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Cell-blocks and immunohistochemistry

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ABSTRACT

The interpretation of results on immunostained cell-block sections has to be compared with the cumulative published data derived predominantly from formalin-fixed paraffin-embedded (FFPE) tissue sections. Because of this, it is important to recognize that the fixation and processing protocol should not be different from the routinely processed FFPE surgical pathology tissue. Exposure to non-formalin fixatives or reagents may interfere with the diagnostic immunoreactivity pattern. The immunoprofile observed on such cell-blocks, which are not processed in a manner similar to the surgical pathology specimens, may not be representative resulting in aberrant results. The field of immunohistochemistry (IHC) is advancing continuously with the standardization of many immunomarkers. A variety of technical advances such as multiplex IHC with refined methodologies and automation is increasing its role in clinical applications. The recent addition of rabbit monoclonal antibodies has further improved sensitivity. As compared to the mouse monoclonal antibodies, the rabbit monoclonal antibodies have 10 to 100 fold higher antigen affinity. Most of the scenarios involve the evaluation of coordinate immunostaining patterns in cell-blocks with relatively scant diagnostic material without proper orientation which is usually retained in most of the surgical pathology specimens. These challenges are addressed if cell-blocks are prepared with some dedicated methodologies such as NextGen CelBloking™ (NGCB) kits. Cell-blocks prepared by NGCB kits also facilitate the easy application of the SCIP (subtractive coordinate immunoreactivity pattern) approach for proper evaluation of coordinate immunoreactivity. Various cell-block and IHC-related issues are discussed in detail.

Keywords: Cell-block, Cellblock, Cell block, CMAS, Monograph, Immunohistochemistry, Subtractive coordinate immunoreactivity pattern, SCIP approach, Standard optimum cell-block protocol, SOCP NextGen CelBloking, Shidham method, IHC.

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The book will be available in *print version* and also as *e-book version* on CytoJournal website (www.CytoJournal.com).

INTRODUCTION

Compared to the immunohistochemical evaluation of surgical pathology specimens, there are multiple challenges which have to be considered for the optimal outcome when interpreting the immunohistochemistry (IHC) of cell-block sections.

The most critical consideration, usually underrecognized, is potential interference associated with exposure to the various physical and chemical factors during cell-block processing. The immunoprofile of a particular neoplasm or category of cells/tissue determined by the interpretation of immunostained cell-block sections is correlated with the cumulative published database derived predominantly from formalin-fixed paraffin-embedded (FFPE) tissue sections. It is important to recognize, if the fixation and processing protocol is different from routinely processed FFPE tissue with exposure to non-formalin fixatives or reagents, the immunoprofile concluded from the immunostaining pattern of IHC on such cell-block sections may not be representative due to the potential for aberrant results.[1-3]

The continued progress in the field of IHC has been significantly advancing with the standardization of many immunomarkers with the availably of multiple novel antibodies. Introduction of various technical advances including multiplex IHCs[4,5] with refined methodologies/automation and recent addition of rabbit monoclonal antibodies has further improved the techniques. Rabbit monoclonal antibodies demonstrate 10 to 100 fold higher antigen affinity as compared to the mouse monoclonal antibodies and have significantly higher sensitivity.[6]

Most approaches for IHC evaluation include an interpretation of coordinate immunoreactivity patterns to decide the immunoprofile of a particular type of cell associated with a given pathology. However, as compared to tissue sections of surgical pathology specimens, the diagnostic cells and groups of cells in cell-block sections are randomly distributed. The diagnostic cells may even be difficult to categorize as the target of interest in some cases, especially in serous fluid cytology and Fine-needle aspiration biopsy (FNAB) samples with higher potential of contamination from the trajectory of the needle during endoscopic ultrasound-guided fine-needle aspiration (EUS-FNAs). Because of this, the randomly scattered cells and microtissue fragments in various random sections may be difficult to follow for coordinate immunostaining pattern in same cells in some cell-blocks.

Most of the tumor cells are large enough to be sliced into multiple 4 μm thick sections and so would be present in at least a few 4 μm thick serial levels [Figure 1]. However, such singly scattered cells may not be easily trackable in adjacent serial levels, unless the relationship of sections and the sequence of individual serial sections are properly designated on the slides and the individual sections on each glass slide are oriented exactly in an identical manner (without flipping and rotating any of the sections while transferring the paraffin sections of cell-block to the glass slides) [Figures 2 and 3].

The interpretation of coordinate immunoreactivity is based on the feature that requires evaluation and correlation of the immunostaining patterns in exactly the same cells (not just similar cells) and microtissue fragments [Figures 4 and 5]. This critical component of evaluating coordinate immunoreactivity pattern cannot be achieved in cytology preparations, because the same cell(s) cannot be present in more than one cytology preparation.[7] This drawback, along with interferences due to the exposure of fixatives and reagents during processing of cytology preparations, does not match with those used in FFPE of surgical pathology specimen. This further discourages their application for evaluation by immunostaining as the routine primary choice. However, in some specific clinicopathological settings with specific requirements, cytology preparations may be the only option after proper standardization and optimization of protocol for a particular clinical scenario.[8-12]

Similar to the interpretation of hematoxylin and eosin (H&E) stained sections, the immunostained cell-block sections should also evaluate the immunomorphology with reference to the appropriate immunostaining patterns – membranous, cytoplasmic, nuclear, paranuclear, bile canalicular pattern etc. [Figures 5-7]. Some of these immunostaining patterns may be difficult to evaluate in cytology preparations as compared to tissue sections. Because of this, the interobserver and interinstitutional reproducibility may be compromised in the absence of methodical standardization.

The final cytopathology report should provide details as part of a standard optimum cell-block protocol (SOCP) [Figure 8] for assessing the integrity of the results on the cell-blocks prepared. Such a component in the report should assist in achieving reproducible interinstitutional results with the goal of matching with the results in published databases generated on FFPE of surgical pathology resection and biopsy specimens.

Challenges related to the evaluation of coordinate immunostaining patterns in cell-blocks are overcome by the application of subtractive coordinate immunoreactivity pattern (SCIP) approach.[13] The remaining challenges are addressed by making cell-blocks with NextGen CelBloking™ (NGCB) kits.[1,2,7,14] Cell-blocks prepared by NGCB kits also facilitate easy application of SCIP approach, various features of which are explained below.

SCIP APPROACH

i. The relationship of each serial section of the FFPE of cell-blocks should be identifiable by sequentially numbering

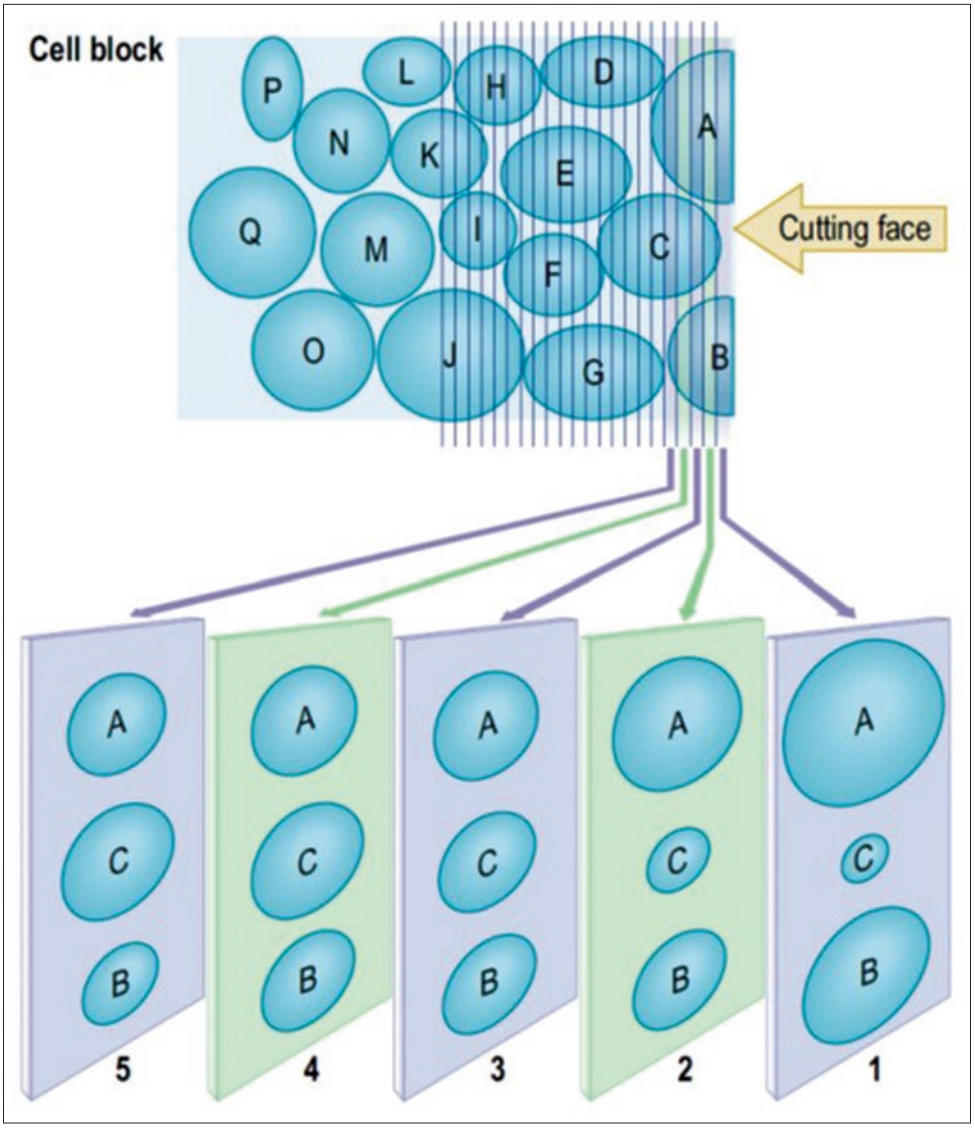


Figure 1: Four micron thick sections with same cells for subtractive coordinate immunoreactivity pattern (©vshidham reproduced from Ref #7).

the glass slides with serial sections microtomed for immunostaining [Figure 1]

ii. The serial levels of the cell-block sections are oriented identically without letting the sections be flipped, rotated, or tilted while mounting on the glass slides during microtoming by the histotechnologist [Figure 3]

iii. Immunostaining strategies to create a basic local map of the cell-block sections [Figure 2]

Depending on the type of specimen, including Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) aspirates with bronchial and gastrointestinal (GI) mucosal contamination, and especially effusion fluids, the exact location of the diagnostic cells under scrutiny may be challenging. This may lead to suboptimal and even false-positive interpretation. For cell-blocks of effusion fluids, the SCIP approach also facilitates the creation of a basic location map of various components in the effusion fluid. SCIP approach along with vimentin immunostaining (either as single color or as dual color with BerEP4) [Figure 9][15] on the first serial section helps in locating all the mesothelial cells and all inflammatory cells as non-neoplastic components.

Thus, if any component which is non-immunoreactive for vimentin (and may be also BerEP4 immunoreactive in dual color immunostain) is equivalent to a second foreign population.[7,15] This approach is a very simple and effective approach for detecting metastatic disease in effusion fluid (but is usually not applicable to washings). With reference to the data in the adult population, most metastases to serous cavities are secondary to adenocarcinoma. The majority of adenocarcinomas (other than from a few sites such as

