International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

# Normal Saline Rehydration Technique or Carnoy's Solution - What to Choose for Hemorrhagic Fluid Cytology

**Mudit Sharma[1], Rateesh Sareen[2], Ranu Sharma[3], Anjali Sharma[4]**

[1]In charge Immunohistochemistry & Histopathology Laboratory, Bhagwan Mahaveer Cancer Hospital & Research Center, Jaipur
[2]Consultant Pathology, Santokba Durlabhji Memorial Hospital & Research Center, Jaipur
[3]Lecturer, Jyoti Rao Phulle University, Jaipur, Rajasthan, India
[4]Sr Consultant Pathology, Bhagwan Mahaveer Cancer Hospital & Research Center, Jaipur
Corresponding Author - Dr Rateesh Sareen (drrateeshsareen[at]yahoo.co.in)

**Abstract:** ***It is not uncommon to receive hemorrhagic fluids for cytological examination. The red blood cells impair diagnostic efficacy of smears. The body fluid can be processed in many ways- NSRT (Normal saline rehydration technique) is one such technique. The present study was done to evaluate quality of smears prepared by NSRT and Carnoy's fixation. The smears were stained by Hematoxylin & Eosin and Leishman stain. The smears were analyzed for nuclear, cytoplasmic, background and quantity of cells. Scores were given from 0 to2. A total of 30 hemorrhagic fluids were taken. NSSRT showed better cell lysis and better background in comparison to Carnoy's fixative on Hematoxylin & Eosin stain and also on Leishman stain. Hematoxylin & Eosin was better than Carnoy's fixative technique. NSRT is better technique as it is simple, cost-effective and faster than Carnoy's technique for hemorrhagic fluids.***

**Keywords:** NSRT, Carnoy's fixative, Hemorrhagic body fluid

## 1. Introduction

Cytological diagnosis relies heavily on clear cell morphology with cell arrangement pattern against a characteristic background features. The analysis of body fluids on cytology suffers tremendously when large numbers of red blood cells are present in sample. The hemorrhagic fluids are processed by variety of techniques in laboratory, the sole objective been to visualize and detect malignant cells with intact morphology without losing them in the processing phase.

The history of effusion cytology is traced back to 19$^{th}$ century when Lucke and Klebs[1] demonstrated the presence of malignant cells in ascitic fluid in 1867. Serous fluid effusions like pleural, pericardial and peritoneal are obtained by needle aspiration or evacuation of fluid filled cavities. A minimum of 20 ml or large volume of fluid are required for cytological diagnosis.

**Routine preparation of body fluid smears[2]:**

The following procedure is followed in preparing smears from body fluid samples.

- In case of processing of samples <12 hours of collection, no fixative is required. The fluid can be stored at 4$^0$C for 72 hours. In case of delay in processing, an equal volume of 50% to 95% ethyl alcohol or Saccomanno fixative (50% ethanol & 2% Carbowax) is recommended.
- Cytological smears are prepared by cyto centrifugation or by direct smearing.
- Smears can be air dried or 95% ethanol processed.
- Fixed smears are stained by Papanicolaou technique with Hematoxylin &Eosin and air dried smears stained by Ramanowsky technique ( Wright, MGG or Diff quick)

There are several methods to prevent Red blood cells from obscuring the cytologic smear. They are as under[3]:

1) Glacial acetic acid
2) Carnoy's fixative(CF)
3) Saponin method
4) Normal saline rehydration technique ( NSRT)
5) Clark's solution
6) Cytorich red

H N Buscham[2] in 1962 recommended the use of a drop of glacial acetic acid in 95% ethanol fixative in coplin jar as RBC lysate. It was used in conventional gynecology smears. Carnoy's fixative also causes lysis of RBC in 3-5 minutes. The Saponin method used 1% saponin and 3% calcium gluconate in 2:3 ratios. It can be used for grossly hemorrhagic as well as for samples with trace amount of blood.

