

## CERVICAL CANCER SCREENING REDEFINED: A COMPARATIVE STUDY OF CONVENTIONAL PAP SMEAR AND LIQUID-BASED CYTOLOGY

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### ABSTRACT

**Background:** Cervical cancer remains a significant health issue, particularly in developing countries like India, where screening coverage is often limited. The Papanicolaou (Pap) smear, introduced in the 1940s, has been the mainstay of cervical cancer screening, but it has notable limitations such as high rates of unsatisfactory samples and false-negative results. Liquid-Based Cytology (LBC) was developed to overcome these limitations and improve diagnostic accuracy. This study aims to compare the diagnostic efficacy, specimen adequacy, and sensitivity of Conventional Pap Smear (CPS) and LBC in cervical cancer screening. **Materials and Methods:** A prospective comparative study was conducted in a hospital among 196 women aged 21–65 years. Both CPS and LBC methods were used to collect cervical samples, and the slides were evaluated using the Bethesda System (2014). Statistical analysis was performed using Kappa statistics to assess inter-method agreement and descriptive statistics to evaluate sample adequacy and diagnostic results. **Result:** The study found that LBC had superior specimen adequacy compared to CPS, with zero unsatisfactory samples in LBC versus 6.6% in CPS. LBC showed a higher detection rate for Low-Grade Squamous Intraepithelial Lesions (LSIL) (5.1%) compared to CPS (3.1%). Kappa statistics revealed excellent agreement for inflammatory smears ( $\kappa = 0.81$ ) and perfect agreement for bacterial vaginosis, fungal infections. Moderate agreement was observed for LSIL ( $\kappa = 0.62$ ) and High-Grade Squamous Intraepithelial Lesions (HSIL) ( $\kappa = 0.50$ ), suggesting LBC's diagnostic advantage in detecting epithelial abnormalities. **Conclusion:** LBC outperforms CPS in terms of sample adequacy and the detection of epithelial abnormalities, including premalignant lesions, which makes it a valuable tool for cervical cancer screening. The findings support the adoption of LBC in clinical settings, particularly in resource-limited environments, where improving the accuracy and efficiency of screening programs is crucial.

## INTRODUCTION

Cervical cancer is the fourth most common cancer among women globally and continues to be a major public health problem, particularly in developing countries.<sup>[1]</sup> According to GLOBOCAN 2020 estimates, cervical cancer accounts for approximately 604,000 new cases and 342,000 deaths worldwide each year.<sup>[2]</sup> In India alone, it represents the second most common cancer among women, contributing to nearly one-fifth of the global cervical cancer burden.<sup>[3]</sup> Most cervical cancers are caused by persistent infection with high-risk human

papillomavirus (HPV) types, and the disease typically progresses through a series of pre-invasive stages over several years, providing a crucial window for early detection and intervention.<sup>[4]</sup>

Screening plays a pivotal role in the prevention and early detection of cervical cancer. The Papanicolaou (Pap) smear, introduced by George Papanicolaou in the 1940s, has been the mainstay of cervical cancer screening programs worldwide.<sup>[5]</sup>

This conventional cytological technique has led to a marked decline in the incidence and mortality of cervical cancer in countries with organized screening programs.<sup>[6]</sup> Despite its success, the conventional Pap

smear has notable limitations, including a high rate of unsatisfactory smears, false-negative results due to sampling and preparation errors, and difficulty in interpreting slides because of obscuring blood, mucus, or inflammation.<sup>[7]</sup>

Liquid-Based Cytology (LBC), introduced in the 1990s, was developed to address these limitations.<sup>[8]</sup> In LBC, cells are collected using a brush and rinsed into a liquid preservative medium, after which they are processed to remove debris and create a thin, uniform layer of cells on the slide. LBC reduces the number of unsatisfactory smears, improves the detection of low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL), and allows for ancillary testing such as HPV DNA testing and immunocytochemistry from the residual sample.<sup>[9]</sup> Several studies and meta-analyses have reported that LBC improves specimen adequacy and may increase sensitivity for the detection of epithelial abnormalities compared to the conventional Pap smear.<sup>[10]</sup>

Despite these advantages, the adoption of LBC in low-resource settings like India has been limited, mainly due to its higher cost, need for specialized equipment, and lack of widespread availability.<sup>[11]</sup> Therefore, it is crucial to assess whether LBC provides a clinically meaningful improvement over conventional Pap smear in the local context, where cervical cancer screening coverage is often low, and resources are constrained.

