

Comparative Analysis of Liquid-Based Cytology and Conventional Smear Cytology in Peritoneal Washings: A Prospective Study

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DOI: <https://doi.org/10.52403/ijshr.20260301>

ABSTRACT

Background: Peritoneal washing cytology is a critical tool for staging gynaecological and gastrointestinal malignancies. Liquid-based cytology (LBC) has emerged as a refined technique over conventional smear (CS) cytology, but its comparative performance in peritoneal washings requires systematic evaluation.

Aim: To compare the cytomorphological characteristics of peritoneal washings processed by LBC (BD SurePath™) and conventional smear cytology with respect to adequacy, cellularity, cell distribution, cytoplasmic and nuclear details, background clarity, and final diagnostic yield.

Methods: A prospective study was conducted over one and a half years at Government Medical College, Patiala, on 50 peritoneal washing samples. Each sample was simultaneously processed by both LBC (BD SurePath™) and conventional smear methods. Smears were stained with Papanicolaou (PAP), May-Grünwald Giemsa (MGG), and Haematoxylin & Eosin (H&E) stains and scored on a validated cytomorphological scoring system for distribution, cellularity, morphology, and background. Statistical analysis was performed using paired t-test; $p < 0.05$ was considered significant.

Results: LBC demonstrated significantly superior cell distribution (70% vs 40%, $p < 0.001$), cytoplasmic detail (80% vs 58%, $p = 0.02$), nuclear detail (88% vs 70%, $p = 0.03$), and background clarity (88% vs 2%, $p < 0.001$), with a significantly higher total cytological score (6.14 ± 2.54 vs 4.24 ± 2.76 , $p < 0.001$). Conventional smear showed better sample adequacy (96% vs 84%, $p = 0.04$) and cellularity (50% vs 24%, $p = 0.02$). Both techniques showed identical final cytological diagnosis ($\kappa = 1.000$).

Conclusion: LBC offers superior smear quality in peritoneal washings, particularly in cell distribution, morphological preservation, and background clarity, while conventional smear yields better adequacy and cellularity. Both techniques are diagnostically equivalent, and their combined use may enhance cytological evaluation.

Keywords: liquid-based cytology; conventional smear; peritoneal washings; cytomorphology; BD SurePath™; gynaecological malignancy

1. INTRODUCTION

Cytological examination of body fluids - including pleural, pericardial, ascitic, cerebrospinal, bronchoalveolar lavage, urine, and fine needle aspiration specimens - is well established in the diagnosis of malignant tumours[1]. Malignant serous effusions are

typically diagnosed through cytological evaluation, making the ability to differentiate malignant from benign effusions crucial for appropriate clinical management. Reactive mesothelial cells are known to pose significant diagnostic challenges, potentially leading to false-positive assessments of malignancy[2].

The serosa lines the pericardial, pleural, and peritoneal cavities. In pathological conditions, increased fluid accumulates as an effusion[3]. Detection of malignant cells in effusions serves both as a primary diagnostic method and a prognostic indicator, and routine cytological preparations offer a straightforward, safe, and cost-effective alternative to biopsy[4].

The peritoneal cavity, lined by visceral and parietal peritoneum, can harbour deposits from various benign and malignant lesions. Peritoneal washings are submitted for cytological analysis to detect such deposits[5,6].

Peritoneal lavage cytology is a standard procedure during initial surgical staging of gynaecological malignancies, particularly ovarian tumours. Positive peritoneal washings carry significant prognostic implications — including higher recurrence rates and decreased survival in endometrial cancer. False-negative cytology may result from insufficient fluid volume, obscuring mesothelial reaction, haemorrhage, excessive dilution, or small metastatic foci. Conventional smears further compound these issues through cell clustering, obscuration by mucus or debris, and multilayering[7,8,9].

