Original Article

DOI: 10.21276/APALM.1432

Cytological Evaluation of Two Methods of Effusion Cell Block Preparations

Deepa Siddappa Masur[1] and Shilpa Somashekhar Biradar[2*]

[1]Department of Pathology, S. Nijalingappa Medical College and HSK hospital and research centre. Bagalkot,India
[2]Department of pathology, Akash Institute of Medical Sciences and research center, Prasannahalli road. Devanahalli, Bangalore, India

ABSTRACT

Background: Cell Block (CB) procedures have now become an established part of cytological diagnostics because of its pivotal role in diagnosis and ancillary studies. Hence the present study was undertaken to emphasize the role of CB technique over Conventional Smear (CS) in serous effusions and to compare the Plasma-Thrombin (PT) block to Formalin Method block (FM) in assessment of morphological preservation and cellularity.

Aim: To obtain simple , cost effective and ideal CB preparation where in maximal number of cells are displayed within a small area

Methods: The sample was divided into three Parts(A,B,C). After centrifugation of all three parts of sample at 3000rpm for 15min – Part A sediment was used to prepare two CS for Papanicolaou (PAP) and May Grunwald Giemsa(MGG) stains. Part B sediment was subjected for 1hr and 24hr fixation in 1:1 solution of 5ml ethylalcohol and 10% formalin. To the Part C sediment 2drops of finger prick plain blood was added, mixed well and allowed to clot. The sediment of Part B and the clot of Part C were then processed for paraffin embedding.

Result: 110 fresh effusion samples were evaluated for cellularity , retention of architectural patterns and volume of background. FM block's were inconclusive in 12 cases due to low cellularity. PT block's were all evaluable with best preservation of architecture and pale background.

Conclusion: The CB technique revealed better architectural patterns and increased the sensitivity of cytodiagnosis. PT block's had sufficient to abundant cellularity with evenly distributed cells in small area. PT preparation is simple and cost effective.

Keywords: *Serous Effusions, Conventional Smear, Cellblock, Plasma Thrombin Method, Formalin Cell Block.*

Introduction

Bahrenberg in 1895 introduced CB technique for routine processing of fluids and Mandelbaum in 1917 devised a technique for the preparation of CB.[1] Ceelen in 1957 demonstrated bright red granules in the cytoplasm of mesothelial cells by CB's.[2] De Girolami developed a technique to cover the sediment with few drops of plasma and few drops of thrombin in order to entrap cells within the artificial clot. The clot was then fixed in 15% formalin processed in paraffin and stained like tissue specimen.[3] Histochemical stains and various special stains such as PAS, Diastase, Ziehl-Neelsen and Gomori-Methenamine Silver nitrate can easily be performed on the sections prepared from CB.[4]

The customary methods of investigation of serous effusions are cytologic examination of the fluid by both CS and CB sections, biopsy of the pleura, peritoneum, pericardium, and supplemented by immunocytochemistry, biochemical, bacteriologic or cytogenetic investigations.[5] Various studies conducted in the past and in recent years strongly advice to process the remainder of the specimen as a CB.[6]

Ideally, cellular components revealed by CB's are expected to exhibit the following features: resemblance to corresponding cells in alcohol-fixed PAP stained smears, adequate preservation of nuclear-cytoplasmic details, easy recoverability, and suitability for ancillary studies such as immunostaining.[7] An ideal method for preparation would be simple, reproducible, and readily adaptable in a routine setting.[8] CB study is valuable in cytopathology because it provides histopathologic correlation and additional material for immunohistochemical studies.[9] Although the CB method is not new, handling of the specimens in a conventional manner results in considerable

loss of material.[6] Instead of separating the cells by preparing smears, the principle of blood clotting can be applied to the sediment obtained. In such a way, the intercellular relationships and morphologic features of tissues in human organs could be maintained.[3]

In today's era of personalized medicine with an increasing array of molecular tests being applied to cytological specimens, there is a need for a standardized protocol for CB optimization to enhance cellularity. This study serves

as a baseline to launch further investigation of the pros and cons of different CB preparation techniques. For this reason, in this study an attempt was made to prepare and analyze both CS and CB from the same specimen. To assess the feasibility of both CB preparations and emphasize the role of PT and FM CB's in assessment of morphological preservation and cellularity which would be useful for immunohistochemistry and other special stains if required.

