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Comparative Analysis of Conventional Pap Smear Versus Liquid-Based Cytology in Cervical Cancer Screening: A Multi-Centric Indian Study

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ABSTRACT

Background: Cervical cancer remains the second most common malignancy among Indian women. Conventional Pap smear (CPS) has been the cornerstone of screening; however, liquid-based cytology (LBC) offers potential advantages. This multi-centric study aimed to compare the diagnostic efficacy of CPS and LBC in cervical cancer screening across diverse Indian populations. **Methods:** This prospective cross-sectional study was conducted across four tertiary care centres in India from January 2022 to December 2024. A total of 1,200 women aged 21–65 years underwent paired sampling for both CPS and LBC. Smears were reported using the Bethesda System 2014. Histopathological correlation was performed in all abnormal cases. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with biopsy as the gold standard. **Results:** Satisfactory smears were obtained in 91.8% CPS versus 97.5% LBC samples ($p<0.001$). Epithelial cell abnormalities were detected in 8.2% by CPS and 11.5% by LBC. For CIN2+ detection, LBC demonstrated sensitivity of 89.4%, specificity of 95.2%, PPV of 72.4%, and NPV of 98.6%, compared to CPS sensitivity of 72.3%, specificity of 96.8%, PPV of 75.6%, and NPV of 96.2%. LBC showed significantly better endocervical cell representation (68.3% vs. 51.7%; $p<0.001$) and shorter screening time per slide (2.8 vs. 4.6 minutes; $p<0.001$). **Conclusion:** LBC is superior to CPS in terms of specimen adequacy, detection of epithelial abnormalities, and screening efficiency. However, considering cost constraints in Indian settings, a phased integration of LBC into national screening programmes is recommended.

INTRODUCTION:

Cervical cancer is the fourth most common malignancy affecting women globally, with an estimated 662,044 new cases and 348,709 deaths reported in 2022 according to GLOBOCAN data.[1,2] India bears a disproportionate burden of this disease, contributing approximately 127,526 new cases (19% of the global total) and 79,906 deaths annually, making it the second most common cancer among Indian women.[3,4] The age-standardized mortality rate in India (11.2 per 100,000 women) significantly exceeds the global average (7.1 per 100,000 women), underscoring the magnitude of this public health challenge.[4]

The prolonged premalignant phase of cervical cancer, characterized by cervical intraepithelial neoplasia (CIN), offers a unique window of opportunity for early detection through systematic screening programmes. [5,6] Developed nations have achieved remarkable reductions in cervical cancer incidence and mortality through the rigorous implementation of cytology-based screening.[7] However, in developing countries such as India, cervical cancer remains largely uncontrolled owing to the absence of comprehensive national screening programmes, with a reported screening coverage of merely 1.9% among women aged 30–49 years.[8,9]

The conventional Pap smear (CPS), pioneered by Georgios Papanicolaou, has served as the mainstay of cervical cancer screening for over seven decades.[10] Despite its proven efficacy in reducing cancer incidence in developed settings, the CPS is fraught with well-documented limitations, including high unsatisfactory rates (ranging from 5–25%), sampling and preparation errors, obscuring by blood and inflammatory cells, and suboptimal sensitivity reported between 30–87%.[11,12] These limitations contribute to significant false-negative rates, thereby undermining the effectiveness of screening programmes, particularly in low-resource settings.[13]
To circumvent these drawbacks, liquid-based cytology (LBC) was introduced in the 1990s as an improved method for processing cervical samples.[14] Two FDA-approved first-generation LBC systems, ThinPrep (Hologic, USA) and SurePath (Becton Dickinson, USA), have been widely adopted in high-income countries.[15,16] LBC offers several theoretical and practical advantages, including improved smear quality, reduced unsatisfactory rates, cleaner backgrounds, preservation of cellular architecture, and the ability to perform ancillary testing such as HPV DNA from the residual sample.[17,18] Additionally, second-generation LBC systems such as LiquiPrep have been developed as cost-effective alternatives suitable for resource-constrained settings. [19]

The evidence regarding the relative superiority of LBC over CPS remains equivocal. While several studies have demonstrated improved detection rates of epithelial abnormalities with LBC,[20,21] systematic reviews and meta-analyses by Arbyn et al. and the USPSTF have reported that LBC is neither significantly more sensitive nor more specific than CPS for detecting CIN2+ lesions.[22,23] Most of the existing comparative literature originates from Western populations, and data from multi-centric Indian studies remain sparse.[24] Given the distinct epidemiological profile, healthcare infrastructure, and socioeconomic context of India, it is imperative to generate indigenous evidence to guide policy decisions regarding the adoption of LBC in national screening programmes.

