



FIXATIVE CHOICE MATTERS: A COMPARATIVE STUDY OF DIFFERENT TYPES OF FIXATIVES IN SEROUS EFFUSION CYTOLOGY

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Abstract:

1. **Objectives:** This study aimed to evaluate and compare the efficacy of Carnoy's, Formalin and Ethanol fixatives in preserving cellular morphology and providing background clarity in serous effusion cytology. The goal of this study is to determine the most effective fixative for optimal nuclear and cytoplasmic preservation and diagnostic quality.

2. **Materials & Methods:** Out of 50 effusion samples, including Pleural (48%), Ascitic (36%), Synovial (12%) and Pericardial (4%) fluids. Samples were centrifuged and smears were prepared using Carnoy's, Formalin and Ethanol fixatives. Papanicolaou (PAP) stained slides were assessed for nuclear and cytoplasmic details, background staining and cellular arrangement.

3. **Statistical Analysis :** The data was tabulated in an MS Excel worksheet and basic statistical analysis such as calculating the mean and percentage was performed.

4. **Results:** Among all three fixatives, Carnoy's fixative demonstrated the best preservation of cellular morphology. For nuclear details, 74% of smears showed excellent features with Carnoy's, 34% of Ethanol and 0% of Formalin. Cytoplasmic features were also best preserved with Carnoy's, 80% showed excellent results. Formalin showed the highest incidence (30%) of residual background staining. Cells were predominantly arranged in singles or in sheets, while 3D clusters were rare (2%).

5. **Conclusion:** Carnoy's fixative proved to be superior in preserving both nuclear and cytoplasmic details with best background clarity making it most effective among the fixatives that are been used in this study. Ethanol was moderately effective, while formalin showed poor cellular preservation and background staining. Hence, Carnoy's is recommended for cytological evaluation of body fluid samples.

Keywords: Ethanol, Carnoy's, Formalin

INTRODUCTION:

Exfoliative and aspiration fluid Cytology became prominent in the late 19th century. Exfoliative cytology especially in body fluid analysis (Pleural, Ascitic, Synovial, Pericardial, Cerebrospinal fluid etc) were fully standardized. When there is any underlying disease abnormal fluid accumulation occurs in the body cavities which is termed an effusion. These fluid can either be a Transudate or Exudate. Evaluation of these effusions needs accurate sample handling and proper fixation [1-4]. Cytological interpretation mostly depends on preserving cellular morphology until processing. Effective fixatives must provide rapid cell penetration, reduce shrinkage, maintain integrity, inactivate autolytic enzymes, support dye diffusion and cell adhesion, also prevent microbial and cellular degradation. Ethanol is flammable and evaporates easily; Carnoy's is acidic in nature which fixes rapidly and preserves nucleic acids and proteins; while formalin is a standard aldehyde fixative in

histopathology^[5,6]. All three fixatives work by coagulating proteins and minimizing shrinkage with their pros and cons.

Most common type of fixative used for cytological smears were alcohol based usually Isopropanolol. In this study, effusion fluids were centrifuged, smeared and fixed using each of the three different fixatives (Ethanol, Formalin & Carnoy's) and we compared their effectiveness in enhancing cellular morphology and background clarity.

MATERIALS AND METHODS:

The present study was an Prospective observational study, conducted in the Department of Pathology, Puducherry, after obtaining Institutional Ethical Clearance. This study included Pleural, Pericardial, Peritoneal and Synovial fluids. The study was conducted over a period of six months, from January 2025 to June 2025. A sample size of 50 cases was taken by the convenient sampling method. Fresh samples were obtained and examined grossly for volume and colour. Detailed clinical history and relevant findings were also noted. The fixation of the smears using three fixatives (Ethanol, Formalin & Carnoy's fixative) were done immediately after cytocentrifugation of the samples at 1500 rpm (Rotation per minute). The Supernatant was discarded and the sediment is then smeared and fixed at room temperature for 15 - 20 minutes. After which the smears were stained using Papanicolaou (PAP) stain and the cytomorphological details of the smears were scored as,

- I. Score 3 (Excellent preservation and sharp nuclear and cytological features),
- II. Score 2 (Good with nuclear and cytological features),
- III. Score 1 (Average – just acceptable for assessment),
- IV. Score 0 (Poor -unsuitable for assessment)^[1,3-5].

