A comparative study of cytological processing techniques in hemorrhagic effusion

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INTRODUCTION

Fluids are the most common sample received in cytology laboratories. The common problem faced by all cytologists is the presence of red blood cells (RBC) that is partially or completely obscuring the morphologic details of the cells in the serous fluid, making it difficult for the cytologist to give an accurate diagnosis.¹ They may be pathological, traumatic, or iatrogenic in origin.² They are usually associated with malignancy. The diagnostic performance of the cytological study of the fluid may be attributable to the fact that the cell population present in the sediment is representative of a much larger surface area than that obtained by needle biopsy.³ Hemorrhagic fluids are processed by a variety of techniques in cytology laboratories. The common goal of each technique is the selection and concentration of an adequate number of tumor cells with intact cell morphology, without losing them during processing. Some of the commonly used reagents and methods are glacial acetic acid (GAA), Carnoy's fixative (CF), Saponin method, normal saline rehydration technique (NSRT) to hemolysis red blood cells (RBC) present in the smear background for better cytological assessment.

ABSTRACT

Objectives: Fluids are one of the most common specimens received in cytology laboratories. The presence of erythrocytes may obscure the cells in the smears, making the diagnosis, and identification of cells difficult. Many techniques are being used by laboratories to eliminate these erythrocytes. The present study was undertaken to improve the quality of cytology smears of hemorrhagic samples by comparing three different techniques, namely, Carnoy's fixative (CF), modified CF; and normal saline rehydration technique (NSRT) to hemolysis red blood cells (RBC) present in the smear background for better cytological assessment. The present study was a prospective study done over 1 year 6 months from November 2012 to March 2014, in the Department of Pathology in a Tertiary Care Rural Medical College.

Materials and Methods: All hemorrhagic effusions received in the department of pathology were processed using CF, modified CF, and NSRT. The background of the smear and cytomorphological details with two different stains was analyzed. The Chi-square test was used to find out the association of different techniques in the reduction of RBC.

Results: More than 60% reduction of RBCs in the smear was noted in 85.40%, 14.60%, and 15.60% by NSRT, modified CF, and CF, respectively. Staining was better and nuclear features were best preserved in NSRT.

Conclusion: NSRT is the best, simple, and cheaper technique to lyse RBC in the hemorrhagic fluid. It also shows better staining and well-preserved cytomorphological features of the cell.

Keywords: Hemorrhagic effusion, Carnoy's fixative, Normal saline rehydration technique

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rehydration technique (NSRT),[4a] cellular fixation, and concentration methods.[4b,5] The present study was undertaken to improve the quality of cytology smears of hemorrhagic samples by hemolysis RBC present in the smear background for better cytological assessment by comparing three different techniques, namely, CF, MCF, and NSRT.

MATERIAL AND METHODS

The present study was a prospective study done for 1 year 6 months from November 2012 to March 2014, in the Department of Pathology in a Tertiary Care Rural Medical College.

All fluids received were subjected to physical and cytopathological examination. Physical characteristics including volume, appearance, and color of the fluid were noted. All hemorrhagic effusions of at least 3 ml volume received are included in the study. All clotted samples and all non-hemorrhagic effusions were excluded from the study.

Processing

The fluids sent to the laboratory were collected under sterile aseptic conditions by the concerned clinicians. No anticoagulant was used during the time of collection. Hemorrhagic effusions received were taken in a clean tube. The fluid was centrifuged at 2000 rpm for 10 min. A total of six smears were prepared from the sediment thus obtained.

CF was prepared by mixing absolute ethanol, chloroform, and GAA in the proportion of 6:3:1, respectively. Modified CF was prepared by mixing 95% ethanol and GAA in the proportion of 6:1, respectively. Two smears each were placed in CF and modified CF for 5 min. Smears were transferred to 95% ethanol. They were stained with Pap and H&E stains.

NSRT

Two smears were dried in an incubator at 37°C for 5 min. Smears were rehydrated by flooding with normal saline for 30 s. Slides were fixed in 95% ethanol. They were stained with Pap and H&E stains.

These slides were then analyzed for
1. Efficiency in reducing the number of RBCs
2. The effects of processing on the nuclear and cytoplasmic staining characteristics
3. Improvement in cellular yield and hence its diagnostic utility.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package, version 16. The Chi-square test was used to find out the association of different techniques in the reduction of RBC. A $P < 0.05$ was considered statistically significant.

