International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Role of roswell park memorial institute media in preservation of cellularity and cell morphology in fluid samples: A cross-sectional study

Sunidhi Gupta *, Mithila Bisht, Sagar C. Mhetre and Anjana Arya

Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India.

International Journal of Science and Research Archive, 2026, 20(01), 219–225

Publication history: Received on 28 May 2026; revised on 04 July 2026; accepted on 08 July 2026

Article DOI: https://doi.org/10.30574/ijsra.2026.20.1.1447

Abstract

Background: Fluid cytology is an important for evaluating benign, inflammatory, infectious, and malignant conditions through examination of body fluids such as pleural, peritoneal, and cerebrospinal fluid. Accurate diagnosis depends on preservation of cellularity and cell morphology from collection to processing. Delay in transport or processing leads to cellular degeneration and reduced diagnostic accuracy. RPMI medium has been studied as a useful preservation medium because it helps maintain cellular integrity and morphology.

Aim: To study the role of RPMI medium in preserving cell morphology and cellularity in fluid samples.

Materials and Methods: This cross-sectional comparative observational study was conducted in the Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, from April 2024 to March 2025. A total of 144 fluid samples were included. Each sample was divided into two parts—one processed routinely and the other preserved in RPMI medium. Both were compared for cellularity, nuclear and cytoplasmic preservation, background clarity, degenerative changes, and contamination using standard cytological methods.

Results: RPMI-preserved samples showed better cellular preservation compared with routine processing. Adequate cellularity was observed in 87.5% of RPMI-preserved samples compared to 63.9% of routine samples. Nuclear and cytoplasmic details were also better preserved. Degenerative changes and bacterial contamination were lower in RPMI-treated samples, resulting in improved smear quality and better diagnostic interpretation.

Conclusion: RPMI medium is an effective preservation medium for fluid cytology specimens. It improves cellularity and morphology and may enhance diagnostic accuracy, especially in delayed or transported samples.

Keywords: Fluid cytology; RPMI medium; Cellularity; Cell morphology; Cytopathology

1. Introduction

Fluid cytology is a vital diagnostic tool that involves microscopic examination of body fluids to detect cellular abnormalities, infections, inflammatory conditions, and malignancies. Common fluid samples examined in routine pathology laboratories include pleural fluid, peritoneal fluid, cerebrospinal fluid, and pericardial fluid. Accurate cytological interpretation depends not only on the adequacy of sample collection but also on appropriate preservation of cellularity and cellular morphology until the time of microscopic examination. Cellular degeneration occurring during transport, delayed processing, and improper storage can substantially reduce diagnostic accuracy and may lead to false-negative or equivocal results. Conventional preservation methods such as refrigeration, alcohol-based fixatives, and formalin preservation have inherent limitations. Refrigeration may delay but not completely prevent cellular degeneration, alcohol fixation can induce cellular shrinkage and cytoplasmic distortion, and formalin fixation may

* Corresponding author: Sunidhi Gupta

obscure nuclear details critical for cytological interpretation. These limitations highlight the need for effective preservation techniques capable of maintaining both cellularity and morphology in cytological specimens. Renshaw SA et al, Boekema B et al, and Roh MH et al highlighted the importance of appropriate preservation methods in maintaining cytological adequacy and diagnostic reliability.[1–4]

Roswell Park Memorial Institute medium, commonly referred to as RPMI medium, was originally developed to support growth and maintenance of human lymphoid cells in vitro. Owing to its balanced salt composition, buffering agents, amino acids, vitamins, glucose content, and physiological osmolarity, RPMI medium provides an environment that supports cellular viability and structural preservation. The medium helps maintain membrane integrity, reduces osmotic stress, minimizes autolysis, and preserves nuclear and cytoplasmic details important for accurate cytomorphological assessment. Preservation of cell morphology is particularly important in malignant effusions because identification of malignant cells often depends upon subtle nuclear irregularities, chromatin alterations, nucleolar prominence, cytoplasmic vacuolation, and architectural arrangements. Sura GH et al, Montañes Sancho L et al, Wangen R et al, and Bartosh Z et al emphasized the importance of preservation media and optimized cell handling techniques in maintaining cellular integrity and improving diagnostic utility.[5–8]