Inclusion Criteria:

Fresh samples of fluids with Volume more than or equal to 2ml; Samples sent for routine processing were included in the study.

Composition of the fixatives used in the study:^[5,6]

- 1. 95% Ethanol (100ml): 95 ml ethanol; 5ml Distilled water.
- 2. Carnoy's: 95% ethanol; 30ml Chloroform; 10ml Glacial acetic acid.
- 3. 10% Neutral Buffered Formalin (100ml): 37% Formaldehyde solution; 0.8g Sodium Chloride; 0.4g Potassium phosphate monobasic; 0.65g Potassium phosphate dibasic.

Statistical Analysis:

The data was tabulated in an MS Excel worksheet and basic statistical analysis such as calculating the mean and percentage was performed.

RESULTS:

In our study, the most commonly received effusion sample was Pleural fluid (48%), followed by ascitic fluid (36%), Synovial fluid (12%) and Pericardial fluid (4%). The majority of the samples were from patients aged 35 to 65 years. On Physical examination most of the fluid appeared straw yellow in colour (50%).

1. Assessment of Morphological Preservation of Cellular Details (Nuclear & Cytoplasmic Features):

Carnoy's fixative showed highest quality preservation for both nuclear and cytoplasmic features. For nuclear features, 74% of samples fixed with Carnoy's were scored excellent [Figure 1], compared to 34% for ether alcohol and 0% for formalin. In formalin fixative 10% were scored unsatisfactory; none were unsatisfactory with Carnoy's or ethanol.

For cytoplasmic features, 80% of Carnoy's cases were scored excellent, which was much superior compared to both ethanol (52%) and formalin (4%). Formalin had 2% unsatisfactory cytoplasmic preservation.

Ethanol showed better results than formalin but was inferior to Carnoy's, especially for nuclear details. Formalin was the least effective, with most of the cases scored only good or average and some unsatisfactory results for nuclear & cytoplasmic details [Table 1].

Among the three fixatives, Carnoy's fixative showed best nuclear and cytoplasmic morphology, followed by ethanol; formalin was less optimal.

Table 1: Comparison of Nuclear & Cytoplasmic features using various fixatives:

	Score	Ethanol	Formalin	Carnoy's
Nuclear Features	3 (Excellent)	34%	0%	74%
	2 (Good)	64%	52%	26%
	1 (Average)	2%	38%	0%
	0 (Poor)	0%	10%	0%
Cytoplasmic Features	3 (Excellent)	52%	4%	80%
	2 (Good)	48%	68%	20%
	1 (Average)	0%	26%	0%
	0 (Poor)	0%	2%	0%

2. Cellular arrangement and Background characteristics:

The pattern of cellular arrangement also includes the nature and presence of background material.

Cellular Arrangement:

The cells in most of the fluids were arranged in singles and in sheets (64%), followed by singles (34%), with rare 3D clusters (2%).

Type of Background Material:

Lymphocytes & proteinaceous (38.3%) were the most commonly seen background material, then proteinaceous alone (19.15%), with least common are the lymphocytes & hemorrhagic, hemorrhagic and neutrophils.

Background Staining:

The fluids fixed with Carnoy's fixative showed clearest background in 90% of cases, indicating optimal slide clarity [Figure 2].

Ethanol showed a clear background in 80% of cases. Formalin demonstrated clear background in 70% of cases. But formalin showed background staining in 30% of cases which was highest when compared with other two types of fixative.

