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## Review Article

# Cellblockistry in serous effusion cytology- A systematic review with recent concepts

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### ABSTRACT

Serous effusion cytology being a minimally invasive, readily accessible and inexpensive diagnostic procedure. Nevertheless, the accuracy of SEC could vary widely due to the multitude factors including the level of experience of the cytologists Conventional smear has its own limitations with varied efficacy and hence warranting ancillary studies. Cell block has emerged as a paramount and robust platform for sample processing techniques in cytology. Research studies have proved that the efficiency of cytological diagnosis increases by significant margin of 15-20 percent when it is done in conjunction with cell block techniques especially in cases of exudative fluids in picking up crucial cases and based on this, we intended with a novel aim to analyse the accuracy of Serous effusion cytology by combining conventional smear and cell block technique with an attempt to assess the effectiveness of the cell block by our indigenous Modified Bouin's method.

Cell block method prepared by our indigenous Modified Bouin's preparation with formalin fixative proved to show high quality significance and hence it could be followed in routine practice across laboratories. Cell block technique is quantitatively superior both standalone as well as in conjunction with conventional smear by improving the effective diagnosis of SEC. Diligent use of cell block technique eliminates the suspicious of malignant category on CS and thereby giving more definite diagnosis and thus it is mandated that combined approach of CB in conjunction with CS should be practiced as binary protocol which proved to have obvious influence on patient management.

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## 1. Introduction

Serous inflammation is a type of reaction of lining epithelium as an immune response to various noxious stimuli and its reflection is marked by the outpouring of a thin fluid that may be derived from the plasma or from the mesothelial cells lining the peritoneal, pleural or pericardial cavities.<sup>1</sup>

Accumulation of fluid other than blood in body cavities is broadly termed as 'effusion'. Body cavity effusions are classified under two categories- transudates and exudates.<sup>2</sup> The transudates are clear, straw-colored fluids characterized

by a low specific gravity often below 1.010 and low protein content (usually below 3g/dl). It is due to mechanism of increased venous pressure as usually seen in congestive cardiac failure or cirrhosis of liver and decreased oncotic intravascular pressure as noted conditions such as hypoproteinemia, nephrotic syndrome. The exudates are characterized by relatively high protein content (usually above 3 g/dl) and therefore a high specific gravity more than 1.015.<sup>3</sup>

Literature analysis postulates that transudates are mostly due to pathological causes of systemic organs of medical etiology whereas exudates are due to systemic infectious causes including metastasis often warranting surgical

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intervention which are solely determined by Pathologist's opinion on the fluid analysis.<sup>1,4</sup>

### 1.1. Role of Laboratory medicine in effusion cytology

Cytology is a clinically oriented speciality that can flourish when a cytologist spends time and energy on the basic initial steps of planning good and meticulous smear preparation.<sup>5</sup> A well prepared cytosmear is a backbone and the most basic step, without which even a knowledgeable and experienced cytologist is at loss and will not be able to comment or cast any opinion.<sup>5</sup>

Cytological examination of body fluids has gained immense acceptance in the clinical medicine to such an extent that a positive diagnosis rendered is considered as a definitive diagnosis.<sup>6</sup> It helps in early diagnosis and treatment and ultimately increases the survival rate of the patients.<sup>6</sup> Effusion cytology pertains to cytological analysis and evaluation of body fluids with various ancillary techniques with variable precision on its diagnostic effectiveness. Body fluids are obtained from sediments representing larger surface areas and clinicians are widely dependent on pathologist's interpretation on body fluid samples for further plan of management both in elective and critical situations.<sup>7,8</sup>

Cytological examination of serous fluids being a commonly performed investigation, the accurate identification of cells either as malignant or reactive mesothelial cells is a perennial diagnostic challenge in conventional cytology smears.<sup>8</sup> The cytodiagnosis by conventional smears have got lower sensitivity due to various confounding factors like overcrowding of cells, artifacts, benign mimickers, background features, inter-observer variations and different laboratory processing methods.<sup>9</sup> Also another major constraints of conventional method is high incidence of technical errors, loss of cells while preparation and lack of provisions for ancillary diagnostic investigations.<sup>1,10</sup>

Cyocentrifugation is preferred as it is less tedious and quicker than the membrane filtration, but at times it may cause cellular distortion. The cell block technique being a pivotal procedure of examining the body fluids, the procedure in toto along with concomitant use of smears has shown an added advantage in figuring out the diagnosis.

Cell block has emerged as a paramount and robust platform for sample processing techniques in cytology and based on this, we intended to draft this present review article to analyse the accuracy of serous effusion cytology by combining Conventional Smear and Cell Block with an attempt to assess the effectiveness of the cell block by our indigenous Modified Bouin's method of cell block preparation and analyzing its efficacy in laboratory medicine

## 2. Literature review

Recently, Indian Academy of Cytologists (IAC)<sup>1</sup> has developed guidelines in consultation with experts across the country for implementation as a standard format in our country for all laboratories providing cytopathology services.<sup>1</sup>

The guidelines are broadly divided as follows:

1. Essential – implying that these are absolute and non-negotiable recommendations and if a laboratory cannot achieve this, then the sample may be referred to a centre which is equipped to fulfil these criteria.
2. Optimal – implying that this is achievable in any good laboratory and should be part of the protocol and can be made mandatory for accreditation.
3. Optional – implying that this recommendation is resource-dependent and is left to the choice of the individual laboratory.