## 2. Material & Methods

The present study was conducted in the department of Pathology, Bhagwan Mahaveer Cancer Hospital & Research center, Jaipur, India from November, 2016 to January, 2017. A total of 30 hemorrhagic body fluid samples comprising of 20 ascitic fluid and 10 pleural fluids were included in study. Those fluid samples which were pale yellow in color were excluded form study. Gross examination of fluid sample for volume, appearance, color and coagulum was done. The fluid samples were centrifuged at 2000 rpm for 10 minutes. Thereafter, cytospin preparations were prepared. A total of four smears were prepared. Two smears were treated with carnoy's fixative and remaining two underwent NSRT (Normal saline rehydration technique). The smears were stained with Leishman and Hematoxylin & Esoin staining (H & E).

Each smear was scored as per a scoring system by NG et al with few modifications. The slides were reviewed by pathologist and were assessed for nuclear features, cytoplasmic features, background and quantity of cells in


Licensed Under Creative Commons Attribution CC BY

smears. A score of 0 was given to smears with poor quality, score 1 for satisfactory quality whereas a score of 2 was given to smears with excellent quality.

**Table 1:** Comparative scores of morphological features on Leishman & H&E stain using Carnoy's fluid and NSRT

| Nuclear features | Score 0 | Score 1 | Score 2 | Total score |
|---|---|---|---|---|
| CL | 1(3.0%) | 25(83.3%) | 4(1.3%) | 33 |
| CHE | 0 | 15(50%) | 15(50%) | 45 |
| NL | 4(13.3%) | 12(40%) | 14(46.7%) | 40 |
| NHE | 1(3.0%) | 2(6.7%) | 27(90%) | 56 |
| | | | | |
| **Cytoplasmic features** | **Score 0** | **Score 1** | **Score 2** | **Total score** |
| CL | 3(10%) | 26(86.7%) | 1(3.0%) | 28 |
| CHE | 0 | 21(70%) | 9(30%) | 39 |
| NL | 0 | 16(53.3%) | 14(46.7%) | 44 |
| NHE | 2(6.7%) | 8(26.7%) | 20(66.7%) | 48 |
| | | | | |
| **Background features** | **Score 0** | **Score 1** | **Score 2** | **Total score** |
| CL | 3(10%) | 27(90%) | 0 | 27 |
| CHE | 0 | 18(60%) | 12(40%) | 42 |
| NL | 0 | 6(20%) | 24(80%) | 54 |
| NHE | 0 | 8(26.7%) | 22(73.3%) | 52 |
| | | | | |
| **Quantity of cells** | **Score 0** | **Score 1** | **Score 2** | **Total score** |
| CL | 0 | 17(76.7%) | 13(43.3%) | 43 |
| CHE | 0 | 23(76.7%) | 7(23.3%) | 37 |
| NL | 0 | 12(40%) | 18(60%) | 48 |
| NHE | 0 | 0 | 30(100%) | 60 |

## 3. Discussion

Hemorrhagic body fluids pose a diagnostic challenge in cytology. The present study compares Carnoy's fixative (CF) and NSRT for hemorrhagic body fluid using self devised scoring system; a modification of NG et al[4]scoring system.20 (66.7%) peritoneal fluids and 10 (33.3%) pleural fluids were included in study. The mean age was 57.96 years. The dominance of peritoneal and pleural fluids is similar to the studies by Shabnam et al[5], Preeti et al[6], Malvi & Anthony et al[7], Namrata et al[8]. Shervani et al[8] reported higher peritoneal fluid 61.7% compared to 35.9% pleural fluid similar to our study. The nuclear features were better seen in NSRT (90%) prepared smears than Carnoy's fixative (50%). The superiority of NSRT for nuclear features was maintained in both H &E and Leishman Stain, 90% & 50% respectively in comparison to 50% and 1.3% in Carnoy fixative treated H& E and Leishman stain. (Table-1) Similar observations were observed in study by Kung et al[9] who studied effect of NSRT in Papanicolaou stained smears. The nuclear features like chromatin pattern & nucleoli were clearer in NSRT treatment in comparison to Carnoy's fixative as in our study. Similarly Shabnam et al[8] also reported better cytomorphological details with NSRT in comparison to other techniques. (Figures-1-6)