Clinical laboratories frequently encounter delays in transport and processing of fluid specimens, especially when samples are collected at peripheral centers and transported to tertiary care institutions for evaluation. RPMI medium may act as a stabilizing medium by preserving cellular integrity and minimizing degenerative changes during storage and transport. In addition to preserving morphology. Preservation of diagnostically significant cells is essential for detection of malignant cells, inflammatory cells, mesothelial cells, and infectious organisms. Restivo G et al, Nybo M et al, Block DR et al, and Wu D et al discussed the significance of specimen transport conditions and optimized preservation methods in maintaining cellular viability and smear quality.[9–12]

The present study was therefore undertaken to evaluate the role of RPMI medium in preserving cell morphology and cellularity in fluid samples and to compare RPMI-preserved samples with routinely processed samples with respect to morphological preservation, cellularity, and bacterial contamination.

Aim

To study the role of RPMI media in preserving cell morphology and cellularity in fluid samples.

Objectives

- To observe changes in cellularity and cell morphology by routine processing.
- To document changes in cellularity and cell morphology by processing in RPMI media added sample.
- To compare the changes in routine processing and RPMI media with respect to:
  - Morphological changes
  - Cellularity
  - Bacterial growth

2. Materials and methods

The present cross-sectional comparative observational study was conducted in the Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, over a duration of one year from April 1, 2024 to March 31, 2025. The study included all pleural, peritoneal, and cerebrospinal fluid samples received in the Department of Pathology for cytological examination for malignant cells during the study period until the desired sample size was achieved. A total of 144 cases were included in the study. Samples that were inadequate, heavily hemolyzed, improperly labeled, or unsuitable for processing were excluded from the study. After receipt in the laboratory, each fluid sample was divided into two aliquots. One aliquot underwent routine processing according to standard laboratory protocols, while RPMI medium was added to the second aliquot for preservation prior to processing. Samples were centrifuged at 2500 rpm for 10 minutes prior to smear preparation. Smears were prepared from centrifuged deposits and stained using routine cytopathological stains according to standard laboratory protocols. Both aliquots were subjected to cytological examination and comparative evaluation. Microscopic examination was carried out to assess preservation of cellularity, nuclear morphology, cytoplasmic morphology, background characteristics, degenerative changes, and bacterial contamination. Morphological preservation was evaluated with reference to nuclear membrane integrity, chromatin preservation, cytoplasmic clarity, cell borders, cellular cohesion, and background artifacts. Cellularity was assessed based on adequacy and quantity of diagnostic cells. Statistical analysis was performed using SPSS software version XX. Chi-square test was applied for comparison between routinely processed and RPMI-preserved samples. A p-value of

<0.05 was considered statistically significant. Comparative analysis between routinely processed samples and RPMI-preserved samples was performed using appropriate statistical methods. Institutional ethical approval was obtained prior to commencement of the study and confidentiality of patient information was maintained throughout the study.

3. Results

A total of 144 fluid samples were included in the present study to evaluate the role of RPMI medium in preservation of cellularity and cellular morphology in fluid cytology specimens. Comparative assessment was performed between routinely processed samples and RPMI-preserved samples with respect to demographic profile, preservation of cellularity, morphological preservation, and bacterial contamination. The findings demonstrated that RPMI medium provided superior preservation characteristics and improved overall diagnostic quality of cytological smears.

3.1. Demographic Profile

The age-wise distribution of study participants revealed that the majority of cases belonged to the 51–60 years age group, accounting for 29.2% of the total study population, followed by the 41–50 years age group comprising 24.3% of cases. Participants aged 31–40 years constituted 16.7% of the study population, while individuals aged 61–70 years represented 16.0% of cases. Younger individuals aged ≤30 years accounted for 8.3% of the study population, whereas participants aged more than 70 years constituted 5.5% of cases. The demographic distribution indicated that fluid cytology evaluation was more frequently required among middle-aged and elderly individuals, reflecting the increased prevalence of inflammatory and malignant conditions in these age groups.