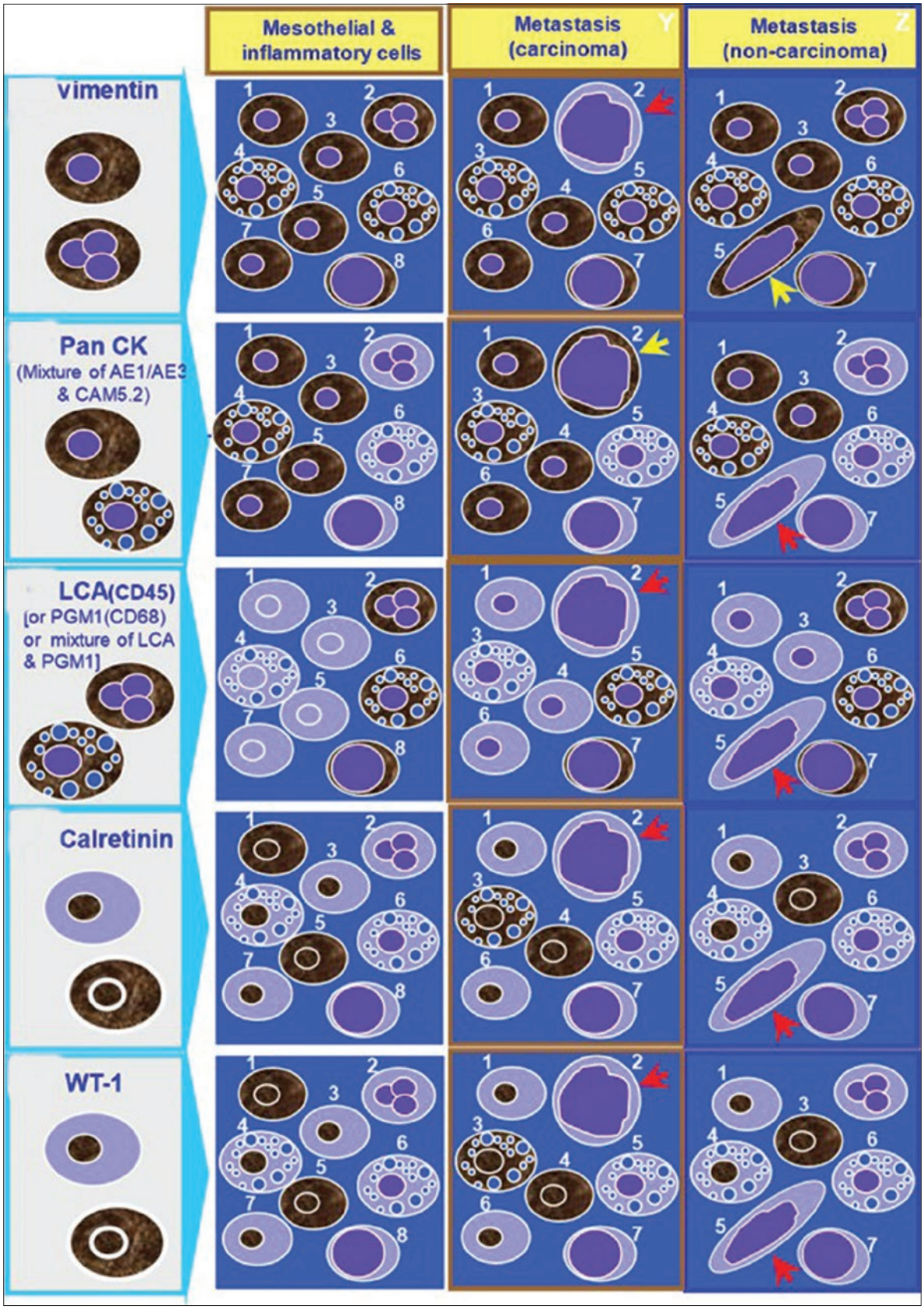


Figure 2: Subtractive coordinate immunoreactivity pattern approach (SCIP) (©vshidham reproduced from Ref #7).

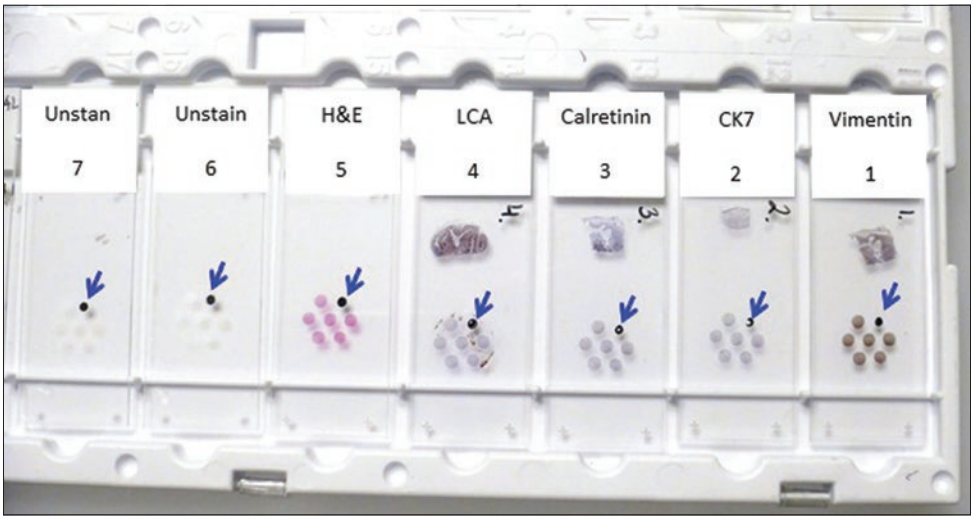


Figure 3: Cell-blocking and AV marker–subtractive coordinate immunoreactivity pattern approach (SCIP). Identical orientation of all sections on glass slides with proper labeling of their exact sequence in relation to each other.

Table 1: Significant vimentin immunoreactivity patterns.

Vimentin immunoreactive carcinomas
Renal cell carcinoma (except the chromophobe variant)
Adrenal cortical carcinoma
Endometrial adenocarcinoma
(Endometrial endometrioid carcinomas immunostain strongly for vimentin, but endocervical carcinomas rarely stain (weak focal staining in up to 13% of endocervical carcinomas).
Malignant mixed Müllerian tumors, serous ovarian carcinomas
Large cell carcinoma of lung
Metaplastic carcinoma of breast
Poorly differentiated adenocarcinoma of stomach (6%)
Sarcomatoid carcinomas (spindle cell carcinomas)
Pleomorphic salivary gland tumors
"Basal-like" breast carcinomas
Follicular and anaplastic thyroid carcinomas
Epithelial and sarcomatoid mesotheliomas
Solid pseudopapillary neoplasm of the pancreas
Vimentin expressing tumor epithelial cells in surgically resected carcinomas including pancreatic adenocarcinomas and hepatocellular carcinoma independently predicted a shorter post-surgical survival
Vimentin non-immunoreactive sarcoma
Alveolar soft part sarcoma

Table 2: BerEP4 immunoreactivity in various tumors.[22]

Immunoreactive	Non-immunoreactive
Adenocarcinomas (most, 50–100% in various studies)	Mesothelioma*
Neuroendocrine tumors, including small cell carcinoma	Lymphoma
Chromophobe renal cell carcinoma (75%), papillary renal cell carcinoma (55%), clear cell renal carcinoma (18%), metastatic renal cell carcinoma (14%)	Most soft-tissue sarcomas
Hepatocellular carcinoma	Adenomatoid tumor
Basal cell carcinomas	Renal oncocytoma
Basosquamous carcinomas	
Synovial sarcoma	

*However, 4–26% of mesotheliomas may show BerEP4 immunoreactivity (which is usually membranous with microvillous pattern, in contrast to the cytoplasmic and membranous pattern in non-mesothelial tumors). Due to this, lack of immunoreactivity for BerEP4 favor mesotheliomas in malignant clinical setting. However, this non-immunoreactivity should be applied with an appropriate immunopanel (to discriminate mesothelioma from other metastases in serous effusions): At least two immunoreactive mesothelial immunomarkers (such as calretinin, vimentin, cytokeratin 7, and WT1) with at least two non-mesothelial immunomarkers (such as BerEP4, B72.3, and relevant lineage-specific immunomarker in particular clinical scenario such as lung primary)

renal cell carcinoma, metastatic hepatocellular carcinoma, endometrial carcinoma, etc.) are non-immunoreactive for vimentin [Table 1].[16-19]

Similarly, most adenocarcinomas are immunoreactive for Ber-EP4 with a few exceptions [Table 2].[20-22] Localization and identification of these metastases are facilitated with improved efficacy by a dual color immunostaining for vimentin (as red color) with BerEP4 (as brown color), especially in cases with a small number of singly scattered metastatic tumor cells.[23]

An important caveat to this approach is that in some effusion fluids, the second foreign population with vimentin immunoreactivity may not be easily detectable. This is applicable to a relatively insignificant proportion of cases belonging to a small subset which includes a few adenocarcinomas (such as renal cell carcinoma, metastatic hepatocellular carcinoma,[24] endometrial carcinoma, some

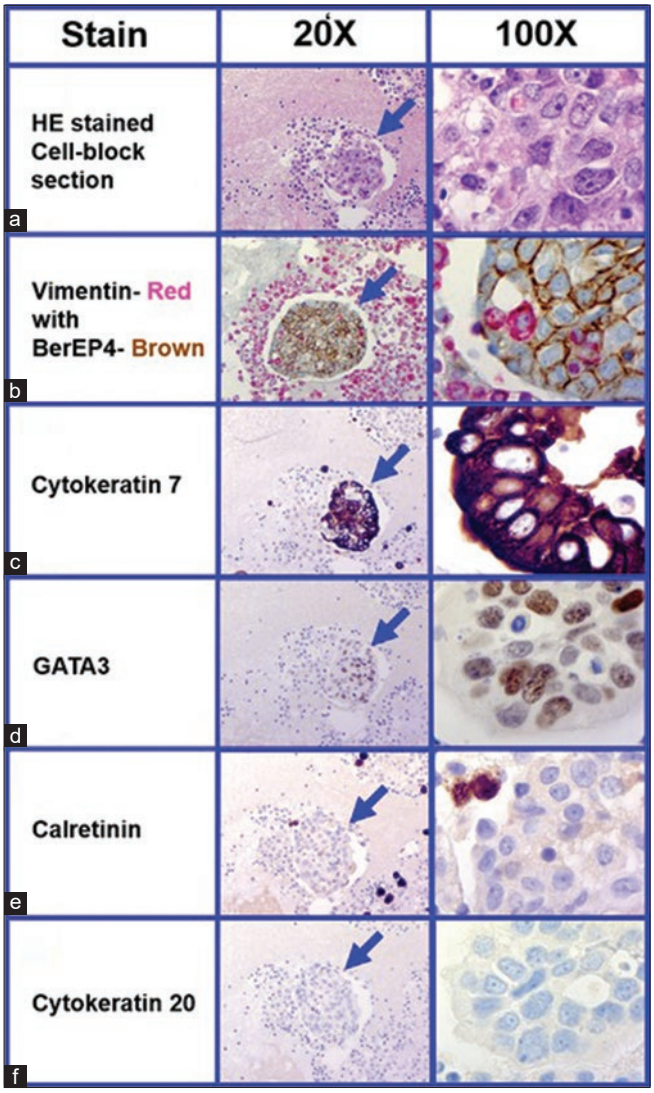


Figure 4: Subtractive coordinate immunoreactivity pattern (SCIP) in cell-block section. Metastatic adenocarcinoma (cell-block sections of pleural fluid. History of triple-negative infiltrating duct carcinoma). Follow the neoplastic cells (arrows) (a) in serial sections to evaluated coordinate immunoreactivity pattern for at least two positive and two negative lineage-specific immunomarkers. The tumor cells (arrows) in dual color immunostained section are immunoreactive for BerEP4 (brown) and non-immunoreactive for vimentin (red) (b). They are also immunoreactive for cytokeratin 7 (c) with nuclear immunoreactivity for GATA3 (d). They are non-immunoreactive for calretinin (a few adjacent reactive mesothelial cells are immunoreactive) (e) and cytokeratin 20 (f).

lung carcinoma, spindle cell carcinomas from various sites, etc.) [Table 1] and most non-carcinoma malignancies (such as sarcomas, lymphomas,[25] and melanomas). In such cases, vimentin immunoreactive tumor cells can be confirmed as non-mesothelial by evaluating their coordinate immunoreactivity in relation to mesothelial immunomarkers such as cytokeratin (CK) 7, WT1, and calretinin (nuclear).

