cellular morphology can be clearly seen.

**The Precancerous lesions are**

Cervical intraepithelial neoplasia (CIN) is the precursor to cervical cancer. Most cases of CIN remain stable, or are eliminated by the host's immune system without intervention. However a small percentage of cases progress to become cervical cancer, usually cervical squamous cell carcinoma, if left untreated. The major cause of CIN is chronic infection of the cervix with the sexually transmitted human papillomavirus (HPV), especially the high-risk HPV types 16 or 18.

Classification for Premalignant Squamous Cervical Lesions

| DYSPLASIA/CARCINOMA IN SITU | CIN | SQUAMOUS INTRAEPITHELIAL LESION(SIL) |
|---|---|---|
| Mild Dysplasia | CIN 1 | Low-grade SIL (LSIL) |
| Moderate dysplasia | CIN II | High-grade SIL (HSIL) |
| Severe dysplasia | CIN III | High-grade SIL (HSIL) |
| Carcinoma in situ | CIN III | High-grade SIL (HSIL) |

In this study, LBC and conventional Pap smear technique are compared and evaluated for adequacy of smear, clarity of background, preservation of morphological features, detection of dysplastic cells and infective organisms to determine whether LBC is superior to the conventional Pap smear.

## REVIEW OF LITERATURE

Bourgin's Comparative study of liquid Based Cytology versus conventional Pap smear, it was found that there was improvement in the quality of the smear using LBC and there was increased diagnosis of ASCUS and LSIL. However there was no increase in the diagnosis of HSIL.

Sulik et al (2001) based their evaluation on five studies – three comparing LBC and Pap smear results with a reference test of colposcopy and biopsy and two using expert consensus as the reference standard, with biopsy for at least 50 per cent of the women showing significant abnormalities on either or both tests. In none of the five studies was there a significant difference between the two methods. Compared to Pap test, LBC showed higher sensitivity (90 versus 79 per cent) and a lower specificity (85 versus 89 per cent). LBC specimens were more likely to be reported as satisfactory. No difference in the number of unsatisfactory test results for Pap smear or LBC was reported. With LBC there was a six per cent higher rate of absence of endocervical cells but a 10 per cent decrease in reports of 'satisfactory but limited by exudates and inflammatory material.'[2]

The LBC pilot study in Wales has demonstrated a significant reduction in the number of inadequate smears and a fall in the number of women referred for colposcopy. Smear takers found LBC simple and easier to use. The significant reduction in the inadequate rate results in fewer repeat smears and hence reduces the workload for all members of the primary care team, with the benefit of less anxiety and discomfort for women. Our study is also correlated with this.

In a study done by Vassilakos *et al*., 2000 sensitivity of conventional was found to be 88.6% and LBC smears 91.0%.The review by Moseley & Paget (2002) identified several split-sample studies reporting increased detection of abnormalities with LBC.

In this study, LBC and the conventional Pap smear are compared by comparing the sensitivity and specificity of both in detecting infective organisms and dysplastic cells.

## AIMS AND OBJECTIVES

**Objective:** To evaluate liquid based cytology over conventional Pap smear with respect to adequacy of smear, preservation of morphological features, clarity of background, detection of infective organisms and dysplastic cells.

Adequacy of smear is evaluated based on whether or not endocervical cells are present. Preservation of morphological feature is graded based on the extent of clumping of cells. Clear background is indicated by the presence or absence of RBCs and mucous.

**Hypothesis**: Liquid Based Cytology offers more advantages over the conventional Pap smear in terms of adequacy of smear, preservation of morphological features, clear background, detecting infecting organisms and dysplastic cells.

## MATERIALS AND METHOD

### MATERIALS

1. LBC kit comprising of sampling brush and vial containing preservative.
2. Patients- 114 women between 18-55 years attending Obstetrics and Gynecology (OG) clinic in which the Pap test is indicated will be included in this study.
3. Research microscope and photo micrographic unit.

