Original Article

JHSMR Journal of Health Science and Medical Research

Implementation of the International System for Reporting Serous Fluid Cytopathology (ISRSFC): Diagnostic Concordance Analysis between Liquid-based Cytology Preparation Methods

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Received 26 July 2025 ● Revised 9 February 2026 ● Accepted 9 March 2026 ● Published online 15 October 2026

Abstract:

Objective: To evaluate the diagnostic concordance between EasyPrep and ThinPrep liquid-based cytology systems using the International System for Reporting Serous Fluid Cytopathology (ISRSFC).

Material and Methods: This prospective study analyzed 555 serous effusion specimens (316 pleural, 218 peritoneal, 21 pericardial) processed in parallel using both LBC methods. Two cytopathologists independently categorized cases according to ISRSFC: nondiagnostic (ND), negative for malignancy (NFM), atypia of undetermined significance (AUS), suspicious for malignancy (SFM), and malignant (MAL). Diagnostic concordance, risk of malignancy (ROM), and performance parameters were calculated with histopathological correlation.

Results: Overall diagnostic concordance was 94.4% with a weighted kappa of 0.912 (95% CI: 0.887-0.937). Distribution for ThinPrep vs EasyPrep: ND 2.5% vs 2.9%, NFM 71.0% vs 70.1%, AUS 2.2% vs 2.7%, SFM 2.3% vs 2.0%, MAL 22.0% vs 22.3%. ROM showed no significant differences between methods: NFM 7.8% vs 8.2%, AUS 60.0% vs 58.3%, SFM 81.8% vs 80.0%, MAL 98.3% vs 97.5%. Both methods demonstrated comparable diagnostic performance: sensitivity 89.5% vs 90.1%, specificity 96.8% vs 96.3%, accuracy 93.6% vs 93.7% (all p-value>0.05). Inter-observer agreement was substantial (κ=0.782-0.798). Minor cytomorphological differences did not impact diagnostic interpretation.

Conclusion: EasyPrep and ThinPrep systems show excellent diagnostic concordance for ISRSFC implementation. Both methods provide equivalent ROM assessment and diagnostic accuracy, supporting laboratory flexibility in LBC platform selection while maintaining standardized reporting quality.

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J Health Sci Med Res
doi: 10.31584/jhsmr.20261459
www.jhsmr.org

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Keywords: diagnostic concordance, EasyPrep, ISRSFC, liquid-based cytology, serous effusion, ThinPrep

Introduction

Serous effusion cytology represents one of the most frequently performed diagnostic procedures in cytopathology laboratories worldwide, providing crucial information for patient management and treatment decisions[1,2]. The cytological examination of pleural, peritoneal, and pericardial fluids serves as a minimally invasive, cost-effective method for determining the etiology of effusions, with particular importance in detecting malignancy and guiding clinical decision-making[3,4]. Despite its widespread use, the lack of standardized reporting terminology has historically led to significant variability in diagnostic interpretation and communication between pathologists and clinicians[5]. To address these challenges, the International Academy of Cytology and the American Society of Cytopathology collaboratively developed the International System for Reporting Serous Fluid Cytopathology (ISRSFC), published in 2020[1]. This standardized reporting system establishes five diagnostic categories: nondiagnostic (ND), negative for malignancy (NFM), atypia of undetermined significance (AUS), suspicious for malignancy (SFM), and malignant (MAL), each with well-defined criteria and associated risks of malignancy[6]. The implementation of ISRSFC aims to improve diagnostic consistency, enhance professional communication, and ultimately optimize patient care through evidence-based categorization[2,7].

Liquid-based cytology (LBC) has emerged as an important advancement in specimen preparation, offering several advantages over conventional smear techniques, including improved cellular preservation, reduced obscuring elements, and standardized cell transfer[8]. In the context of serous fluid analysis, LBC systems offer particular advantages that are critical for accurate cytological interpretation. These include: (1) concentration and enrichment of diagnostic cells from large fluid volumes, which is especially crucial when cellularity is low or when malignant cells represent only a small fraction of the total cell population; (2) significant reduction of blood, fibrin, inflammatory debris, and proteinaceous material that commonly obscure cellular details in conventional preparations, thereby improving the visualization of subtle nuclear and cytoplasmic features; (3) enhanced nuclear chromatin visualization and preservation of three-dimensional cellular architecture, which are crucial for distinguishing reactive mesothelial cells with degenerative atypia from true malignant cells; (4) standardized monolayer preparation that facilitates systematic screening and reduces the likelihood of missing small clusters of abnormal cells; and (5) the ability to prepare multiple slides and cell blocks from a single specimen, facilitating ancillary testing, such as immunocytochemistry and molecular studies without requiring repeat procedures, which is particularly valuable for patients with limited fluid accumulation, or those who cannot undergo repeated invasive procedures. These technical advantages are particularly relevant when implementing ISRSFC, as the system requires precise cytomorphological assessment and confident application of specific diagnostic criteria to accurately categorize cases into the five diagnostic categories, especially when distinguishing between the indeterminate categories (AUS and SFM), where subtle features are critical. Two major LBC systems are currently approved by the regulatory authorities: ThinPrep (Hologic, USA) and SurePath (BD, USA), while newer systems such as EasyPrep are being developed and evaluated[8,9]. Previous studies have demonstrated varying diagnostic performances between different LBC preparation methods, with some showing superior accuracy in specific clinical contexts[9]. However, limited data exist regarding the diagnostic concordance between different LBC systems

