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Short Communication
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# CellBlockistry: Science of Cell-Block Making as Ancillary Cytopathology Component in the Era of Minimally Invasive Techniques with Increasing Role of Molecular Pathology

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## Abbreviations

CPT Code: Current Procedural Terminology Code; FFPE: Formalin-Fixed Paraffin-Embedded; FNA: Fine Needle Aspiration; IHC: Immunohistochemistry; RVUs: Relative Value Units; SOCP: Standard Optimum Cell-block Protocol

## Introduction

Cytology specimens can be processed for preparation of paraffin embedded material called cell block/cell-block. The process for achieving this is called cell-block making. This terminology for process of cell-block making may be simplified further as cell-blocking. The cell-blocks are comparable to the Formalin-Fixed Paraffin-Embedded (FFPE) tissue bocks from surgical pathology specimens. They facilitate performance of various tests including Immunohistochemistry (IHC), special stains for detection with confirmation of various microbials and deposits, molecular tests etc. However, term 'cellblock' and 'cell block' in general literature is usually identified with prison cells. Because of this, any attempt for internet search results in data is predominantly related to 'prison cells' with only a few searches related to cytopathology. As recommended previously, this distinction may be refined if the word is hyphenated and spelled as 'cell-block' [1].

The role of cell-blocks in cytopathology is already established. However, this role is increasing continually with ongoing advances including addition of novel IHC markers with technical refinements including evolving sophistication in multicolor Immunohistochemistry (IHC) and the Subtractive Coordinate Immunoreactivity Pattern (SCIP) approach [2,3]. Similarly, many new molecular markers are being standardized on FFPE tissue. All these molecular pathology tests could also be performed on properly prepared cell-blocks. It may be highlighted that, because the cell-blocks can be archived, they are important material available retrospectively at later date if any new tests are introduced in future when the diagnostic material from tumor may not be obtainable. Further cytopathology material extends many benefits of minimally invasive procurement at relatively lower cost. The cell-blocks predominantly have concentrated diagnostic tumor cells without significant proportion of stroma. As compared to this the tissue biopsies contain significant proportion of stroma as non-tumor tissue component which may interfere with molecular tests. Thus, properly prepared cell-blocks should be preferred over the core biopsies for molecular pathology tests.

It is important to emphasize the significant role of cell-blocks in the tissue diagnosis protocols with ongoing advances and refinements for continued excellence in patient care. Dedicated scientific efforts are expected to be extended to study the complexity of this science at qualitative and quantitative level for proper innovations. CellBlockistry as the science of cell-blocking to study the chemistry and the art for quantitative and qualitative enhancement of cell-blocks for maximum outcome for best patient care is evolving [1]. This science to study morphological and qualitative preservation of diagnostic components during processing for cell-block making would prevent compromisation of results on various elective ancillary tests performed on such cell-blocks. This is critical for various interpretation decisions which ultimately affect management and prognostic decisions.

The results of any ancillary tests performed on cell-blocks would be compared ultimately with the published data, which is predominantly based on the results obtained on FFPE of surgical

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Every cytology report on *cytology specimens with cell block* should have following minimum details communicated in it under gross description section or other designated section such as quality details.
This would allow proper decision making in relation to various quality related aspects when any ancillary tests are performed.

**Number of cell-blocks prepared with their designation** (with any descriptive comments similar that in surgical pathology report): _____
Eg.
A1 (prepared from the clot in fresh unfixed specimen)
A2 (from sediments after lysis of red blood cells with
(mention method with reference if possible)
Etc.

**Specimen collected in**:
*Isotonic media*:
Saline / RPMI / Hanks balance solution / Isotonic Medium S/Other _____
*Non-isotonic media / fixative*:
10% formalin
Other (*Not Recommended* due to potential interference with the results of variety of IHC and molecular test results): CytoLyt / Saccamanno's fixative / CytoRich Red / Other alcohol based or acid based non-formalin reagents
**Duration of specimen in the collection medium and temperature**
(prior to actual fixation in 10% formalin):
*Duration*: _____ Hours / minutes
*Temperature*: 2-8ºC / Room temperature / Other _____
**Any processing prior to making the cell-block**
(and prior to final fixation in 10% formalin):
*Lysis of red blood cell contamination*: with (mention method used)
CytoRich Red®, BloodLyz™, Other lyzing reagent _____
**Fixation time in 10% formalin**
(prior to start of actual tissue processing):
*Duration*: _____ Hours / minutes