3.2. Cellularity Preservation

Comparative evaluation of cellularity demonstrated significantly better preservation in RPMI-treated samples compared with routine processing methods. Adequate cellularity was maintained in 87.5% of RPMI-preserved samples, whereas only 63.9% of routinely processed specimens demonstrated satisfactory cellularity. Mild reduction in cellularity was observed in 19.4% of routinely processed samples compared with 8.3% of RPMI-preserved specimens. Moderate reduction in cellularity was identified in 11.1% of routine samples, while only 3.5% of RPMI-treated samples showed similar findings. Severe reduction in cellularity was observed in 5.6% of routinely processed samples compared to only 0.7% of RPMI-preserved specimens. These findings clearly indicated that RPMI medium effectively minimized cellular degeneration and significantly improved preservation of diagnostic cellular yield.

3.3. Morphological Preservation

Evaluation of cytomorphological preservation further highlighted the superiority of RPMI medium in maintaining cellular integrity and smear quality. Preserved nuclear details were observed in 91.0% of RPMI-preserved samples compared with 61.1% of routinely processed specimens. Similarly, preserved cytoplasmic morphology was identified in 88.9% of RPMI-treated samples, whereas only 58.3% of routinely processed samples retained satisfactory cytoplasmic preservation. Background clarity was maintained in 84.7% of RPMI-preserved specimens compared to 54.9% of routine samples. Degenerative changes including nuclear smudging, cytoplasmic vacuolation, cellular fragmentation, and loss of cellular cohesion were significantly more common in routinely processed specimens, being present in 36.1% of cases compared with only 9.7% of RPMI-preserved samples. Cellular distortion was observed in 32.6% of routinely processed specimens, whereas only 7.6% of RPMI-treated samples demonstrated similar alterations. These observations established that RPMI medium effectively preserved nuclear membrane integrity, chromatin pattern, cytoplasmic details, and overall smear architecture, thereby improving interpretability of cytological specimens.

3.4. Bacterial Contamination

The present study also demonstrated reduced bacterial contamination in RPMI-preserved samples compared with routinely processed specimens. Bacterial growth was identified in 20.1% of routinely processed samples, whereas only 6.3% of RPMI-preserved samples demonstrated bacterial contamination. Absence of bacterial growth was observed in 93.7% of RPMI-treated specimens compared with 79.9% of routine samples. Reduced bacterial contamination contributed to improved smear clarity, better background preservation, and enhanced identification of diagnostically significant cells. The lower incidence of bacterial growth in RPMI-preserved samples further supported the role of RPMI medium in maintaining smear quality and improving overall cytological interpretation.

3.5. Statistical Analysis

Statistical analysis was performed using SPSS software version XX. Chi-square test was applied for comparison between routinely processed samples and RPMI-preserved samples. A p-value of <0.05 was considered statistically significant.

Table 1 Distribution of Study Participants According to Age Group (n = 144)

Age Group (Years)	Number of Cases	Percentage (%)
≤ 30	12	8.3
31–40	24	16.7
41–50	35	24.3
51–60	42	29.2
61–70	23	16.0
> 70	8	5.5
Total	144	100.0

Most study participants belonged to the 51–60 years age group, accounting for 29.2% of the total study population, followed by the 41–50 years age group comprising 24.3% of cases. Participants aged 31–40 years constituted 16.7% of the study population, while individuals aged 61–70 years represented 16.0% of cases. Younger participants aged ≤30 years accounted for 8.3% of the study population, whereas patients aged more than 70 years constituted the smallest proportion with 5.5% of cases. The findings indicated that fluid cytology evaluation was more commonly required among middle-aged and elderly individuals, reflecting the increased prevalence of inflammatory and malignant conditions in these age groups.

Table 2 Comparison of Cellularity in Routine Processing and RPMI-Preserved Samples (n = 144)

Cellularity Assessment	Routine Processing n (%)	RPMI-Preserved Samples n (%)	p-value
Adequate Cellularity	92 (63.9%)	126 (87.5%)	<0.001
Mild Reduction in Cellularity	28 (19.4%)	12 (8.3%)	0.004
Moderate Reduction in Cellularity	16 (11.1%)	5 (3.5%)	0.011
Severe Reduction in Cellularity	8 (5.6%)	1 (0.7%)	0.036
Total	144 (100%)	144 (100%)	—

RPMI-preserved samples demonstrated significantly better preservation of cellularity compared to routine processing. Adequate cellularity was maintained in a substantially higher proportion of RPMI-treated samples, while severe cellular loss was markedly reduced. The differences observed between routine processing and RPMI-preserved samples were statistically significant.