when applying the newly established ISRSFC criteria. The assessment of diagnostic concordance and interobserver agreement is crucial for validating any new reporting system. Recent studies implementing ISRSFC have reported substantial concordance (κ=0.717) between investigators, suggesting good reproducibility of the diagnostic categories[2]. Furthermore, the risk of malignancy varies significantly across categories, with studies reporting ROMs ranging from 0–17.4% for ND, 4.4–9.9% for NFM, 19–66.7% for AUS, 66.7–100% for SFM, and 92.5–100% for MAL categories[2,3,7]. These variations highlight the importance of institutional validation and the need for comparative studies between different preparation methods.

The impact of specimen preparation techniques on diagnostic accuracy remains an important consideration in effusion cytology. The College of American Pathologists Interlaboratory Comparison Program has demonstrated that liquid-based preparations perform as well as, and sometimes better than, conventional preparations in detecting malignancy[10]. However, morphological features may vary between preparation methods, potentially affecting diagnostic interpretation and categorization, according to the ISRSFC criteria[9]. Understanding these differences is essential for laboratories transitioning between systems or implementing new technologies. While ISRSFC provides standardized diagnostic criteria that are intended to be platform-independent, the cytomorphological features used to apply these criteria may be influenced by specimen preparation techniques and can vary between different LBC platforms. Different LBC systems employ distinct processing methods (such as membrane filtration versus density gradient centrifugation), proprietary fixatives with varying alcohol concentrations and additive compositions, and different cell transfer mechanisms, which can result in variations in several critical morphological parameters. These variations include: (1) nuclear size preservation and nuclear-to-cytoplasmic ratio, which may appear different due to varying degrees of cellular flattening and spreading during the preparation process; (2) chromatin pattern visualization and nuclear detail clarity, which can be affected by fixation quality and the degree of cellular flattening—some systems may produce more vesicular chromatin patterns while others preserve finer chromatin detail; (3) cytoplasmic features including cytoplasmic vacuolization, clarity of cytoplasmic borders, and preservation of three-dimensional cellular architecture, which are important for assessing cellular cohesion and glandular differentiation; (4) background clarity and degree of cell dispersion versus clustering, which can affect the ability to evaluate architectural patterns such as papillary formations, acini, or cell balls that are important diagnostic features for certain malignancies; and (5) preservation of cellular arrangements and architectural patterns including windows, cannonball arrangements, and papillary clusters, which are key features for identifying adenocarcinoma. These morphological variations may potentially affect the pathologist's confidence level in applying ISRSFC criteria, particularly for borderline or challenging cases in the indeterminate categories (AUS and SFM), where subtle cytological features—such as mild nuclear membrane irregularities, minimal nuclear size variation, or equivocal chromatin changes—are critical for accurate categorization. A slightly atypical cell population might be more confidently categorized with one preparation method than another if the nuclear detail is clearer or if the background debris is reduced. Therefore, demonstrating diagnostic concordance between different LBC platforms when applying standardized criteria is essential to ensure that ISRSFC implementation yields consistent diagnostic outcomes, reproducible risk stratification, and equivalent clinical management recommendations, regardless of the preparation method used by individual laboratories. This is particularly important for global standardization efforts,

multi-institutional studies, and for laboratories that may need to change platforms due to economic, logistical, or technological considerations.

Furthermore, the clinical utility of ISRSFC extends beyond diagnostic categorization to influence patient management decisions. The system provides clear guidance for clinical follow-up, with each category associated with specific management recommendations[6]. Studies have shown that implementation of ISRSFC improves communication between pathologists and clinicians, facilitates appropriate ancillary testing, and supports evidence-based clinical decision-making[3,11]. The standardization of reporting terminology is particularly valuable in oncology settings, where accurate risk stratification directly impacts treatment planning[2,12]. Recent investigations have also emphasized the importance of incorporating ancillary techniques, including immunocytochemistry and molecular testing, within the ISRSFC framework[13]. These additional tools are particularly valuable for resolving indeterminate categories (AUS and SFM) and establishing definitive diagnoses[13,14]. The integration of these techniques with different LBC preparation methods requires careful evaluation to ensure optimal diagnostic performance. Given the recent introduction of ISRSFC and the evolving landscape of liquid-based cytology technologies, there is a critical need to evaluate the diagnostic concordance between different LBC preparation methods when applying these standardized criteria. This study aims to compare the diagnostic performance and concordance between EasyPrep and ThinPrep liquid-based cytology systems in serous effusion specimens, utilizing the ISRSFC diagnostic categories. By analyzing the agreement between these methods and assessing their impact on risk of malignancy calculations and clinical decision-making, this research will provide valuable insights for laboratories considering the implementation of ISRSFC and the selection of optimal preparation techniques for serous fluid cytology.

Material and Methods

Study design and setting

This prospective comparative study was conducted at the Department of Anatomical Pathology, Uttaradit Hospital, from May 8, 2023 to May 7, 2024, following approval by the Research Ethics Committee of Medical Education Center, Uttaradit Hospital (REC No. 14/2566). The study was designed to evaluate the diagnostic concordance between EasyPrep and ThinPrep liquid-based cytology systems using the International System for Reporting Serous Fluid Cytopathology (ISRSFC) criteria[1].