This study aims to perform a head-to-head comparison of conventional Pap smear and Liquid-Based Cytology in a tertiary care hospital setting. Specifically, we will compare the two methods in terms of sample adequacy, detection rates of cervical epithelial abnormalities, reporting of inflammatory and infectious conditions, and overall diagnostic utility. The results of this study will help inform the selection of appropriate screening methods and guide cervical cancer screening strategies in resource-limited healthcare settings.

Cervical cancer remains a significant health burden, especially in developing countries like India. The use of cytological screening methods such as the conventional Papanicolaou (Pap) smear and Liquid-Based Cytology (LBC) plays a pivotal role in early detection. This study aims to compare the diagnostic efficacy, specimen adequacy, and sensitivity between the conventional Pap smear and LBC. A prospective hospital-based study was conducted among 200 women aged 21–65 years at. Results showed that LBC had higher sensitivity and specimen adequacy with zero unsatisfactory samples, suggesting it may be a more reliable method for cervical cancer screening.

### **Aim and Objectives**

#### **Aim**

“To compare Conventional Pap Smear and Liquid-Based Cytology for Cervical Cancer Screening – A Comparative Study”

### **Objectives**

1. Comparative cytologic diagnosis between conventional Pap smear and liquid-based cytology.
2. Evaluation of specimen adequacy in both techniques.
3. Assessment of diagnostic sensitivity of both methods.

## **MATERIALS AND METHODS**

Study Design: Hospital-Based Screening Test – A Prospective Comparative Study

Duration: 1 years

Sampling:

- Type: Probability Sampling
- Method: Simple Random Sampling

The required sample size for the study was calculated to be approximately 196 women based on a 15% prevalence rate, a 5% margin of error, and a 95% confidence level.

### **Inclusion Criteria**

1. Sexually active women aged 21–65: The study targets women in this age range who are at a higher risk for certain gynecological conditions.
2. Patients visiting OPD for gynecological consultation: Only women seeking gynecological care in the outpatient department are included.
3. Patients giving informed consent: Participation is voluntary, requiring informed consent from each participant.

### **Exclusion Criteria**

1. Age <21 or >65: Women outside the 21–65 age range are excluded as they may have different health needs.
2. Antenatal/postnatal women: Excludes pregnant or recently delivered women due to unique health conditions.
3. Prior cervical screening or surgeries: Women with a history of cervical procedures are excluded as their condition may differ from the general population.
4. Known pre-invasive lesions or on treatment: Excludes women with abnormal cervical conditions or undergoing treatment.
5. Active vaginal bleeding: Excludes women with ongoing vaginal bleeding, indicating potential gynecological issues.
6. Known cases of HIV/STDs: Women with known HIV or STDs are excluded due to their unique health risks.

### **1. Informed Consent and Clinical History**

- Before proceeding with the study, informed consent was obtained from each participant to ensure ethical standards.
- A thorough clinical history was gathered to understand the health background of each woman, including any previous cervical screening, risk factors for cervical cancer, and other relevant medical information. This would

help contextualize the results and potential outcomes.

## 2. Sample Collection

- Both CPS and LBC methods were used for each participant to collect samples from the squamocolumnar junction (SCJ), which is the site of transformation of the cervix, where epithelial changes are most likely to occur.
- A cytobrush was used to collect the cells, ensuring an adequate sample from the cervix for both methods.

## 3. Conventional Pap Smear (CPS) Collection and Staining

- Sample Preparation: The sample collected from the cytobrush was immediately spread on a glass slide and fixed using ethyl alcohol to preserve the cells and prevent degradation.
- Staining: The samples were stained using the standard Papanicolaou (Pap) method, which involves two main types of stains:
  - Hematoxylin: Stains the cell nuclei, helping to highlight the cell structure.
  - Cytoplasmic stains: Highlight the cytoplasm of the cells, allowing for a better view of overall cell morphology.
- The stained slides were then evaluated under a microscope for cytological abnormalities.