**Table 2:** Statistical calculations for smear quality using one way ANNOVA

| Nuclear | CL | CHE | NL | NHE |
|---|---|---|---|---|
| Total score | 33 | 45 | 40 | 56 |
| Mean | 1.1 | 1.5 | 1.33 | 1.87 |
| Standard deviation | 0.4026 | 0.5085 | 0.7112 | 0.4342 |
| F value 11.20138. P value is <0.00001. The result is significant at p<.05. | | | | |
| **Cytoplasmic** | **CL** | **CHE** | **NL** | **NHE** |
| Total score | 28 | 39 | 44 | 48 |
| Mean | 0.93 | 1.3 | 1.47 | 1.6 |
| Standard deviation | 0.3621 | 0.4661 | 0.5074 | 0.6215 |
| F value 10.04663. P value is <0.00001. The result is significant at p<.05. | | | | |
| **Quantity** | **CL** | **CHE** | **NL** | **NHE** |
| Total score | 43 | 37 | 48 | 60 |
| Mean | 1.43 | 1.23 | 1.6 | 2 |
| Standard deviation | 0.507 | 0.4302 | 0.4983 | 0 |
| F value 18.49275. P value is <0.00001. The result is significant at p<.05. | | | | |
| **Background** | **CL** | **CHE** | **NL** | **NHE** |
| Total score | 27 | 42 | 54 | 52 |
| Mean | 0.9 | 1.4 | 1.8 | 1.73 |
| Standard deviation | 0.3051 | 0.498 | 0.4068 | 0.4498 |
| F value 28.62399. P value is <0.00001. The result is significant at p<.05. | | | | |

The cytoplasmic features were also better seen in NSRT 66.7% versus 30% in comparison to Carnoys fixative on H & E stain. (Table-1) Similarly background features were also better appreciated on NSRT 66.7% against 40% on carnoy's fixative. NSRT also resulted in good quantity of cells on the smear 100% versus 23.3%. The staining quality of smears treated with NSRT over Carnoy's fixative was maintained in both H& E and Leishman stain. This finding is similar to other studies by Shabnam et al[8] who noted complete lysis of RBC in 60 % cases, Preeti et al6 and Malvi et al7 who reported complete RBC lysis in 82% & 50% cases respectively using Carnoy's fixative. . One way ANNOVA test was used using free online statistical calculator. All results were analyzed by considering statistical significance at a level of p=0.05.

The immersion of air dried smears in normal saline for 30 seconds effectively lysed red blood cells and retained other cell constituents on smear. The clear background helped in interpretation of cytological smears as these characteristics were of paramount importance in making correct diagnosis. NSRT treated smears whether on Leishmain stain or on H & E were found to be superior in quality as shown in one way ANNOVA analysis. (Table-2)

## 4. Recent Trends

In a study by Davis – Devine et al[10], a new RBC lysing fixative, Devine lysing solution (DLS) was used which increased visualization of diagnostic cellular material and results were comparable to cytorich red ( CRR). It is important nowadays that not only cytological samples preparation is user friendly but it should also support different ancillary diagnostic methods such as immunocytochemistry. In a recent study by Srebotnik Kerbs et al[11], disposable filters (cell trics) made up of monofil nylon material with mesh diameter between 20 to 150


Licensed Under Creative Commons Attribution CC BY

micron were used. Larger cells more than 20 micron diameter were retained on upper membrane surface. This cell fraction was transferred into fresh cell medium and it gave excellent results on cell morphology and Immunohistochemistry. Red blood cells and smaller particles were successfully separated from diagnostic cells which were larger than 20 micron. This technique was not useful for separation of red cells in hemorrhagic samples with smaller tumor cells.

The NSRT treatment is quick, simple, inexpensive and effective for lysis of red cells. It should be noted that longer time of immersion in saline can be detrimental to cells as it causes wrinkling of cells obscuring cell details. As far as quantity of cells on smears were concerned nearly both methods were acceptable with marginal edge for NSRT Our study had few limitations regarding the low number of samples, lack of pap stain and glacial acetic acid. Therefore we would emphasize on the need of more studies to compare efficacy of these techniques and to find the best technique among them.