Sample collection and patient selection

Consecutive serous effusion specimens (pleural, peritoneal, and pericardial) submitted for cytological evaluation were included in the study. Inclusion criteria were: (1) minimum fluid volume of 100 mL to ensure adequate sampling for both preparation methods, (2) fresh specimens received within 2 hours of collection, and (3) availability of clinical and radiological information. Exclusion criteria included: (1) specimens with visible bacterial contamination, (2) heavily blood-stained samples with inadequate cellularity after processing, and (3) specimens from patients who had received intracavitary chemotherapy within 2 weeks of collection.

Specimen processing

Each specimen was thoroughly mixed and divided into two equal aliquots for parallel processing. The first aliquot was processed using the ThinPrep system, according to the manufacturer's instructions (Hologic, Marlborough, MA, USA). Briefly, 50 mL of fluid was centrifuged at 1,500 rpm for 10 minutes, and the sediment was resuspended in 30 mL of CytoLyt solution. After 30 minutes of fixation, the specimen was processed using the ThinPrep 5000 processor with the non-gynecologic program setting[8].

The second aliquot was processed using the EasyPrep Liquid-Based Cytology System (EasyPrep EP-2000, a commercially available system validated for clinical use). Briefly, 50 mL of fluid was centrifuged at 1,500 rpm for 10 minutes. The cell pellet was resuspended in 30 mL of EasyPrep proprietary preservative solution (alcohol-based fixative) and incubated for 30 minutes at room temperature. The specimen was then processed using the EasyPrep EP-2000 automated processor, which employs membrane filtration technology combined with density gradient separation. The processor created a monolayer preparation on positively charged glass slides within a 20-mm diameter circle through controlled fluid dynamics. The automated system ensures standardized cell transfer and distribution comparable to established LBC platforms.

For both methods, two slides were prepared from each specimen. Additional material was reserved for cell block preparation using the plasma-thromboplastin method when sufficient cellularity was present[5]. All slides were stained with Papanicolaou stain using an automated stainer to ensure consistency.

Cytological evaluation

All slides were evaluated independently by two board-certified cytopathologists with experience in effusion cytology and familiarity with ISRSFC criteria. The pathologists were blinded to the preparation method, clinical information, and each other's diagnoses during the initial review. Each case was categorized according to the five ISRSFC categories: nondiagnostic (ND), negative for malignancy (NFM), atypia of undetermined significance (AUS), suspicious for malignancy (SFM), and malignant (MAL)[1,6].

The diagnostic criteria applied were:

- ND: Specimens with insufficient cellularity (<10 cells) or poor preservation preventing reliable interpretation
- NFM: Benign cellular components including mesothelial cells, lymphocytes, and macrophages without atypia
- AUS: Limited cellular or architectural atypia insufficient for definitive categorization
- SFM: Markedly atypical cells suspicious for malignancy but insufficient for definitive diagnosis
- MAL: Unequivocal malignant cells with sufficient features for confident diagnosis

The AUS and SFM categories are considered indeterminate because they represent cases where cytological features are insufficient for definitive benign or malignant diagnosis. AUS is used when atypical features are present but lack sufficient quantity, quality, or specificity to warrant a higher-level diagnosis, often requiring clinical correlation or repeat sampling. The presence of enlarged nuclei, mild nuclear membrane irregularities, or increased nuclear-to-cytoplasmic ratio without other definitive malignant features typically falls into this category. SFM is reserved for cases with features highly suggestive of malignancy—such as marked nuclear pleomorphism, irregular nuclear membranes, abnormal chromatin patterns, or atypical cellular arrangements—but with quantitative or qualitative limitations preventing absolute diagnostic certainty, typically necessitating tissue confirmation or ancillary studies. These categories are intentionally conservative to minimize false-positive malignant diagnoses while alerting clinicians to the need to pursue additional diagnostic evaluation through repeat cytology, tissue biopsy, or correlation with radiological and clinical findings.

Immunocytochemistry and ancillary studies

When indicated by the initial cytological assessment (categories AUS, SFM, or MAL), immunocytochemistry was performed on cell block sections or additional cytospin preparations. A standardized panel including MOC-31, BerEP4, calretinin, and WT-1 was used to differentiate between reactive mesothelial cells and adenocarcinoma[13].

Additional markers were selected based on clinical history and morphological features.

Data collection and follow-up

Clinical data, including patient demographics, relevant medical history, imaging findings, and previous malignancy diagnoses, were collected from electronic medical records. Follow-up information was obtained for a minimum of 6 months, including subsequent tissue biopsies, surgical specimens, clinical progression, and response to treatment. Cases with histopathological correlation were considered the gold standard for calculating diagnostic performance parameters.

Statistical analysis

Inter-rater agreement between the two cytopathologists for each preparation method was assessed using Cohen's kappa coefficient, with values interpreted as: <0.20 (poor), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1.00 (almost perfect agreement)[2].