**Figure 1:** Recommended to include Standardized Optimum Cell-block Processing (SOCP) details in cytology report (From 00).

pathology specimens. Due to this, the cell-blocking protocol should be comparable to FFPE of surgical pathology specimens. Recently, a dedicated review article on CellBlockistry highlights the current limitations and reports a few recent advances to overcome conventional limitations so that the excellence in cell-blocking is attained for the best patient outcome [1]. With reference to this consideration, the cell-block should be made with tracking of various features mentioned under Standard Optimum Cell-block Protocol (SOCP) (Figure 1 and 2). These details should be mentioned in the final cytopathology report under gross description as quality parameters. This would facilitate proper assessment of the results of any tests such as IHC performed on any cell-block to compare with the results in published data predominantly based on FFPE.

Although generally not required, the cell-block also extend additional benefit related to improved sampling with some benefits of tissue biopsy sections including evaluation of some diagnostic architectural patterns such as papillary, acinar, duct-like formations, psammoma bodies, and evaluation of tumor invasion if sampled.

**Number of cell-blocks prepared with their designation** : 2
A1 Prepared from the clot in fresh unfixed specimen
A2 From sediments of the residual specimen
**Specimen collected in**:
*Isotonic medium*: IsotonicMediumS™
**Duration of specimen in the collection medium and temperature**
(prior to actual fixation in 10% formalin):
*Duration*: 3 hours, 40 minutes
*Temperature*: 2-8ºC (on ice)
**Any processing prior to making the cell-block**
(and prior to final fixation in 10% formalin):
*Lysis of red blood cell contamination*: Lysed with BloodLyz™
**Fixation time in 10% formalin**
(prior to start of actual tissue processing):
*Duration*: 6 Hours

**Figure 2:** Sample cytology report showing cell-block details (From 00).

The morphological evaluation of cell-block sections is particularly of increased significance during evaluation of peritoneal/serous cavity washings by facilitating histomorphological comparison with associated primary tumor [4].

Preparation of direct cytology smears with other cytology preparations for optimal cytomorphological evaluation is the primary goal during processing of cytology specimens [1,5]. After achieving the best cytology preparations, the residual specimens have been routinely processed for cell-blocking with variety of conventional methods which have been practiced historically (Figure 3 and 4) [1]. With any protocol, the sediments in the cytology specimen are conglomerated so that the sediments can be manipulated for tissue processing and for making paraffin embedded block. However, depending on a particular method, there are different challenges at various steps in addition to the procedure-related issues. The problems due to the indiscriminate distribution of diagnostic cells in the cell-block and exposure to various fixatives/reagents other than direct fixation in 10% formalin may compromise the results of some ancillary studies especially IHC and molecular tests (Table 1). Because of this, the most of the conventional methodologies lead to production of quantitatively and qualitatively suboptimal cell-blocks.

Conventionally prepared cell-blocks suffer from lack of reproducibility due to indiscriminate distribution of diagnostic cells in the cell-blocks without control over the depth of cutting of paraffin block by histotechnologists (Figure 5). Recent advances include special efforts for quantitative and qualitative improvements with enhanced cell-blocks. Although some of these enhanced cell-block