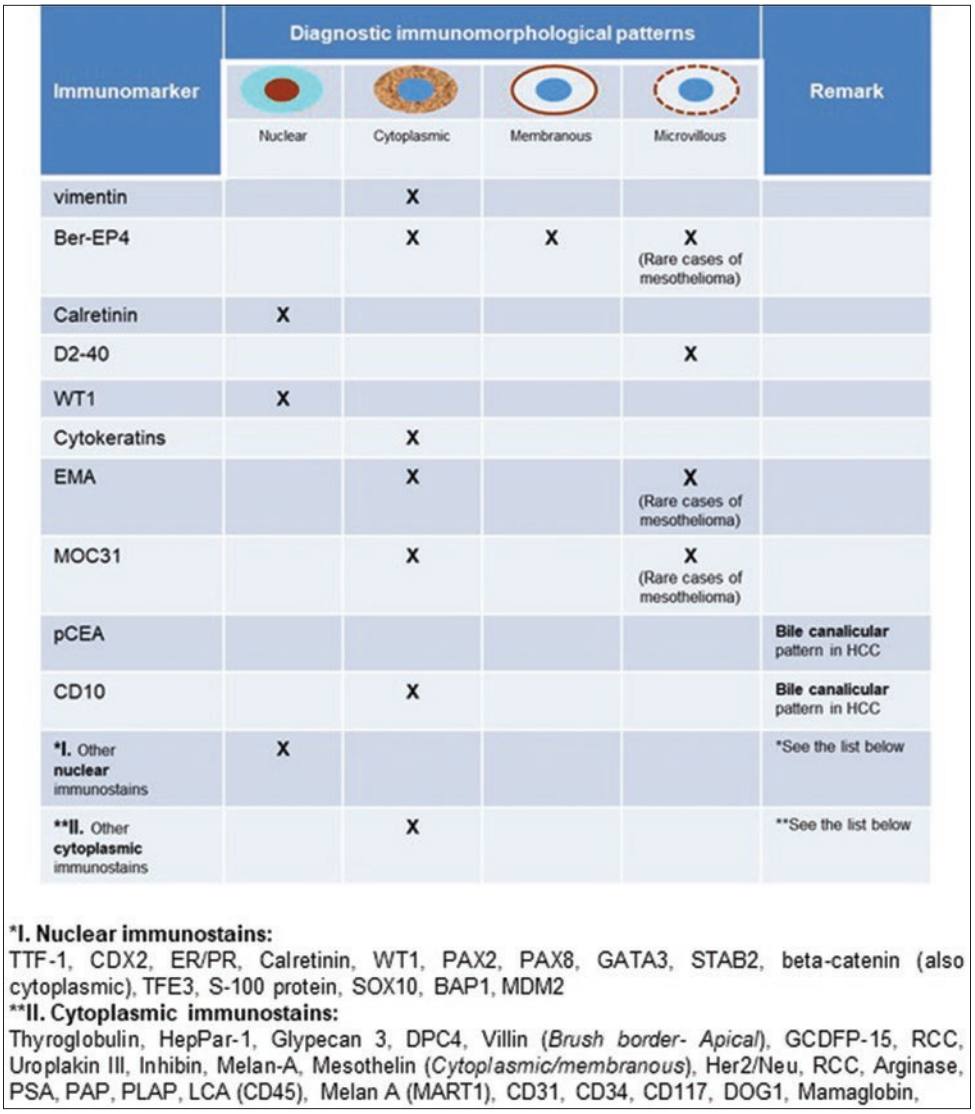
The immunomarkers usually applied for the lineage specific evaluation include broad-spectrum anti-pan-CK antibody cocktails for carcinomas, lymphoid antigens (CD45 or CD20 and CD3) for lymphoproliferative lesions, and melanocyte differentiation antigens S100 protein (S100), Melan-A/MART-1, tyrosinase, and SOX10) for melanoma [Tables 3 and 4].[7] Further evaluation of carcinomas generally includes broad categorization based on coordinate immunoreactivity for CK7 and CK20 followed by the application of lineage-specific immunomarkers [Tables 3 and 4].[16,26]

iv. Orientation marker: Application of SCIP approach is facilitated further by the AV marker reported initially with Shidham's method and included in the cell-blocks prepared by NGCB kits as a built in feature.[27] AV marker assistance is critical while transferring various serial sections from the paraffin ribbon floated on the warm water bath. The histotechnologist during microtoming of the FFPE of cell-block can easily see the dark colored AV marker in each of the sections in the paraffin ribbon to ensure their proper orientation on sequentially numbered glass slides.[1]

POSITIVE CONTROLS FOR IHC

As a routine approach, the sections of known positive controls[28] are mounted on the same glass slide adjacent to the cell-block sections to be immunostained for the respective immunomarker. Different types of positive controls are described below:

a. Archived known tissues with positive immunoreactivity[28]
These are the routine and most commonly used type of positive controls in most of institutions with availability of a wide range of archived tissues and neoplasms. However, the reliability of this type of positive control depends on various storage conditions including duration, storage temperature, and ambient humidity.
b. Multitumor tissue block (sausage method)[29]
Multitumor tissue block prepared usually from archived FFPE with different types of neoplasms which cover a wide range of immunomarkers. Sections of such positive controls can be used for screening of hybridoma monoclonal antibodies in production settings in addition to rapid screening of novel immunomarkers in an economical manner. These, in addition to routine clinical application, can also be useful for large-scale interlaboratory quality control programs (with limitations related to the reliability applicable to the positive control mentioned above as #a).
c. Tissue microarray (TMA) of various positive controls[30-32]
TMA is paraffin blocks which include up to 1000 tissue cores from various types of tissues and neoplasms arranged as an array for multiplex histological analysis. Such positive controls from archived FFPE may be used to validate cancer biomarkers with large numbers of



Immunomarker	Diagnostic immunomorphological patterns				Remark
	Nuclear	Cytoplasmic	Membranous	Microvillous	
vimentin		X			
Ber-EP4		X	X	X (Rare cases of mesothelioma)	
Calretinin	X				
D2-40				X	
WT1	X				
Cytokeratins		X			
EMA		X		X (Rare cases of mesothelioma)	
MOC31		X		X (Rare cases of mesothelioma)	
pCEA					**Bile canalicular** pattern in HCC
CD10		X			**Bile canalicular** pattern in HCC
***I.** Other **nuclear** immunostains	X				*See the list below
****II.** Other **cytoplasmic** immunostains		X			**See the list below

***I. Nuclear immunostains:**
TTF-1, CDX2, ER/PR, Calretinin, WT1, PAX2, PAX8, GATA3, STAB2, beta-catenin (also cytoplasmic), TFE3, S-100 protein, SOX10, BAP1, MDM2
****II. Cytoplasmic immunostains:**
Thyroglobulin, HepPar-1, Glypecan 3, DPC4, Villin (*Brush border- Apical*), GCDFP-15, RCC, Uroplakin III, Inhibin, Melan-A, Mesothelin (*Cytoplasmic/membranous*), Her2/Neu, RCC, Arginase, PSA, PAP, PLAP, LCA (CD45), Melan A (MART1), CD31, CD34, CD117, DOG1, Mamaglobin,

Figure 5: Immunomorphological patterns of some common immunomarkers (×, the immunomorphology of the immunomarker).

cancer samples for the evaluation of protein expression patterns by immunostaining simultaneously with identical protocols with minimal inconsistency and technical variability. TMAs can be used as IHC positive controls for prognostic, therapeutic, and diagnostic immunomarkers related to a variety of cancers (with limitations related to the reliability applicable to the positive controls mentioned above as #a and b).

d. Preparing FFPE of cell-blocks of formalin-fixed known standard cell lines with NGCB™ kits:[33] Uniplex (one positive control in one section) or Multiplex (multiple immunomarkers on one section – each well in the gel disc in NextGen CelBloking™ kit with different immunomarkers (personal experience).[33a] The benefits of such positive controls are relatively consistent integrity of individual immunomarkers with better control over processing and storage conditions. This approach overcomes the limitations and problems related to the availability of a large pool of positive controls from a clinical setting in a safe and controlled manner. In comparison, the cell lines can be grown commercially as needed at production level to provide high-quality, well-standardized positive IHC controls.

IMMUNOHISTOCHEMICAL EVALUATION OF DIFFERENT TYPES OF CYTOLOGY SPECIMENS

Application of IHC to cell-blocks of Fine-needle aspiration (FNA) aspirates is relatively straight forward. It is comparable to IHC on tiny surgical pathology specimens as micro-biopsies with a few additional factors which have to be taken into consideration for the best outcome with quantitatively

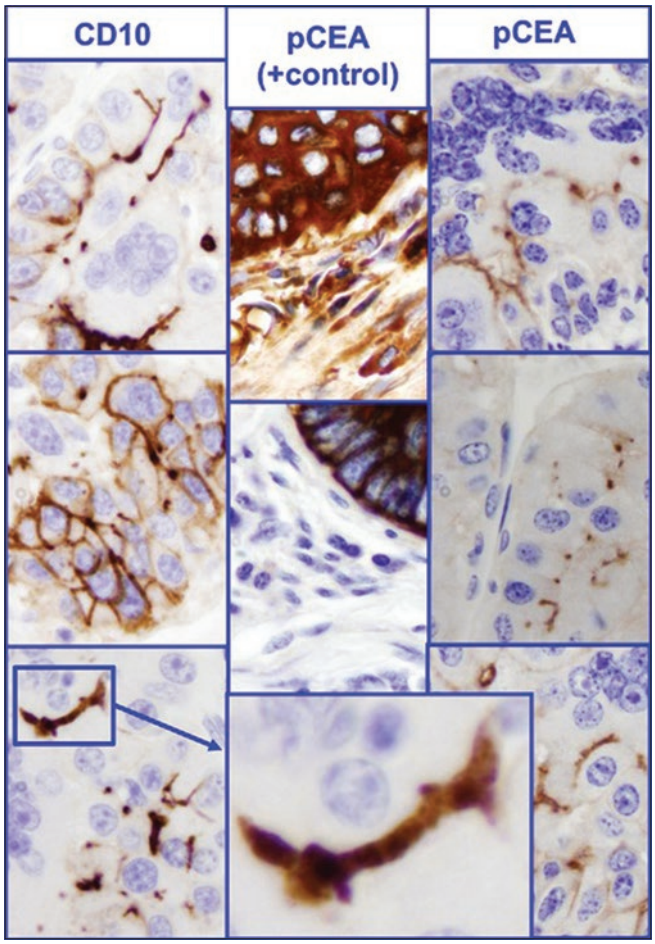


Figure 6: Immunomorphology – bile canalicular pattern (Hepatocellular carcinoma). The immunostaining pattern highlights the bile canaliculi [by CD10 on left side or polyclonal CEA (pCEA) on right side] between different cells. The immunostaining should be seen as longitudinal sections with or without branching, Some may be seen as cross section and appear as dot between two cells. Nonspecific random immunostaining such as cytoplasmic immunostaining seen in the positive control in the center is interpreted as negative for bile canalicular pattern. The preferred positive control is liver tissue or hepatocellular carcinoma with reference to evaluation of bile canalicular immunostaining pattern.

small amounts of diagnostic material in properly made cell-blocks.[1,2,34] Another important consideration is the potential contamination of cells/tissue material present along the track of the FNA during the procedure. This feature is significantly more frequent in procedures where the needle has to be longer such as with various EUS-FNAs. Depending on the procedure, the FNA rinses from such procedures may show variable proportions of bronchial mucosal, GI mucosal (gastric, esophageal, or duodenal), oral, and other tissue contaminants. Although these contaminations are usually easy to weed out morphologically during the interpretation, IHC interpretations on such cell-blocks could be challenging with potential pitfalls.[2] For example, some cases with relatively few tumor cells diluted by bronchial mucosal contamination, may show many TTF-1 immunoreactive cells. This may lead to misinterpretation of the neoplasm as TTF-1 immunoreactive with potential miscategorization of tumor as adenocarcinoma. Similarly, other immunomarkers may have specific limitations in some clinicopathological situations.

A slightly different challenge is the presence of some secondary elements in the lesion with the diagnostic component of the lesion. An important example in this category is the presence of reactive lymphocytes in neuroendocrine tumors (NET) during grading of these tumors based on Ki-67 proliferation index. Some of the reactive lymphocytes aspirated during the procedure may also be immunoreactive (nuclear) for Ki-67 and may be counted as NET cells and lead to a higher count with false upgrading of NET, especially for tumors in borderline categories.[5] This challenge can be overcome by applying dual color IHC (Ki-67: Brown, nuclear with LCA: Red, cytoplasmic).[5]

Immunohistochemical evaluation of hematolymphoid pathologies

Similarly for lymphoproliferative neoplasms and pathologies, the interpretation is dependent on the immunoprofile of various components in the lesion. In general, flowcytometry is the preferred approach for the evaluation of immunoprofiles in these pathologies. However, availability of cell-blocks in these situations is crucial for the evaluation of some immunomarkers which are better evaluated by IHC. In addition, cell-block may be required to perform some molecular pathology tests (for more details refer to other review articles on this topic).