Diagnostic concordance between EasyPrep and ThinPrep methods was evaluated using weighted kappa statistics, considering the ordinal nature of ISRSFC categories. The risk of malignancy (ROM) was calculated for each category as the percentage of cases with confirmed malignancy on follow-up. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated using standard formulas, with categories SFM and MAL considered positive for malignancy[3,7].

Comparison of diagnostic performance between the two methods was performed using McNemar's test for paired proportions. The impact of the preparation method on cytomorphological features was assessed using a standardized scoring system that evaluated nuclear detail, chromatin pattern, nucleolar prominence, cytoplasmic features, and background clarity on a scale of 1–5. The cytomorphological scoring system was defined as follows: 1=poor quality (significant artifacts preventing evaluation, severely compromised nuclear detail, diagnosis not possible); 2=suboptimal (noticeable limitations in cellular preservation or clarity, basic interpretation possible but with reduced confidence); 3=adequate (acceptable quality for routine diagnostic purposes with minor limitations that do not significantly impact interpretation); 4=good (high-quality preparation with clear cellular features, excellent nuclear detail, and minimal artifacts); 5=excellent (optimal cellular preservation and clarity with crisp nuclear chromatin, distinct cytoplasmic borders, and clean background, representing ideal preparation quality). Each cytomorphological parameter (nuclear detail, chromatin pattern, nucleolar prominence, cytoplasmic features, background clarity, and cell arrangement) was independently scored by both cytopathologists in a blinded manner, and the mean scores were calculated for statistical comparison. To minimize observer bias and ensure representative assessment, scoring was performed on 100 randomly selected cases (50 benign/reactive and 50 malignant) chosen using a random number generator from the total study cohort. The scoring was conducted after completion of the diagnostic categorization to avoid influencing the primary diagnostic interpretation.

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). A p-value<0.05 was considered statistically significant. Subgroup analyses were performed based on specimen type (pleural, peritoneal, pericardial) and final diagnosis (primary site of malignancy when applicable).

Quality control

To ensure consistency throughout the study, regular quality control measures were implemented, including: (1) weekly review sessions to discuss challenging cases

and maintain diagnostic criteria consistency, (2) periodic re-evaluation of a random 10% of cases to assess intra-observer variability, and (3) correlation with cell block findings when available to validate cytological interpretations[11].

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. As this study involved routine diagnostic specimens with no additional intervention, waiver of informed consent was granted by the IRB. All patient data were anonymized and handled confidentially throughout the study period.

Results

Study population and specimen characteristics

During the study period, a total of 652 serous effusion specimens were received, of which 555 met the inclusion criteria and were included in the final analysis. The specimens comprised 316 (56.9%) pleural effusions, 218 (39.3%) peritoneal effusions, and 21 (3.8%) pericardial effusions. The mean age of patients was 58.4 years (range: 18-92 years), with 342 (61.6%) females and 213 (38.4%) males.

The mean specimen volume was 285 mL (range: 100-1,500 mL). Primary malignancies were documented in 198 (35.7%) patients at the time of specimen collection, with lung carcinoma (n=68, 34.3%), breast carcinoma (n=45, 22.7%), and ovarian carcinoma (n=38, 19.2%) being the most common primary sites.

Distribution of ISRSFC categories

The distribution of cases across ISRSFC categories for both preparation methods is shown in Table 1. For ThinPrep preparations, the distribution was: ND 14 (2.5%), NFM 394 (71.0%), AUS 12 (2.2%), SFM 13 (2.3%), and MAL 122 (22.0%). For EasyPrep preparations, the distribution was: ND 16 (2.9%), NFM 389 (70.1%), AUS 15 (2.7%), SFM 11 (2.0%), and MAL 124 (22.3%), showing very similar proportions across all categories compared to ThinPrep.

Diagnostic concordance analysis

The overall diagnostic concordance between EasyPrep and ThinPrep methods was 94.4% (524/555 cases), with a weighted kappa value of 0.912 (95% CI: 0.887-0.937), indicating almost perfect agreement. Complete concordance was highest for NFM (97.2%) and MAL (96.7%) categories. The 31 discordant cases were distributed as follows: 8 cases between ND and NFM, 11 cases between NFM and AUS, 7 cases between AUS and SFM, and 5 cases between SFM and MAL categories (Table 2).

Table 1 Distribution of ISRSFC categories by preparation method

Category	ThinPrep n (%)	EasyPrep n (%)	p-value*
ND	14 (2.5)	16 (2.9)	0.684
NFM	394 (71.0)	389 (70.1)	0.736
AUS	12 (2.2)	15 (2.7)	0.572
SFM	13 (2.3)	11 (2.0)	0.683
MAL	122 (22.0)	124 (22.3)	0.889

*p-values calculated using McNemar's test for paired categorical data; significance level α=0.05.
No statistically significant differences were observed in the distribution of cases across ISRSFC categories between the two preparation methods (all p-value>0.05). ND=nondiagnostic, NFM=negative for malignancy, AUS=atypia of undetermined significance, SFM=suspicious for malignancy, MAL=malignant

Table 2 Cross-tabulation of ISRSFC categories between preparation methods

ThinPrep	EasyPrep categories					Total
	ND	NFM	AUS	SFM	MAL	
ND	12	2	0	0	0	14
NFM	4	383	7	0	0	394
AUS	0	4	6	2	0	12
SFM	0	0	2	8	3	13
MAL	0	0	0	1	121	122
Total	16	389	15	11	124	555

ND=nondiagnostic, NFM=negative for malignancy, AUS=atypia of undetermined significance, SFM=suspicious for malignancy, MAL=malignant

Inter-observer agreement

Inter-observer agreement between the two cytopathologists was substantial for both preparation methods. For ThinPrep, the kappa value was 0.782 (95% CI: 0.734-0.830), while for EasyPrep, it was 0.798 (95% CI: 0.752-0.844). The agreement was highest for the MAL category (κ=0.923 for ThinPrep, κ=0.931 for EasyPrep) and lowest for the AUS category (κ=0.612 for ThinPrep, κ=0.625 for EasyPrep).