**Table 1:** Issues related to fixatives in relation to cell-blocking [1].

| Fixatives | Histology | Immunocytochemistry | Molecular testing |
|---|---|---|---|
| Formalin | Sections of resultant FFPE would show histomorphology comparable to that with formalin fixed biopsies and resections. | IHC results would be comparable to that with published data predominantly based on FFPE studies. | The limiting factor with FFPE is fragmentation of DNA with associated artefacts during sequencing with potential interference.<br>RNA-based test (other than miRNA) may be affected due to low yield. However, most of the methodology are standardized on FFPE. |
| Chemical based fixatives: fixatives with heavy metal (B5, Zenker's fixative). or Acidic solutions (Picric acid, Bouin's fixative) | Histomorphology is not affected significantly and is comparable to that with formalin fixed biopsies and resections. Toxicity hazard (Eg-mercury poisoning with Zenker's fluid) | Morphologically good immunostaining, but results may NOT be comparable to that with FFPE with which the results will be compared. This may lead to aberrant immunoprofile with liability due to potential compromization of patient care. | Little data related to stability related to nucleic acid stability (Some such as picric acid results in DNA damage) |
| Alcohol: Methanol in PreservCyt and CytoLyt used in LBC Ethanol in SurePath LBC CellientTM CB | Histomorphology is not affected significantly and is comparable that with formalin fixed biopsies and resections. Shrinkage related artifacts may inerfere. | Immunoreactivity may be affected with erroneous immunoprofiles resulting in suboptimal interpretation outcome. This is especially applicable to nuclear immunomarkers including ER/PR, Ki-67, PCNA, p53, S100 protein, S-100 protein, etc. including other [9]. | Standardized tests/protocols may be required. |

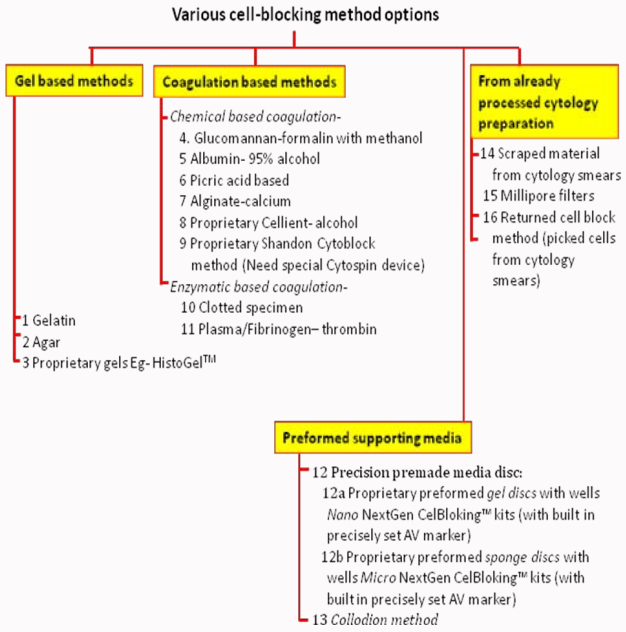


**Figure 3:** Different types of approaches in cell blocking with limitations with each [1].

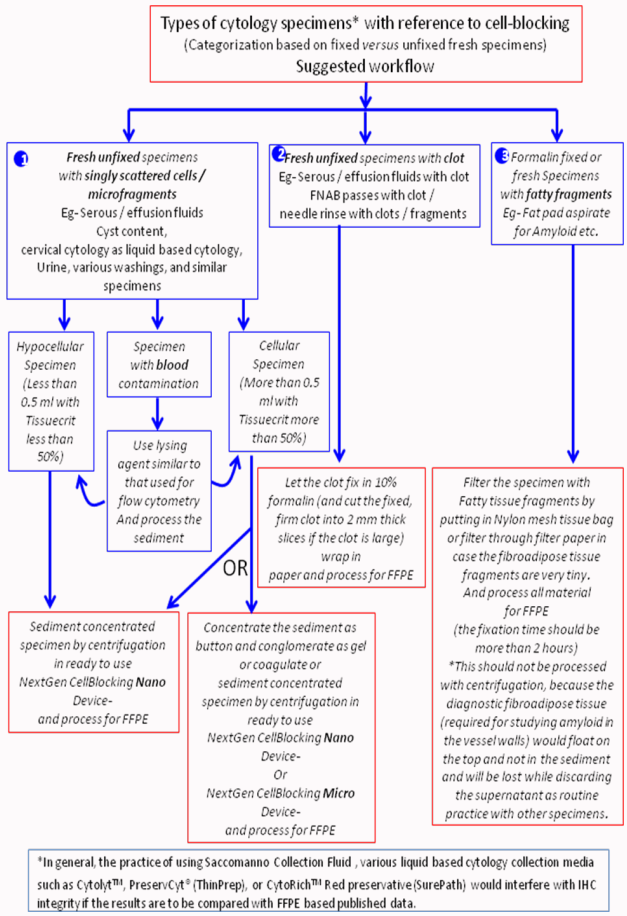


**Figure 4:** The specimens may be divided into various categories (From 00).

making methodologies may achieve quantitative improvements, they may not have qualitative integrity comparable to FFPE of formalin-fixed surgical pathology specimens [6,7]. Shidham's method was