Table 3 Comparison of Morphological Preservation in Routine Processing and RPMI-Preserved Samples (n = 144)

Morphological Parameter	Routine Processing n (%)	RPMI-Preserved Samples n (%)	p-value
Preserved Nuclear Details	88 (61.1%)	131 (91.0%)	<0.001
Preserved Cytoplasmic Morphology	84 (58.3%)	128 (88.9%)	<0.001
Background Clarity Maintained	79 (54.9%)	122 (84.7%)	<0.001
Degenerative Changes Present	52 (36.1%)	14 (9.7%)	<0.001
Cellular Distortion Present	47 (32.6%)	11 (7.6%)	<0.001

RPMI medium demonstrated statistically significant superiority in preservation of cellular morphology with better

maintenance of nuclear and cytoplasmic details. Degenerative alterations and cellular distortion were considerably lower in RPMI-preserved samples compared to routine processing.

Table 4 Comparison of Bacterial Contamination in Routine Processing and RPMI-Preserved Samples (n = 144)

Bacterial Growth Status	Routine Processing n (%)	RPMI-Preserved Samples n (%)	p-value
Bacterial Growth Present	29 (20.1%)	9 (6.3%)	0.002
No Bacterial Growth	115 (79.9%)	135 (93.7%)	0.002
Total	144 (100%)	144 (100%)	—

RPMI-preserved samples showed significantly lower bacterial contamination compared to routinely processed samples. Reduced bacterial growth contributed to improved smear quality, enhanced background clarity, and superior cytological interpretability.

4. Discussion

Preservation of cellularity and cellular morphology is one of the most important determinants influencing the diagnostic accuracy of fluid cytology. Cytological interpretation largely depends upon the integrity of nuclear and cytoplasmic details, background clarity, and maintenance of adequate cellular yield. Degenerative changes occurring during transportation, delayed processing, and inappropriate storage conditions may significantly compromise cytological interpretation and result in false-negative or indeterminate diagnoses. The present study evaluated the role of Roswell Park Memorial Institute (RPMI) medium in preservation of fluid cytology specimens and demonstrated markedly improved preservation characteristics in RPMI-treated samples compared with routine processing methods.

The present study included 144 fluid samples processed by conventional methods and after addition of RPMI medium. Comparative assessment revealed superior preservation of cellularity, nuclear morphology, cytoplasmic morphology, and smear background in RPMI-preserved samples. Adequate cellularity was maintained in 87.5% of RPMI-treated specimens compared with 63.9% of routinely processed samples. Preserved nuclear details were observed in 91.0% of RPMI-preserved samples compared to 61.1% in routine processing, while satisfactory cytoplasmic preservation was identified in 88.9% of RPMI-treated specimens compared with 58.3% of routinely processed samples. Degenerative alterations and cellular distortion were considerably lower in RPMI-preserved specimens, clearly indicating the beneficial role of RPMI medium in maintaining cytomorphological integrity.

The findings of the present study are consistent with observations reported by Rani U et al, who demonstrated improved preservation of cellular morphology in pleural and peritoneal fluid cytology specimens processed with RPMI medium. Similar observations were noted in the present study, where RPMI-treated samples demonstrated superior preservation of nuclear details, cytoplasmic integrity, and background clarity. Renshaw SA et al also reported significantly improved cellularity in cerebrospinal fluid cytology specimens preserved with RPMI medium, emphasizing the importance of maintaining diagnostic cellular yield during delayed processing. Improved preservation of cellularity is particularly important in fluid cytology because malignant cells may often be scanty and prone to degeneration during transport and storage. Loss of diagnostic cells can significantly reduce sensitivity and compromise accurate cytological interpretation.1,2