Immunohistochemical evaluation of effusion fluids

As compared to IHC on other type of specimens, application and interpretation of IHC on cell-block sections of effusion fluids is relatively complex due to a wide morphological spectrum of reactive mesothelial cells.[7,35-37] Although the cytomorphological clues helping to identify the second foreign population of tumor cells may be facilitated by Diff-Quik stain,[35] interpretation and confirmation are relatively objective with IHC. The SCIP approach with vimentin (one color or dual color) immunostaining with BerEP4 detects the second foreign population of tumor cells in most of the cases with significant ease and reproducibility.[7]

However, the interpretation of immunostained cell-block sections of effusion specimens with relative hypocellularity of diagnostic cells diluted with reactive mesothelial and inflammatory cells is challenging. This is further accentuated if the metastasis is from a well to moderately differentiated

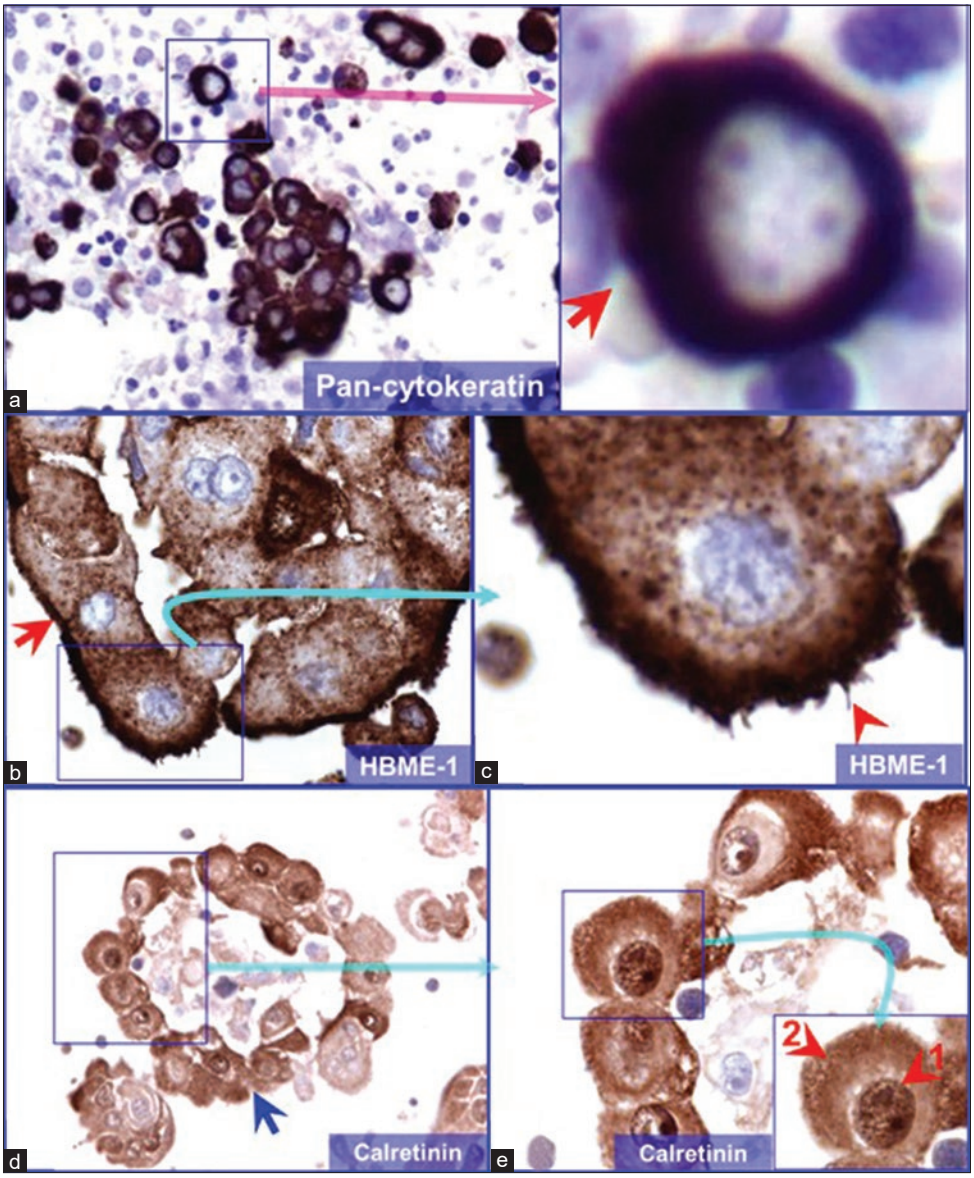


Figure 7: (a) Pancytokeratin immunoreactivity pattern (pleural fluid). Reactive mesothelial cells with cytoplasmic immunostaining (arrow in inset). Some reactive mesothelial cells may show a concentric immunostaining pattern around the nucleus better appreciated by adjusting fine focus. (b and c) HBME-1 immunoreactivity pattern (epithelioid mesothelioma, pleural fluid). Mesothelioma cells with membranous (arrow in a) and cytoplasmic immunostaining. Note the microvilli (arrowhead in b). (d and e) Calretinin immunoreactivity pattern (epithelioid mesothelioma, pleural fluid). Mesothelioma cells (arrow in a) show nuclear (arrowhead 1) immunoreactivity usually with cytoplasmic immunostaining (arrowhead 2) imparting the so-called "fried-egg" appearance (©vshidham reproduced from Ref #7).

carcinoma presenting as scattered isolated tumor cells such as in metastatic mammary carcinoma (especially lobular carcinoma), ovarian carcinoma, and some lung carcinomas. The same challenge may also compromise the selective microdissection of the tumor cells for molecular pathology studies.

Conventionally, vimentin is dismissed as a non-specific immunomarker. However, it has a very critical role in identification of a second foreign population of metastatic tumor cells in serous fluid cytology in the adult population.[7] This benefit of vimentin immunostaining in concert with the SCIP approach may be applied as single color or dual color technique (vimentin red with Ber-EP4 brown) for objective detection of a second foreign population or tumor cells in cell-block sections of effusion fluid. In most of the cases, they would be detected as a vimentin non-immunoreactive population with Ber-EP4 immunoreactivity [Figure 9].[38,39]

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™ [33]
Duration of specimen in the collection medium and temperature
(prior to actual fixation in 10% formalin):
Duration: 3 hours, 40 minutes
Temperature: 2-8°C (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™ [33]
Fixation time in 10% formalin
(prior to start of actual tissue processing):
Duration: 6 Hours

Figure 8: Sample SOCP in cytology report highlighting important details about the cell-block (reproduced from open access publication, ref #1). (see also Table 6).

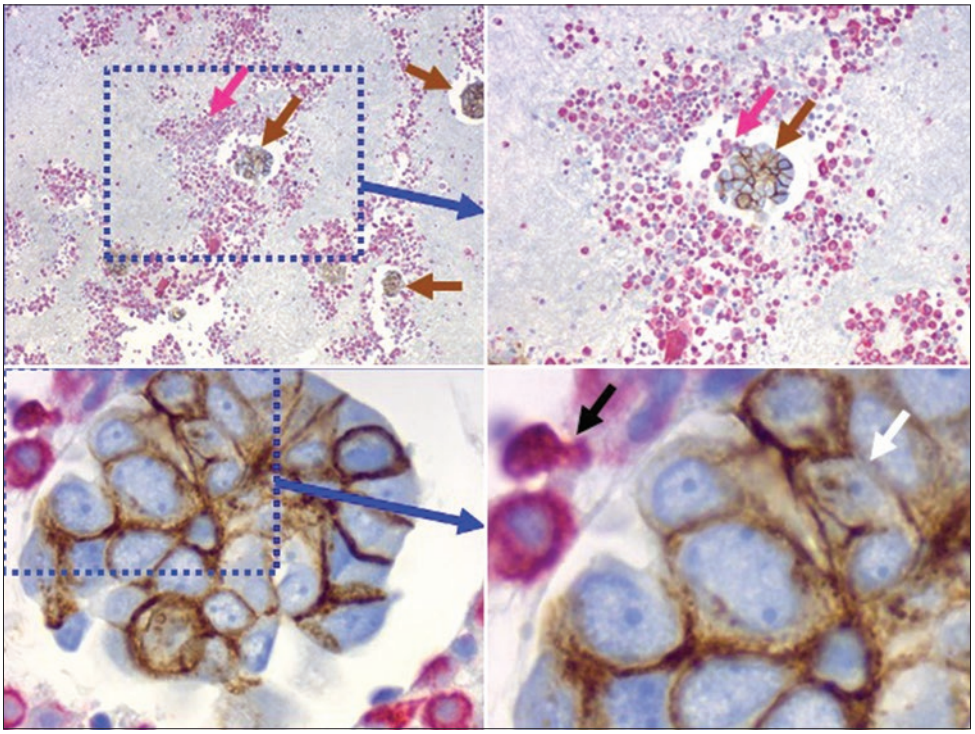


Figure 9: Dual color immunostaining (vimentin: Red, BerEP4: Brown) (Pleural effusion fluid with history of mammary carcinoma). All the usual components of effusion fluids (reactive mesothelial cells and inflammatory cells) are immunoreactive for vimentin (red). Second foreign population is highlighted and easily detectable as vimentin non-immunoreactive component, consistent with metastatic tumor cells. In the adult population, most of these are metastatic adenocarcinoma which are usually immunoreactive for BerEP4 with cytoplasmic and membranous immunostaining (brown). (Red arrows show vimentin immunoreactive red immunostained mesothelial cells and inflammatory cells as usual components of effusion fluids. Brown arrows show BerEP4 immunoreactive brown immunostained second foreign population of adenocarcinoma cells.).

Depending on the cytomorphology and clinical setting, the differential interpretations for effusion fluid IHC should also include mesothelioma, primary peritoneal carcinoma, and primary effusion lymphoma.[40,41] The approach of identifying the second foreign cell population will not assist in effusions from cases with the epithelioid variant of

Table 3: Algorithmic immunohistochemical analysis of undifferentiated carcinomas.[16,38]

Metastatic carcinoma (SCIP approach)			
Broad categorization based on: Carcinoma: PanCytokeratin + Not sarcoma: vimentin - ; Not melanoma: S100/HMB45/MelanA - ; Not hematolymphoid (lymphoma): LCA(CD45) - Differential based on coordinate immunoreactivity pattern for CK7 and CK20			
CK7+/CK20+	**CK7+/CK20-**	**CK7-/CK20+**	**CK7-/CK20-**
Gastric AdCa (subset) **Pancreatic AdCa (subset)** **Cholangiocarcinoma** (minor subset) **Ovarian mucinous Ca** PAX8,MUC5-AC+ WT1, MUC2 CDX2 (variable) **Urothelial Ca** Uroplakin, Thrombomodulin GATA3,P63+ CK5/6 (-. ½+) **Bladder AdCa** Thrombomodulin+ CDX2 (variable)	**Breast Ca** ER/PR+ GCDFP, Mamaglobin, GATA-3+ **Lung AdCa** TTF1, Napsin, BAP1+ mCEA, CK5/6, p63, p40- **Lung SmCC** NE markers (2 of Synaptophysin, chhromogranin,CD56), TTF1, Napsin+ P63, p40- **Endometrial AdCa** mCEA- Vimentin, ER/PR+ **Endocervical AdCa** mCEA+ Vimentin, ER/PR- **Cervical SqCC** mCEA- P16,vimentin, ER/PR+ **Ovarian (serous) Ca** PAX8, PAX2, WT, mesothelin, ER/PR+ CDX2 (variable) mCEA- **Ovarian (clear cell) Ca** PAX8, pVHL, HNF-1β, Napsin A+ AFP, WT1, ER, GPC3- PAX2+/- **Cholangiocarcinoma** CK19,mCEA+ CDX2 (variable) **Pancreatic AdCa** DPC4,CK17+/− Maspin, S100P, MUC5AC+ pVHL− **Gastric AdCa** mCEA+ TTF1- CDH17+/− CDX2, MUC1, MUC5AC−/+ **Esophageal AdCa** mCEA, CDH17+ CDX2+/− MUC1, MUC5AC−/+ SATB2- **Mesothelioma** Calretinin, vimentin, CK7, CK5/6, D240, WT1	**Colorectal AdCa** CDX2, STAB2MUC2, mCEA, villin+ MUC5AC- **Merkel cell Ca** (paranuclear dot CK20) NE immunomarkers (2 of Synaptophysin, Chhromogranin,CD56), Ber/EP4+ S100,TTF1,Napsin- **Gastric AdCa** (subset)	**Colon** (medullary) SATB2+, CDH17+, CDX2,TFF3,Calretinin+/− **Prostate AdCa** PSA, PAP, NKX3.1, B72.3, AR+ TTF1, villin, GATA3 WT1, p63- **Hepatocellular Ca** pCEA and CD10 with canalicular pattern vimentin,HepPar1,Glypecan3, Arginase1+ BerEP4, CK19- **Renal (clear cell) Ca** PAX8, PAX2, vimentin, RCC, CD10,pVHL+ Inhibin,TTF1,mCEA- **Adrenocortical Ca** Vimentin, inhibin, calretinin, Melan-A, synaptophysin+ chromogranin, mCEA- **Non-seminoma GCT** PLAP+ EMA- Yolk sac tumor AFP+ Embryonal Ca OCT+(3/4) CD30+ **Mesothelioma** (subset) **Lung- SmCC** (subset) Gastric AdCa (subset) **Head and Neck Ca**

(Contd...)