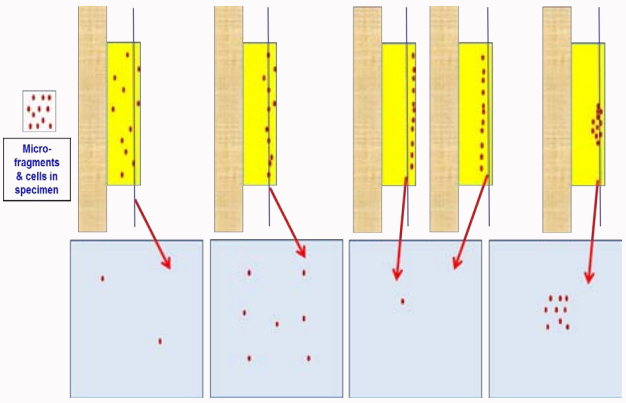


**Figure 5:** Conventional Cell-blocking- Randomness of depth of cutting, leading to suboptimum cellularity of final tissue sections.

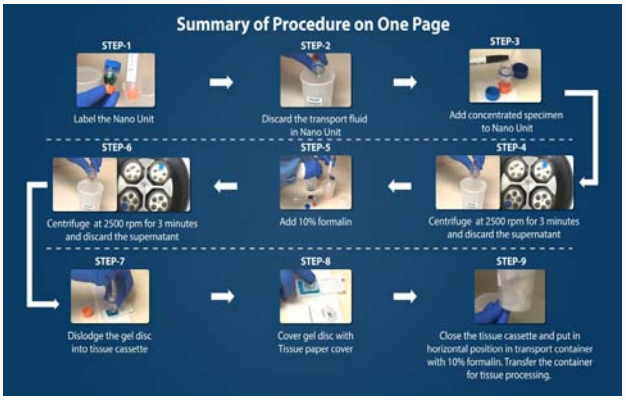


**Figure 6:** Summary of cell-block preparation protocol for Nano NextGen CelBloking™ unit [53]. Manufacturer has suggested an approach for processing multiple specimens simultaneously [60] (Courtesy: www.AVBioInnovation.com).

standardized and reported to achieve these features including a way to monitor the depth of cutting of the final paraffin-embedded cell-block by the histotechnologists with AV Marker as dark colored guiding beacon [8]. However, this method may be difficult to practice with demand for significant skills with difficulty in adapting to the routine workflow of the cytology laboratory. Ready-to-use, low cost, and easy-to-use commercially available kits which extend all the benefits of initially published Shidham's method in addition to the precisely set built-in AV Marker. These Next Gen CelBloking™ kits [9] including Nano (Figure 6) [10,11] and Micro [12,13] versions are simple to be used and do not demand significant skill. These kits can be used by any standard cytology laboratory for making quantitatively and qualitatively enhanced cell-blocks from any specimen with tiny fragments and loosely/singly scattered cells. The processing matches with FFPE prepared from formalin fixed surgical pathology specimens (Figures 7-10). They do not require capital investment for special machines [10-13].

Thus it is critical to maximize the diagnostic outcome of the cytology specimens by enhancing the cell-blocks both quantitatively and qualitatively. However, the extra efforts and resources invested in preparation of such enhanced cell-blocks should be endorsed for the future ongoing innovations for further progress in the field of CellBlockistry. The enhancement technologies also recommend urgently introduced dedicated CPT code (Current Procedural Terminology code) with higher RVUs (Relative Value Units) [14]. In