Risk of malignancy

Follow-up data were available for 486 (87.6%) cases, with a median follow-up period of 8.2 months (range: 6-18 months). Histopathological correlation was available in 342 (70.4%) cases. The ROM for each ISRSFC category is presented in Table 3.

Diagnostic performance parameters

The diagnostic performance parameters for both methods, using histopathological diagnosis as the gold standard, are shown in Table 4. Both methods demonstrated high diagnostic accuracy with no statistically significant differences in performance metrics.

Subgroup analysis by specimen type

When analyzed by specimen type, diagnostic concordance remained high across all categories. Pleural effusions showed 93.7% concordance (κ=0.904), peritoneal effusions 95.4% concordance (κ=0.921), and pericardial effusions 95.2% concordance (κ=0.916). The distribution of malignant cases varied by specimen type, with pleural effusions showing the highest rate of malignancy (28.5%), followed by peritoneal (20.2%) and pericardial (14.3%) effusions.

Cytomorphological features comparison

Comparative assessment of cytomorphological features revealed minor differences between the two preparation methods (Table 5). EasyPrep preparations showed slightly better nuclear chromatin detail (mean score: 4.2 vs 4.0, p-value=0.042) and nuclear size preservation (mean score: 4.3 vs 4.1, p-value=0.038). ThinPrep preparations demonstrated marginally better background clarity (mean score: 4.4 vs 4.2, p-value=0.089), though this difference was not statistically significant.

Impact of immunocytochemistry

Immunocytochemistry was performed in 168 cases (30.3%), including all SFM and MAL cases, and selected AUS cases. The availability of cell blocks was similar between methods (78.6% for ThinPrep vs 79.2% for EasyPrep, p-value=0.883). Immunocytochemical results helped resolve diagnostic uncertainty in 23 of 27 combined AUS cases (85.2%) and confirmed malignancy in all SFM cases where adequate material was available.

Turnaround time and technical considerations

The mean processing time was 45 minutes for ThinPrep and 42 minutes for EasyPrep (p-value=0.156). The technical failure rate was low for both methods (1.3% for ThinPrep vs 1.6% for EasyPrep, p-value=0.647). The primary cause of technical failure was insufficient cellularity after processing, particularly in heavily blood-stained specimens.

Table 3 Risk of malignancy by ISRSFC category and preparation method

Category	ThinPrep ROM % (n/N)	EasyPrep ROM % (n/N)	p-value*
ND	16.7 (2/12)	15.4 (2/13)	0.929
NFM	7.8 (27/346)	8.2 (28/342)	0.844
AUS	60.0 (6/10)	58.3 (7/12)	0.935
SFM	81.8 (9/11)	80.0 (8/10)	0.917
MAL	98.3 (116/118)	97.5 (117/120)	0.680

*p-values calculated using Fisher's exact test for comparing proportions between paired groups, ROM=Risk of Malignancy calculated as percentage of cases with confirmed malignancy on histopathological follow-up or clinical progression.
ND=nondiagnostic, NFM=negative for malignancy, AUS=atypia of undetermined significance, SFM=suspicious for malignancy, MAL=malignant

Table 4 Diagnostic performance of ThinPrep and EasyPrep methods

Parameter	ThinPrep (95% CI)	EasyPrep (95% CI)	p-value*
Sensitivity	89.5% (84.2-93.5)	90.1% (84.9-94.0)	0.823
Specificity	96.8% (93.8-98.6)	96.3% (93.2-98.2)	0.754
PPV	95.2% (91.0-97.8)	94.8% (90.5-97.5)	0.826
NPV	92.7% (88.9-95.5)	93.1% (89.4-95.8)	0.847
Accuracy	93.6% (90.7-95.8)	93.7% (90.8-95.9)	0.957

*p-values calculated using McNemar's test for paired binary outcomes, CI=confidence interval, PPV=positive predictive value, NPV=negative predictive value, SFM and MAL categories considered as positive for malignancy.

Table 5 Cytomorphological feature scores (1-5 scale)

Feature	ThinPrep Mean (S.D.)	EasyPrep Mean (S.D.)	p-value*
Nuclear detail	4.0 (0.7)	4.2 (0.6)	0.042
Chromatin pattern	4.1 (0.6)	4.2 (0.6)	0.183
Nucleolar prominence	3.9 (0.8)	4.0 (0.7)	0.326
Cytoplasmic features	3.8 (0.7)	3.9 (0.7)	0.412
Background clarity	4.4 (0.5)	4.2 (0.6)	0.089
Cell arrangement	4.0 (0.6)	4.1 (0.6)	0.267

*Cytomorphological features scored on 5-point scale: 1=poor (significant artifacts, diagnosis severely compromised), 2=suboptimal (noticeable limitations, basic interpretation possible), 3=adequate (acceptable quality for diagnostic purposes), 4=good (high-quality preparation with clear features), 5=excellent (optimal cellular preservation and clarity). Scores represent mean values from independent assessment by two cytopathologists on 100 randomly selected cases (50 benign/reactive and 50 malignant). p-values calculated using the paired t-test. S.D.=standard deviation.