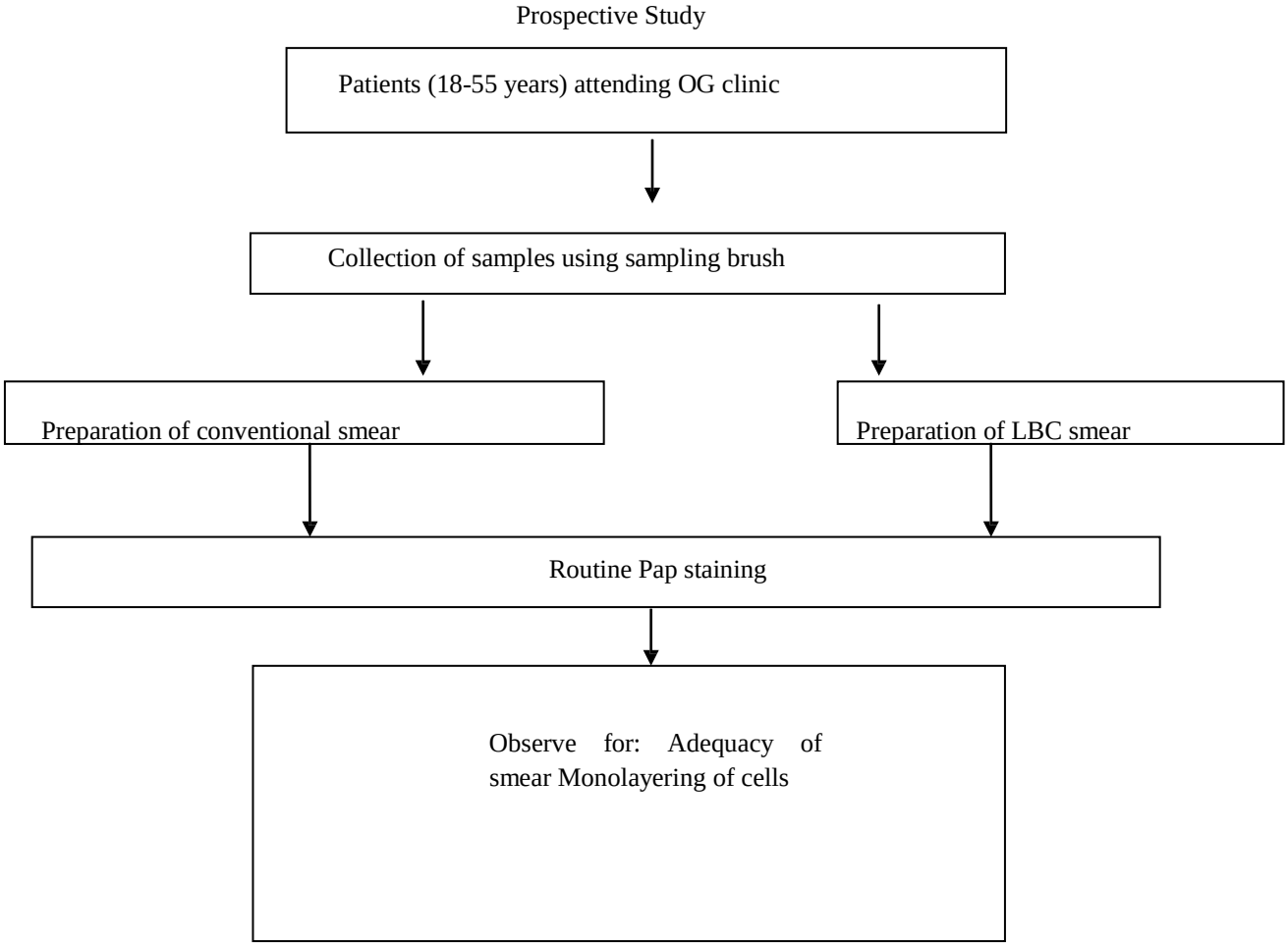
We excluded the pregnant women and women having their menstrual cycle.