Materials and Methods

This is a one year prospective study performed in Department of Pathology of Shri. Nijalingappa. Medical. College and HSK hospital Bagalkot, with approval from the institutional ethics committee. Written informed consent was also obtained while sample collection. Serous effusions from the body cavities comprising of pleural, peritoneal and pericardial fluids were included. All fluids other than pleural, peritoneal, pericardial fluids were excluded.

Fresh serous fluid samples received were first submitted for naked eye examination for physical characteristics and divided into three parts.

Part A sample was centrifuged at 3000 rpm for 15 min and two CS were prepared for PAP and MGG stains.

Part B sample was processed for FM CB. The sample was added to 1:1 solution of 5 ml of ethanol and 10% formalin and fixed for 1 hr. The mixture was agitated often for uniform fixation of the material. After fixation, the specimen was centrifuged at 3000rpm for 5min. The supernatant was decanted and the sediment completely drained off by inverting tube over Whatman filter paper. To this sediment 1:1 mixture of ethanol and 10% eosin tinted formalin was added and kept for fixation for 24hrs. Then after discarding the supernatant fixative, the pellet formed was removed with a pointed spatula and placed on top of the lens paper inside the tissue cassette and processed for paraffin embedding.

Part C sample was processed for PT CB (Fig 1). The fluid was centrifuged at 3000 rpm for 5min. The supernatant was decanted and the excess fluid was removed by inverting over Whatman filter paper. To this sediment 2 drops of finger prick plain blood was added and mixed well and allowed to clot for 30 seconds. Then the clot was dislodged from the test tube and fixed in 1:1 mixture of ethanol and 10% eosin tinted formalin for 1hr. Further the clot was transferred to the lens paper in the tissue cassette and then processed for paraffin embedding. Instead of plasma and commercial available thrombin separately we have used 2 drops of finger prick blood to make the method cost effective.

Interpretation of Conventional Smears and Cellblocks. After evaluating the available clinical data, the CS and CB sections were than objectively analyzed for cellularity, architectural arrangement and background. The point scoring system (Table 1) as described by Mair et.al [10]was used to group the cases into following three categories

difficult for evaluation, b) sufficient for evaluation, and c) superior **.**

Result

A total of 110 body cavity fluid samples were studied, of which 54 were pleural fluids, 51 were peritoneal fluids and 5 were pericardial fluids. 77 fluid samples were from male patients and 33 were from female patients. Male : Female ratio was 2.3:1. The maximum number of samples were from patients in the age group of 41 - 50 years. (Table 2)

Cellularity in Cell Blocks (PT & FM) and Conventional Smears The cellularity in both the CB's preparation was sufficient to abundant for evaluation (Table 3). FM CB's did not contain cellular material in 12 cases and were inconclusive for evaluation (Fig 4). But the same samples had low cellularity and not easy for evaluation in CS (Fig 2) but evaluable in PT block (Fig 3). So not a single case was inconclusive or difficult for evaluation by PT method.

Volume of the Obscuring Background : As per the criteria described in Table 1 the volume of the obscuring background was considered to be large when the diagnosis was greatly compromised, moderate when diagnosis was possible and minimal when the background was clear. The CS were difficult for evaluation due to large and obscuring background of hemorrhage and degenerating cells (Fig 5). In FM CB's, the background was clear in all the samples but had artifactually crowded cells which was scattered in various parts of the slides (Fig 7). The PT CB's (Fig 6) revealed uniform distribution of the cells with clear nuclear and cytoplasmic preservation in pale background.

Preservation of Architectural Patterns. The CB's concentrated material in smaller fields with more frequent appearance of organoid pattern and cells in the same focal plane. The serial sections of even minute amount of cellular material from various types of sample showed high cellularity with better morphologic preservation (Table 4). Architectural pattern preservation was excellent in all the PT blocks(Fig 3 &6) and only sufficient for evaluation in half of FM blocks and CS.

Malignant Effusions Diagnosed by Cellblock Method. Even though the primary aim of the present study was not to detect the diagnostic accuracy for malingnancy, additional 15.5% malignancies were diagnosed by cellblock

preparations. Total number of 27 body fluid samples were diagnosed as malignant effusions by CB's and diagnosis of metastatic malignant effusion was rendered. Primary was identified in 12 cases and was unknown in 15 cases. 3 cases expired before diagnosis and 3 cases discontinued the treatment. The remaining 4 unknown cases were cases of alcoholic liver disease and cirrhosis and primary was diagnosed by immunohistochemistry from the liver. In another 2 cases primary was not known, as patients lost for follow up study.