Table 3: *(Continued)*

Metastatic carcinoma (SCIP approach)			
Broad categorization based on: Carcinoma: PanCytokeratin + Not sarcoma: vimentin - ; Not melanoma: S100/HMB45/MelanA - ; Not hematolymphoid (lymphoma): LCA(CD45) - Differential based on coordinate immunoreactivity pattern for CK7 and CK20			
CK7+/CK20+	**CK7+/CK20-**	**CK7-/CK20+**	**CK7-/CK20-**
	GATA3,Thrombomodulin,mesothelin+ BerEP4, B72.3, MOC31, mCEA TTF1, Napsin, p63, p40, BAP1 **Thyroid Ca (Non-medullary)** TTF1, Thyroglobulin,PAX8+ mCEA- **Thyroid Ca (medullary)** mCEA,neuroendocrine markers,calcitonin+ TTF1 (week to moderate) PAX8 (variable weak) Thyroglobulin- **Salivary gland (ductal) tumors** GATA3,AR,GCDFP-15+ **Thymic primary** CD5+/−, p63+/−, PAX8+/−, CD117+/−, Glut1+/- **Renal (papillary) Ca** vimentin,CA-IX,RCC(Clone PN15 anti-gp200)+ PAX2,PAX8+(>85%) **Urothelial Ca** (subset)		

AdCa: Adenocarcinoma, Ca: Carcinoma, GCT: Germ cell tumor, HCC: Hepatocellular carcinoma, mCEA: Monoclonal carcinoembryonic antigen, NE: Neuroendocrine, pCEA: Polyclonal carcinoembryonic antigen, pVHL: von Hippel-Lindau tumor suppressor, RCC: Renal cell carcinoma, S100P: Placental S100, SmCC: Small cell carcinoma (3); SqCC: Squamous cell carcinoma; reproduced after some modifications from open access publication – Bahrami *et al*. Arch. Pathol. Lab. Med. 2008, 132, 326–348[16]

mesothelioma because of the overlap of immunoprofile with that of reactive mesothelial cells. In such cases, additional quantitative (numerous vs. a few) and qualitative (numerous large groups vs. a few small groups) features would help[40] along with clinical and radiologic findings of mesothelioma with approach to rule out metastatic adenocarcinoma and application of some recently available molecular pathology tests for mesothelioma.[42]

In contrast to most of the adenocarcinomas (except a few tumors such as renal cell carcinoma, some lung adenocarcinoma, Mullerian tumors, and metastatic hepatocellular carcinoma), most of the mesothelioma cells and reactive mesothelial cells are immunoreactive for vimentin [Table 1]. The mesothelial cells are also immunoreactive for CK 7 and calretinin (nuclear and cytoplasmic). However, the immunostaining pattern of mesothelioma for vimentin immunostaining may be heterogeneous with focal vimentin non-immunoreactivity. Other immunomarkers for mesothelial cells include WT1, D2-40, and CK 5/6 in positive category.[7] In addition to confirming the cells as mesothelial cells, depending on a particular clinical setting, potential metastasis from other primary sites in the differential diagnosis should be ruled out with the immunomarkers in the *negative category* for mesothelial cells (including some relatively organ-specific immunomarkers such as CDX2 (colorectal and upper GI), TTF-1 (lung), Napsin 1 (lung), PAX8 (Mullerian and TTC), GATA3 (breast), ER (breast and Mullerian), PSA (prostate), PAP (prostate), AFP (germ cell tumors), TFE (alveolar soft part sarcoma), HCG (choriocarcinoma), S100 (melanoma and MPNST), and synovial sarcoma (beta-catenin and SYT-SSX) [Tables 3 and 4].[43]

IHC ON CELL-BLOCKS TREATED WITH FORMIC ACID FOR PRIONS

The cell-block sections of formic acid treated FFPE will require pretreatment or combination of pretreatments for IHC analysis for the best results.[44] Based on the study of IHC for the prion protein on brain tissue of CJD patients,

Table 4: Lineage-specific immunomarkers (modified from 43).

Lineage category and subtypes	Immunomarkers
Lung adenocarcinoma	TTF-1, Napsin A
Breast carcinoma	GATA3, ER, GCDFP-15, Mamaglobin
Ovarian serous carcinoma	PAX-8, ER, WT1, vimentin
Ovarian clear cell carcinoma	pVHL, HNF-1B, KIM-1, PAX-8
Endometrial adenocarcinoma	PAX-8/PAX-2, ER, vimentin
Endocervical adenocarcinoma	PAX-8, p16, CEA, HPV *in situ* hybridization, loss of PAX-2
Thyroid follicular cell origin	TTF-1, PAX-8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF-1, mCEA, chromogranin
Salivary duct carcinoma	GATA3, AR, GCDFP-15, HER2
Renal cell carcinoma, clear cell type	PAX-8/PAX-2, RCCma, pVHL, KIM-1
Papillary renal cell carcinoma	P504S, RCCma, pVHL, PAX 8, KIM-1
Translocation renal cell carcinoma	TFE3
Upper gastrointestinal tract	CDH17, CDX2, CK20
Lower gastrointestinal tract	SATB2, CDX2, CK20, CDH17
Hepatocellular carcinoma	Arginase-1, CISH for albumin mRNA, Bile canalicular pattern (pCEA, CD10)
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Pancreas, acinar cell carcinoma	Trypsin, chymotrypsin, lipase, elastase, BCL10
Pancreas, ductal adenocarcinoma	MUC 5AC, CK17, Maspin, S100, IMP3
Pancreas, neuroendocrine tumor	PR, PAX-8, PDX1, CDH17, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear beta-catenin, vimentin, E-cadherin-Neg, PR, CD10
Prostate, adenocarcinoma	NKX3.1, PSA, PSAP, ERG
Urothelial carcinoma	GATA3, uroplakin II, S100P, CK5/6, p63, CK20
Adrenal cortical neoplasm	SF-1, Mart-1, inhibin-a, calretinin
Seminoma	SALL4, LIN28, OCT4, CD117, D2-40
Yolk sac tumor	SALL4, LIN28, glypican-3, AFP
Embryonal carcinoma	SALL4, LIN28, OCT4, NANOG, CD30, SOX2
Choriocarcinoma	GATA3, b-HCG, CD10
Sex cord stromal tumor	SF-1, inhibin-a, calretinin, FOXL2
Thymic origin	PAX-8, p63, CD5
Gastrointestinal stromal tumor	CD117, DOG1, PGDFRA
Solitary fibrous tumor	STAT6, CD34, Bcl2, CD99
Vascular tumor	ERG, CD31, CD34, Fli-1
Synovial sarcoma	TLE1, CK, nuclear beta-catenin
Chordoma	CK, S100, Brachyury
Desmoplastic small round cell tumor	CK, CD99, desmin, WT1 (N-terminus)
Alveolar soft part sarcoma	TFE3
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Smooth muscle tumor	SMA, MSA, desmin, calponin
Ewing sarcoma/PNET	NKX2.2, CD99, Fli-1
Myxoid and round cell liposarcoma	NY-ESO-1
Low-grade fibromyxoid sarcoma	MUC4
Epithelioid sarcoma	CD34, loss of INI1
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Langerhans cell Histiocytosis	CD1a, S100, Langerin (CD207)
Angiomyolipoma	HMB-45, SMA, Mart-1 (S100 non-immunoreactive)

(Contd...)

Table 4: (*Continued*).

Lineage category and subtypes	Immunomarkers
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers (may lose INI1)
Myeloid sarcoma	CD43, CD34, MPO
Follicular dendritic cell tumor	CD21, CD35
Merkel cell carcinoma	CK20 (paranuclear dot staining), MCPyV
Mesothelial origin	Calretinin (nuclear), WT1, D2-40, CK5/6, mesothelin, CK7, vimentin
Neuroendocrine origin	Chromogranin, synaptophysin, CD56
Mast cell tumor	CD117, tryptase
Squamous cell carcinoma	p40, CK5/6, p63, SOX2
Melanoma	S100, Mart-1, tyrosinase, HMB-45, MiTF, SOX10, PNL2

hydrated steam autoclaving (HA) for 10 min at 121°C in 10 mM citric acid recovery buffer at pH 6 showed best results.[44] However, for other immunomarkers, it is recommended to standardize this antigen retrieval protocol with a positive control processed in the same manner with formic acid treatment similar to the formic acid treated FFPE of the cell-block.

DIFFERENTIAL DIAGNOSIS OF UNKNOWN PRIMARY[38]

Unknown primary may be defined as a clinicopathologic scenario when the exact site of origin of a neoplasm cannot be determined based on routine evaluation with clinical and imaging studies.[16,23,39,41,45-50] Knowing the exact nature of the primary site of origin is critical for proper treatment and management of the malignancy. In brief, it is an occult primary with metastatic spread and needs a tissue diagnosis with the help of ancillary studies such as IHC and molecular pathology testing. There are numerous publications which discuss pathological evaluation of unknown primary.[43]

In general, the nature of the primary site is usually obvious in cases with metastases to serous cavities as Stage IV phenomenon. Because of this, in majority of cases, cytopathologic and IHC evaluation may usually require confirmation of a second foreign population as a metastasis. However, in some cases, IHC may have to be extended to confirm the primary site of an unknown primary or for differential diagnosis between two or more possible primaries in some rare cases. The challenge may be complex in such cases with an approach to locate the metastatic tumor cells and track them by additional immunomarkers (under SCIP approach) depending on possible differential diagnosis for the evaluation of the primary site [Tables 3 and 4].

NGCB™ kits[33] which has a built-in AV marker result in cell-blocks which allow improved interpretation with the SCIP approach [Figure 2]. Combination of various immunomarkers including site-specific immunomarkers [Tables 3 and 4] which allow interpretation of coordinate immunoreactivity for two and non-immunoreactivity for two tissue-specific immunomarkers for the same tumor cells would result in higher accuracy.

Most of the other specimens, including various FNAs, require methodical application of IHC for the evaluation of an unknown primary. Additional methods, including molecular techniques, may be needed as indicated.[38] The approach begins with simple initial steps to broadly categorize the malignancy as carcinoma, sarcoma, hematolymphoid process – lymphoma, or melanoma [Tables 3 and 4].[25,48] This step is relatively straightforward in most cases with IHC.

Continued progress in IHC has significantly advanced with availability of many organ- and tumor-specific immunomarkers including multiplex IHCs.[4,5] Determination of primary site without any prognostic or therapeutic advantages would equate with expensive and unnecessary testing.[51] However, diagnosing the primary site is critical for delivering specific treatment for an increasing number of primary sites. Due to this, precision in determination of primary site is very critical in the current era with ongoing progress in molecular and immuno-based targeted therapies.[51]

Site-specific treatment options are relatively more effective for some metastases such as from breast, ovary, prostate, and thyroid with improved prognosis[52] with ongoing additions of other sites including colorectal and lung. IHC in correlation with clinical and imaging findings usually identifies the primary in more than 90% of the cases. In the remaining cases, application of molecular analysis may help to identify the primary sites.

There are many novel site-specific immunomarkers with ongoing additions for interpretation of a primary site [Tables 3 and 4].[53] Many publications review and suggest various algorithms and guidelines.[16,38,39,41,43,49,50,54] Many more immunomarkers will be added in future with an ever increasing number of site-specific and therapy-specific immunomarkers [Table 5]. However, in some cases, the results may not be conclusive because of scant or poorly

preserved or improperly processed specimen. In such cases, performing IHC on repeat specimen with adequate proportion of diagnostic tumor cells may be conclusive in correlation with clinical and imaging findings. In some unresolved cases, other ancillary tests such as molecular studies may be required.[45,48,55-60] (see chapter #5).

SOME IHC-RELATED PITFALLS

Non-specific immunostaining due to a proteinaceous background around the diagnostic components in the cell-block sections may interfere with proper evaluation of the immunoreactivity pattern in immunostained cell-block sections.

Distribution pattern of diagnostic components in the cell-block sections (tiny cell groups *vs.* single cells), fixative(s) used, choice of antigen retrieval protocol (i.e., heat-induced epitope retrieval, enzyme digestion, etc.), titer of antibody used, clone of the antibody, and other similar potential variables in the protocols may interfere with the immunostaining pattern.

Immunoexpression of CKs, although usually associated with epithelial neoplasms, is known to occur in some non-carcinomas. The list of such tumors includes synovial sarcoma, adamantinoma, chordoma, epithelioid sarcoma, myoepithelial neoplasms, and desmoplastic small round cell tumor. Similarly, focal aberrant immunoexpression for CK may be observed in some tumors which are not expected to demonstrate CK immunoexpression. There are a relatively significant number of tumors in the latter category. To mention, a few of relative significance are melanomas, peripheral nerve sheath tumors, angiosarcomas, meningiomas, clear cell sarcomas, undifferentiated pleomorphic sarcomas, epithelioid hemangioendotheliomas, epithelioid leiomyosarcomas, plasmacytomas, rhabdomyosarcomas, malignant rhabdoid tumors, and even lymphomas such as diffuse large B-cell lymphomas and anaplastic large cell lymphomas.[61]

As compared to the primary tumor, the site-specific immunomarkers in metastases may show a complete loss or relatively weak immunoreactivity for the immunomarker. This feature of commonly observed heterogeneous immunoexpression pattern in some tumors may cause sampling of non-immunoreactive areas leading to misinterpretation due to sampling artifact. This challenge has a relatively higher significance in cell-block sections with relatively a few diagnostic cells and if interpretation is based on a single or few immunomarkers. Thus, it is important to practice methodologies achieving best cellularity and qualitative integrity of cell-blocks possible with NGCB™ kits[33] along with the practice of applying multiple immunomarkers sufficient to evaluate at least two positive and two negative immunomarkers for a particular tumor in the differential interpretation.