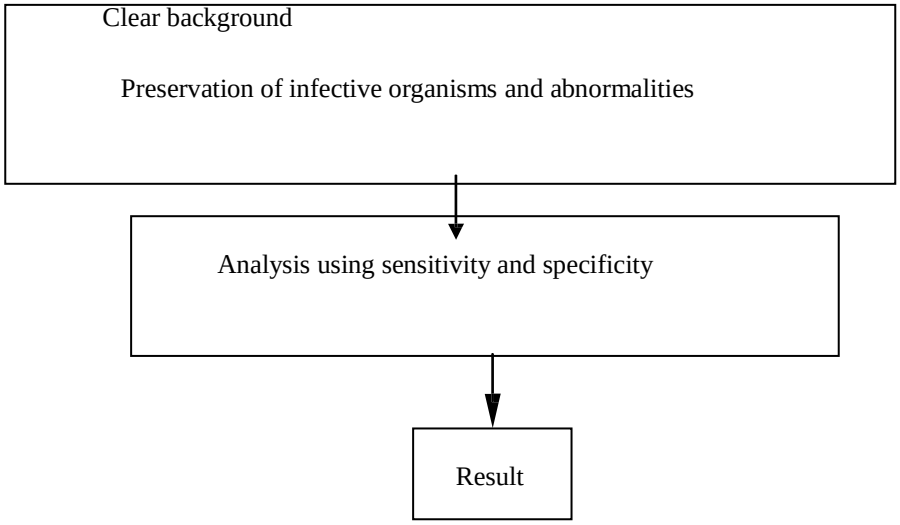
### METHOD

In this study the Pap smear sample from the same patient was used to prepare the conventional Pap and LBC smears (FIG-1). The samples were collected at the Obstetrics and Gynecology clinic, and Pap staining and analysis was done in the Department of Pathology. The sample size was

114.

Under speculum examination, the cytobrush was introduced into the external cervical os and scraped to collect the cells from the endocervix and ectocervix. The material was smeared onto a slide to prepare a conventional Pap smear. The brush was then dropped into a vial containing the preservative fluid. This sample was then subjected to processing which dispersed the sample to separate the representative cells and unwanted cell debris. A smear was then prepared using the required cells. Both smears were stained by the routine Pap stain and the slides examined under a microscope by the researcher along with a Pathologist. Our findings were recorded and the differences were analyzed.






**OBSERVATION AND RESULTS**

In this study, LBC and the conventional Pap smear (of the same patient) were compared for adequacy of smear, clarity of background, preservation of morphological features, detection of dysplastic cells and infective organisms.

| Total number of slides(sample size)=114 | | |
|---|---|---|
| | **CONVENTIONAL PAP SMEAR** | **LIQUID BASED CYOLOGY (LBC)** |
| Number of smears lacking a clear background(obscured by mucin) | 114 | 2 |
| Number of adequate smears | 105 | 112 |
| Number of smears where cellular morphology was obscured due to clumping of squamous cells(more than 75% of the slide) | 23 | 2 |
| Number of smears where cellular morphology was obscured by inflammatory cells(more than 75% of the slide | 8 | 5 |
| Number of smears positive for Bacterial Vaginosis(BV) | 8 | 8 |
| Number of smears positive for *Trichomonas vaginalis* | 4 | 5 |
| Number of smears positive for *Candida* | 4 | 6 |
| Number of smears in which dysplastic cells(LSIL/ASCUS) were detected | 0 | 2 |

LSIL-Low grade squamous Intraepithelial Lesion; ASCUS-Atypical Squamous Cell of Undetermined Significance

| | **CONVENTIONAL PAP SMEAR** | | **LIQUID BASED CYOLOGY (LBC)** | |
|---|---|---|---|---|
| | Sensitivity | Specificity | Sensitivity | Specificity |
| Bacterial Vaginosis | 100% | 100% | 100% | 100% |
| *Trichomonas vaginalis* | 80% | 99.9% | 83.33% | 98% |
| *Candida* | 66.67% | 99% | 75% | 96% |

**DISCUSSION**

In this study, LBC and the conventional Pap smear (of the same patient) were evaluated for adequacy of smear, clarity of background, preservation of morphological features, detection of dysplastic cells and infective organisms. In LBC, the brush which was used to obtain the sample was subjected to processing so that the required cells were separated from unwanted material like mucin etc. This also ensured that the entire sample of cervical squamous cells is used up in contrast to the conventional smear where over 90% of the collected cells were discarded with the sampling device. Hence using LBC it was possible to have a greater number of representative cells than the conventional smear.