The superior preservation of cellular morphology observed in the present study can be attributed to the balanced nutrient composition and buffering capacity of RPMI medium. Boekema B et al evaluated RPMI and other preservation media for tissue storage and reported improved preservation characteristics due to maintenance of physiologic osmolarity and reduction of cellular degeneration. Similar findings were observed in the present study, where RPMI-preserved samples demonstrated better maintenance of nuclear membrane integrity, chromatin pattern, and cytoplasmic details. Wangen R et al also emphasized that optimized preservation methods minimize cellular stress and improve maintenance of morphologic architecture essential for microscopic interpretation.3,4

Preservation quality is increasingly important because cytological specimens are frequently utilized for ancillary diagnostic techniques including immunocytochemistry and molecular testing. Roh MH et al highlighted the importance of appropriate preservation techniques for successful molecular diagnostic applications in cytological specimens. Sura GH et al further emphasized that preservation of viable and morphologically intact cells improves the diagnostic utility of cytology effusion specimens in modern pathology practice. In the present study, RPMI-preserved samples

demonstrated superior cellular integrity and background preservation, thereby potentially improving applicability for ancillary investigations.5,6

Delayed transportation and prolonged storage are common preanalytical challenges encountered in pathology laboratories. During delayed processing, cellular degeneration may occur due to osmotic imbalance, enzymatic degradation, and bacterial proliferation. The present study demonstrated significantly lower degenerative alterations in RPMI-preserved specimens compared with routinely processed samples. Montañes Sancho L et al similarly reported improved preservation and cellular stability in cerebrospinal fluid specimens preserved using RPMI medium. Nybo M et al and Wu D et al also highlighted the importance of optimized transport and preservation protocols in maintaining cellular integrity and diagnostic accuracy during storage and transportation of biological specimens.7–9

Another important finding of the present study was significantly reduced bacterial contamination in RPMI-preserved specimens. Bacterial growth was identified in 20.1% of routinely processed samples compared with only 6.3% of RPMI-preserved samples. Reduced bacterial contamination contributed to improved background clarity and enhanced interpretability of cytological smears. Restivo G et al discussed the importance of optimized preservation conditions in maintaining structural integrity and reducing degradation of biological specimens. Similar principles may explain the improved smear quality observed in RPMI-treated samples in the present study.10

Block DR and Algeciras-Schimnich emphasized the importance of maintaining specimen quality in body fluid analysis because accurate cytological interpretation depends heavily upon preservation of cellular architecture and nuclear details. In malignant effusions, subtle alterations in chromatin pattern, nuclear membrane irregularity, and cytoplasmic morphology are essential for diagnosis. The present study demonstrated superior preservation of these cytomorphological features in RPMI-treated samples, thereby improving diagnostic reliability.11

Bhanvadia VM et al and Shivakumarswamy U et al highlighted the importance of specimen preservation and processing quality in improving diagnostic accuracy in body fluid cytology. Their studies demonstrated that improved preservation methods significantly enhance identification of malignant cells and reduce interpretative errors. Similar observations were noted in the present study, where RPMI medium substantially improved smear quality, preservation of cellular details, and overall interpretability.12,13 Tan WJ et al also emphasized the expanding role of cytological specimens in molecular diagnostics and advanced ancillary investigations, further underscoring the importance of maintaining cellular integrity during specimen processing.14

Moore GE et al originally developed RPMI medium for maintenance and growth of human leukemic cells in vitro. Subsequent studies by McCoy RE et al and Sanchez-Rosario Y et al emphasized the importance of chemically defined media in maintaining cellular stability and viability under laboratory conditions. The balanced content of RPMI medium likely contribute to its effectiveness in preserving cytological specimens by minimizing osmotic stress and reducing autolytic degeneration.15–17

The present study therefore strongly supports the role of RPMI medium as an effective preservation medium for fluid cytology specimens. Improved preservation of cellularity, nuclear morphology, cytoplasmic details, and smear background significantly enhanced diagnostic reliability and interpretability. Reduced degenerative alterations and lower bacterial contamination further contributed to improved smear quality. The incorporation of RPMI medium into routine laboratory protocols may substantially improve the overall quality and diagnostic utility of fluid cytology specimens.

5. Conclusion

RPMI medium demonstrated significant superiority over routine processing methods in preservation of cellularity, nuclear morphology, cytoplasmic details, and smear background in fluid cytology specimens. Reduced degenerative alterations and bacterial contamination further enhanced diagnostic interpretability. Incorporation of RPMI medium into routine cytopathology protocols may therefore improve diagnostic accuracy, particularly in delayed and transported fluid samples.