RESULTS

A total of 942 samples were received out of which 178 were hemorrhagic. Of these, 96 hemorrhagic samples fulfilled the inclusion criteria and were included in the study.

Effect of reduction of RBCs on smear background

CF and MCF showed very similar results of 30–60% reduction [Figures 1 and 2], whereas more than 60% reduction was seen in 85.40% cases by NSRT method [Figure 3]. The reduction has been found out to be statistically significant with a $P < 0.001$ [Table 1].

Effect on staining characteristics

SRT showed better staining of cells compared with other fixatives and staining results with both Pap and H&E stains were similar as shown in [Table 2]. Focal changes included intense

![Figure 1: (a) Reactive mesothelial cells in hemorrhagic effusion treated with MCF $\times 40$ (Pap) (b) Reactive mesothelial cells in hemorrhagic effusion treated with MCF $\times 40$ (H&E).](image1)

![Figure 2: (a) Reactive mesothelial cells in hemorrhagic effusion treated with CF $\times 40$ (Pap). (b) Reactive mesothelial cells in hemorrhagic effusion treated with CF $\times 40$ (H&E).](image2)

![Figure 3: (a) Reactive mesothelial cells in hemorrhagic effusion treated with NSRT $\times 40$ (Pap). (b) Reactive mesothelial cells of hemorrhagic effusion treated with NSRT $\times 40$ (H&E).](image3)
staining of the cytoplasm and light staining of the nucleus. With saline rehydration technique, stain muck and RBCs were absent with clear background, but the nuclear and cytoplasmic staining was lighter in a few areas which did not affect the diagnosis.

**Effect on nuclear chromatin**

Nuclei at places showed pale staining compared with other cells. Most of these changes were noted with CF on H&E stain. The techniques used had no significant bearing on the nuclear chromatin in most of the cases as shown in Table 3. The nuclear borders or their size were not affected by any of the fixatives. Two cases showed shrinkage which might be because of acetic acid.

**Effect on cell borders**

NSRT showed the effect on cell borders only in few numbers of cases compared with Carnoy’s and Modified CF [Table 4].

**Effect on cytoplasmic staining**

Most of them showed fraying of the cytoplasm which is mainly because of acetic acid; also centrifugation itself could cause such effect on cell borders.

**Effect on special morphological features**

Special features were preserved in all the cases of saline rehydration technique but with CF and MCF the background which had RBCs treated with acetic acid itself caused obscuring of the special features [Table 6].
DISCUSSION

The relative ease for diagnostic approach and comparatively less painful procedure has made aspiration cytology a diagnostic modality for body effusions to point out the etiology for effusions.\textsuperscript{[6]} The cellular population present in the sediment smear of the fluid is representatively much larger than that obtained by needle biopsy. Therefore, cytology has a greater opportunity to retrieve malignant cells compared to biopsy specimen.\textsuperscript{[9]}

Cytomorphological evaluation of hemorrhagic samples can be very difficult and is often not successful. RBCs may partially or totally obscure the diagnostic cells, making morphological interpretation more difficult.\textsuperscript{[7]} The common goal of each technique is selection and concentration of adequate number of cells with intact cell morphology without losing them during processing. To achieve the best possible smears, precaution should be taken at the level of sample collection, preservation, or immediate processing of the sample. This is most important to prevent the coagulum formation or an anticoagulant can be used.\textsuperscript{[3]}

The most common hemorrhagic effusion received in this study was pleural fluid. Pericardial fluid sample was not received during our study period.

Clear background was seen in 85.4\% of cases with NSRT in the present study which correlated with most of the studies. Ng \textit{et al.} assessed 11 grossly hemorrhagic samples and noted complete lysis in all cases. Immersion of air dried smears in normal saline for 30 s effectively lysed all the RBCs but retained epithelial and mesothelial cells. This resulted in a clean background, optimal for cytological assessment. Most probably rehydration leads to more osmotically active intracellular medium in red cells leading to lysis. Breaking up hemoglobin chains into smaller molecules with drying and rehydration may be a possibility. For nucleated cells an equilibrium is maintained with no major influx of water occur with normal saline rehydration. Cytosol in such cells more readily retains osmotic integrity with drying and rehydration. Hypotonic saline, tap water, and aqueous glycerin were proved to lyse nucleated cells as well as RBCs.\textsuperscript{[9]} Rehydration offers the advantage of cleaner background with more vivid morphological interpretation, especially in malignant cases.\textsuperscript{[10]}

Saline rehydration technique in the present study showed no effect on cells stained with H&E (58.3\%) or Pap stain (56.8\%). There are no studies to compare the staining effect of H&E and Pap stain but Pap stain after air drying is highly unsatisfactory for cytological assessment,\textsuperscript{[9]} several other studies on cervical cytology have used Pap stain after saline rehydration and reported satisfactory staining features for interpretation. In the present study, Pap stained smears of fluid samples was satisfactory and the nuclear features were also better observed.