The smears prepared using LBC were monolayer preparations. Hence clumping and overlapping of cells were almost absent in LBC (FIG-3). This ensured that the cellular and nuclear morphology was preserved which helped in accurate diagnosis. However, since the conventional smears were not monolayer preparations, they were not of uniform thickness, i.e., there were thicker and thinner areas of the smear resulting in clumping and overlapping of cells (FIG-2). This obscured the morphology of the cell thereby making it difficult to read the smear.

According to the Bethesda System Categories for Specimen Adequacy, an adequate smear is defined as 'well defined and well visualized squamous epithelial cells should cover more than 10% of the slide surface.' A smear that is satisfactory for evaluation is one in which squamous epithelial cells cover more than 10% of the slide surface and obscuring elements are less than 50-75%. A smear may be unsatisfactory for evaluation because of insufficient squamous component and obscuring elements cover more than 75% epithelial cells.

It is essential that the background of the cells in the smear is clear and free of mucous and other artifacts so that sufficient light can penetrate the slide and illuminate the cells clearly. In LBC, mucin and other artifacts were removed in the procedure of processing. Hence only the representative cells were present and the background was completely free of mucin and other obscuring artifacts(FIG-5).

This ensured that the cells were clearly seen against a bright background. In the conventional smear, mucous along with the required cells were smeared on the slide(FIG-4). In addition, drying artifacts were also present. This obscured the background and also the cellular morphology resulting in poor illumination of the thereby making it difficult to study the smear. Therefore using LBC, it was possible to overcome these shortcomings of a conventional smear.

Since a greater number of representative cells were present in LBC and the cellular morphology is preserved there was an increase in the detection dysplastic cells. Also it was possible to detect infective organisms even when their load was low. Hence the rates of unsatisfactory smears were low and it was possible to avoid recalling the patient to repeat the smear.

However, in case of the conventional smear, due to decreased number of representative cells, lack of clear background, overlapping and clumping of cells, it was difficult to detect dysplastic cells. Since over 90% of the material was discarded along with the sampling device it was difficult to detect infective organisms when their load is low.

| | LBC | COVENTIONAL PAP SMEAR |
|---|---|---|
| SAMPLING DEVICE | Cytobrush | Ayre's spatula |
| COLLECTED MATERIAL | Subjected to processing to remove mucous and other unwanted artifacts | Directly smeared onto slide |
| SMEAR | Contains representative cells only | Contains representative cells, mucous and artifacts |
| THICKNESS OF SMEAR | Monolayer | Uneven; thinner and thicker areas may be present |
| READING TIME | Shorter due to better layout and better presentation of smear | Longer due to clumping and overlapping of cells and presence of artifacts |

However the disadvantage of LBC is its high cost- cost of transport of vials, cost of the equipment, cost of processing etc. This must be taken into consideration, especially when screening on a large scale.

| ADVANTGES OF LBC | DISADVANTAGES OF LBC |
|---|---|
| Better presentation on slide | Cost of transport, equipment and processing |
| Increased detection of infective organisms and dysplastic cells | |
| Shorter screening time | |
| Lower rate of unsatisfactory smears | |

Hence the benefits and disadvantages must be taken into consideration in order to consider a shift from conventional Pap smear to LBC.

The detection of dysplastic cells and CIN can be further studied more extensively using a larger sample size.

## CONCLUSION

The safety or risks associated with collection of cervical cells for an LBC test for cervical screening have not been evaluated and reported in the literature. None of the appraised studies reported these outcomes or technical problems arising for women undergoing primary screening using LBC technologies, although any risk is likely to be associated with collection of cervical cells and not the tests themselves.

Liquid Based Cytology has been found to be superior to the conventional Pap smear with respect to adequacy of smear, clearity of background, and absence of interfering debris. Also diagnosis of *Trichomonas vaginalis* and *Candida* is better using LBC.