Conventional smears, prepared by spreading material directly onto a slide, are economical and quick but are prone to compression artefacts, uneven cell distribution, cell overlap, and high false-negative rates[10,11]. Liquid-Based Cytology (LBC), first introduced in 1991, was developed to overcome these limitations. It employs specialised collection devices to preserve cells in liquid fixative, followed by centrifugation or filtration to remove non-diagnostic material, and deposits a thin

monolayer of cells onto a slide. LBC offers several advantages over conventional cytology: improved sensitivity and specificity; reduced unsatisfactory sample rates; capacity for ancillary testing (HPV DNA, molecular studies, immunocytochemistry); standardisation and automation; and prolonged sample preservation for retrospective analysis [12,13,14].

In recent years, LBC has emerged as a valuable alternative to traditional cytopreparatory techniques, enabling more accurate cytological evaluation through superior fixation, preserved nuclear detail, and concentrated cellular distribution within a defined circular area[15].

The present study was therefore undertaken to compare the utility of liquid-based cytology with conventional smear cytology in the analysis of peritoneal washings.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This prospective, comparative study was conducted in the Department of Pathology, Government Medical College and Rajindra Hospital, Patiala, over a period of one and a half years. The study was approved by the institutional ethics committee Baba Farid University of Health Sciences Vide No. BFUHS/2K26p-TH/304, and patient confidentiality was maintained throughout. No additional financial burden was placed on study participants.

2.2 Sample Collection and Processing

A total of 50 consecutive peritoneal washing samples received in the department were included. All age groups were eligible (inclusion criteria). Cases already diagnosed or currently on treatment were excluded. Each sample was divided and processed simultaneously by both techniques.

Conventional Smear (CS): Material was spread onto clean glass slides, air-dried, fixed with alcohol, and stained with Papanicolaou (PAP), May-Grünwald Giemsa (MGG), and Haematoxylin & Eosin (H&E) stains.

Liquid-Based Cytology (LBC – BD SurePath™): The sample was rinsed into a tube containing 12 ml of BD CytoRich™ Red preservative and allowed to stand for 60 minutes. After centrifugation at 600g for 10 minutes, the supernatant was discarded and the pellet vortexed. The suspension was processed using the BD PrepStain™ automated slide processor. Slides were stained with PAP and MGG stains.

2.3 Observer Assessment Methodology

All cytological slides were independently evaluated by 3 cytopathologists. The study was conducted in a double-blind manner, the observers were blinded to the radiological and histopathological data at the time of slide assessment, and the patients were blinded to the individual observer's interpretations and the interim findings of study. Each cytopathologist reviewed the slides independently without prior knowledge of

the other observer's interpretation. Discordant cases were resolved by simultaneous review at a multihead microscope to reach a consensus diagnosis. Interobserver agreement was assessed using Cohen's kappa (k) statistic, where a k value of less than 0.2 was considered slight agreement, 0.2-0.4 fair, 0.4-0.6 moderate, 0.6-0.8 substantial, greater than 0.8 near-perfect agreement.

2.4 Cytomorphological Scoring

Smears were evaluated using a validated four-parameter cytomorphological scoring system[4], shown in Table 1, for: (i) background cleanliness, (ii) cellularity, (iii) cell distribution, and (iv) cell morphology (cytoplasmic and nuclear detail). Each parameter was scored 0–2, yielding a maximum total score of 8. Scores were assigned independently for LBC and CS slides.

Table 1 : cytomorphological scoring system

PARAMETER	QUANTITATIVE DESCRIPTION	SCORE
Background blood or mucus	Larger amount, great compromise in diagnosis	0
	Moderate amount, diagnosis possible	1
	Minimal, diagnosis easy	2
Cellularity	Minimal, diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant	2
Cell Morphology	Marked cellular degeneration, diagnosis not possible	0
	Moderate cellular degeneration, diagnosis possible	1
	Minimal cellular degeneration, easy diagnosis	2
Distribution of Cells	In Periphery or sparsely distributed	0
	Combination	1
	Evenly distributed	2

2.4 Statistical Analysis

Data were compiled and analysed using Microsoft Excel 2021. Paired t-test was used for continuous variables; chi-square test was applied for categorical variables. Intermethod agreement was assessed using Cohen's kappa (κ) statistics. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Patient Demographics

The mean age of the study population was 43.24 ± 16.24 years (range: 11–80 years). The most frequently represented age group

was 51–60 years (24.0%), followed by 31–40 years (20.0%). Female patients predominated, constituting 82.0% of cases ($n = 41$) and males 18.0% ($n = 9$), yielding a male-to-female ratio of 1:4.6. The majority of samples were gynaecological in origin (66.0%), while non-gynaecological samples constituted 34.0%.