Limitations

The study was conducted at a single tertiary care center with a limited study duration. Evaluation was restricted to selected fluid samples and broader multicentric studies may further validate the findings.

Recommendations

Further large-scale studies should be conducted to evaluate the utility of RPMI medium in different cytological specimens and advanced ancillary techniques. Routine incorporation of RPMI medium may be considered in pathology laboratories handling delayed or transported fluid cytology samples.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Institutional ethical approval was obtained prior to commencement of the study.

Statement of informed consent

Patient confidentiality and data privacy were maintained throughout the study.

References

[1] Rani U, Singh M, Saith A, Jain SL, Aggarwal A, Aggarwal S. Evaluation of Use of RPMI Medium to Preserve Cell Morphology for Pleural/Peritoneal Fluid Cytology. J Cytol. 2022;39(1):26-9.

[2] Renshaw SA, Gupta S, Campos M, Hodes L, Renshaw AA, Gould EW. The addition of RPMI significantly improves the cellularity of cerebrospinal fluid cytology specimens over time. Cancer Cytopathol. 2013;121(5):271-4.

[3] Boekema B, van der Valk J, de Jong W, et al. Evaluation of saline, RPMI and DMEM/F12 for storage of human skin biopsies. J Dermatol Sci. 2015;78(1):72-4.

[4] Roh MH, Lee JH, Lee H, et al. The utilization of cytologic fine-needle aspirates of lung cancer for molecular diagnostic testing. J Pathol Transl Med. 2015;49(4):300-9.

[5] Sura GH, Mino-Kenudson M, Mino-Kenudson M, et al. Molecular testing opportunities on cytology effusion specimens. J Thorac Oncol. 2023;18(1):1-9.

[6] Montañes Sancho L, O'Neill D, McNeill CJ. Diagnostic utility of canine cerebrospinal fluid preserved in RPMI medium. J Small Anim Pract. 2022;63(10):1-5.

[7] Wangen R, Fuchs M, Dufresne M, et al. Preservation method and phosphate buffered saline washing of acute myeloid leukemia patient samples. Int J Mol Sci. 2018;19(1):296.

[8] Bartosh Z, Weiskirchen S, Weiskirchen R. New approaches to old techniques in cell handling for functional assays. Cells. 2025;14(16):1271.

[9] Restivo G, Sorrentino G, Gagliardi M, et al. Live slow-frozen human tumor tissues viable for 2D, 3D, ex vivo cultures and drug testing. Commun Biol. 2022;5(1):1-11.

[10] Nybo M, Cadamuro J, Cornes MP, et al. Sample transportation – an overview. Diagn Pathol. 2019;14(1):1-12.

[11] Block DR, Algeciras-Schimnich A. Body fluid analysis: Clinical utility and applicability of published studies to guide interpretation of today's laboratory testing in serous fluids. Crit Rev Clin Lab Sci. 2013;50(2):107-24.

[12] Wu D, Xu Z, Zhang X, et al. How long can we store blood samples: A systematic review and meta-analysis. EBioMedicine. 2017;15:43-9.

[13] Bhanvadia VM, Desai S, Shah S, et al. Analysis of diagnostic value of cytological smear method versus cell blocks method in body fluid cytology: Study of 150 cases. Ethiop J Health Sci. 2014;24(2):125-31.

[14] Shivakumarswamy U, Kaur M, Sood S, et al. Diagnostic utility of the cell block method versus conventional smear in pleural effusions. J Cytol. 2012;29(3):167-71.

[15] Tan WJ, Tan SH, Tan G, et al. Cytologic and molecular diagnostics for vitreoretinal lymphoma. Mol Biosyst. 2021;17(6):1424-35.

[16] Moore GE, Gerner RE, Franklin HA. Culture of human leukemic cells in a medium supporting normal cell growth. JAMA. 1967;199(6):519-22.

[17] McCoy RE, Costa NA, Morris AE. Factors that determine stability of highly concentrated chemically defined production media. Biotechnol Prog. 2015;31(2):493-502.