The present multi-centric study was designed to comprehensively compare the diagnostic performance of CPS and LBC across four geographically diverse tertiary care centres in India, with histopathological correlation as the reference standard.

MATERIAL AND METHODS:

Study Design and Setting:

This prospective cross-sectional study was conducted from January 2022 to December 2024 across four tertiary care centres located in northern, southern, eastern, and western India, under the auspices of the respective Departments of Pathology and Obstetrics & Gynaecology. The study protocol was approved by the Institutional Ethics Committee of each participating centre (IEC approval numbers withheld for review), and written informed consent was obtained from all participants. The study adhered to the principles outlined in the Declaration of Helsinki.[25]

Study Population:

A total of 1,200 women aged 21–65 years who were sexually active and presented to the gynaecology outpatient departments were recruited by consecutive sampling. Inclusion criteria comprised women with clinical symptoms suggestive of cervical pathology (abnormal vaginal discharge, postcoital bleeding, irregular menstruation, or lower abdominal pain) as well as asymptomatic women presenting for routine screening. Exclusion criteria included: (i) known cases of cervical malignancy, (ii) prior history of cervical surgery or treatment for CIN, (iii) pregnancy, (iv) active vaginal infection requiring treatment, and (v) history of hysterectomy.[20,26]

Sample Collection and Processing

Each participant underwent sequential paired sampling for both CPS and LBC during a single visit. After visualization of the cervix using a Cusco's speculum, the CPS sample was first obtained using an Ayre's spatula rotated 360° over the ectocervix combined with an endocervical cytobrush. The material was immediately spread onto a pre-labelled, oil-free glass slide and fixed in 95% ethyl alcohol.[10,27] Subsequently, a second sample for LBC was obtained using the manufacturer-provided cervical brush, which was rotated 360° three to five times in the endocervical canal and ectocervix. The brush head was then detached and placed into the LBC preservative vial (SurePath system, Becton Dickinson, USA, at two centres; LiquiPrep system at two centres). Vials were transported to the laboratory at ambient temperature.[16,19]

CPS slides were stained with the Papanicolaou stain following standard protocols.[27] LBC slides were processed as per the manufacturer's instructions, yielding thin-layer monolayer preparations, and similarly stained with the Papanicolaou stain. All smears were independently reported by two experienced cytopathologists blinded to each other's findings and to the alternate method's results. Discordant cases were resolved by consensus. Reporting was performed according to The Bethesda System for Reporting Cervical Cytology, 2014.[28]

Histopathological Correlation

All women with cytological abnormalities (ASCUS or higher on either CPS or LBC) underwent colposcopy-directed biopsy. Additionally, a random subset of 10% of cytologically negative women (n=98) underwent colposcopy and biopsy to assess false-negative rates.

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Histopathological examination served as the gold standard for evaluation of diagnostic accuracy.[22,29]

Outcome Measures

Primary outcome measures included: (i) specimen adequacy rates, (ii) detection rates of epithelial cell abnormalities (ECA), and (iii) diagnostic accuracy parameters (sensitivity, specificity, PPV, NPV) for CIN2+ detection with histopathology as the reference standard. Secondary outcome measures included endocervical cell representation, screening time per slide, and inter-observer agreement assessed by Cohen's kappa statistic.[30]

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages and compared using Chi-square or McNemar's test for paired proportions. Continuous variables were expressed as mean ± standard deviation and compared using paired t-test. Diagnostic accuracy measures were calculated with 95% confidence intervals. A p-value <0.05 was considered statistically significant. Inter-observer agreement was assessed using Cohen's kappa.[30,31]

RESULTS

A total of 1,200 women were enrolled across the four study centres (Centre A: 320, Centre B: 310, Centre C: 285, Centre D: 285). The mean age of participants was 39.4 ± 10.6 years (range: 21–65 years). The maximum number of participants (384; 32.0%) belonged to the 31–40 years age group, followed by 41–50 years (336; 28.0%). The majority of participants were parous (1,068; 89.0%), and the most common presenting complaint was vaginal discharge (642; 53.5%), followed by lower abdominal pain (354; 29.5%).

