INDIAN ACADEMY OF CYTOLOGISTS
GUIDELINES FOR COLLECTION, PREPARATION, INTERPRETATION AND REPORTING OF SEROUS EFFUSION FLUID SAMPLES

Introduction

Serous effusion indicates accumulation of excess fluid in the body cavities namely, pleural, pericardial and peritoneal, the latter also referred to as ascites. Effusion invariably indicates an underlying pathology and constitutes an important diagnostic sample in clinical practice, including oncology. Fluid specimen from various body cavities can be evaluated by cytopathological examination.

The techniques for collection, transportation and preparation of fluid specimen are of utmost importance, as an adequate, well prepared, well stained smear helps in the ultimate goal of an accurate cytopathological diagnosis.

Methods of collection and processing of effusion specimen for cytological diagnosis vary from laboratory to laboratory. In order to achieve uniformity in this technique across our country, the Indian Academy of Cytologists (IAC) has developed these guidelines in consultation with experts across the country for implementation as a standard format in our country for all laboratories providing cytopathology services.

The guidelines are broadly divided as follows:

i. **ESSENTIAL** – implying that these are absolute and non-negotiable recommendations and if a laboratory cannot achieve this, then the sample may be referred to a center which is equipped to fulfill these criteria.

ii. **OPTIMAL** – implying that this is achievable in any good laboratory and should be part of the protocol and can be made mandatory for accreditation.

iii. **OPTIONAL** – implying that this recommendation is resource-dependent and is left to the choice of the individual laboratory.

Aim: - It is our aim to develop guidelines in order to achieve appropriate practice across all laboratories offering cytopathology services, for delivery of an accurate diagnosis in order to achieve good clinical practice in the interest of patient management. It is also envisaged that there is a general uniformity in the recommendations made by the laboratories in the same situation.

1. SAMPLE COLLECTION AND TRANSPORTATION

1.1 Requisition form

ESSENTIAL

Clinical Details

The sample should accompany a requisition form with the following necessary details:

- Name, age, gender, unique Hospital identification (i.d) number.
- Clinical diagnosis, including purpose
- Date and time of collection
- Date and time of receiving sample in the laboratory
- Temperature conditions in case the sample is stored – Refrigerated or Room temperature
- Clinical symptoms
- Imaging finding
- Endoscopic findings, if any
- Previous diagnoses and pertinent treatment history
- Anticoagulant used or not, including type.

OPTIONAL

- Tobacco & alcohol habits
- Occupational history

Note on Effusion Sample Collection Guidelines

Fluid collection is performed by the treating clinicians. The procedure consists of inserting a wide bore needle (under local anesthesia) through the body wall into the fluid containing cavity. Pleural fluid is removed by thoracocentesis, peritoneal fluid by paracentesis and pericardial fluid by pericardocentesis.

Peritoneal washing samples may be obtained by instilling normal saline solution into the various recesses of peritoneal cavity and then withdrawing the fluid. This is done in patients undergoing abdomino-pelvic surgical exploration to detect peritoneal dissemination of cancer cells.

1.2 Collection of Fluid sample

Types of Samples

The various samples include pleural fluid, peritoneal / ascitic fluid, pericardial fluid, peritoneal washing and rarely pleural washing.

ESSENTIAL

The fluid is collected in a **Sterile / Non-sterile clean dry container with proper labeling and identification which includes Name of the patient and hospital i.d**

The laboratory has a right to reject any improperly labeled samples and those in dirty, cracked, broken containers without proper lid.

The laboratory should have a rejection policy for any improperly labeled samples and those in dirty, cracked, broken containers without proper lid

1.3 Recommended volume for Cytological evaluation:

At least 20-30mL is OPTIMAL; There is no minimum or maximum limit.

If cell block preparation is required, then at least 30-50mL is optimal; Low celluarity specimen would need larger volume of fluid.

1.4 Transportation

ESSENTIAL

Fluid sample must be transported to the laboratory as soon as possible as freshly tapped samples are preferred for cytological examination. Up to 2 hours transportation time is achievable in most centers.

If for unavoidable reason processing cannot be done immediately or within 2 hours, the fluid should be refrigerated at $4^{\circ}C$ and transported on ice / cool box, to arrest degeneration of cells. Routine cytological evaluation is possible up to 48 hours under these conditions but cell morphology gets compromised after 24 hours.

1.5 Anticoagulation – OPTIMAL

In samples with high protein content such as exudates and those which are blood-tinged or hemorrhagic, addition of an anticoagulant prevents clotting of sample and can provide accurate cell counts if required and also achieve optimal smear quality.

Choice of anticoagulants is as follows:

i. Heparin – easily available in OPDs /wards – 3units /mL (can be titrated depending on strength of heparin). Usually the syringe used for paracentesis or collection container is rinsed in heparin.
ii. Citrate
iii. Ethylenediaminetetraacetic acid (EDTA)
iv. Ammonium oxalate-1% to be added in a ratio of 9 parts fluid to 1 part anticoagulant. This is a cheap option.