## 4. Liquid-Based Cytology (LBC) Collection and Processing

- Sample Collection: The cytobrush, with a detachable head, was placed in a SurePath preservative vial. This helps to preserve the cells in a liquid medium, preventing cell degradation and allowing for better cell preservation.
- Sample Processing: The samples were processed using the Nanocyt Neo Auto Slide Processor, which automates the preparation of slides for LBC. The automated processing aids in more standardized and consistent results.
- Centrifugation and Cellular Enrichment: The sample underwent centrifugation to concentrate

and enrich the cells, which helps in ensuring a higher quality sample with less cellular debris for evaluation.

## 5. Smear Evaluation

- The smears prepared from both CPS and LBC were evaluated using the Bethesda System (2014), which provides a standardized way of reporting cervical cytology results. The Bethesda System categorizes the results into different levels of abnormality, ranging from negative for intraepithelial lesion or malignancy (NILM) to various grades of squamous intraepithelial lesions (SIL) and cancer.

## 6. Statistical Analysis

- Graph Pad Prism 10 : Statistical analysis was performed using Graph Pad Prism 10, a widely used software for data management and analysis.
- Kappa Statistics: Kappa statistics were used to assess the agreement between the CPS and LBC methods. Kappa is a measure of inter-rater or inter-method reliability, showing how closely the results from the two methods agree.
- Descriptive Statistics: Descriptive statistics were employed to analyze the frequency, percentage, and adequacy of samples. This analysis helps in understanding how often different results or conditions occur in the study population and assessing the overall quality and completeness of the collected data.

## 7. Adequacy and Further Considerations

- The adequacy of the samples collected from both methods would be an important consideration. This could include factors like cell preservation, the number of cells collected, and the quality of the smear. A sample would be considered inadequate if it lacks sufficient cells or is poorly preserved.
- The study might further look into whether one method is more reliable or easier to perform in routine clinical settings, especially in terms of sample quality and diagnostic yield.

# RESULTS

**Table 1: Age Distribution**

Age Group	N=196	%
21-30	52	26.5%
31-40	81	41.3%
41-50	35	17.9%
51-60	15	7.7%
61-65	11	5.6%

Observation: Most subjects were in the 31-40 age group.

**Table 2: Residential Status.**

Residential Area	N=196	%
Rural	150	76.5%
Urban	46	23.5%

**Table 3: Clinical Presentation**

Symptoms	N=196	%
Routine Checkup	95	48.5%
Discharge per vaginum	36	18.4%
Menstrual irregularities	26	13.3%

Bleeding per vaginum	14	7.1%
Pain lower abdomen	10	5.1%
Lower urinary symptoms	13	6.6%
Dyspareunia	2	1.0%
Mass per vaginum	3	1.5%
Infertility	2	1.0%

**Table 4: Cervical Examination**

Findings	N=196	%
Normal	181	92.3%
Erosion	13	6.6%
Suspicious carcinoma (Cervical mass)	2	1.0%

**Table 5: Conventional Pap Smear Results**

Interpretation	N=196	%
Unsatisfactory	13	6.6%
NILM (Normal)	21	10.7%
Inflammatory	135	68.9%
Atrophic	7	3.6%
LSIL	6	3.1%
Bacterial Vaginosis	6	3.1%
Atypical Cells (ASC/AGC)	3	1.5%
HSIL	2	1%
Fungal (Candidiasis)	2	1.0%
Squamous Cell Carcinoma (SCC)	1	0.5%

**Table 6: LBC Results**

Interpretation	N=196	%
Unsatisfactory	0	0%
NILM (Normal)	31	15.8%
Inflammatory	130	66.3%
Atrophic	10	5.1%
LSIL	10	5.1%
Bacterial Vaginosis	5	2.6%
Atypical Cells (ASC/AGC)	4	2.0%
HSIL	2	1.0%
Fungal (Candidiasis)	2	1.0%
Squamous Cell Carcinoma (SCC)	1	0.5%