Table 1: Criteria for the assessment of the quality of CS and CB preparations.

Criterion	Quantitative description	Point score
Amount of cellular material	Low (5-10 nucleated cells /hpf)	1
	Sufficient (10-100 nucleated cells/hpf)	2
	Abundant (>100 nucleated cells /hpf)	3
Retention of appropriate architectural pattern	Minimal or Absent (evaluation not possible)	1
	Sufficient for evaluation	2
	Abundant	3
Volume of obscuring background	Large amount, evaluation greatly compromised	1
	Moderate amount, evaluation possible	2
	Clear	3

Table 2: Showing Number of patients in each Age Group

Age group in yrs	No. of males	No. of females	Total No.	Percentage (%)
0 – 10	1	3	4	3.6
11 – 20	2	1	3	2.7
21 – 30	6	4	10	9.1
31 – 40	13	10	23	21
41 – 50	25	7	32	29.1
51 – 60	16	6	22	20
61 – 70	8	1	9	8.2
71 – 80	4	1	5	4.5
81 – 90	2	0	2	1.8

Table 3: Morphology of PT CB's (H&E stain) and CS (PAP and MGG stain)

Method	Cellularity		Preservation		Background	
	Sufficient/ abundant	low	Sufficient / abundant	Minimal/ absent	Large/ obscuring	Clear / pale
CS	52	58	39	71	58	52
PT-CB	98	12	87	23	00	110
FM-CB	87	09	65	33	00	98

Table 4: Categories depending upon the scoring system

Method	Difficult for evaluation	Sufficient for evaluation	Superior
CS	58	50	02
PT-CB	00	30	80
FM-CB	21	59	27

Table 5: Architectural Patterns in CS and CB's (PT & FM)

Architectural pattern	CS		PT		FM	
	No	%	No	%	No	%
Singly scattered	56	50.9	21	19.09	28	25.5
Cell balls	18	16.4	00	00	00	00
Cell clusters	21	19.09	11	10	38	34.5
Papillae	02	18.2	07	63.6	05	45.4
Glands	00	00	10	9.09	06	54.5
Sheets	13	11.8	61	55.5	33	30