3.2 Sample Adequacy and Cellularity

Conventional smear demonstrated significantly better adequacy than LBC — as mentioned in Table 2, adequate samples were identified in 96.0% of CS preparations

compared to 84.0% of LBC preparations ($p = 0.04$). High cellularity was also more frequently observed in CS smears (50.0%)

than in LBC (24.0%; $p = 0.02$). Moderate cellularity, however, was more common in LBC (66.0% vs 40.0%).

Table 2 Comparison of adequacy and cellularity between LBC and Conventional Smear cytology

Parameter	LBC (n=50)	Conventional Smear (n=50)	p-value
Sample adequacy	84.0% (42/50)	96.0% (48/50)	0.04
High Cellularity	24.0% (12/50)	50.0% (25/50)	0.02
Moderate Cellularity	66.0% (33/50)	40.0% (20/50)	-
Scanty Cellularity	10.0% (5/50)	10.0% (5/50)	-

3.3 Cell Distribution, Morphology, and Background

As mentioned in Table 3, LBC provided significantly more uniform cell distribution. Evenly distributed cells were seen in 70.0% of LBC smears versus only 40.0% of conventional smears ($p < 0.001$). Cytoplasmic details were good in 80.0% of LBC preparations compared to 58.0% for CS

($p = 0.02$), and nuclear details were superior in LBC (88.0%) over CS (70.0%; $p = 0.03$). The most striking difference was in background clarity — a clean background was observed in 88.0% of LBC smears but in only 2.0% of CS smears ($p < 0.001$), where blood, inflammation, necrosis, and mixed patterns were prevalent.

Table 3 Comparison of cytomorphological parameters between LBC and Conventional Smear

Parameter	LBC (n=50)	Conventional Smear (n=50)	p-value
Even cell distribution	70.0% (35/50)	40.0% (20/50)	<0.001
Good cytoplasmic detail	80.0% (40/50)	58.0% (29/50)	0.02
Good nuclear detail	88.0% (44/50)	70.0% (35/50)	0.03
Clean background	88.0% (44/50)	2.0% (1/50)	<0.001

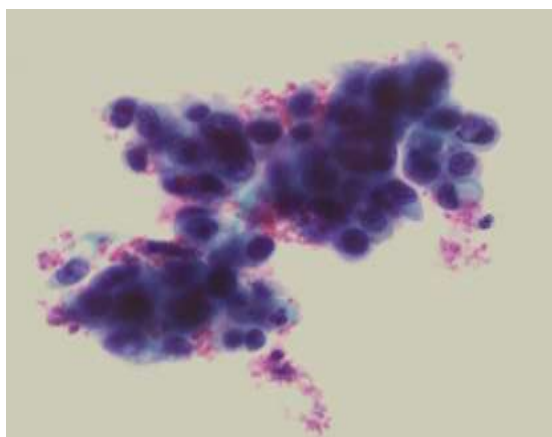


Figure 1: High power view of adenocarcinoma with cells showing irregular nuclear contour and prominent nucleoli (LBC; PAP X 400)X

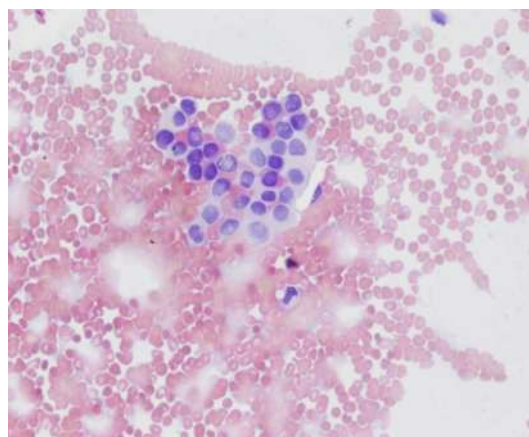


Figure 2: High power view of adenocarcinoma with cells exhibiting nuclear pleomorphism (CS; H&E X 400)