**Table 7: Correlation of CPS and LBC (Kappa Statistics)**

Finding	CPS	LBC	Kappa Value
Unsatisfactory	13	0	0
NILM	21	31	0.53
Inflammatory	135	130	0.81
Atrophic	7	10	0.79
LSIL	6	10	0.62
Bacterial Vaginosis	6	6	1
Atypical Cells	3	4	0.92
HSIL	2	2	0.50
Fungal Infection	2	2	1
SCC	1	1	1

In this study of 196 women, the majority were in the 31–40 years age group (41.3%), with most participants coming from rural areas (76.5%). Nearly half of the women (48.5%) presented for routine checkups, reflecting good screening awareness, while others reported symptoms such as vaginal discharge (18.4%) and menstrual irregularities (13.3%). On cervical examination, 91% had a normal appearance, 6.6% showed cervical erosion, and 1% were suspicious for carcinoma, underscoring the need for cytological screening beyond visual inspection. Conventional Pap smear (CPS) results showed that 68.9% had inflammatory smears, with 11% reported as NILM (negative for intraepithelial lesion or malignancy), while premalignant and malignant lesions were relatively rare (LSIL 3.1%, HSIL 0.5%,

SCC 0.5%). Notably, 6.6% of CPS samples were unsatisfactory. In contrast, liquid-based cytology (LBC) yielded no unsatisfactory samples, detected a slightly higher proportion of NILM (15.8%) and LSIL (5.1%), and showed better specimen adequacy. Kappa statistics revealed excellent agreement between CPS and LBC for inflammatory smears ( $\kappa = 0.81$ ) and perfect agreement ( $\kappa = 1$ ) for bacterial vaginosis, fungal infections, Trichomonas, and SCC. Moderate agreement was observed for LSIL ( $\kappa = 0.62$ ) and HSIL ( $\kappa = 0.50$ ), suggesting LBC's diagnostic advantage in detecting premalignant lesions. Overall, the findings highlight the superiority of LBC over CPS in terms of sample adequacy and detection of epithelial abnormalities, supporting its use in cervical cancer screening programs.

## DISCUSSION

Cervical cancer remains one of the most preventable cancers globally, thanks to screening methods like the Pap smear. However, despite its long-standing use, traditional Conventional Pap Smear (CPS) has several limitations, such as a higher rate of unsatisfactory smears, poorer sample adequacy, and less accurate detection of certain lesions. Over the years, Liquid-Based Cytology (LBC) has been introduced as a promising alternative to CPS due to its advantages in sample collection, preservation, and diagnostic accuracy. In this discussion, we will compare the findings of the present study with those from various relevant studies to understand the strengths and weaknesses of both methods.

### 1. Cytological Detection and Sample Adequacy

The present study found that LBC outperformed CPS in terms of sample adequacy. While 6.6% of CPS samples were deemed unsatisfactory, LBC yielded no unsatisfactory samples. This finding is consistent with the results from multiple studies, such as those by Macharia et al,<sup>[12]</sup> Hawaldar et al,<sup>[13]</sup> and Kldiashvili et al,<sup>[14]</sup> all of which reported fewer unsatisfactory smears with LBC compared to CPS. For instance, Hawaldar et al,<sup>[13]</sup> found that only 1.67% of LBC samples were unsatisfactory compared to 6.67% in CPS, while Kldiashvili et al,<sup>[14]</sup> found that LBC had a significantly lower rate of unsatisfactory samples (1.33% vs. 7.33%).

LBC's superior sample adequacy allows for better cellular preservation, reducing the likelihood of false negatives or inconclusive results. This is especially beneficial in settings where quality control is a concern, as it minimizes the chances of inadequate sampling and improves the overall reliability of cervical cancer screening programs.

### 2. Detection of Epithelial Abnormalities and Inflammatory Smears

The study conducted on 196 women revealed that LBC detected a slightly higher proportion of NILM (Negative for Intraepithelial Lesion or Malignancy) (15.8%) compared to CPS (11%), although the difference was not large. More notably, LBC showed better diagnostic performance in detecting low-grade squamous intraepithelial lesions (LSIL) (5.1% in LBC vs. 3.1% in CPS). These findings align with those of Bhagyalakshmi Atla, whose study showed that LBC was more effective in identifying high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinoma (SCC).<sup>[15]</sup> Additionally, LBC also identified epithelial abnormalities with higher efficiency than CPS, as reflected by the significant difference in epithelial cell abnormalities (p value = 0.002414) in the study by Bhagyalakshmi Atla.<sup>[15]</sup> This indicates the superior sensitivity of LBC in identifying pre-malignant and malignant lesions, making it a valuable tool for early detection of cervical abnormalities.