Many studies have scored retention of epithelial/mesothelial cells by different methods. Ng \textit{et al.} observed that with air drying and rehydration technique, cell retention could be increased to 78\%. The cell loss with wet fixation appeared more marked in the cytocentrifuged smears (50% loss) as compared with the dried smears (31% loss).\textsuperscript{[10]} Preeti \textit{et al.} observed with NSRT 86.65\% cases scored 4 (same as control smears) followed by Carnoy’s technique 72.02\% and by GAA in 56.65\%.\textsuperscript{[11]} Shabanam \textit{et al.} observed retention of cells in 70.5\% cases with NSRT followed by Carnoy’s technique 57.8\% and by GAA in 50.9\%. High cellularity was noted in NSRT 58.9% compared to WF 56.4%.\textsuperscript{[13]} Gupta \textit{et al.} observed that retention of cells in Pap smears was 80.2\% with conventional group and 78.2\% with air dried rehydration technique.\textsuperscript{[10]} In the present study, no significant loss of cells was observed in the samples processed from all the three methods [Table 7].

Malavi \textit{et al.} showed nuclear shrinkage in 76.92\% cases with CF and 72\% cases with GAA whereas present study only one case showed nuclear shrinkage with CF.

Gupta \textit{et al.} observed distinct nuclear borders in 78.6\% cases by conventional group and 76.1\% cases by air dried rehydration group on Pap smears.\textsuperscript{[10]} Distinct nuclear borders in squamous and endocervical cells were, respectively, seen in 88.95\%, 79.76\% cases in air dried group and 97.09\%, and 86.48\% in wet fixed group.\textsuperscript{[6]} Nuclear border was distinct in all the Pap smears with ARF and WF.\textsuperscript{[13]} Nuclear details were superior in ARF smears compared to WF smears in gastric mucosal cell smears.\textsuperscript{[14]}

Smears stained with Cytorich red exhibited crisp nuclear details while smears stained with cytospin collection fluid showed smudging with less nuclear details.\textsuperscript{[15]} The chromatin pattern was similar in both ARF and WF.\textsuperscript{[13]} The present study does not show much difference on nuclear features in all the three techniques and Pap stain showed better nuclear details [Table 8]. CF showed 7.3\% of cases with hazy nuclear feature in the present study. Smudging of chromatin was seen in 68\% of cases with dirty and distracting background.\textsuperscript{[11]} It is an excellent fixative in Pap smears for fixation and reduction of RBC in the background which also helps in identifying squamous cells, columnar cells, inflammatory cells and pathogenic organism for easy diagnosis.\textsuperscript{[8]} GAA showed smudging of chromatin in

| Technique | Stain | Preserved | | Obscured |
|-----------|------|-----------|-----------|
|           | No. of cases | % | No. of cases | % |
| CF        | Pap    | 94 | 97.9 | 2 | 2.1 |
|           | H&E    | 94 | 97.9 | 2 | 2.1 |
| MCF       | Pap    | 94 | 97.9 | 2 | 2 |
|           | H&E    | 95 | 99 | 1 | 1 |
| SRT       | Pap    | 96 | 100 | 0 | 0 |
|           | H&E    | 96 | 100 | 0 | 0 |
73% with dirty and distracting background with decrease in proportion of correct diagnosis.\cite{1}

Smears treated with NSRT, 11.8% cases showed suboptimal staining and blurring with loss of sharpness of nuclear feature and 3.9% cases were not suitable for assessment.\cite{11} However, smudged chromatin was noted in 7.14% of cases with no nuclear artifacts and morphology was retained in various cavity fluids. However, nuclear artifacts were noted in crisp nuclei by saline rehydration technique.\cite{11}

The present study showed well preserved cell borders in 90.6% by NSRT [Table 9].

