

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

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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 to 2. A total of 30 hemorrhagic fluids were taken. NSRT 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 19th century when Lucke and Klebs¹ 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 smears²:

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 4⁰C for 72 hours. In case of delay in processing, an equal volume of 50% to 95% ethyl alcohol or Saccomanno fixative (50% ethanol & 2% Carbomax) 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 under³:

- 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 Buscham² 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 from 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 & Eosin 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

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's 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 al⁵, Preeti et al⁶, Malvi & Anthony et al⁷, Namrata et al⁸. Shervani et al⁸ 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 al⁹ 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 al⁸ 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 Carnoy's 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 al⁸ 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 Leishman 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 al¹⁰, 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 al¹¹, disposable filters (cell trics) made up of monofil nylon material with mesh diameter between 20 to 150

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.

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

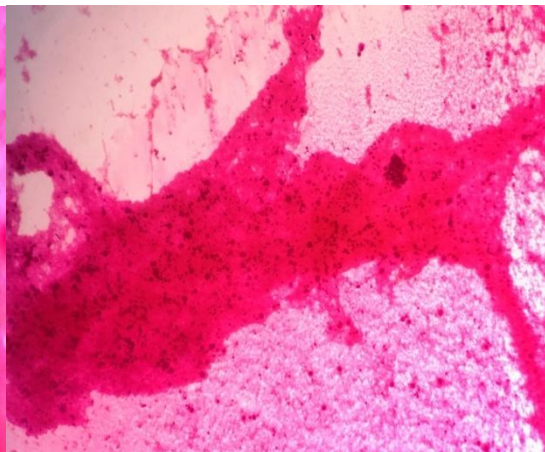


Figure 2: Ascitic fluid Hemorrhagic (H&E, 40x)

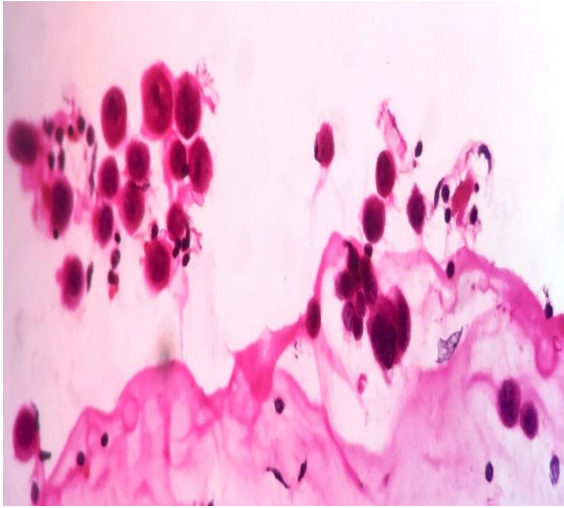


Figure 3: Pleural fluid –NSRT (H&E, 40x)

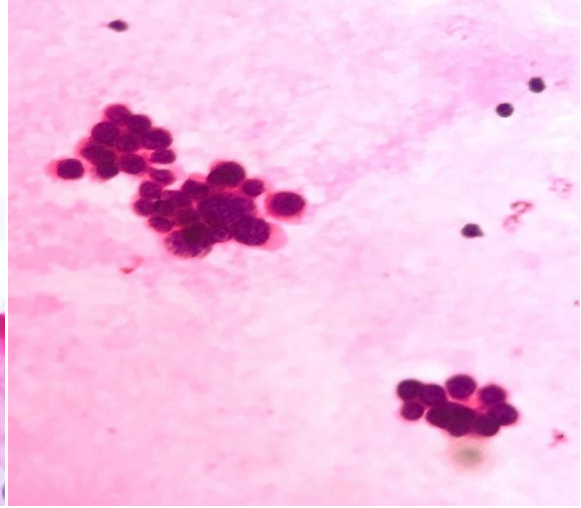


Figure 4: Pleural –Carnoy's fluid (H&E, 40x)

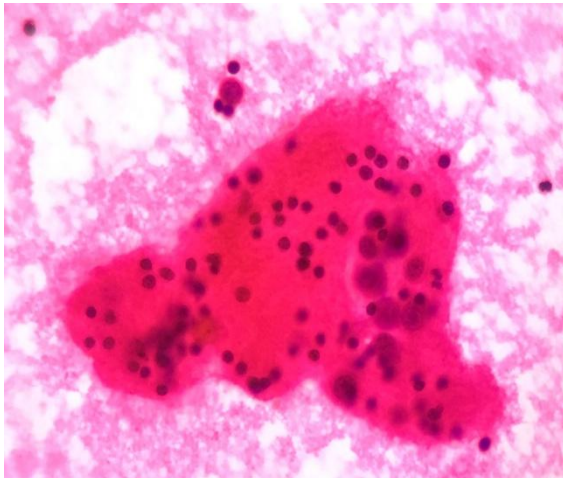


Figure 3: Ascitic fluid –NSRT (H&E, 40x)

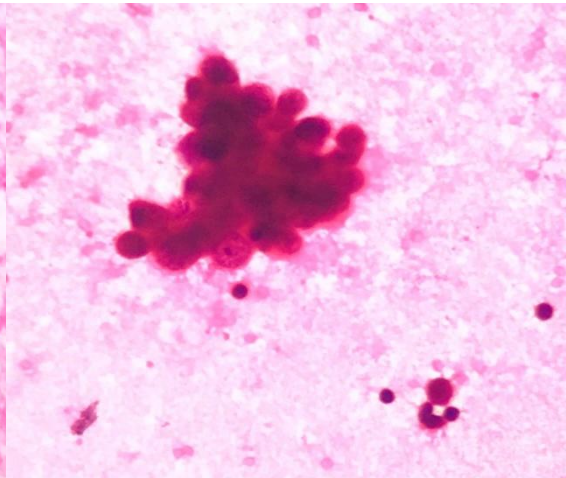


Figure 4: Ascitic fluid–Carnoy's fluid (H&E, 40x)