Relatively underestimated but an equally significant issue is improper processing related aberrations leading to the potential compromisation of patient care with related liability. The study evaluating effects of fixation and the time in relation to IHC on cell-block sections[62] summarized that formalin preservation should be the preference for proper results of IHC. Most importantly, alcohol preservation/fixation introduces an unpredictability of immunoreactivity usually with reduced immunostaining depending on how long the cells/tissue is exposed to alcohol. The pre-analytic variables are critical for proper IHC results with an important role of proper positive controls. A study evaluating PD-L1 immunostaining of cell-block sections with reference to various processing approaches, reported that formalin, RPMI, and saline demonstrated stronger cytoplasmic immunostaining as compared to Cellient cell-blocks, with the poorest immunostaining with CytoLyt.[63-66]

Formaldehyde has preferential interaction with primary amines (such as lysine in proteins) and primary amides (asparagine and glutamine) present in tissue for intra- and intermolecular covalent cross-linking of various biomolecules, which may impair the enzymatic activity and also immunoreactivity.[67,68] Due to this, it requires proper application of the antigen retrieval step on FFPE sections of cell-blocks similar to FFPE sections of surgical pathology specimens.

The immunoprofile of the tumor cells or diagnostic components in cell-blocks prepared with methodologies which do not match with FFPE processing would be interpreted by comparing with immunoprofiles in published data performed on FFPEs of surgical pathology biopsy specimens. Because of this, the results generated would be non-representative and aberrant with ultimate compromisation of delivery of appropriate therapy with a direct impact on patient care and related liability for the testing institution.[1] However, it is not uncommon to apply protocols in which the specimens are collected in various alcohol-based fixatives such CytoLyt™, PreservCyt® (ThinPrep),[69] or CytoRich™ Red preservative (SurePath).[2,63,70,71] As mentioned previously, this introduces significant liability including malpractice and other legal concerns in some settings such as mesothelioma claims.[72] This pitfall can be easily prevented by applying the protocol similar to that used for surgical pathology specimens which can be achieved with the help of recently available NGCB™ kits.[1,33]

IHC pitfalls and challenges with reference to effusion fluid cell-blocks

As compared to the interpretation of immunostained cell-block sections of FNA specimens, evaluation of immunostained cell-block sections of effusion fluids poses a significant number of special challenges. Some unexpected aberrant immunoreactivity may lead to incorrect interpretations.

Table 5: Some therapy and prognosis-related immunomarkers*.

Immunomarker	Remark
Estrogen receptor (ER) Progesterone receptor (PR)	The ER and PR status should be tested on the primary tumor and/or metastases for all newly diagnosed invasive breast cancer or recurrence For ductal carcinoma *in situ*, ER status is required to decide hormone therapy to reduce the risk of future breast cancer. Testing DCIS for PR status is optional
HER2/neu (ERBB2; c-erbB-2; erb-b2 receptor tyrosine kinase 2; human epidermal growth factor receptor 2)	For application of HER2-targeted therapy Tested in invasive or recurrent breast cancers, both lobular and ductal (Joint ASCO and CAP) Similarly, inoperable, locally advanced, recurrent or metastatic stomach and esophagus cancers (Joint ASCO and CAP) HER2 status tested by: (a) Immunohistochemistry to measures HER2 protein. (b) Fluorescent *in situ* hybridization evaluates amplification (increased number of gene copies)[73]
CD117 DOG1 PGDFRA SDHB	Majority of GISTs overexpress CD117 (c-KIT) and *PDGFRA*. DOG1 (anoctamin-1/ANO1, a voltage-gated calcium-activated anion channel) immunoexpression may be noted even in CD117 non-immunoreactive GISTs. A minority of GISTs only shows PDGFRA immunoreactivity SDHB immunohistochemical staining should be considered for any tumors that lack CD117 or PDGFRA immunoreactivity to identify SDH-deficient GIST
EGFR	EGFR is highly expressed in a variety of solid malignant tumors (including non-small cell lung cancer, pancreatic cancer, breast cancer, medullary thyroid cancer, salivary gland carcinoma, squamous cell carcinoma of the head and neck, colorectal cancer, chordoma, and malignant gliomas) and its expression has been correlated with disease progression and poor survival With increasing role of targeted therapies such as Anti-EGFR monoclonal antibody (IMC-C225: Cetuximab) IHC-based EGFR screening methods using FFPE of tumor assists selection of cancer patients eligible for cetuximab treatment
p16	p16 immunohistochemistry for risk stratification in oropharyngeal SCC with significantly better outcome of p16-positive oropharyngeal SCC than for p16 negative tumors[74]
p40 TTF1	Thyroid transcription factor-1 (TTF1) and p40 (an isoform of p63) are immunomarkers for adenocarcinoma (ADC) and squamous cell carcinoma (SCC) respectively for objective categorization (especially in cases with solid/squamoid morphology which may be misclassified as SCC with therapy (with bevacizumab) related potentially lethal pulmonary hemorrhage[75]
p53	p53 is altered gene in human cancers in approximately 50% of all invasive tumors. The most difficult-to-treat cancers (such as high-grade serous ovarian cancers, triple-negative breast cancers, esophageal cancers, small-cell lung cancers and squamous cell lung cancers) show p53 mutation in at least 80% of cases In such cases, mutant p53 protein is a candidate target for the new anticancer therapies[76]
Ki67	For grading of neuroendocrine tumors, GIST, pheochromocytoma, lymphoma As proliferation marker could be important in many other tumors including breast carcinoma[77]
Ber-EP4	Cases with metastasis of Ber-EP4 immunoreactive (epithelial cell adhesion molecule -positive tumors) may be treated with intraperitoneal catumaxomab antibody[17]
MMR proteins (MLH1, MSH2, MSH6, and PMS2)	To identify patients at risk for *Lynch syndrome* (hereditary non-polyposis colorectal cancer) and patients with sporadic microsatellite instable colorectal cancer Lynch syndrome is associated with increased risk of carcinoma at various sites including: Gastrointestinal: Colon, small intestine, stomach, hepatobiliary, pancreas Genitourinary: Kidney, bladder, prostate Gynecological: Endometrium, ovary
PDL1	Therapeutic monoclonal antibodies that target either PD-1 or PD-L1 have been FDA approved for use in various malignancies (including metastatic melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, bladder cancer, head and neck cancer, Merkel cell carcinoma, Hodgkin lymphoma, gastric cancer, hepatocellular carcinoma, and microsatellite instability-high cancer regardless of histology). Approval is pending in other diseases However, testing for PD-L1 positivity is required for therapy of NSCLC with pembrolizumab (PD-1/PD-L1 antibody). However, there are four FDA registered PD-L1 IHC assays based on four different PD-L1 antibodies (22C3, 28-8, SP263, and SP142) used on two different IHC platforms (Ventana and Dako) with different scoring systems[78]

*Qualitative and quantitative integrity of the tumor cells in cell-blocks (and surgical pathology specimens) is more critical than routine application of immunohistochemistry

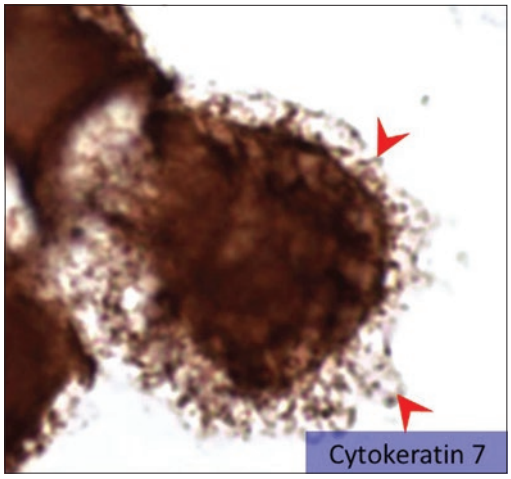


Figure 10: Membranous microvillus immunostaining pattern (cytokeratin 7, ×100 zoomed) (©vshidham reproduced from Ref #7).

BerEP4 immunostaining in some mesothelioma cells may lead to their interpretation as metastatic adenocarcinoma.[7] BerEP4 immunostaining in mesothelioma cells if present is usually membranous with microvillous pattern. This could be evaluated more clearly under an oil immersion lens at higher magnification [Figures 7 and 10].[7] Immunomorphological evaluation, instead of just observing positive or negative immunostaining, is critical in such cases. Contrarily, a few metastases may be Ber-EP4 non-immunoreactive [Table 2] but detectable as a foreign second population due to vimentin non-immunoreactivity. Such cells in effusion fluids are consistent with metastatic tumor cells, which should be evaluated properly by following their coordinated immunostaining pattern for other relevant immunomarkers.

Similarly, focal, faint immunoreactivity for Napsin A in some reactive mesothelial cells may be observed with

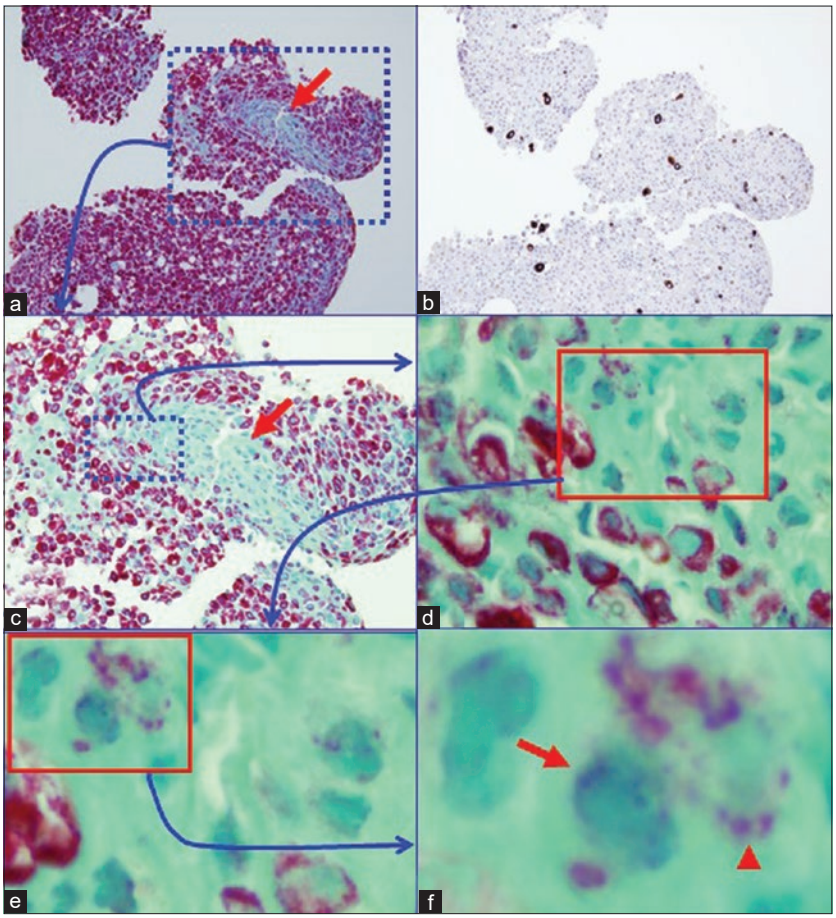


Figure 11: Pleural fluid, negative for malignant cells (history breast carcinoma) immunostained with SCIP approach. (a) The cell-block section showed vimentin immunoreactive (red cytoplasmic) mixed inflammatory cells admixed with a few reactive mesothelial cells (which were immunoreactive for cytokeratin 7 (b). These cells were immunoreactive for calretinin). Some focal areas (arrows in a and c) may appear to be non-immunoreactive for vimentin (without immunoreactivity for BerEP4, GATA3, and estrogen receptor) if evaluated casually. However, on careful morphological examination at higher magnification, these may be lymphocytes with scant cytoplasm (arrow in f) with scant red vimentin immunoreactivity or other inflammatory cells (arrowhead in f) with some focal vimentin immunoreactivity (d-f). (a, c through f) Dual color vimentin (red cytoplasmic) with BerEP4 (brown cytoplasmic). (b) Cytokeratin 7 (a and b: ×10; c: ×20; d: ×100 oil; e and f: Zoom of d).

dual color immunostaining without immunoreactivity for nuclear TTF1 in rare cases (personal experience). However, this could be avoided with the inclusion of an adequate number of immunomarkers in the panel with a coordinate immunostaining pattern as two negatives and two positives in the same cells evaluated by the SCIP approach.[7]

As observed in some cases, without a known malignancy or metastasis (personal experience), the predominance of small chronic inflammatory cells with a scant amount of cytoplasm may show negligible vimentin immunoreactivity which may be misinterpreted as a vimentin non-immunoreactive population with potential for false-positive interpretation as metastasis. Vimentin non-immunoreactivity in some cells interpreted as a second foreign population can be avoided by a careful morphological evaluation at higher magnification. The small cells can be confirmed as lymphocytes with minimum amount of cytoplasm with scant focal vimentin immunoreactivity in at least a few cells which can be distinguished from the larger tumor cells with relatively more cytoplasm [Figure 11].