The present study showed good staining of cytoplasm by smears treated with NSRT by both staining methods which correspond with other studies [Table 10]. Cytomorphological scoring was done in few studies where cytoplasmic and nuclear features were considered together and scored with Ng et al. scoring system proposed by Ng et al. This showed excellent preservation of cytological features by CF 60.6%, GAA 58.8%, and NSRT 52.9%.\cite{11} Preeti et al. observed excellent cytological preservation using the same scoring system in 77.33% of CF, 68% of GAA, and 46.67% of samples processed by NSRT.\cite{15} Ng et al. also proved that excellent cytological details were seen by wet fixation method compared with rehydration technique.\cite{9} Where the above three studies showed CF as best technique for cytological preservation but the present study and Malvi and Anthony have shown that cytological features are well preserved in smears treated with NSRT.\cite{1,9,11,15}

Malvi and Antony had difficulty in diagnosis in five malignant untreated hemorrhagic effusion cases. They reported that three cases could be diagnosed by CF, four cases after treatment

### Table 7: Comparative table for reduction of RBC in the background.

| Technique | Malvi and Anthony, 2000\cite{11} (%) | Shabnam, 2013\cite{12} (%) | Jaiwong et al., 2006\cite{6} (%) | Preeti, 2011\cite{11} (%) | Weidmann, 1997\cite{15} (%) | Kirib, 2006\cite{10} (%) | Shamsi, 2007\cite{16} (%) | Mirzaie et al., 2006\cite{17} (%) | Present study 2014 (%) |
|-----------|----------------------------------|--------------------------|-------------------------------|--------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| GAA       | 10                               | 3.9                      | 53.33                         |                          |                 |                 | 94              | 85.4            |
| CF        | 20                               | 60.8                     | 82                            | 51.7                     | 15.6            |                 |                 |                 |
| NSRT      | 93                               | 72.5                     | 96.5                          | 91.33                    |                 | 94              | 85.4            |
| MCF       |                                  |                          |                               |                          |                 |                 | 14.6            |
| 95% ethane|                                  |                          |                               |                          |                 | 0               | 70.9            |
| Cytorich red|                                 |                          |                               |                          |                 | 92.5            |
| Cytospin  |                                  |                          |                               |                          |                 | 22.5            |
| Filtration|                                  |                          |                               |                          |                 |                 | 88              |

### Table 8: Comparative table for effect on nuclear chromatin.

| Technique | Malvi and Anthony, 2000\cite{11} (%) | Jaiwong, 2006\cite{6} (%) | Gupta et al., 2003\cite{18} (%) | Dahlstrom et al., 1999\cite{16} (%) | Present study 2014 (%) |
|-----------|----------------------------------|--------------------------|-------------------------------|------------------|------------------|
| Crisp     | GAA                             | 26.93                    |                               |                 | 92.7            |
|           | CF                              | 32                       |                               |                 | 96.9            |
|           | NSRT                            | 92.86                    | 87.9                          | 71.7            | 25              |
|           | MCF                             |                          |                               | 96.9            | 96.9            |
|           | 95% alcohol                     | 96.51                    | 73.6                          | 27              |
| Satisfactory|                                 |                           |                               |                 |                 |
|           | 95% alcohol                     | 63                       |                               |                 |                 |
|           | NSRT                            | 29                       |                               |                 |                 |
| zHazy     | GAA                             | 73.07                    |                               |                 |                 |
|           | CF                              | 68                       |                               | 7.3             | 3.1             |
|           | NSRT                            | 7.14                     | 12.20                         | 28.3            | 33              |
|           | MCF                             |                          |                               | 4.2             | 3.1             |
|           | 95% alcohol                     | 3.48                     | 26.4                          | 0               | 3.1             |
with GAA, and all five cases after treatment with NSRT. In the present study, out of 96 cases, ten were malignant of which only one was suspected clinically and others were diagnosed on cytological examination of fluids. NSRT helped in confirming these cases with clear background and well preserved, stained cytomorphological features [Figures 4-6]. The present study showed with all three techniques, the microorganism was well preserved and with NSRT special stain PAS positive pseudohyphae was noted [Figure 7]. Immunocytochemistry can be performed on direct cytology smears; however, direct smears will not show the same population of cells in all the smears or there might be absence of cells in the smears. PAP stained smears without destaining or after destaining may be used for immunocytochemistry. Because of numerous technical and quality control issues, it is ideal to have cell-block for immunocytochemistry[19-21]. Smears treated with fixative containing GAA has shown high false positive results on hybrid capture 2 human papillomavirus testing.[17] Ammonium chloride-based erythrocyte lysing reagent, similar to that used for flow cytometry is relatively simpler and inexpensive without compromising the immunoprofile integrity in preparing cell-block of hemorrhagic effusions.[19-21]