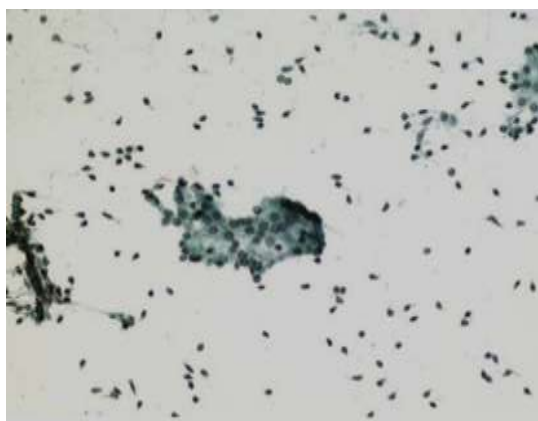


Figure 3: Low power view of serous cystadenoma - ovary (LBC; PAP X 100)

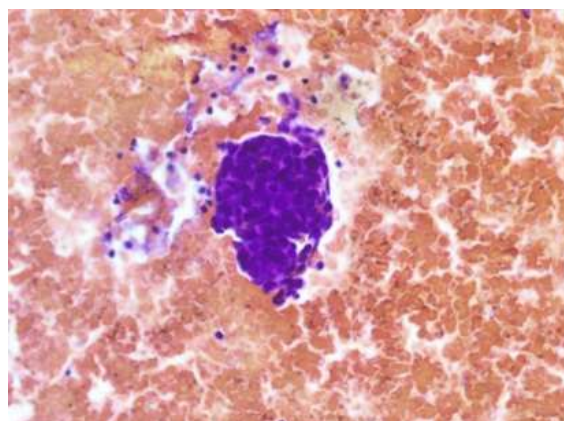


Figure 4: High power view of adenocarcinoma with smear showing tightly packed 3-D cell cluster (CS; H&E X 400)

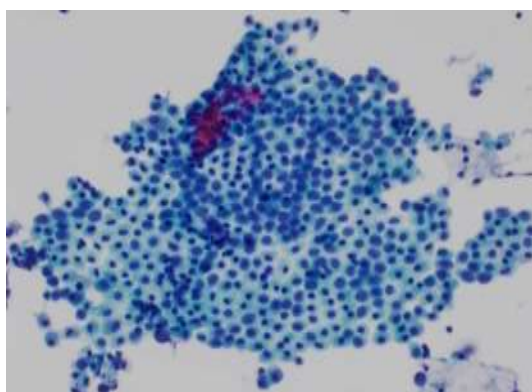


Figure 5: High power view of smear showing sheet of benign mesothelial cells-honeycomb pattern (LBC; PAP X 400)

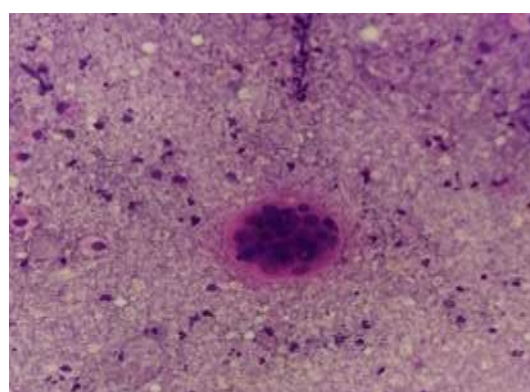


Figure 6: High power view of smear showing tumour giant cell in a necrotic background (CS; H&E X 400).