Scant cellularity may compromise the ability to interpret and follow the same cell (or small groups of cells) for the evaluation of coordinate immunoreactivity, primarily due to a lack of identical orientation between the serial sections. Routine application of the SCIP approach would overcome this pitfall in a significant proportion of cases.[7]

Predominance of singly scattered tumors cells simulating a single population (especially in cases with tumors cells showing cytomorphology overlapping with reactive mesothelial cells) may lead to a false-negative interpretation of H&E stained cell-block sections of effusion fluids. This is significantly more challenging with metastases from lower grade tumors. The list of such primary sites includes the breast (lobular carcinoma) and low-grade ovarian neoplasms. These scenarios can be avoided by the SCIP approach with IHC of the first section for vimentin as one color or as dual color (vimentin: Red, cytoplasmic with BerEP4: Brown, cytoplasmic) immunostaining. This approach would easily achieve objective confirmation in most of cases, even those with relatively scant numbers of diagnostic tumor cells. With reference to the dual color immunostaining (vimentin: Red, cytoplasmic with BerEP4: Brown, cytoplasmic), in some rare cases, the tumor cells may show immunoreactivity for both vimentin and BerEP4 which may be difficult to interpret. In such cases, IHC may be repeated as single color immunostaining for vimentin and BerEP4, respectively.[7]

SUMMARY

CellBlockistry, the science and art of art of cell-block making, refines cell-blocking of variety of cytology specimens.[79] However, currently, multiple methods have been practiced with significant variation with lack of reproducibility in

Table 6: Recommended to include standardized optimum cell-block processing (SOCP) details in cytology report (reproduced from Ref. 1). (see also Figure 8).

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): _____
E.g.
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™[33]/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: 2–8°C/room temperature /Other _____
Any processing before making the cell-block (and before final fixation in 10% formalin)
Lysis of red blood cell contamination: With (mention method used)
  CytoRich Red® (potential interference with IHC), BloodLyz™[33], other lysing reagent _____
Fixation time in 10% formalin (before start of actual tissue processing)
  Duration: _____Hours/minutes

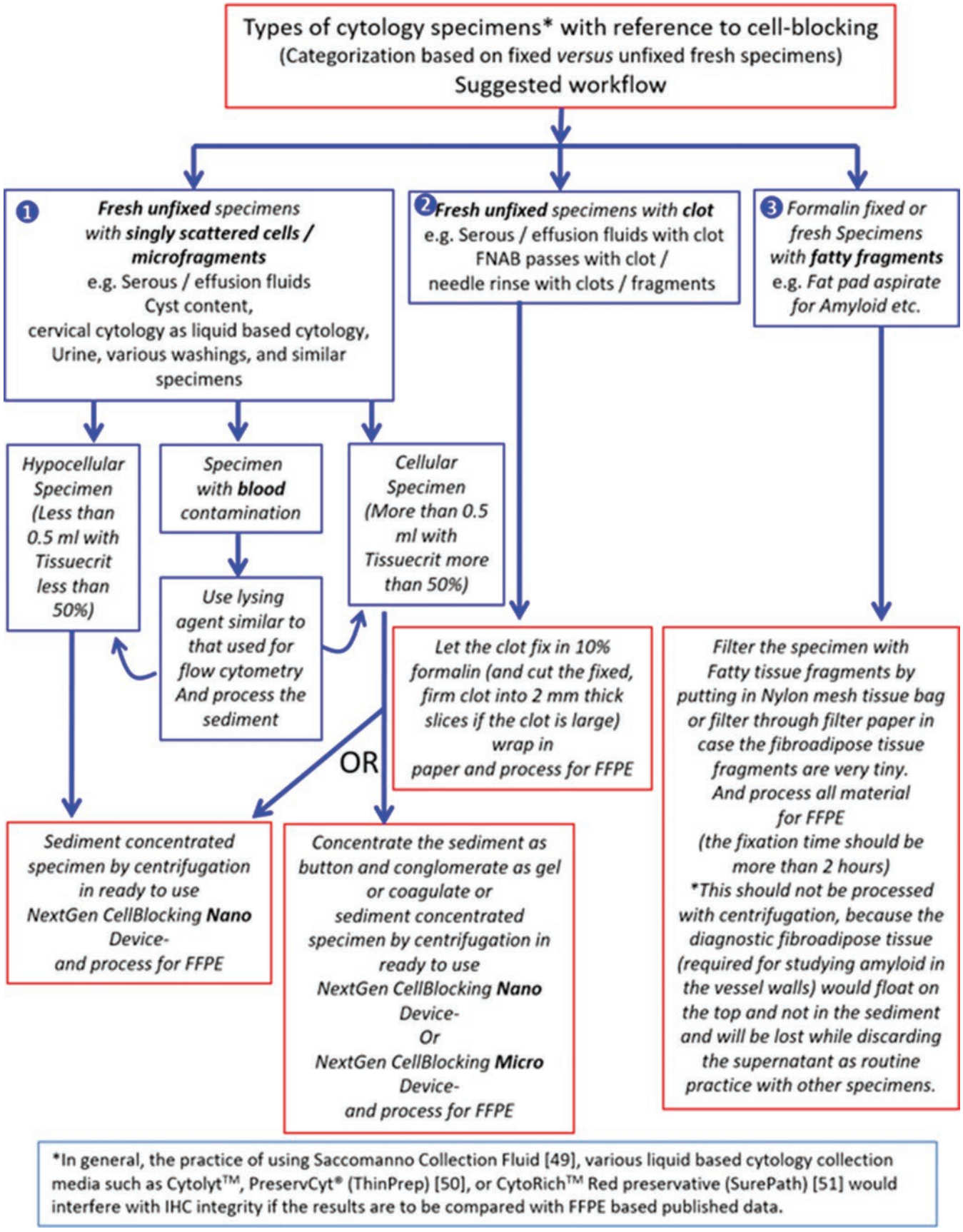


Figure 12: The specimens may be divided into various categories (reproduced from open access publication, ref #1).

qualitative and quantitative parameters of final cell-blocks. For the best outcome, the fixation and processing of cell-block should be comparable to FFPE of biopsy and surgical pathology specimens. Such a practice in universal fashion would result in reproducible results with interinstitutional comparability. FFPE of such cell-blocks would yield results of ancillary studies (especially IHC and molecular tests) comparable to those of FFPE of biopsy and surgical pathology specimens. This is highly significant due to the increasing number of tests with direct impact on selection of targeted therapy in the era of personalized medicine.

As a routine, cell-block is recommended to be made from the residual cytology specimens after making cytology preparations. Cell-blocks are critical for performing various elective ancillary studies including IHC, special stains for organisms or chemical components, and the molecular studies. Some of these elective studies may not be required immediately during the time of cytopathology reporting, but such tests may be indicated at later stage of the disease. In such situations, cell-block will be an excellent archival material which could be used any time at a later date.

The entire process starts with a proper specimen collection. The preferred approach is to collect it as fresh and unfixed specimen which permits the flexibility of using the recommended protocol matching that used for biopsy/surgical pathology specimens [Figure 12]. Shidham's method[33] addresses many of the quantitative concerns including guidance to histotechnologists for the selection of sections with diagnostic material with the help of AV marker. However, this method as in its original published protocol is heavily dependent on skill and labor. Due to this, it is difficult to be adopted as a routine by individual laboratories. Recently, a ready-to-use, low cost, alternative based on Shidham's method is available as NGCB™ kits which overcome the limitations with initial protocol. The cell-block making units in the kits include a built-in, precisely set, dark colored AV Marker to monitor the depth of cutting objectively by the histotechnologists microtoming the FFPE of cell-blocks.[33]

In addition to standardization for making quantitatively and qualitatively optimum cell-blocks, the final cytopathology report should document the critical SOCP information in the form of quality indicator component in the final cytology report [Table 6]. The introduction of this practice is critical to increase the impact of cell-blocks in patient care with enhanced reproducibility and comparability of results of important ancillary tests such as IHC performed on the cell-blocks.

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COMPETING INTERESTS STATEMENT BY ALL AUTHORS

VS (the first author) and LL (the second author) are the coeditors-in-chief of CytoJournal. They do not have any competing interests. The spouse of VS has stakes in AV BioInnovation LLC product(s) cited in this review.

LIST OF ABBREVIATIONS (In alphabetic order)

CK – Cytokeratin
CMAS – CytoJournal Monograph & Atlas Series
EUS-FNA – Endoscopic ultrasound-guided fine-needle aspiration
FFPE – Formalin-fixed paraffin embedded
FNA – Fine-needle aspiration
FNAB – Fine-needle aspiration biopsy
HA – Hydrated steam autoclaving
GI – Gastrointestinal
H&E – Hematoxylin and eosin
IHC – Immunohistochemistry
NET – Neuroendocrine tumor
NGCB – NextGen CelBloking™
S100 – S100 protein
S100P – Placental S100
SCIP – Subtractive coordinate immunoreactivity pattern
SOCP – Standard optimum cell-block protocol
TMA – Tissue microarray

REFERENCES

1. Shidham VB. CellBlockistry: Chemistry and art of cell-block making-a detailed review of various historical options with recent advances. Cytojournal 2019;16:12.
2. Shidham VB. CellBlockistry: Science of cell-block making as ancillary cytopathology component in the era of minimally invasive techniques with increasing role of molecular pathology (short communication). Clin Surg 2019;4:2510. Available from: http://www.clinicsinsurgery.com/pdfs_folder/cis-v4-id2510.pdf. [Last accessed on 2021 Jan 06].
3. CellBlockistry-IAC 2019 (May 6-Monday 16-1500 to-1800) Chemistry and Art of Cell-Block Making (Part I); 2019. Available from: https://www.slideshare.net/vshidham1/01-cellblockistry-iac-2019-may-6monday-16-1500-to-1800. [Last accessed on 2021 Jan 06].
4. Sethi S, Geng L, Shidham VB, Archuletta P, Bandyophadhyay S, Feng J, *et al*. Dual color multiplex TTF-1+Napsin A and p63+CK5 immunostaining for subcategorizing of poorly differentiated pulmonary non-small carcinomas into adenocarcinoma and squamous cell carcinoma in fine needle