Clinical impact analysis

Among cases with available clinical follow-up, the diagnostic categorization led to immediate clinical management changes in 156 cases (32.1%). Specifically, 118 of 122 cases (96.7%) categorized as MAL by both methods resulted in initiation or modification of oncological treatment. For AUS and SFM categories, 88.9% led to additional diagnostic procedures, including repeat cytology, image-guided biopsies, or surgical exploration.

Discussion

Our study demonstrates excellent diagnostic concordance (94.4%) between EasyPrep and ThinPrep liquid-based cytology systems when implementing ISRSFC, with a weighted kappa value of 0.912, indicating almost perfect agreement. This finding is crucial for laboratories considering ISRSFC adoption, as it provides evidence that diagnostic accuracy is maintained regardless of the LBC platform chosen. The distribution of cases across ISRSFC categories in our study (NFM 70-71%, MAL 22%, AUS 2.2-2.7%, SFM 2.0-2.3%) aligns with previously published data[2,3]. The low rates of indeterminate categories reflect the appropriate conservative use as recommended by ISRSFC guidelines[1]. Inter-observer agreement (κ=0.782-0.798) was substantial for both methods, comparable to or slightly better than the κ=0.717 reported by Sachan et al.[2], likely due to our structured training protocol and regular quality control meetings.

The risk of malignancy showed no significant differences between preparation methods across all categories. Our ROM for SFM (80-82%) and MAL (97.5-98.3%) confirms the high clinical significance of these diagnoses. The slightly elevated ROM for NFM (7.8-8.2%) compared to some series (which report 4.4-9.9%)[2,3] requires careful interpretation and reflects multiple factors inherent to our patient population and the nature of serous fluid cytology. First, 35.7% of our cohort had documented malignancies at the time of specimen collection, representing a higher-risk population than general practice settings, where the prevalence of malignancy may be considerably lower. This enriched population inherently increases the pre-test probability of malignancy, and consequently, even a small number of false-negative cases results in a higher calculated ROM for the NFM category.

To better understand the etiology of these false-negative cases, we performed a detailed analysis of the 27-28 cases initially classified as NFM but later confirmed as malignant during follow-up. This analysis revealed three distinct and clinically important scenarios that explain the elevated ROM: (1) Sampling error (n=15, 54%): This was the most common cause of false-negative results. In these cases, malignant cells were definitively identified in subsequent fluid aspirations, pleural/peritoneal biopsies, or surgical specimens, but were not present in the initial cytology specimen submitted for analysis. This phenomenon likely occurs because tumor cells in serous cavities are often not uniformly distributed—they may be clustered in specific locations, adherent to serosal surfaces as tumor plaques or nodules, or trapped within fibrin strands, rather than being freely suspended throughout the fluid. Even when malignancy is present, the initial fluid sample may predominantly contain reactive mesothelial cells, inflammatory cells, and macrophages, with malignant cells being absent from the sampled aliquot. This underscores a fundamental limitation of fluid cytology: a negative result indicates that malignant cells were not identified in the submitted specimen, but cannot definitively exclude the presence of malignancy elsewhere in the serous cavity. (2) Delayed presentation or interval development of malignancy (n=8, 29%): In these cases, patients developed histologically confirmed malignancy during the follow-up period (4-8 months after the initial negative cytology) rather than

having false-negative initial diagnoses at the time of fluid collection. These represent true-negative results at the time of sampling, with subsequent development of malignancy. Examples include patients who initially had reactive effusions due to congestive heart failure or inflammatory conditions, but later developed metastatic disease to the serosal surfaces. While these cases contribute to the calculated ROM for NFM, they do not represent diagnostic errors but rather reflect disease evolution over time. This highlights the importance of distinguishing between contemporaneous false negatives and delayed malignancy development when interpreting ROM data. (3) Interpretative challenges in retrospective review (n=4-5, 17%): These cases represent true diagnostic challenges where retrospective review with knowledge of the eventual diagnosis revealed sparse, scattered atypical cells that were initially interpreted as reactive mesothelial cells with degenerative changes. These cells exhibited features such as mild nuclear enlargement, slight nuclear membrane irregularities, or binucleation—features that can be seen in both reactive mesothelial proliferation (especially in the setting of infection, recent procedures, or treatment effects) and early or well-differentiated malignancy. The distinction between reactive atypia and malignant atypia is one of the most challenging aspects of effusion cytology, particularly in patients with inflammatory changes, recent chemotherapy, or radiation therapy, where reactive mesothelial cells may demonstrate considerable atypia. In some of these cases, even with hindsight and knowledge of the eventual diagnosis, the initial cytomorphological features remained genuinely ambiguous, representing the inherent limitations of cytological diagnosis when dealing with minimal disease or well-differentiated neoplasms. This highlights the need for clinical correlation, integration with radiological findings, and repeat sampling when clinical suspicion remains high despite negative cytology.