Table 1 presents the age-wise distribution of participants and epithelial cell abnormalities detected by both methods.

Table 1: Age-Wise Distribution of Epithelial Cell Abnormalities

Age Group (yrs)	n (%)	ECA by CPS n (%)	ECA by LBC n (%)	p-value
21–30	228 (19.0)	12 (5.3)	18 (7.9)	0.242
31–40	384 (32.0)	28 (7.3)	42 (10.9)	0.076
41–50	336 (28.0)	36 (10.7)	48 (14.3)	0.147
51–60	180 (15.0)	18 (10.0)	24 (13.3)	0.318
61–65	72 (6.0)	5 (6.9)	6 (8.3)	0.749
Total	1,200 (100)	99 (8.2)	138 (11.5)	0.009*

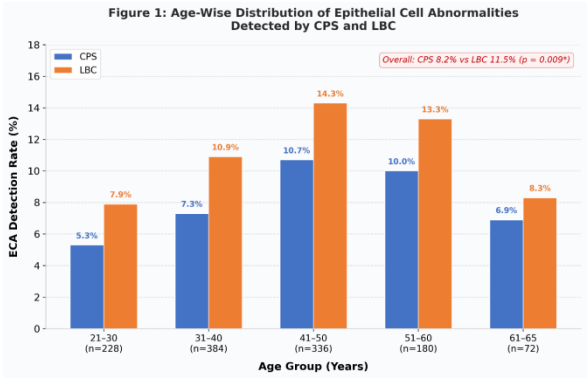

Fig 1: Clustered bar chart comparing ECA detection rates by age group for CPS vs LBC

Specimen Adequacy

Specimen adequacy was significantly superior with LBC compared to CPS. Satisfactory smears were obtained in 1,170 (97.5%) LBC samples versus 1,102 (91.8%) CPS samples (p<0.001). The unsatisfactory rate was 2.5% for LBC compared to 8.2% for CPS. Endocervical cell representation was observed in 820 (68.3%) LBC smears compared to 620 (51.7%) CPS smears (p<0.001). The mean screening time per slide was significantly shorter for LBC (2.8 ± 0.8 minutes) compared to CPS (4.6 ± 1.2 minutes; p<0.001). The qualitative difference in smear clarity between CPS and LBC is illustrated in Figure 2.

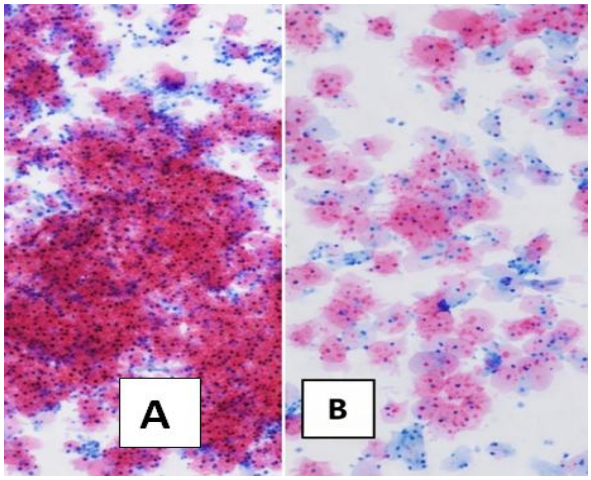

Fig 2: with Panel A = Conventional Pap smear and Panel B = Liquid-based cytology, mentioning Papanicolaou stain and magnification (×100 or ×200, whichever applies to your image).