aspiration specimens. Cytojournal 2012;9:10.
5. Mejías-Badillo L, Jeanty J, Khalid K, Bhalla A, Salem N, Thomas S, *et al*. Dual-color immunocytochemistry (Ki-67 with LCA) for precise grading of pancreatic neuroendocrine tumors with applicability to small biopsies and cell-blocks. Cytojournal 2020;17:6.
6. Nguyen TT, Lee JS, Shim H. Construction of rabbit immune antibody libraries.Methods Mol Biol 2018;1701:133-46.
7. Shidham VB, Atkinson BF, editors. Immunocytochemistry of effusion fluids: Introduction to the SCIP approach. In: Cytopathologic Diagnosis of Serous Fluids. 1st ed., Ch. 5. Amsterdam: Elsevier, WB Saunders Company; 2007. p. 55-78.
8. CellBlockistry-IAC 2019 (May 6-Monday 16-1500 to-1800) Chemistry and Art of Cell-Block Making (Part II); 2019. Available from: https://www.slideshare.net/vshidham1/02-cellblockistry-iac-2019-may-6monday-16-1500-to-1800. [Last accessed on 2021 Jan 06].
9. Fitzgibbons PL, Bradley LA, Fatheree LA, Alsabeh R, Fulton RS, Goldsmith JD, *et al*. Principles of analytic validation of immunohistochemical assays: Guideline from the college of american pathologists pathology and laboratory quality center. Arch Pathol Lab Med 2014;138:1432-43.
10. Thunnissen E. How to validate predictive immunohistochemistry testing in pathology? A practical approach exploiting the heterogeneity of programmed death ligand-1 present in non-small cell lung cancer. Arch Pathol Lab Med 2019;143:11-2.
11. Choi SY, Kim KH, Suh KS, Yeo MK. Diagnostic significance of dual immunocytochemical staining of p53/cytokeratin20 on liquid-based urine cytology to detect urothelial carcinoma. Cytojournal 2020;17:3.
12. Østbye KM, Pedersen MK, Sauer T. Immunocytochemical expression of Ki-67/p16 in normal, atypical, and neoplastic cells in urine cytology using BD SurePath™ as preparation method. Cytojournal 2019;16:26.
13. Shidham VB, Hunt B, Jardeh SS, Barboi AC, Devata S, Hari P. Performing and processing FNA of anterior fat pad for amyloid. J Vis Exp 2010;44:1747.
14. Monzon FA, Lyons-Weiler M, Buturovic LJ, Rigl, CT, Henner WD, Sciulli C, *et al*. Multicenter validation of a 1550-gene expression profile for identification of tumor tissue of origin. J Clin Oncol 2009;27:2503-8.
15. Noorsaeed A, Khan M, Shidham V. Efficiency and Effectivity of Using Dual-Color Immunostaining with Combination of BerEP4 and Vimentin as Compared to BerEP4 Alone on Serous Effusion. 68th Virtual Annual Scientific Meeting, American Society of Cytopathology; 2020.
16. Bahrami A, Truong LD, Ro JY. Undifferentiated tumor: True identity by immunohistochemistry. Arch Pathol Lab Med 2008;132:326-48.
17. Spizzo G, Fong D, Wurm M, Ensinger C, Obrist P, Hofer C, *et al*. EpCAM expression in primary tumour tissues and metastases: An immunohistochemical analysis. J Clin Pathol 2011;64:415-20.
18. Ordóñez NG. Value of the Ber-EP4 antibody in differentiating epithelial pleural mesothelioma from adenocarcinoma. The M.D. Anderson experience and a critical review of the literature. Am J Clin Pathol 1998;109:85-9.
19. Wang B, Li D, Ou X, Yi Q, Feng Y. Diagnostic accuracy of Ber-EP4 for metastatic adenocarcinoma in serous effusions: A meta-analysis. PLoS One 2014;9:e107741.
20. Azami S, Aoki Y, Ogura K, Kojima K, Matsumoto T. Application of returned cell-block method (cell-block from a papanicolaou staining smear on a glass slide) for the evaluation of fine needle aspiration cytology of tumors of the breast. Diagn Cytopathol 2016;44:505-11.
21. Processing of Single Specimen of Any Cellularity to Make a Cell-block with Nano Unit. Available from: https://youtu.be/y29SS1NwO_8. [Last accessed on 2021 Jan 06].
22. Pernick N. BerEP4/EpCAM; 2020. Available from: https://www.pathologyoutlines.com/topic/stainsepcam.html. [Last accessed on 2020 Nov 27].
23. Benderra MA, Ilie M, Hofman P, Massard C. Standard of care of carcinomas on cancer of unknown primary site in 2016. Bull Cancer 2016;103:697-705.
24. Hu L, Lau S, Tzang CH, Wen JM, Wang W, Xie D, *et al*. Association of Vimentin overexpression and hepatocellular carcinoma metastasis. Oncogene 2004;23:298-302.
25. Sanchez SR, Chang CC. Hematolymphoid disorders. In: Shidham VB, Atkinson BF, editors Cytopathologic Diagnosis of Serous Fluids. 1st ed., Ch. 12. Amsterdam: Elsevier, WB Saunders Company; 2007. p. 171-93.
26. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology 2002;40:403-39.
27. Varsegi GM, Shidham V. Cell-block preparation from cytology specimen with predominance of individually scattered cells. J Vis Exp 2009;29:1316.
28. IHC World-Life Science Products and Services, Positive Control Tissue List for Immunohistochemistry; 2020. Available from: http://www.ihcworld.com/_technical_tips/positive_control_tissue_list.htm; https://www.archive.is/ksw4T. [Last accessed on 2020 Apr 18].
29. Battifora H. The multitumor (sausage) tissue block: Novel method for immunohistochemical antibody testing. Lab Invest 1986;55:244-8.
30. Sharma SK, Deka L, Gupta R, Gupta S, Singh DK, Singh S. Tissue microarray construction from gross specimens: Development of a novel simple technique. J Clin Pathol 2010;63:782-5.
31. Packeisen J, Buerger H, Krech R, Boecker W. Tissue microarrays: A new approach for quality control in immunohistochemistry. J Clin Pathol 2002;55:613-5.
32. Tissue Microarray (From Wikipedia, the Free Encyclopedia). Available from: https://en.wikipedia.org/wiki/Tissue_microarray. [Last accessed on 2021 Jan 06].
33. AV BioInnovation, USA. Available from: https://www.avbioinnovation.com. [Last accessed on 2021 Jan 06].
33a. Shidham VB. Specimen-specific cell-blocking approaches. Cytojournal 2020;17:28.

34. Shidham VB, Kajdacsy-Balla AA. Immunohistochemistry: Diagnostic and prognostic applications. In: Detrick B, Hamilton RG, Folds JD, editors. Manual of Molecular and Clinical Laboratory Immunology. 7th ed., Ch. 47. Washington, DC: American Society of Microbiology Press; 2006. p. 408-13. Available from: https://www.tetondata.com/TitleInfo.cshtml?id=274. [Last accessed on 2021 Jan 06].
35. Shidham VB, Falzon M. Serous effusions: Reactive, benign and malignant. In Gray W, Kocjan G, editors. Diagnostic Cytopathology. 3rd ed., Ch. 3. Amsterdam: Elsevier; 2010.
36. Naylor B. Pleural, peritoneal, and pericardial fluids. In: Bibbo M, editor. Comprehensive Cytopathology. 2nd ed. Philadelphia, PA: WB Saunders; 1997. p. 551-621.
37. Shandon™ CytoRich™ Red Collection Fluid. Available from: https://www.thermofisher.com/order/catalog/product/b9990802#/b9990802. [Last accessed on 2021 Jan 06].
38. Selves J, Long-Mira E, Mathieu MC, Rochaix P, Ilié M. Immunohistochemistry for diagnosis of metastatic carcinomas of unknown primary site. Cancers (Basel) 2018;10:108.
39. Lin F, Liu H. Immunohistochemistry in undifferentiated neoplasm/tumor of uncertain origin. Arch Pathol Lab Med 2014;138:1583-610.
40. Rao RN. Mesothelioma. In: Shidham VB, Atkinson BF, editors. Cytopathologic Diagnosis of Serous Fluids. 1st ed., Ch. 8. Amsterdam: Elsevier, WB Saunders Company; 2007. p. 107-13.
41. Chang A, Amin A, Gabrielson E, Illei P, Roden RB, Sharma R, *et al*. Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. Am J Surg Pathol 2012;36:1472-6.
42. Chapel DB, Churg A, Santoni-Rugiu E, Tsujimura T, Hiroshima K, Husain AN. Molecular pathways and diagnosis in malignant mesothelioma: A review of the 14th international conference of the international mesothelioma interest group. Lung Cancer 2019;127:69-75.
43. Kandukuri SR, Lin F, Gui L, Gong Y, Fan F, Chen L, *et al*. Application of immunohistochemistry in undifferentiated neoplasms: A practical approach. Arch Pathol Lab Med 2017;141:1014-32.
44. Everbroeck BV, Pals P, Jean-Jacques M, Cras P. Antigen retrieval in prion protein immunohistochemistry. J Histochem Cytochem 1999;47:1465-70.
45. Varadhachary GR, Spector Y, Abbruzzese JL, Rosenwald S, Wang H, Aharonov R, *et al*. Prospective gene signature study using microRNA to identify the tissue of origin in patients with carcinoma of unknown primary. Clin Cancer Res 2011;17:4063-70.
46. Varadhachary GR, Raber MN. Cancer of unknown primary site. N Engl J Med 2014;371:757-65.
47. Barker EV, Cervigne NK, Reis PP, Goswami RS, Xu W, Weinreb I, *et al*. microRNA evaluation of unknown primary lesions in the head and neck. Mol Cancer 2009;8:127.
48. Hainsworth JD, Rubin MS, Spigel DR, Boccia RV, Raby S, Quinn R, *et al*. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: A prospective trial of the Sarah cannon research institute. J Clin Oncol 2013;31:217-23.
49. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: A survey of 435 cases. Mod Pathol 2000;13:962-72.
50. Kandalaft PL, Gown AM. Practical applications in immunohistochemistry: Carcinomas of unknown primary site. Arch Pathol Lab Med 2016;140:508-23.
51. Ross JS, Wang K, Gay L, Otto GA, White E, Iwanik K, *et al*. Comprehensive genomic profiling of carcinoma of unknown primary site: New routes to targeted therapies. JAMA Oncol 2015;1:40-9.
52. Greco FA. Cancer of unknown primary site: Evolving understanding and management of patients. Clin Adv Hematol Oncol 2012;10:518-24.
53. Conner JR, Hornick JL. Metastatic carcinoma of unknown primary: Diagnostic approach using immunohistochemistry. Adv Anat Pathol 2015;22:149-67.
54. Stelow EB, Yaziji H. Immunohistochemistry, carcinomas of unknown primary, and incidence rates. Semin Diagn Pathol 2018;35:143-52.
55. CancerTYPE ID®. San Diego, CA, USA: bioTheranostics, Inc. Available from: https://www.cancertypeid.com/hcp-the-technology. [Last accessed on 2021 Jan 06].
56. Moran S, Martinez-Cardus A, Sayols S, Musulen E, Balana C, Estival-Gonzalez A, *et al*. Epigenetic profiling to classify cancer of unknown primary: A multicentre, retrospective analysis. Lancet Oncol 2016;17:1386-95.
57. Genomic Testing, FoundationOne®. Cambridge, MA, USA: Foundation Medicine. Available from: https://www.foundationmedicine.com/genomic-testing. [Last accessed on 2021 Jan 06].
58. Weiss LM, Chu P, Schroeder BE, Singh V, Zhang Y, Erlander MG, *et al*. Blinded comparator study of immunohistochemical analysis versus 92-gene cancer classifier in the diagnosis of the primary site in metastatic tumors. J Mol Diagn 2013;15:263-9.
59. Hainsworth JD, Greco FA. Gene expression profiling in patients with carcinoma of unknown primary site: From translational research to standard of care. Virchows Arch 2014:464:393-402.
60. Oien KA, Dennis JL. Diagnostic work-up of carcinoma of unknown primary: From immunohistochemistry to molecular profiling. Ann Oncol 2012;23 Suppl 10:x271-7.
61. Ordonez, N.G. Broad-spectrum immunohistochemical epithelial markers: A review. Hum Pathol 2013;44:1195-215.
62. Thomson TA. Immunohistochemistry on Cell-block Specimens Effects of Fixation and Time (PowerPoint Presentation of a Study). British Columbia, Canada: BC Cancer Agency Clinical Laboratory Vancouver; 2014. Available from: http://www.cpqa.ca/main/wp-content/uploads/2014/06/2014-thomson.pdf. [Last accessed on 2020 Apr 20].
63. Lloyd IE, Wenhua Z, Witt BL, Chadwick BE. Characterization of PD-L1 immunohistochemical expression in cell-blocks with different specimen fixation and processing methods. Appl Immunohistochem Mol Morphol 2019;27:107-13.
64. Mathur A, Sharma A, Sharma M, Maurya A, Yadav A, Sethi N. Immunocytochemistry on scrape cellblock: An aid in the diagnosis of metastatic neoplasm with unknown primary: A series of four cases. Cytojournal 2020;17:9.
65. Satturwar S, Malenie R, Sutton A, Dai D, Aly FZ. Validation

of immunohistochemical tests performed on cytology cell-block material: Practical application of the college of American pathologists' guidelines. Cytojournal 2019;16:6.
66. Xu H, Bratton L, Nead M, Russell D, Zhou Z. Comparison of programmed death-ligand 1 (PD-L1) immunostain for nonsmall cell lung carcinoma between paired cytological and surgical specimens. Cytojournal 2018;15:29.
67. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. J Histochem Cytochem 1985;33:845-53.
68. Fowler CB, Cunningham RE, O'Leary TJ, Mason JT. Tissue surrogates as a model for archival formalin-fixed paraffin-embedded tissues. Lab Invest 2007;87:836-46.
69. ThinPrep Non-Gyn. Available from: http://www.thinprep.com/hcp/lab_professionals/thinprep_non_gyn.html. [Last accessed on 2021 Jan 06].
70. Gruchy JR, Barnes PJ, Haché KA. CytoLyt® fixation and decalcification pretreatments alter antigenicity in normal tissues compared with standard formalin fixation. Appl Immunohistochem Mol Morphol 2015;23:297-302.
71. Sauter JL, Grogg KL, Vrana JA, Law ME, Halvorson JL, Henry MR. Young investigator challenge: Validation and optimization of immunohistochemistry protocols for use on cellient cell-block specimens. Cancer Cytopathol 2016;124:89-100.
72. National Mesothelioma Claims. Available from: https://www.nationalmesotheliomaclaims.com. [Last accessed on 2021 Jan 06].
73. Hanna W, O'Malley FP, Barnes P, Berendt R, Gaboury L, Magliocco A, *et al*. Updated recommendations from the Canadian national consensus meeting on HER2/neu testing in breast cancer. Curr Oncol 2007;14:149-53.
74. Lewis JS Jr., Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, *et al*. p16 positive oropharyngeal squamous cell carcinoma:an entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol 2010;34:1088-96.
75. Spinelli M, Khorshad J, Viola P. When tumor doesn't read textbook. Third case of TTF1 and p40 co-expression in the same tumour cells in a non-small cell carcinoma. A potential new entity to consider? Pathologica 2019;111:58-61.
76. Duffy MJ, Synnott NC, Crown J. Mutant p53 as a target for cancer treatment. Eur J Cancer 2017;83:258-65.
77. Sheri A, Dowsett M. Developments in Ki67 and other biomarkers for treatment decision making in breast cancer. Ann Oncol 2012;23 Suppl 10:x219-27.
78. Hunter KA, Socinski MA, Villaruz LC. PD-L1 testing in guiding patient selection for PD-1/PD-L1 inhibitor therapy in lung cancer. Mol Diagn Ther 2018;22:1-10.
79. Shidham VB, Layfield LJ, Pantanowitz L. Webinar on Science of CellBlockistry (CBK-20). Available from: https://www.vimeo.com/showcase/7492760. [Last accessed on 2020 Jul 11].

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