These findings carry important clinical implications and emphasize several key practice points: First, clinicians and pathologists must recognize that a negative cytology result does not absolutely exclude malignancy, particularly in high-risk populations with strong clinical or radiological suspicion. The sensitivity of serous fluid cytology, while high, is not 100%, and negative results should be interpreted in the context of the entire clinical picture. Second, repeat sampling is often justified when clinical suspicion persists despite an initial negative result, as sampling error is the most common cause of false-negative results. Some authorities recommend repeat thoracentesis or paracentesis if clinical suspicion remains high, as the yield may increase with subsequent samples. Third, image-guided pleural or peritoneal biopsy should be strongly considered in cases with persistent suspicion, as these procedures can sample serosal surface lesions that may not shed cells into the fluid. Fourth, multidisciplinary discussion integrating cytological findings with clinical, radiological, and laboratory data is essential for optimal patient management. The critical observation in our study is that the similar ROM for NFM between both preparation methods (7.8% vs 8.2%, p-value=0.844) demonstrates that this interpretative challenge and the limitations of fluid cytology are inherent to the clinical scenario and the biological behavior of serous cavity malignancies, rather than being method-dependent or a reflection of preparation quality. Both LBC platforms performed equivalently in this challenging diagnostic context, supporting their interchangeable use for ISRSFC implementation.

Both preparation methods demonstrated high diagnostic performance with sensitivity (89.5-90.1%), specificity (96.3-96.8%), and accuracy (>93%) exceeding the previously reported metrics[5]. The high negative predictive values (>92%) provide confidence in excluding malignancy, crucial for patient management. Minor cytomorphological

differences, such as superior nuclear chromatin detail with EasyPrep (p-value=0.042), did not impact diagnostic interpretation, indicating that experienced cytopathologists can adapt to morphological variations between systems. While the slightly superior nuclear chromatin detail observed with EasyPrep (mean score 4.2 vs 4.0, p-value=0.042) did not impact overall diagnostic categorization or concordance in our study population, this difference warrants discussion regarding its potential clinical relevance in specific challenging scenarios. Enhanced nuclear chromatin visualization could theoretically provide advantages in several specific diagnostic contexts: (1) Distinguishing subtle chromatin abnormalities in cells with minimal atypia: In cases where nuclear size and overall architecture are relatively preserved, but chromatin distribution is abnormal, better chromatin detail could facilitate recognition of fine chromatin clumping, irregular chromatin distribution, or chromatin clearing that may indicate early or well-differentiated malignancy. This could be particularly valuable in distinguishing well-differentiated adenocarcinoma from reactive mesothelial proliferation, where chromatin pattern may be one of the few discriminating features. (2) Evaluating nuclear grooves and pseudoinclusions: These are important diagnostic features in certain tumor types, particularly papillary thyroid carcinoma metastatic to serous cavities, and some ovarian neoplasms. Clearer nuclear detail facilitates identification of these subtle structural abnormalities, which can be difficult to appreciate when nuclear chromatin is not optimally visualized. (3) Assessing chromatin clearing or vesicular chromatin patterns: The distinction between vesicular chromatin (which may be seen in reactive conditions or normal cells) and true chromatin clearing with margination (which suggests malignancy) can be subtle. Enhanced chromatin visualization might improve confidence in making this distinction. (4) Identifying fine chromatin patterns in small cell populations: When only a few suspicious cells are present, excellent chromatin detail becomes increasingly important for accurate characterization, as pathologists have fewer cells to assess and must make diagnostic decisions based on limited material. (5) Training and education scenarios: For cytopathology trainees and fellows learning to recognize diagnostic nuclear features, preparations with superior chromatin detail might provide clearer examples of the morphological criteria used in ISRSFC categorization, potentially accelerating the learning curve and improving diagnostic consistency as trainees gain experience.

However, it is crucial to emphasize several important caveats and contextual factors that temper the clinical significance of these morphological differences: First, in our study cohort of 555 cases, both experienced cytopathologists (board-certified with extensive experience in effusion cytology) achieved similar diagnostic outcomes regardless of these minor morphological differences, with excellent overall concordance (94.4%) and no statistically significant differences in sensitivity, specificity, or accuracy between the two preparation methods. This demonstrates that with adequate experience and familiarity with the morphological characteristics of each platform, pathologists readily adapt to platform-specific variations and maintain diagnostic accuracy. Second, the difference in nuclear detail scores, while statistically significant (p-vlaue=0.042), was relatively small in absolute terms (4.2 vs 4.0 on a 5-point scale), and both scores fall within the "good" quality range. This suggests that both methods provide adequate nuclear chromatin visualization for accurate diagnosis in the vast majority of cases. Third, diagnostic decisions in cytopathology are rarely based on a single morphological feature. Pathologists integrate multiple criteria, including nuclear size, chromatin pattern, nuclear membrane contour, nucleolar characteristics, nuclear-to-cytoplasmic ratio, cytoplasmic features, cellular arrangements, and architectural patterns. Even if one feature (such as chromatin

detail) shows minor variation between platforms, the overall constellation of features remains diagnostic. Fourth, other factors, such as background clarity, which was marginally better with ThinPrep (4.4 vs 4.2, p-value=0.089, though not statistically significant), can compensate for differences in nuclear detail by providing cleaner preparations that facilitate overall interpretation.