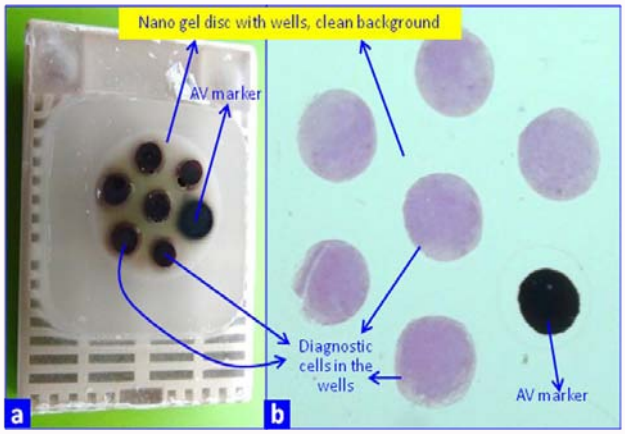


**Figure 7:** a. Final paraffin block; b. Scanning power view of HE stained section of cell-block prepared with *Nano* NextGen CelBloking™ kit. The preformed Nano gel disc is made of proprietary medium which allows the processing reagents to be exchanged freely but the diagnostic cells are retained and concentrated in the wells. The gel medium has clean transparent property as a clean background (pleural fluid).

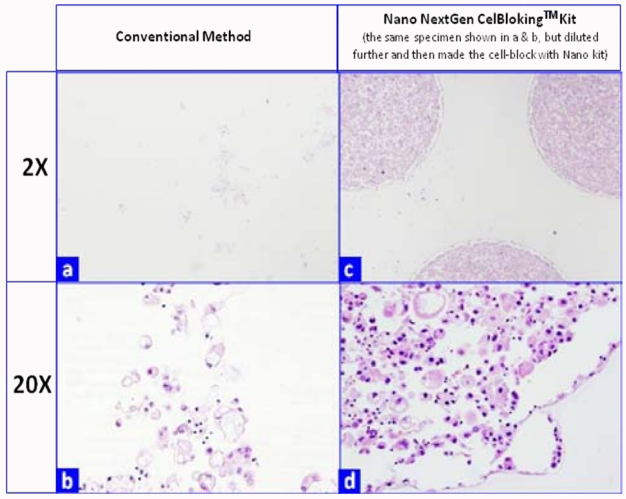


**Figure 8:** Comparison of the morphological details and quantitative enhancement by Nano NextGen CelBloking™ kit (Metastatic adenocarcinoma, pleural fluid). a & b: Cell-block section with very scant cellularity (conventional random, indiscriminatory, plasma-thrombin method); c & d: very cellular cell-block section with many diagnostic cells in the wells (cell-block prepared with enhancement method- Nano NextGen CelBloking™ kit (AV BioInnovation, based on Shidham method http://www.jove.com/index/Details.stp?ID=1316).

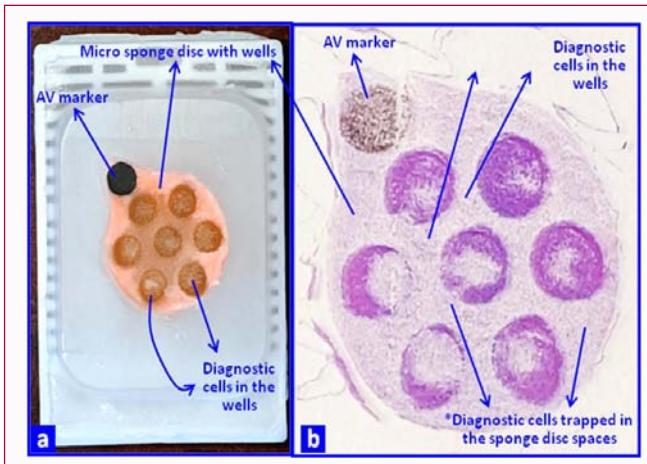


**Figure 9:** a. Final paraffin block; b. Scanning power view of HE stained section of cell-block prepared with Micro NextGen CelBloking™ kit. The preformed Micro sponge disc is made of proprietary porous medium which concentrates the diagnostic cells predominantly in the wells but the small groups of cells and singly scattered cells wandered around during concentration process may also be seen in the sponge spaces*. The sponge disc medium stains faintly (pleural fluid).