Smears stained with the routine Papanicalou technique generally have good definition of malignant cellular and nuclear changes.<sup>11</sup> Even then, in certain conditions cytological findings of fluids on smear preparation could be misleading, for e.g. differentiating reactive mesothelial cells from a mesothelioma, differentiating an exuberant reactive mesothelial hyperplasia from peritoneal metastasis etc., thereby warranting additional technical procedures to enhance the diagnostic efficacy of effusion cytology.<sup>11,12</sup>

### 2.1. Cell Block

Cell block preparation is a technique (in addition to smears) of cell concentration without compromising the cellular content and preserving the tissue architecture.<sup>11</sup> Preparation of Cell Block from the Effusion Sediment is desirable and is being frequently and increasingly requested by the clinician.<sup>12</sup>

### 2.2. Advantage

The main advantage is in the application of immunohistochemistry for various situations including the distinction between reactive mesothelial cells and adenocarcinoma, mesothelioma versus adenocarcinoma, characterization and typing of lympho-proliferative disorders and poorly differentiated malignancies and to elucidate the primary site of tumour when occult at presentation

Cell blocks permits storage of the cellular material as formalin-fixed paraffin embedded material that can be used at any later time for any molecular technique such as FISH, sequencing, genomics, and further research, including translational projects, including for predictive marker testing.

Methods for preparing Cell block:<sup>1,12,13</sup>

1. Direct Sedimentation.
2. Agar Embedding.
3. Plasma-Thrombin or Thromboplastin Clot method.

Shidam described atypical cells from stained smears have pleural and ascitic fluid exhibiting striking variations in size, shape and nuclear arrangement and many atypical mitotic configurations.<sup>8</sup> Shidam also described spindle cells floating free in the pleural fluid in a case of fibro sarcoma of the pleura. Quensel emphasized the presence of deep staining nucleoli as evidence of the neoplastic nature of the cells in an effusion.<sup>8</sup> In 2009, Benecke reported pigment-containing cells in the peritoneal fluid from a case of melanocarcinoma.

A study of 183 serous effusions by Takagi F concluded that the most reliable and useful criteria for diagnosis of malignancy in fluids were:<sup>14</sup>

1. Clusters of malignant cells with an organoid arrangement,
2. Individually lying malignant cells and
3. Cells staining positive for mucin.

### 2.3. Literature review- conventional smear versus cellblock

Malignant cells in pleural or ascitic fluids were almost always indicative of metastatic tumors as primary malignancy arising from mesothelial cell lining these spaces were rare.<sup>12,13</sup> When mesothelial primary was present, tumor cells were usually numerous and seen in clusters.

Shidam in 2017 retrospectively studied 117 malignant effusions from various sites in an attempt to determine the primary site of malignancy.<sup>8</sup> Their study comprised of well-documented cases of pleural and peritoneal serous effusions containing malignant tumor cells derived from the carcinoma of the breast, lung, ovary, endometrium and stomach. Sufficient cytologic similarities occurred to permit classification into three distinct patterns.

Udasimath et al opined that the highest degree of accuracy would be obtained by utilizing smears as principal diagnostic weapon and supplementing with cell blocks of material remaining after preparation of the smears.<sup>4</sup> According to various studies, more than 55% of original smear diagnosis was improved after the cell block was examined.

The overall accuracy rate with cell block technique was 97% with a sensitivity of 95% and specificity of 100% for malignant lesions.

With combined techniques of conventional smears and cell block method in reporting of malignant effusions the primary site could be determined with 81% accuracy. On correlating clinical, radiological and cytological features primary site could be determined with 90% accuracy.<sup>4</sup>

Nathan et al studied 286 cell blocks sections prepared from pleural and pericardial fluid and found that pericellular

lacunae were seen in majority of the neoplasms originating in the breast, ovary, lung and GIT which helped in distinguishing adenocarcinoma from reactive mesothelial cells.<sup>11</sup> Pericellular lacunae were seen in about 2/3rd of the cells, more often (75%) in CBs of malignant cases. Neoplasms with large cell clusters showed pericellular lacunae.

The cell block technique is a valuable method particularly when IHC staining for a battery of markers is required. IHC applied to cell block preparations provide the same accuracy, as do histological specimens. Zito et al reported that the diagnosis on cell block was as definitive as conventional biopsy.

Udasimath S et al<sup>4</sup> studied 60 pleural fluid samples with combined conventional smears and cell block technique, reported 15% increased yield for malignancy by cell block method.<sup>29</sup> They concluded the CB method provided high cellularity, better architectural patterns, morphological features and additional yield of malignant cells hence thereby increasing the sensitivity of cytodiagnosis.

A study by Kulkarni et al.<sup>10</sup> of 100 pleural effusions suspected of malignancy found an additional yield of 46.15% more by modified cell block method when compared to CS.