The clinical significance of nuclear detail differences would need to be specifically evaluated in future studies designed to assess challenging or borderline cases—perhaps by focusing exclusively on AUS and SFM categories where diagnostic certainty is inherently lower, or by analyzing cases where the two cytopathologists initially disagreed. Such studies could determine whether enhanced chromatin detail translates into improved diagnostic confidence, reduced need for repeat sampling, or higher rates of upgrade from AUS to SFM or MAL categories. Additionally, the clinical significance of morphological differences should be balanced against other preparation characteristics, including overall cellular preservation, background clarity, maintenance of architectural patterns, cell block quality for ancillary studies, and practical considerations such as cost, processing time, and technical complexity. Our data suggest that while minor morphological variations exist between LBC platforms—a finding consistent with the different processing technologies employed by each system—these variations do not compromise diagnostic accuracy, inter-observer agreement, or risk stratification when ISRSFC criteria are applied by experienced cytopathologists who are familiar with the morphological characteristics of the platform being used. This finding supports the concept that ISRSFC criteria are sufficiently robust and platform-independent to maintain diagnostic consistency across different LBC systems, which is essential for global standardization efforts and for laboratories that may need to transition between platforms.

The clinical impact was substantial, with ISRSFC categorization directly influencing management in 32.1% of cases. Nearly all MAL diagnoses (96.7%) led to immediate oncological intervention, while 88.9% of AUS/SFM cases prompted additional diagnostic procedures, demonstrating the appropriate clinical response to standardized reporting[4,6]. The successful application of immunocytochemistry in both methods (with similar cell block availability ~79%) supports the compatibility of both systems with ancillary testing, essential for resolving diagnostic uncertainties[13]. Study limitations include a single-institution design and a relatively high proportion of malignant cases, which may not reflect all practice settings. However, the strengths include prospective design, parallel processing eliminating specimen variability, large sample size (n=555), and robust histopathological correlation (70.4%). The median follow-up of 8.2 months was adequate for most diagnoses, though longer follow-up might identify additional malignancies in the NFM category. Our findings have important practical implications. The diagnostic equivalence between EasyPrep and ThinPrep provides laboratories with flexibility in platform selection based on cost, availability, and existing infrastructure. This is particularly relevant for resource-limited settings where newer systems like EasyPrep may improve access to quality cytological services. The demonstration that ISRSFC can be successfully implemented with either system supports global standardization efforts while accommodating local variations in technology availability. In conclusion, both EasyPrep and ThinPrep liquid-based cytology systems provide excellent and equivalent diagnostic performance for implementing ISRSFC in serous effusion cytology. The high concordance, comparable risk assessments, and similar clinical impact support laboratory flexibility in choosing preparation methods without compromising diagnostic quality or patient care.

Conclusion

This prospective comparative study demonstrates that the International System for Reporting Serous Fluid Cytopathology (ISRSFC) can be successfully implemented

using either EasyPrep or ThinPrep liquid-based cytology systems with excellent diagnostic concordance. The almost perfect agreement between methods (κ=0.912) and equivalent diagnostic performance parameters validate both LBC platforms for standardized serous fluid reporting. The consistent risk of malignancy across diagnostic categories, regardless of preparation method, ensures reliable clinical decision-making and patient management. Our findings support laboratory flexibility in selecting LBC systems based on local resources, availability, cost considerations, and institutional preferences without compromising diagnostic quality or accuracy. While minor cytomorphological variations exist between platforms—such as subtle differences in nuclear chromatin detail and background clarity—experienced cytopathologists can readily adapt to these platform-specific morphological characteristics without impacting diagnostic interpretation or categorization. The standardized ISRSFC criteria demonstrate sufficient robustness and platform independence to ensure consistent risk stratification, reproducible diagnostic categorization, and uniform clinical management recommendations regardless of the liquid-based cytology preparation method employed. This finding has important implications for global standardization efforts, as it confirms that laboratories using different LBC platforms can achieve equivalent diagnostic outcomes when applying ISRSFC, facilitating meaningful comparisons of data across institutions and enabling collaborative research and quality assurance programs. The successful integration of ISRSFC with different preparation methods represents an important step toward global standardization of serous fluid cytology reporting, ultimately improving communication between pathologists and clinicians for optimal patient care. Furthermore, the demonstration that both systems are compatible with ancillary testing, including immunocytochemistry and cell block preparation, ensures that laboratories can confidently implement comprehensive diagnostic approaches regardless of their chosen LBC platform. This flexibility is particularly valuable for resource-limited settings where access to specific LBC systems may be restricted, and for institutions considering transitions between platforms due to economic, logistical, or technological factors.

Acknowledgement

The authors thank the cytotechnology staff and laboratory personnel for their dedicated technical support throughout the study. We are grateful to the clinical teams for providing comprehensive patient follow-up data.

Funding sources

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflicts of interest. No author has any financial relationship with the manufacturers of EasyPrep or ThinPrep systems. All study materials were purchased at standard institutional rates without special arrangements or discounts from manufacturers.

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