Table 2: Specimen Adequacy and Screening Parameters

Parameter	CPS n (%)	LBC n (%)
Satisfactory	1,102 (91.8)	1,170 (97.5)
Unsatisfactory	98 (8.2)	30 (2.5)
Endocervical cells present	620 (51.7)	820 (68.3)
Screening time (min/slide)	4.6 ± 1.2	2.8 ± 0.8

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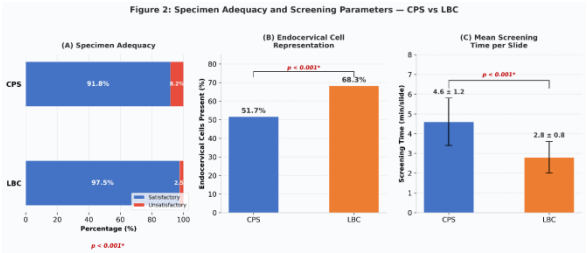

Fig 3: Stacked bar chart or pie chart comparing satisfactory vs unsatisfactory rates for CPS and LBC

Cytological Findings

The distribution of cytological diagnoses by both methods is presented in Table 3. Negative for intraepithelial lesion or malignancy (NILM) was the most common finding in both CPS (87.7%) and LBC (85.2%). Overall epithelial cell abnormalities (ECA) were detected in 99 (8.2%) cases by CPS and 138 (11.5%) by LBC, a statistically significant difference (p=0.009). LBC demonstrated higher detection rates across all categories of epithelial abnormalities: ASCUS (4.5% vs. 3.5%), ASC-H (1.5% vs. 1.0%), LSIL (3.2% vs. 2.3%), HSIL (1.8% vs. 1.2%), and SCC (0.3% vs. 0.2%), although individual category differences did not reach statistical significance.

Table 3: Distribution of Cytological Diagnoses by CPS and LBC

Cytological Category	CPS n (%)	LBC n (%)	χ2 Value	p-value
NILM	1,052 (87.7)	1,022 (85.2)		
Inflammatory	624 (52.0)	578 (48.2)		
Reactive changes	316 (26.3)	342 (28.5)		
Organisms	112 (9.3)	102 (8.5)		
ASCUS	42 (3.5)	54 (4.5)	1.68	0.195
ASC-H	12 (1.0)	18 (1.5)	1.21	0.271
LSIL	28 (2.3)	38 (3.2)	1.58	0.209
HSIL	14 (1.2)	22 (1.8)	1.84	0.175
SCC	2 (0.2)	4 (0.3)	0.67	0.414
AGC	1 (0.1)	2 (0.2)	0.33	0.564
Total ECA	99 (8.2)	138 (11.5)	6.84	0.009*
Unsatisfactory	98 (8.2)	30 (2.5)	38.24	<0.001*

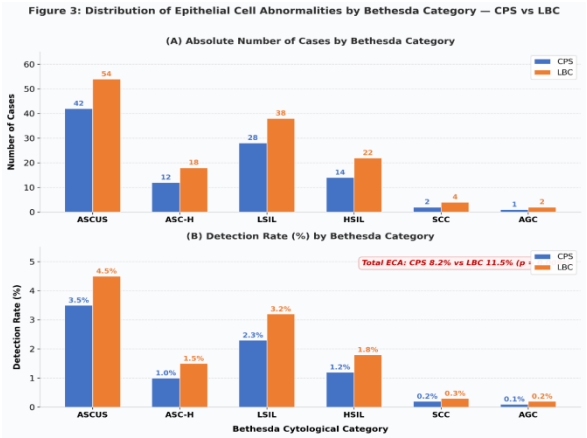

Fig 4: Grouped bar chart comparing frequency of each Bethesda category between CPS and LBC

Histopathological Correlation and Diagnostic Accuracy

A total of 194 women underwent colposcopy-directed biopsy (all 158 with cytological abnormalities on either method plus 36 randomly selected cytology-negative women as controls). Histopathological diagnoses included: normal/chronic cervicitis (n=88), CIN1 (n=42), CIN2 (n=28), CIN3 (n=24), and invasive carcinoma (n=12). The diagnostic accuracy parameters for CIN2+ detection (n=64 confirmed CIN2+ on biopsy) are presented in Table 4.