Frale D et al. studied 40 patients with lung cancer and exudative pleural effusions by both CS and CB and found increased ratio of diagnosis of malignancy by 10%. They could also subtype lung cancer as adenocarcinoma in 35% of cases. Thus, CB combined with CS increased the diagnostic yield in exudative pleural effusions accompanying lung cancer in their study.

### 2.4. Hypothesis-Need for further study

Cell-block techniques are neither new nor distinctive. Even though various authors have reported the utility of cell block methods over decades, only a few have documented satisfactory technique. Method of cell-block preparation differs from institution to institution and it had been advocated to prepare an in-house method indigenous to our laboratory.<sup>1,2,7</sup>

Based on the hypothesis and Research gap analysis, the study was conducted to assess the feasibility and implication of creating a framework of reporting body fluid cytology.<sup>1,8</sup> The study included with a scope to analyse the diagnostic performance of the conventional smears, cell block technique (Modified Bouin's method) with special emphasis on IHC and to determine their respective accuracy in terms of statistical analysis to check if the findings could impart effective information in diagnostic armamentarium of effusion cytology.

For the unique of its kind, a distinctive trial was carried out with a novel attempt to stratify the cytomorphological features with subsequent cell block sections in reference to graded scoring system in neoplastic as well as

**Table 1:** Indian Academy of Cytology(IAC)- Diagnostic Categories for Reporting Serous Effusion Cytology samples<sup>1</sup>

IAC reporting category	Cytopathology Diagnosis	Remarks
1		No cell seen/Obscuration by blood, artifacts, extensive degenerative changes
2A	No malignant cells detected	Correlate clinically and with imaging studies and microbiological studies
2B	Benign Changes seen. Reactive mesothelial cell, Inflammatory cells seen. Lymphocyte-rich effusion. Specific infections Tuberculosis, Microfilaria, Fungal infection, Hydatid cyst, any other	Repeat cytology Correlate clinically and with imaging studies Ancillary techniques-Optional
3	Atypical cells, Not Otherwise Specified	
4	Atypical cells, Suspicious for malignancy	
5	Malignant cells seen	Subtype the malignancy wherever possible on cytomorphology and ancillary techniques of immunocytochemistry

non-neoplastic /reactive conditions. Being first of its identity the study domain, the analysis also included immunohistochemistry marker expression as well as special stains on cell block section thereby proposing cell block (Modified Bouin's method) to fill the gap and serving as a bridge between cytology and histopathology so that obviating the need for redundant surgical procedures.

In Indian context, studies on effusion cytology that has been rolled upon with a sample size of above 150 composition are proved to draw a definite conclusion and observed to have power of the study above 80%.<sup>1,2</sup> In the present study composed of 189 samples size and based on statistical observations, the power of the present study was calculated to be 92% and thus the present study deemed with high statistical significance. The following table shows the comparative analysis of prior studies by Indian authors with sample size and distribution of effusion fluid samples.

**Table 2:** Comparison of power of present study with similar other studies in terms of cell block versus conventional smears.

Author's study on effusion cytology	Sample size
Sujathan et al <sup>12</sup>	163
Shivakumarasami Udisamath, et al <sup>4</sup>	155
Sujoy, et al <sup>1</sup>	167
Radhika et al <sup>12</sup>	174
Our study	180

### 3. Recommendations from the present review study

The study validated and standardized the unique and first of its kind of cell block preparation method -Modified Bouin's technique with ideal formalin fixative, indigenous to our cytology laboratory in the department. While majority of the studies in Literature on cell block were carried upon with Thromboplastin and agar methods with alcohol fixatives and commercially available kits in the market for research

projects, the present study was empowered by newer in-house, simple and very cost-effective method

The study also validated the usage of IHC marker expressions on CB preparations from Modified Bouin's method which proved to be imperative making provisions to implement in practical applications Bouin's method of CB has increased significance with modestly higher sensitivity and specificity when compared to other methods of CB preparation as postulated in Literature.<sup>1,11,12</sup>

Sensitivity and specificity of CB proved to be substantially higher than proposed in the existing Literature and Tumour representation on CB appeared to be caricature of Histopathological features. Practice of Mair's scoring system on reporting Cell Block sections added value to study and the statistical analysis revealed its significance and serves as frame work system for CB reporting at par with other standard reporting system in Cytopathology

In point of practicing pathologists, the involves in depth insights about IHC on CB and architecture analysis on CB with convention stain, while the former add few information to the existing IHC and ICC knowledge armoury while the later could aid to revisit their knowledge in the light of scoring system in categorization CB sections

### 4. Conclusions

Cell block method prepared by our indigenous Modified Bouin's preparation with formalin fixative proved to show high quality significance and hence it could be followed in routine practice across laboratories.

Cell block technique is quantitatively superior both standalone as well as in conjunction with conventional smear by improving the effective diagnosis of SEC. Diligent use of cell block technique eliminates the suspicious of malignant category on CS and thereby giving more definite diagnosis and thus it is mandated that combined approach of CB in conjunction with CS should be practiced as binary

protocol which proved to have obvious influence on patient management.

## 5. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

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None.

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