CONCLUSION

Hemorrhagic fluids are most problematic and cause difficulty in diagnosis. The availability of lysing agents in different combinations has led to several studies of these agents on hemorrhagic samples. The common goal of all the techniques is background free of RBC, retention of epithelial/mesothelial

| Table 9: Comparative table for cell border preservation. |
|-----------------------------------------------|
| Technique       | Jaiwong et al., 2006[6] (%) | Gupta et al., 2003[10] (%) | Dahlstrom et al., 1999[14] (%) | Present study 2014 (%) |
|-----------------|-----------------------------|-----------------------------|-------------------------------|-------------------------|
|                 | H&E                         | Pap                         |                               |                         |
| Preserved       |                             |                             |                               |                         |
| CF              | 86.5                        | 86.5                        |                               |                         |
| NSRT            | 81.8                        | 20                          | 89.6                          | 90.6                    |
| MCF             | 85.4                        | 90.6                        |                               |                         |
| 95% alcohol     | 83.1                        | 3.7                         |                               |                         |
| Satisfactory    |                             |                             |                               |                         |
| 95% alcohol     | 54                          |                             |                               |                         |
| NSRT            | 36                          |                             |                               |                         |
| Disrupted       |                             |                             |                               |                         |
| CF              | 13.5                        | 13.5                        |                               |                         |
| NSRT            | 18.2                        | 31                          | 10.4                          | 9.4                     |
| MCF             | 14.6                        | 9.4                         |                               |                         |
| 95% alcohol     | 16.9                        | 33                          |                               |                         |

| Table 10: Comparative results for cytoplasmic staining. |
|--------------------------------------------------------|
| Technique       | Weidmann, 1997[15] (%) | Jaiwong et al., 2006[6] (%) | Gupta et al., 2003[10] (%) | Present study 2014 (%) |
|-----------------|-------------------------|-----------------------------|-----------------------------|-------------------------|
|                 | H&E                     | Pap                         |                             |                         |
| Unaffected      |                         |                             |                             |                         |
| CF              | 82.3                    | 86.5                        |                             |                         |
| NSRT            | 98.83                   | 87.8                        | 90.6                        | 92.7                    |
| MCF             |                         |                             | 81.2                        | 87.5                    |
| 95% alcohol     | 95.34                   | 79                          |                             |                         |
| Cytorichred     | 97                      |                             |                             |                         |
| Cytospin        | 48                      |                             |                             |                         |
| Affected        |                         |                             |                             |                         |
| CF              | 17.7                    | 13.5                        |                             |                         |
| NSRT            | 1.16                    | 12.2                        | 9.4                         | 7.3                     |
| MCF             |                         |                             | 18.7                        | 12.4                    |
| 95% alcohol     | 4.65                    | 21                          |                             |                         |
| Cytorichred     | 3                       |                             |                             |                         |
| Cytospin        | 52                      |                             |                             |                         |
cells and well preserved cytomorphological features. The present study highlights the simple, cost-effective, and user friendly saline rehydration technique in the interpretation of hemorrhagic smears. It also emphasizes that cytomorphic evaluation of smears is highly dependent on the quality of the smears prepared and on the appropriate use of processing technique. Any deviation from the same can lead to difficulties in diagnostic interpretation. In a resource poor countries, saline rehydration can serve as a boon in the processing of hemorrhagic samples especially in the context of screening malignancies by cytological examination. It is a subject of further research in the field of immunocytochemistry and molecular diagnostics whether saline rehydration technique can be useful or hinder the interpretation of these tests.

**COMPETING INTEREST STATEMENT BY ALL AUTHORS**

The authors declare that they have no competing interests.

**AUTHORSHIP STATEMENT BY ALL AUTHORS**

Each author has participated sufficiently in the work and takes public responsibility for appropriate portions of the content of this article. All authors read and approved the final manuscript. Each author acknowledges that this final version was read and approved.

**ETHICS STATEMENT BY ALL AUTHORS**

As this is case without identifiers, is not required approval from the Institutional Review Board (IRB) (or its equivalent).

**LIST OF ABBREVIATIONS** (In alphabetic order)

- ARF – Airdried rehydration fixative
- C – Celsius
- CF – Carnoys fixative
- FNAC – Fine needle aspiration cytology
- GAA – Glacial acetic acid
- H&E – Hematoxylin & Eosin
- MCF – Modified carnoys fixative
- Min – Minute
- NSRT – Normal saline rehydration technique
- Pap stain – Papanicolaou stain
- Pap smear – Papanicolaou smear
- RBC – Red blood cell
- rpm – rotation per minute
- WF – Wet fixation
EDITORIAL/PEER REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (authors are blinded for reviewers and vice versa) through automatic online system.