DISCUSSION:

The human body cavities are lined by two mesothelial layers- Visceral and Parietal. The main body cavities which includes the Pleural cavity (surrounding the lungs), Peritoneal cavity (around gastrointestinal organs) and Pericardial cavity (enclosing the heart). Normally, a thin layer of lubricating fluid exists between these layers of the cavities to allow smooth movement of the membranes. These effusion fluids can either be a transudate or exudate ^[1-4].

Cytological examination of the effusion fluid plays a vital role in diagnosing diseases and ruling out malignancies. By examining smears prepared from centrifuged fluid samples, clinicians can avoid unnecessary and major surgical procedures. Due to this makes cytology is considered most valuable and a minimally invasive diagnostic tool ^[5,6]. In the present study we analysed various body fluids including Pleural, Pericardial, Synovial and Ascitic fluids.

It is important to document the volume and gross appearance of the fluid as soon as it is received, as these features helps in diagnosis. Key parameters were:

1. Volume - Helps to determine the cytopreparation method.
2. Colour - Provides clues towards the cytological diagnosis.

In this study, Pleural fluid was the most commonly received sample (48%) which was in concordance with the study done by Sonti Sulochana et al and Preeti et al [7,8] and in the same study they also mentioned that the primary breast and lung carcinoma mainly spread to the pleural cavity.

Though many malignant effusions appear blood stained but all are not positive for cancer cells [9]. In our study, among the five hemorrhagic samples, two cases were Positive for malignant cells out of which one is Pleural fluid and the other is Ascitic fluid.

Samples processed immediately retained significantly better cellular morphology. Fresh samples gives us multiple benefits over those stored in preservatives. The choice of an appropriate fixate can be done when they are fixed using different fixatives, they become easier to manage, cells tend to be more stickier and better adhesion to the glass slides^[10]. These observations were also noticed in our study, where smears showed good nuclear and cytoplasmic details in specific fixatives especially with carnoy's which are processed immediately.

Formalin - fixed samples showed poor cellular morphology due to contraction of the cell and less intense staining. The background was unclear and the smears prepared with formalin were mostly scored unsatisfactory [Figure 3]. This finding was in concordance with the study done by Shidham VB, in which the author stated that Formalin-fixed cells do not adhere to the slide and the staining quality is also poor for both Papanicolaou (PAP) and Diff-Quik (DQ) stains^[10]. Too short fixation time with formalin can also lead to underfixed cells and dirty background^[11].

Notably, smears treated with Carnoy's fixative showed excellent nuclear (74%) & cytoplasmic (80%) preservation along with a clear background. This supports Carnoy's role as an effective fixative for body fluids, and it was in concordance with the study done by Mirza Shabnam et al [9]. Six cases (12%) showed score 2 in both cytoplasmic and nuclear features with Carnoy's fixative, this could be due to delay in transportation of the sample from the ward after collection. A delay between collecting the fresh fluid sample and its microscopic examination can result in cellular degeneration, leading to suboptimal or inaccurate findings^[11].

Ethanol showed excellent nuclear (34%) and cytoplasmic (52%) of the cases. Few smears fixed in ethanol also displayed cytoplasmic vacuolation and fraying. Although nuclear staining was adequate, the nucleus appeared shrunken due to excessive cell contraction. Ethanol is best suited for short term preservation, samples should be refrigerated in sealed containers.

Our study assesses nuclear and cytoplasmic features, background staining and cell arrangement in detail, providing a more holistic evaluation of diagnostic efficacy compared to prior studies that focus mainly on cellular details.

LIMITATIONS:

Small sample size; Cerebrospinal fluid could not be included since the most of the samples received are less than 2 ml; Interobserver variability.

CONCLUSION:

In summary, Carnoy's fixative showed superior preservation of cellular details and produced the cleanest, most interpretable slides for serous effusion cytology. Ethanol performed moderately well, while formalin was least effective in all observed parameters. Although Formalin demonstrated inferior cytomorphological preservation compared to ethanol and carnoy's fixative, it remains more economical option, which may influence its use in resource limited settings.

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