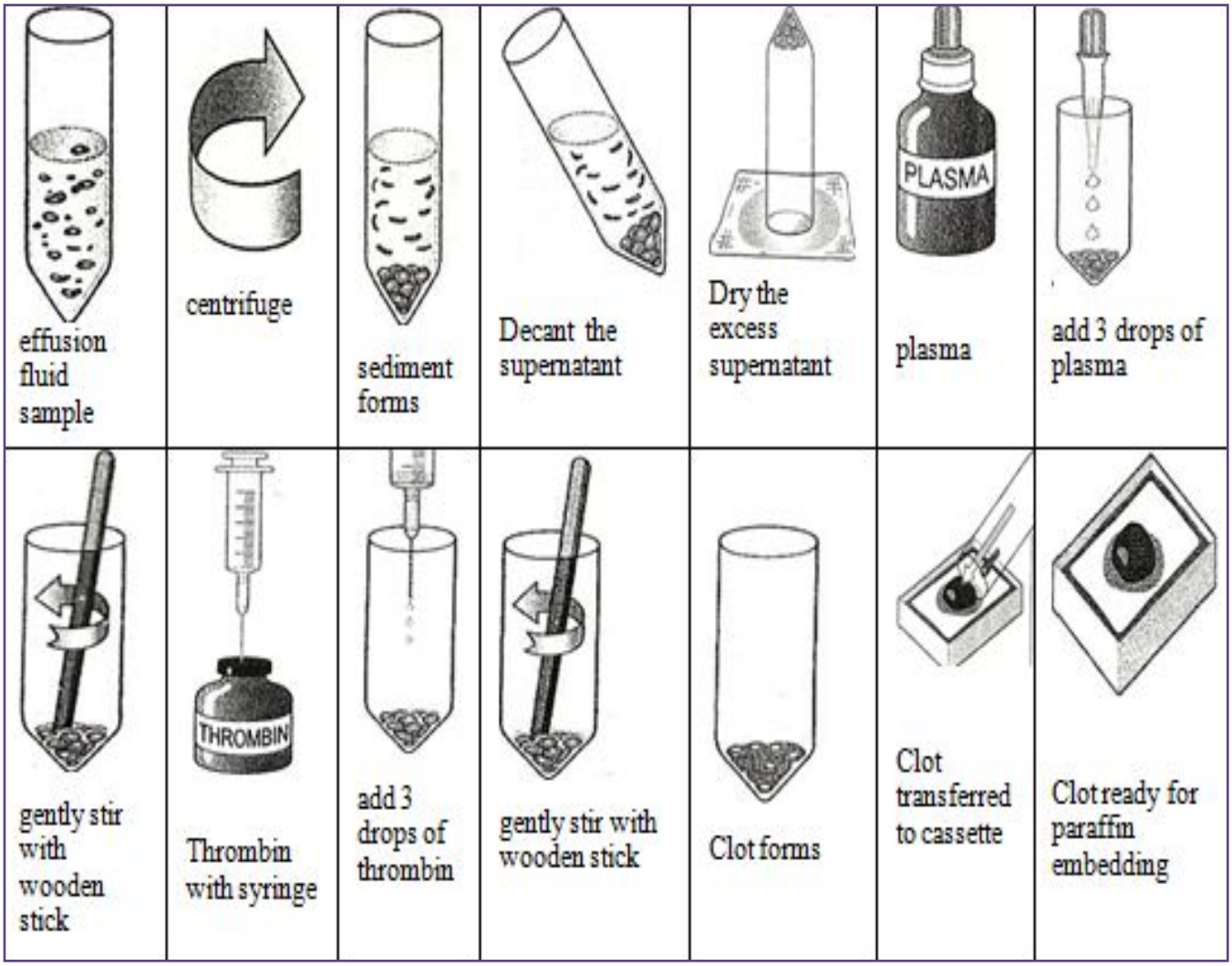

Fig. 1: Flow chart for preparation of PT CB's. (The figure is constructed and derived from various textbooks and sources.).

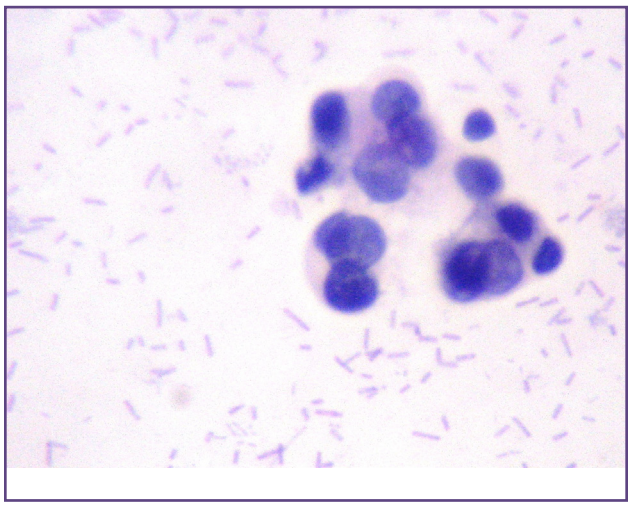

Fig. 2: Small cluster of suscpicious cells on CS (PAP stain 40X).

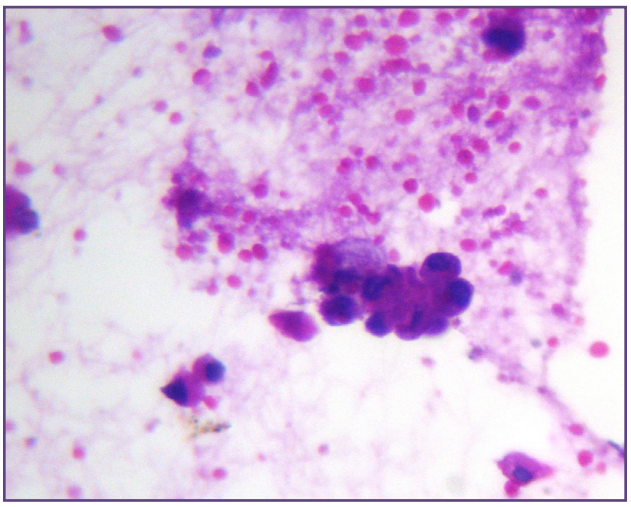

Fig. 3: Single acinar pattern in PT CB (H&E stain 40X).