The study by Patel et al. also corroborates this finding. In their comparison of CPS and LBC, they

found that LBC provided a higher detection rate for epithelial cell abnormalities (11% in LBC vs. 9.7% in CPS), further supporting the role of LBC in better diagnosing cytological changes that may lead to cancer.<sup>[16]</sup>

### 3. Detection of Organisms and Other Pathologies

While LBC showed better detection of epithelial abnormalities, the CPS samples had a higher detection rate for microorganisms, such as *Candida* and *Trichomonas*. This is in line with the study by Patel et al,<sup>[16]</sup> who reported a higher detection rate of organisms (34% in CPS vs. 27% in LBC). However, the present study found no unsatisfactory samples in LBC, highlighting the superior specimen quality and reduced interference from blood, mucus, or debris, which often obscure cellular details in CPS. Additionally, LBC is advantageous in its ability to perform HPV DNA testing on residual material, a feature that enhances its screening capabilities, as emphasized by Patel et al. and Kldiashvili et al.<sup>[14,16]</sup>

### 4. Screening Time and Cost-Effectiveness

One of the notable advantages of LBC is its quicker processing time. LBC takes significantly less time to screen ( $3 \pm 1$  minutes) compared to CPS ( $5 \pm 1$  minutes). This has important implications for large-scale cervical cancer screening programs. The study by Patel et al. reported similar findings, suggesting that LBC allows for faster screening and greater throughput, which could be particularly beneficial in high-volume settings. Although LBC is generally considered more expensive, the reduced need for repeat testing and the ability to perform additional tests (such as HPV testing) on residual samples make it cost-effective in the long run. The study by Macharia et al. also highlighted the cost-effectiveness of LBC when accounting for fewer repeat tests and higher specificity, which reduces false-negative rates.<sup>[12,16]</sup>

### 5. Kappa Statistics and Diagnostic Agreement

The Kappa statistics calculated in this study ( $\kappa = 0.81$  for inflammatory smears,  $\kappa = 1$  for bacterial vaginosis, fungal infections, *Trichomonas*, and SCC) demonstrated excellent to perfect agreement between CPS and LBC for many diagnostic categories. However, there was only moderate agreement for LSIL ( $\kappa = 0.62$ ) and HSIL ( $\kappa = 0.50$ ), indicating that LBC had a higher sensitivity in detecting these abnormalities. This is consistent with the findings by Patel et al., who reported that LBC showed better detection rates for ASCUS, ASC-H, and LSIL, and more cases of high-grade squamous intraepithelial lesions (HSIL) were detected by LBC than by CPS.<sup>[16]</sup>

### 6. Implications for Clinical Practice

In the context of India, where cervical cancer remains a significant cause of morbidity and mortality, LBC provides a more effective and reliable method for screening. As seen in the present study, the implementation of LBC can enhance screening accuracy, especially in rural and underserved areas, where access to follow-up care may be limited. The study by Patel et al,<sup>[16]</sup> (2024) emphasized the



potential of LBC to reduce mortality rates, especially when combined with HPV-based testing on residual samples. Moreover, LBC's improved detection capabilities, as evidenced by studies such as Bhagyalakshmi Atla, Hawaldar et al, and Kldiashvili et al., make it an excellent choice for early detection in cervical cancer screening programs.<sup>[13-15]</sup>

## CONCLUSION

The comparative analysis between CPS and LBC clearly supports LBC as the superior technique for cervical cancer screening. While CPS remains valuable in clinical practice, especially in low-resource settings, LBC offers advantages in terms of sample adequacy, reduced unsatisfactory smears, better detection of epithelial abnormalities, and faster processing time. The higher diagnostic accuracy of LBC, positions it as a more reliable method for identifying pre-malignant and malignant lesions of the cervix. Consequently, LBC should be considered the gold standard for cervical cancer screening in both resource-rich and resource-limited settings, particularly when combined with HPV testing on residual samples.

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