REFERENCES

1. 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.
2. Agarwal PK. Cytologic examination of hemorrhagic fluid by capillary centrifugation: A new technique. Acta Cytol 2009;52:7-32.
3. Frist B, Kahan AV, Koss LG. Comparison of diagnostic value of biopsies of the pleura and cytologic evaluation of pleural fluid. Am J Clin Pathol 1979;48:45-51.
4a. Shidham V, Kampalath B, England J. Routine air drying of all the smears prepared during fine needle aspiration and intraoperative cytology studies: An opportunity to practice a unified protocol, offering the flexibility of choosing variety of staining methods. Acta Cytol 2001;45:60-8.
4b. Nalyor B. Pleural, peritoneal and pericardial fluids. In: Bibbo M editor. Comprehensive Cytopathology. 2nd ed. Philadelphia, PA: Saunders Company; 1997. p. 551-620.
5. Bales CE. Laboratory techniques. In: Koss LG, Melamed MR, editors. Koss Diagnostic Cytology and its Histopathologic Bases. 5th ed. New York: Lippincott Williams and Wilkins; 2006. p. 1570-634.
6. Jaiwong K, Nimmanhaeminda K, Siriaree S, Khunamornpong S. The cytomorphologic comparison between rehydrated air-dried and conventional wet-fixed Pap smears. J Med Assoc Thai 2006;89:1811-5.
7. Kushwaha R, Sashikala P, Hiremath S, Basavaraj HG. Cells in pleural fluid and their value in differential diagnosis. J Cytol 2008;25:138-43.
8. Ng VF, Choi FB, Cheung LH. Rehydration in air-dried smears with normal saline application in fluid cytology. Acta Cytol 1994;38:56-64.
9. Qiu L, Crapanzano JP, Saqi A, Vidhun R, Vazquez MF. Cell block alone as an ideal preparatory method for hemmorhagic thyroid nodule aspirates procured without onsite cytologists. Acta Cytol 2008;52:139-44.
10. Gupta S, Sudhani P, Chachra KL. Rehydration of air dried cervical smears: A feasible alternative to conventional wet fixation. Obstet Gynaecol 2003;102:761-4.
11. Preeti, Mittal S, Alka. Hemorrhagic effusions: Comparison of methods for better cytological assessment. Asian J Crit Care 2011;7:19-22.
12. Shabnam M, Sharma S, Upreti S, Bansal R, Saluja M, Khare A, et al. Comparative study of processing of hemorrhagic body fluids by using different techniques. J Clin Diagn Res 2013;7:2186-8.
13. Mirzaie AZ, Alam KK, Abolhasani M. Rehydration of air dried cervical smears: An alternative to routine wet fixation. Acta Med Iran 2007;45:365-8.
14. Dahlstrom JE, Holdsworth J, Basert ML, Jain S. Rehydration of air dried smears. An alternative method for cytologic examination of exfoliative cells. Acta Cytol 1999;43:214-7.
15. Weidmann J, Chaubal A, Bibbo M. Cellular fixation, a study of cytorichred and cytopin collection fluid. Acta Cytol 1997;41:182-87.
16. Shamsi M, Abdali K, Montazer NR, Kamar PV, Tabatabaei HR. Comparison of carnoys solution and 96% ethyl alcohol fixation in bloody Pap smears. Acta Cytol 2008;52:187-90.
17. Agoff SN, Dean T, Nixon KB, Severn KL, Rinker L, Greico SV. The efficacy of reprocessing unsatisfactory cervicovaginal thinprep specimens with and without glacial acetic acid effect on hybrid capture ii human Papillomavirus testing and clinical follow-up. Am J Pathol 2002;118:727-32.
18. Kirbis S, Flezar SM, Lavrenecak J, Marinsek ZP. Hemorrhagic cytology samples to get the best diagnostic results. Cytopathology 2007;18:175-9.
19. Shidham VB, Janikowski B. Immunocytochemistry of effusions: Processing and commonly used immunomarkers. CytoJournal 2022;19:6.
20. Shidham VB, Layfield LJ. Approach to diagnostic cytopathology of serous effusions. CytoJournal 2021;18:32.
21. Shidham VB. Collection and processing of effusion fluids for cytopathologic evaluation. CytoJournal 2022;19:5.

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