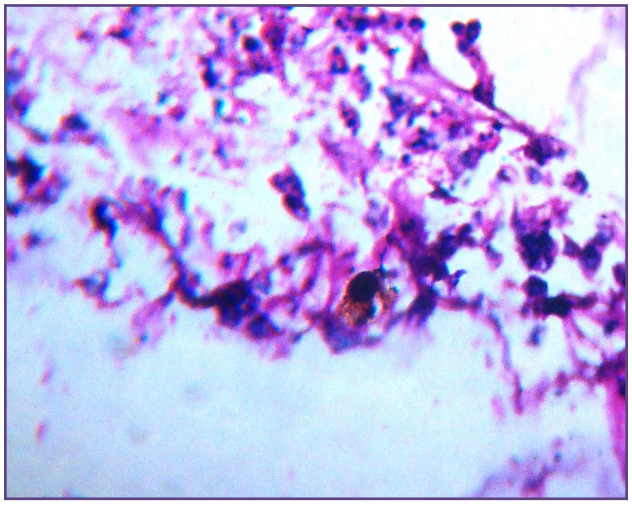

Fig. 4: Inconclusive FM CB when cellurity was less(H&E stain X40).

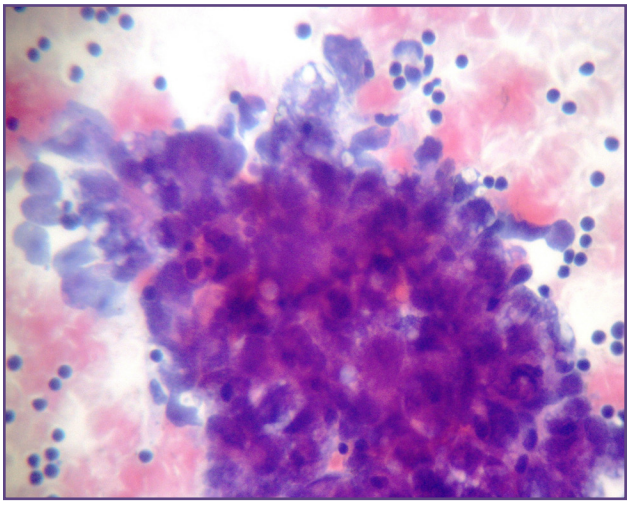

Fig. 5: Suscpicious Papillae on CS (PAP stain 40X).

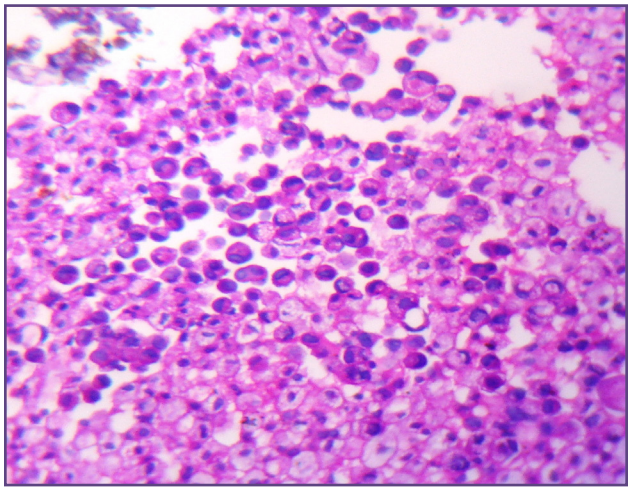

Fig. 6: Uniform distribution of cells in small area in PT CB (H&E stain 40X)

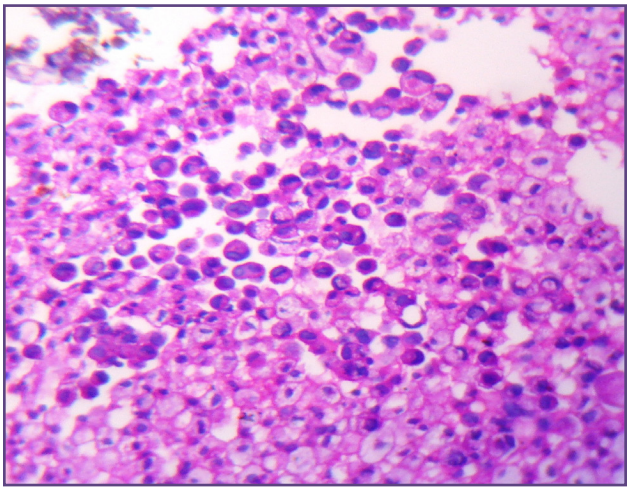

Fig. 7: Shrunken scattered cells all over the fields in FM CB (H&E stain40X).