3.4 Mean Cytological Scores

On paired t-test analysis results shown in Table 4 below, the mean distribution score (1.60 ± 0.66 vs 1.20 ± 0.75 ; $p = 0.004$), morphology score (1.66 ± 0.65 vs 1.28 ± 0.83 ; $p = 0.010$), and background score (1.74 ± 0.66 vs 0.36 ± 0.52 ; $p < 0.001$) were all significantly higher in LBC. Conventional

smear yielded a significantly higher mean cellularity score (1.40 ± 0.66 vs 1.14 ± 0.57 ; $p = 0.035$). The overall mean total cytological score was significantly higher in LBC (6.14 ± 2.54) than in CS (4.24 ± 2.76 ; $p < 0.001$), confirming overall superior smear quality with LBC.

Table 4: Mean cytological scores — LBC vs Conventional Smear (Paired t-test, $n=50$)

Parameter	LBC Mean $\pm$ SD	CS Mean $\pm$ SD	p-value
Distribution Score	1.60 ± 0.66	1.20 ± 0.75	0.004
Cellularity Score	1.14 ± 0.57	1.40 ± 0.66	0.035
Morphology Score	1.66 ± 0.65	1.28 ± 0.83	0.010
Background Score	1.74 ± 0.66	0.36 ± 0.52	<0.001
Total Score	6.14 ± 2.54	4.24 ± 2.76	<0.001

Diagnostic Yield and Agreement

After comparing all the cytological features (nuclear, cytoplasmic, background,

cellularity, distribution), in both techniques, out of 50 cases, Table 5, shows the overall final cytological diagnosis:

Table 5: Final cytological diagnosis

Final cytological diagnosis	LBC (n=50)	Conventional Smear (n=50)
Inflammatory	35 (70.0%)	35 (70.0%)
Malignant neoplasm	5 (10.0%)	5 (10.0%)
Unsatisfactory	5 (10.0%)	5 (10.0%)
Benign neoplasm	4 (8.0%)	4 (8.0%)
Tubercular pathology	1 (2.0%)	1 (2.0%)

The final cytological diagnosis was identical in both techniques: inflammatory lesions 70.0%, malignant neoplasm 10.0%, benign neoplasm 8.0%, unsatisfactory 10.0%, and tubercular pathology 2.0% ($p = 1.000$). Among neoplastic cases ($n = 9$), malignant lesions slightly outnumbered benign lesions (55.6% vs 44.4%) in both groups. Kappa statistics revealed fair agreement for adequacy ($\kappa = 0.322$), cytoplasmic details ($\kappa = 0.365$), and nuclear details ($\kappa = 0.337$), moderate agreement for cellularity ($\kappa = 0.505$), poor agreement for cell distribution ($\kappa = -0.054$), and perfect agreement for final diagnosis ($\kappa = 1.000$).

4. DISCUSSION

The present study provides a comprehensive prospective comparison of LBC and conventional smear cytology for peritoneal washing specimens. Our findings corroborate and extend the existing literature while offering data specifically derived from a tertiary-care setting in northern India.

The mean age of 43.24 ± 16.24 years in this study aligns closely with the findings of Naz et al. (2015), who reported the mean age of 49.9 ± 14.8 , showing a trend toward slightly older age group undergoing peritoneal washing cytology[16].

The female predominance (82.0%) and gynaecological origin of the majority of samples (66.0%) in this study aligns closely with the findings of Rossi et al. (2017), who reported 81.9% females and 64% gynaecological samples in a large series of 10,348 peritoneal washing specimens. This reflects the primary clinical utility of

peritoneal washing cytology in gynaecological oncology staging[17,18].

Conventional smear demonstrated better adequacy (96% vs 84%) and cellularity (50% vs 24%) in our series, a finding consistent with Amiri et al. (2019), who similarly observed higher adequacy in CS (60.4%) compared to LBC (45.8%) in peritoneal fluid from gynaecological malignancies[19]. The higher cellularity in conventional smears is attributable to direct cell transfer without processing-related cell loss. In contrast, Siddiqui et al. (2020) reported marginally higher adequacy in LBC (98.2% vs 97.3%), likely reflecting operator-dependent variability in conventional smear preparation [4].