Hence LBC is a better alternative to conventional smear because of lower rate of unsatisfactory smears and short screening time. Also, due to better quality of the smear helps to avoid repeating the smear and easier to read. Due to lower rates of unsatisfactory smears and decreased incidence of recalling the patient, LBC can also prove to be cost effective.

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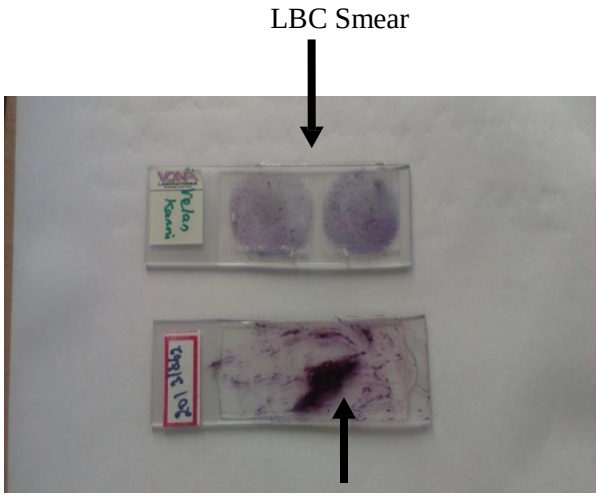


**Fig-1: LBC and conventional smear**

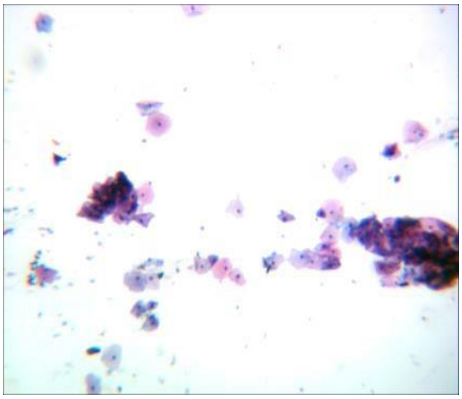

**Fig-2: Clumping of cells seen in conventional smear**

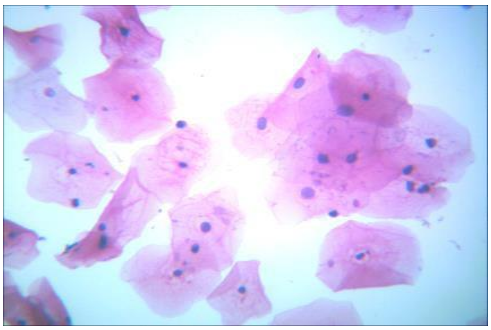

**Fig-3: Cellular and nuclear morphology retained in LBC smear**

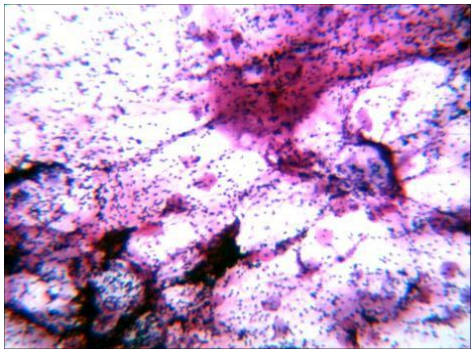

**Fig-4: Conventional smear showing abundant mucin**

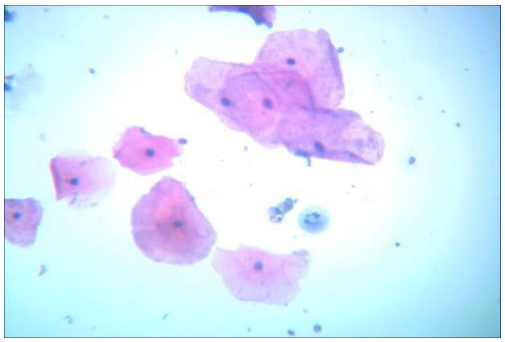

**Fig-5: LBC smear showing clear background**