Discussion

In the present study, we evaluated CS and two types of CB's – PT block and FM block preparations for cellularity, volume of obscuring background and the cellular preservation of morphology.

The CB's concentrated the cellular material into a small area which was useful in screening the material in lesser time.[1,9,11,12,13] The cellularity, in present study denotes the number of cells per field and not the diagnostic tumour or metastatic cells. Similar criteria were used by Mair S et.al,[10] Thapar et.al,[12] and Nigro et.al.[14] These studies categorised the cellularity into Minimal or Absent - when the cells were not sufficient for evaluation, Adequate - when sufficient for evaluation, and Abundant - when increased number of cells were present. In our study we denote positive for cells and also the number of cells. The primary aim of the present study was not to detect the diagnostic accuracy of the preparations, but additional 15.5% malignancies were diagnosed by CB preparations.

PT CB's had better cellularity when compared to CS and FM CB. The agitation of plasma and thrombin present in blood facilitates the trapping of cells by the fibrin strands, which coalesce into a fibrinous clot. Thus all the cellular material present will be efficiently captured into a clot. The clot is also very easy to embed and will not be lost during processing. The eosin tint helps in easy location of the clot during processing. Similar type of blood clot principle was used by Yang et al [9], De.Girolami et.al,[3] Jing et.al,[7] Nigro et.al,[14] Karnauchow et.al,[15] Burt et.al,[16] Kulkarni et.al,[17] Mahzouni et.al,[18] Rowe et.al,[19] and Bhatia et.al.[20] PT block procedure is applied since 1964 and emphasizes the advantage of clustering the cells rather than isolated cells in the diagnosis of neoplastic or pathologic processes.

The advantages of cellblocks are

1) Concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane. PT method is successful in introducing such blocks.
2) Preservation of architectural pattern like cell balls, papillae and three dimensional clusters.
3) Intact cell membranes and crisp chromatin details
4) It bridges the gap between cytology and histology.

The major disadvantage of the CB preparations is time, the delay in diagnosis when compared with CS. Another disadvantage is the loss of the cellular material and cytologic detail during processing.[21,22,23] PT preparation is cost effective, relatively easy and optimal for IHC.[24] They are most useful in CB preparation particularly in effusions with minimal cellularity.[10] Advantages of this method include the assessment of an additional sample volume and thus reduction of sampling error, the possibility for unlimited storage and molecular testing similar to histological samples.[24]

Additional studies with an in depth analysis to determine the appropriate method(s) is necessary. Determining and standardizing the most effective technique may alleviate the variability and provide consistency. As it is adapted to the introduction of immunohistochemistry and flow cytometry, cytology has to align itself with the multitude of molecular diagnostic tests.

Conclusion

PT block's were highly cellular and demonstrated pale background with evenly distributed cells. The preparation is simple, cost effective and reproducible. Thus despite the fact that in majority of cases, the diagnosis can be made on the bases of either CS or CB alone, the study conclude that using the combined technique on the same specimen leads to a more accurate diagnosis.

Based on these data, the PT method showed superior quality compared to CB's prepared in a variety of other ways. Our study illustrates that there are a variety of ways to prepare CB's and that subtle differences in technique can have an impact on important parameters and the overall quality of these specimens. Optimal CB preparation is important as the demand to do more with small specimens increases.

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***Corresponding author:**
Dr. Shilpa Somashekhar Biradar, Department of Pathology. Akash Institute of Medical Sciences and research center.Bangalore, India
Phone: +91 9480716323
Email: shilpa.biradar@yahoo.co.in

Financial or other Competing Interests: None.

Date of Submission : 23.03.2017
Date of Acceptance : 25.04.2017
Date of Publication : 31.08.2017