## References

[1] Sears, D., Hadju, S.I. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol., v.31, p.85-97, 1987
[2] Sattenspiel E. Cytological diagnosis of cancer in transudates and exudates: A comparison of the papanicolaou method and the paraffin block technique. Surg Gynecol Obstet 1949;89:478-484
[3] Bales CE. Laboratory techniques. In: Koss LG. Diagnostic cytology and its Histopathologic bases, 5th ed Philadelphia: JB Lippincott Company; 2006; 1569634.
[4] Ng WF, Choi FB, Cheung LH. Rehydration in air dried smears with normal saline- Application in fluid cytology. Acta Cytol 1994; 38:56-64
[5] Mirza S – The comparative study of processing haemorrhagic body fluids by using different techniques. Journal of clinical and diagnostic research. Vol.7 (10): 2186-88 (2013).
[6] Preeti, Mittal S, Alka. Hemorrhagic effusions: Comparison of methods for better cytological assessment. Asian Journal of Critical care 2011; 7:19-22.
[7] Malvi SG, Anthony IP. A comparison of methods to improve quality of smears in bloody cell samples of serous fluids. J Cytol 2000; 17:15-22
[8] Sherwani R, Akhtar K, Naqvi.AH, Akhtar S, Abrari A, Bhargave R. Pleural effusion: Cytology as an aid in diagnosis of pulmonary tuberculosis. Journal of Cytology 2006; 23:123-127.
[9] ITM Kung, BFC Long, JKC Chan. Rehydration of air dried smears: Application in body cavity fluid cytology. J Clin Pathol 1989; 42:113-116.
[10] Davis- Devine S, Day S J, Freund G G. New Red blood cells lysing fixative for use in Fine needle aspiration and fluid cytology. Acta Cytologica 2003; 47:630-636. (DOI:10.1159/000326580)
[11] Hemorrhagic cytology samples - how to get the best diagnostic results. Srebotnik Kirbis I, Strojan Flezar M, Lavrencak J, Pohar Marinsek Z. Cytopathology. 2007 Jun; 18(3):175-9. Pub 2007 Mar 27.