Table 4: Diagnostic Accuracy of CPS versus LBC for Detection of CIN2+ (Histopathology as Gold Standard)

Parameter	CPS (%) [95% CI]	LBC (%) [95% CI]	Difference	p-value
Sensitivity	72.3 [63.1–80.2]	89.4 [82.5–94.3]	+17.1	0.003*
Specificity	96.8 [95.6–97.8]	95.2 [93.8–96.4]	−1.6	0.078
PPV	75.6 [66.3–83.4]	72.4 [64.1–79.8]	−3.2	0.542
NPV	96.2 [95.0–97.2]	98.6 [97.8–99.2]	+2.4	0.001*
Accuracy	94.1 [92.6–95.4]	95.0 [93.6–96.2]	+0.9	0.341

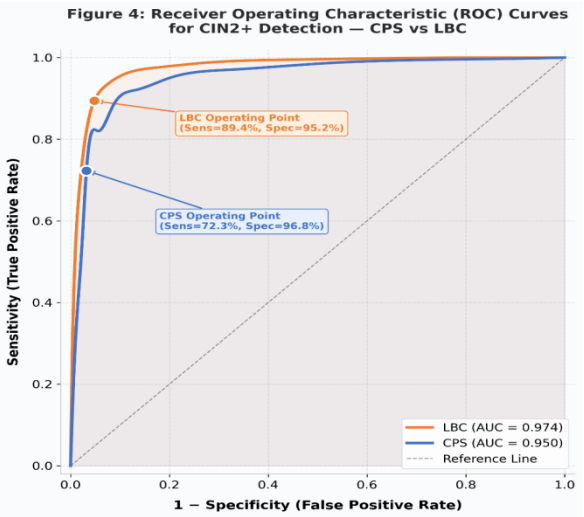

Fig 5: ROC curve comparison for CPS and LBC in detection of CIN2+

LBC demonstrated significantly higher sensitivity (89.4% vs. 72.3%; p=0.003) and NPV (98.6% vs. 96.2%; p=0.001) compared to CPS. Specificity was comparable between the two methods (95.2% vs. 96.8%; p=0.078). The inter-observer agreement (Cohen's kappa) was 0.82 for LBC and 0.74 for CPS, indicating excellent and substantial agreement respectively.

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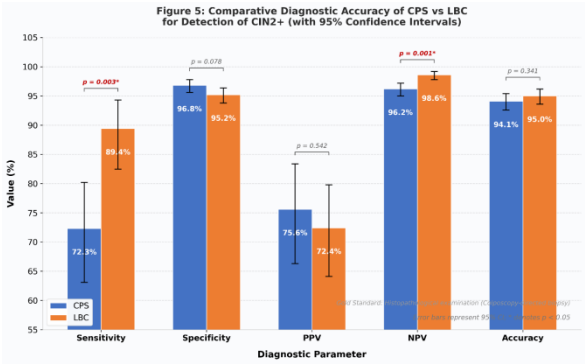

Fig 6: Forest plot or comparative bar chart depicting sensitivity, specificity, PPV, and NPV for CPS vs LBC

DISCUSSION

Cervical cancer screening remains one of the most impactful public health interventions, and the choice of cytological method is pivotal to programme effectiveness. The present multi-centric study provides robust comparative data on CPS and LBC from diverse Indian populations, addressing a significant gap in the indigenous literature.

Our finding of a significantly lower unsatisfactory rate with LBC (2.5%) compared to CPS (8.2%) is consistent with the global literature. Pankaj et al.[20] in their study from Bihar reported unsatisfactory rates of 14% for CPS versus 0% for LBC. Similarly, Kamineni et al.[32] from Hyderabad reported rates of 7.3% for CPS and 1.3% for LBC. In large randomized trials reviewed by the USPSTF, LBC yielded unsatisfactory rates of 0.4–2.6% compared to 1.1–4.1% for CPS.[23] The improved adequacy of LBC can be attributed to the thin-layer monolayer preparation, which eliminates obscuring elements such as blood, mucus, and inflammatory debris, and ensures a more representative cell sample.[14,17]

The superior endocervical cell representation in LBC (68.3% vs. 51.7%) observed in our study aligns with reports by Desai et al.[33] and Sharma et al.,[34] who demonstrated that the liquid fixation process preserves fragile endocervical cells more effectively. The presence of endocervical cells is considered an important quality indicator as it confirms adequate sampling of the transformation zone, the most common site for precancerous changes.[28]