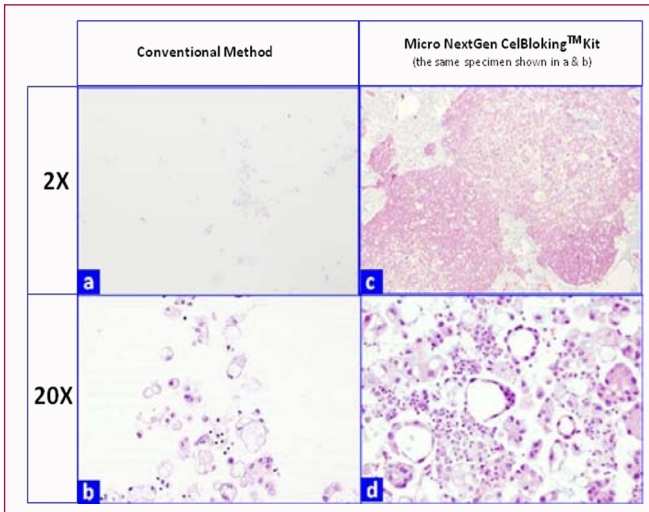


**Figure 10:** Comparison of the morphological details and quantitative enhancement by Micro NextGen CelBloking™ kit (Metastatic adenocarcinoma, pleural fluid). a & b: Cell-block section with very scant cellularity (conventional random, indiscriminatory, plasma-thrombin method); c & d: Relatively cellular cell-block section with many diagnostic cells in the wells and in small spaces in the sponge disc (cell-block prepared with enhancement method- Micro NextGen CelBloking™ kit.

future, many of these technologies with enhanced cell-blocking may be used to process specimens generated from procedures producing very tiny tissue fragments and/or small cell groups. These enhanced cell-blocks would improve the results with such procedures including minimally invasive brush biopsy concept from various sites [15]. To encourage a better patient care, this would allow reimbursement of a deservingly higher technical component to compensate the extra cost invested for making enhanced cell-blocks as compared to the routinely processed cell-blocks or surgical biopsies.

Freshly submitted unfixed cytology specimens who are collected in isotonic media with protein milieu such as Isotonic Medium STM allow flexibility of applying methodologies with final FFPE comparable to that with surgical pathology FFPE [16]. The needle rinses of Fine Needle Aspiration (FNA) collected in Isotonic medium may be processed for cell-blocking.

## Summary

Cell-blocks can be prepared from almost any cytology specimen and are easily archived as paraffin embedded tissue. They are important tissue resource for elective ancillary studies such as IHC and molecular tests related to various prognostic biomarkers and targeted therapy related markers.

Shidham's method addresses most of the issues related to the qualitative and quantitative integrity of final cell-blocks [8]. But this method to be standardized and performed in individual cytology laboratory is labor intensive and relatively non-reproducible due to skill related issues. These limitations of the method are overcome with recently introduced ready-to-use kits which makes the principle used

in this method to be adopted easily in any routine cytology laboratory [9]. These kits allow preparation of qualitatively and quantitatively enhanced cell-blocks from any specimen with tiny fragments and loosely/singly scattered cells with processing when matches with FFPE from formalin fixed surgical pathology specimens (Figures 7-10).

New CPT code with higher RVUs is urgently recommended to encourage a better patient care [14]. A deservingly higher technical component reimbursement for enhanced cell-blocking is encouraged to compensate any extra cost to promote innovations in CellBlockistry [1]. The final cytopathology report should include SOCP details (Figure 1 and 2) related to the cell-blocking as quality indicator. This would allow proper evaluation of results of any ancillary studies if performed on the cell-blocks for comparing the results reproducibly with published database.

## Acknowledgement

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## Glossary of Terminologies

Cell-block- Recommended to use instead of arbitrary conventional pattern as 'cell block' or 'cellblock' in an effort to separate out 'cell block' and 'cellblock' as prison related terminologies.

Cell-blocking- process of preparing cell-block.

CellBlockistry- the art and chemistry of achieving capability to handle the tiny components in different types of cytology specimens.

Needle-rinses-Rinsing of the residual material in FNA needles after preparing direct cytology smears for cytomorphological evaluation.

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12. Processing of single sediment rich* specimen at a time to make a cell block with Micro Unit.
13. Simultaneous processing of multiple concentrated cellular specimens (with Tissuecrit* more than 50%) for making cell blocks with Micro units.
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