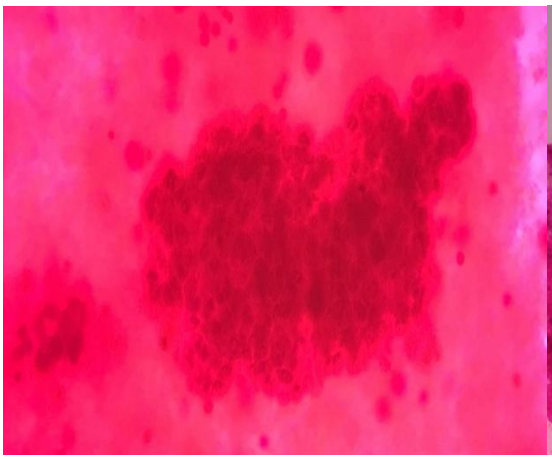

**Figure 1:** Pleural fluid Hemorrhagic (H&E, 40x)

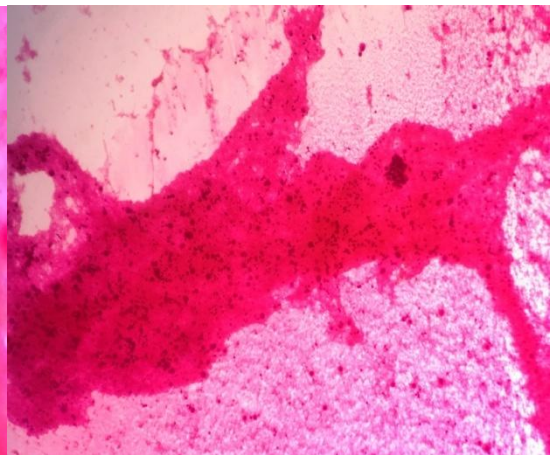

**Figure 2:** Ascitic fluid Hemorrhagic (H&E, 40x)


Licensed Under Creative Commons Attribution CC BY

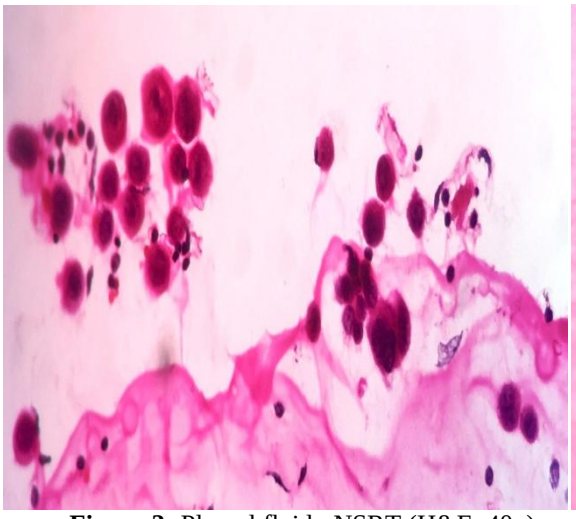

**Figure 3:** Pleural fluid –NSRT (H&E, 40x)

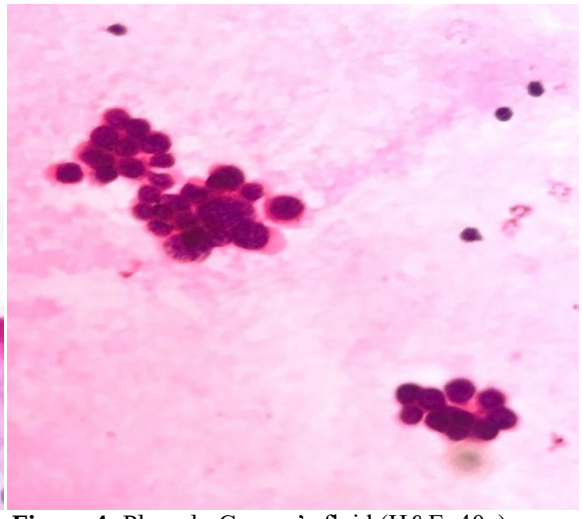

**Figure 4:** Pleural –Carnoy's fluid (H&E, 40x)

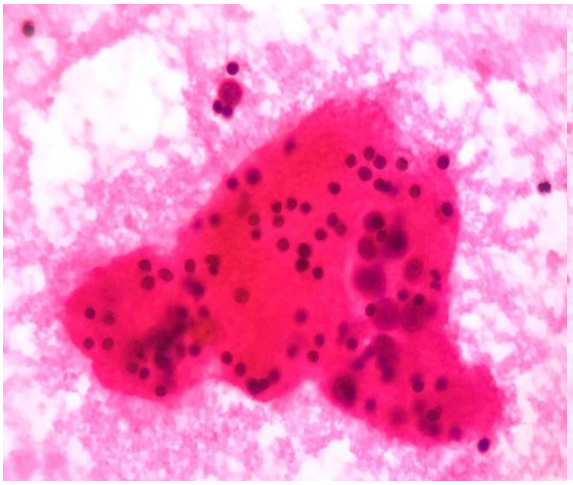

**Figure 3:** Asitic fluid –NSRT (H&E, 40x)

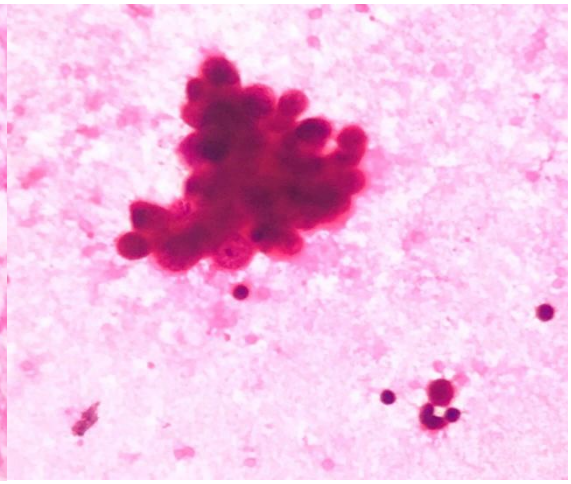

**Figure 4:**Asitic fluid–Carnoy's fluid (H&E, 40x)


Licensed Under Creative Commons Attribution CC BY