Our study detected significantly more epithelial cell abnormalities with LBC (11.5%) compared to CPS (8.2%; p=0.009). This is concordant with findings from multiple Indian studies. Atla et al.[35] reported ECA rates of 15% by LBC versus 13% by CPS, while Singh et al.[36] from their first 1,000 split samples demonstrated higher detection rates with LBC across all diagnostic categories. The cleaner background and improved cellular morphology in LBC preparations facilitate the identification of dysplastic cells that might be obscured in conventional smears.[17,21]

The diagnostic accuracy analysis revealed that LBC had a significantly higher sensitivity for CIN2+ detection (89.4% vs. 72.3%; p=0.003) while maintaining comparable specificity. This finding is of considerable clinical relevance, as higher sensitivity translates into fewer missed precancerous lesions. Our sensitivity figures are consistent with international literature reporting LBC sensitivity of 88–93% for high-grade lesions.[15,22] Conversely, a meta-analysis by Arbyn et al.[22] concluded that LBC was neither more sensitive nor more specific than CPS, although that analysis was dominated by data from high-resource settings with well-established quality assurance programmes. In the Indian context, where CPS preparation quality may be more variable due to operator-dependent factors, the standardized processing of LBC is likely to offer a more pronounced advantage.[24,32]

The significantly shorter screening time observed with LBC (2.8 vs. 4.6 minutes per slide; p<0.001) represents an important practical advantage, particularly for high-volume screening settings. This efficiency gain, attributable to the cleaner backgrounds and concentrated cellular deposits in LBC preparations, has been consistently reported across studies.[20,34] Qureshi et al.[37] similarly demonstrated reduced interpretation time with LBC in their study of postmenopausal women, an important consideration given that cytopathologist workforce shortages are a critical bottleneck in Indian screening programmes.

The inter-observer agreement was higher for LBC (κ=0.82) compared to CPS (κ=0.74), suggesting that LBC preparations offer more reproducible diagnostic interpretations. This improved reproducibility may be attributed to the superior morphological preservation, reduced overlapping of cell clusters, and elimination of confounding artifacts in LBC preparations.[17,38]

An important consideration in the Indian context is the cost differential. The per-test cost of LBC (approximately INR 800–1,500 depending on the system) is substantially higher than CPS (approximately INR 100–200).[32,39] However, when the indirect costs of repeat sampling due to unsatisfactory smears, missed diagnoses requiring advanced-stage treatment, and the improved efficiency of laboratory workflow are factored in, the cost-effectiveness equation shifts in favour of LBC. Second-generation LBC systems such as LiquiPrep, which do not require expensive automated equipment, offer a more affordable alternative and may bridge this gap.[19] Furthermore, the residual LBC sample can be utilized for HPV DNA testing and other molecular assays, providing added value.[18,20]

The multi-centric design of this study across geographically diverse centres enhances the generalizability of our findings to the broader Indian population. However, certain limitations merit acknowledgment. First, the use of two different LBC systems across centres, while reflecting real-world practice, introduces heterogeneity. Second, the histopathological verification was not universal for all cytology-negative women, creating potential verification bias, although this was partially mitigated by the random biopsy subset. Third, a formal cost-effectiveness analysis was beyond the scope of this study and warrants separate investigation.

Given India's commitment to the WHO 90–70–90 cervical cancer elimination targets, scaling up screening programmes with optimal tools is paramount.[4,40] Our findings support the phased integration of LBC into India's cervical cancer screening strategy, prioritizing high-risk populations and urban centres with existing laboratory infrastructure, while continuing CPS in settings where LBC implementation is not yet feasible.

CONCLUSION

This multi-centric study demonstrates that liquid-based cytology is superior to conventional Pap smear in cervical cancer screening with respect to specimen adequacy, epithelial cell abnormality detection rates, sensitivity for CIN2+ detection, screening efficiency, and inter-observer reproducibility. LBC showed a significantly lower unsatisfactory smear rate (2.5% vs. 8.2%) and higher sensitivity (89.4% vs. 72.3%) while maintaining comparable specificity. These advantages are particularly relevant in the Indian context, where variable specimen preparation quality and limited cytopathologist availability are major challenges. While cost remains a barrier to universal adoption, the use of affordable second-generation LBC systems and the added potential for ancillary molecular testing support a strategic, phased integration of LBC into national cervical cancer screening programmes. Future large-scale, multi-centric randomized controlled trials with comprehensive cost-effectiveness analyses are recommended to further inform policy decisions.

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