LBC demonstrated markedly superior cell distribution, with evenly distributed cells in 70.0% of LBC smears versus 40.0% of CS smears ($p < 0.001$). Comparable results were reported by Priyanka and Shivashekar (2025), with 83% even distribution in LBC versus 6.5% in CS, and by Nemade and Kamal (2023), who found 46.4% even distribution in LBC versus 25.0% in CS [2,20]. The monolayer preparation in LBC, achieved through controlled deposition of cells after removal of obscuring elements, is the primary mechanistic explanation for this advantage.

The superiority of LBC in cytoplasmic detail (80% vs 58%, $p = 0.02$) and nuclear detail (88% vs 70%, $p = 0.03$) is consistent with multiple published studies. Nemade and Kamal (2023) reported good cytoplasmic and nuclear details in 73.2% of LBC versus 46.4% of CS preparations[2]. Alwahaibi et

al. (2014) similarly found 61% good cytoplasmic and nuclear details in LBC versus 41% in CS for peritoneal and pleural samples[21]. The superior morphological preservation in LBC results from immediate fixation in liquid preservative, preventing air-drying artefacts and reducing cell overlap.

The most striking finding in our study was background clarity. A clean background was present in 88.0% of LBC smears but only 2.0% of CS smears ($p < 0.001$). This background obscuration in LBC is explained by the density gradient centrifugation or filtration step, which removes blood, mucus, inflammatory exudate, and proteinaceous material before slide preparation. Dadhich et al. (2016) reported a similar magnitude of difference (92% clean LBC vs 9% clean CS), as did Priyanka and Shivashekar (2025) 57.3% vs 1.6%) [20,22]. Clean background in LBC facilitates unobstructed cell identification and reduces interpretative error, particularly in cases with prominent inflammatory or haemorrhagic backgrounds. The significantly higher total cytological score in LBC (6.14 ± 2.54 vs 4.24 ± 2.76 , $p < 0.001$) underscores its overall technical superiority in slide quality. This is of practical importance in routine diagnostic settings where cleaner, better-distributed slides reduce screening time and interpretive difficulty, particularly for trainees and in high-volume laboratories. Moreover, the residual cell suspension in LBC provides the option for ancillary studies - immunocytochemistry, molecular tests, cell block preparation - from a single specimen, a significant operational advantage in modern diagnostic cytopathology.

Limitation

Despite its several advantages, the liquid-based cytology (LBC) technique employed in this study is not without limitations. First, LBC is associated with a significantly higher cost compared to conventional smear preparation, which may limit its widespread applicability in resource-constrained settings such as ours, where affordability and

accessibility remain critical concerns in routine diagnostic practice. Second, in a subset of cases, histopathological correlation could not be performed, as tissue biopsy specimens were not available or were not submitted concurrently — this precluded definitive confirmation of cytological diagnoses and represents a methodological constraint inherent to purely cytology-based study designs. Finally, the present study was limited by a relatively small number of malignant cases in the study cohort, which restricts the statistical power of the analysis and limits the generalizability of findings, particularly with respect to the sensitivity and specificity of LBC in detecting malignancy. Future studies with larger sample sizes and mandatory histopathological follow-up would be necessary to validate and strengthen the conclusions drawn from this work.

CONCLUSION

Liquid-based cytology using the BD SurePath™ platform is superior to conventional smear cytology for peritoneal washing specimens with respect to cell distribution, cytoplasmic and nuclear detail, background clarity, and overall total cytological score. Conventional smear, however, retains advantages in sample adequacy and cellularity. Despite technical differences, both methods demonstrate equivalent final diagnostic yield. Combined or selective use of both techniques, informed by sample characteristics and clinical context, is recommended to optimize the cytological evaluation of peritoneal washings. LBC should be considered a valuable alternative or adjunct to conventional smear cytology, particularly in settings where enhanced slide quality and the capacity for ancillary testing are priorities.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Shefali Gupta, Harpal Singh, Kanwardeep Kaur. Comparative analysis of liquid-based cytology and conventional smear cytology in peritoneal washings: a prospective study. *Int. J. Sci. Healthc. Res.* 2026; 11(3): 1-9. DOI: <https://doi.org/10.52403/ijshr.20260301>