Important Note: No fixative should be added to the fluid[1]. Formalin prevents cells from adhering to the slide and interferes with the quality of Pap staining. Alcohol should not be added as it causes precipitation of proteins and thus interferes with the adherence of the cells to slide [1, 2].

2. CYTOPREPARATION OF EFFUSION SAMPLES

2.1 Receipt of sample in Cytopathology Laboratory

ESSENTIAL: Upon receipt of the sample, the laboratory generates its identification number as per individual laboratory / hospital policy and the sample is accepted for processing after checking of patient identifiers on the request form and on the sample label.

OPTIMAL: Barcoding of sample containers is common practice.

2.2 Gross Evaluation

ESSENTIAL

Gross or macroscopic evaluation must be performed by the laboratory technician / cytotechnician and the following points are to be noted:

i. Quantity- ….mL / L
ii. Color – Clear / straw colored / yellow, / brown / red / chylous / purulent / hemorrhagic / other (please specify)
iii. Consistency: Serous/ Mucoid / Gelatinous / thick copious / tar-like / other
iv. Temperature of sample: Room temperature / refrigerated
v. Any evidence of clotting: Yes/No

2.3 **Equipment & Reagents**

Essential:

- Gloves and masks
- Laboratory Centrifuge
- Centrifuge tubes: Disposable, transparent plastic screw capped
- Glass marking colored pencil
- Applicator sticks / Pasteur pipette with rubber teat
- Clean glass slides -….x….x….mm
- Cover slips
- A wide mouth specimen bottle with fixative
- Normal saline
- Glass marking diamond pencil
- Mayer's egg albumin

OPTIMAL:

Biosafety cabinets
Cytocentrifuge machine, funnels, clips and blotting cards
Applicator sticks with thin tightly wound cotton

2.4 Processing

Essential

Technician handling fluid specimen should wear adequate personal protective gear.
Centrifuge all samples with a desktop laboratory centrifuge.
In case fibrin clot is formed, it should be thoroughly smashed with an applicator stick. In case a large clot remains, it may be processed as cell-block.
A representative volume of the fluid (10-15 ml) should be centrifuged using capped plastic tubes, 2000 rpm for 10 minutes.
Subsequently, most of the supernatant should be gently decanted / removed. Care should be taken not to disrupt the intact sediment while decanting, as it may require repeat centrifugation. From the sediment, smears are made.

- A small volume of the sediment using an applicator / disposable Pasteur pipette should be placed onto a pre-labeled albuminized slide. Thereafter, the sediment should be spread gently using the flat surface of another glass slide or optimally by rolling the cotton tipped applicator stick gently in a rotatory motion on the slide, so as to make a thin evenly spread smear. Note that the cotton tipped applicator should be moistened by the last drop of fluid at the time of decantation before picking up the sediment for good preparation.

- Minimum two smears should be prepared from each sample – one is air-dried and another is wet-fixed / fixed immediately in a coplin jar / jar containing fixative.
- Ideal fixative: 95% ethanol
- Stains:
    - Single Romanowsky stain – Giemsa / May-Grunwald Giemsa can be performed on the air-dried smear and
    - Papanicolaou stain performed on the wet-fixed smears

Optimal
1 air-dried smear and 1-2 unstained alcohol fixed slides may be prepared at the outset (to attempt immunocytochemistry in case) in low volume samples where a cell block might not be possible.

2.4.1 Processing of hemorrhagic samples
There are several methods of processing hemorrhagic samples. The laboratory may choose from any technique that is well-standardized locally.
2.4.1.1 Pre-smearing techniques

- About 10-15 ml sample is taken in a plastic conical centrifugation tube. one percent acetic acid (1ml) is added for 10 mins. The sample is centrifuged at 2000 rpm for 5 mins. Supernatant is decanted. The sediment cells are washed twice with PBS. Three or more smears may be prepared from washed cells as above.

2.4.1.2 Post-smearing techniques

- In haemorrhagic samples, an extra smear for haemolysis is prepared.

Haemolysis

Haemolysis can be achieved by any one of the following methods:

- Carnoy's fixative
- Glacial acetic acid
- Saline re-hydration
- Saponin

2.4.1.2.1 Glacial acetic Acid

15ml sample + 1ml of 1% Glacial acetic acid. Keep at Room temp for 5-10 minutes. Centrifuge, wash the deposit with PBS, centrifuge and make smear with deposit.

2.4.1.2.2 Saline Re-hydration Technique

This technique is employed in order to lyse RBCs in haemorrhagic fluids. It improves the yield of diagnostic cells thus increases the accuracy and also reduces the eyestrain while screening.

This technique is simple and cost-effective, compared to other techniques, such as Carnoy's fixative, glacial acetic acid, saponin etc. Normal saline is readily available and needs no preparation and has an unlimited shelf life. However, the method causes blotting artifacts which may be observed more in non-epithelial cells.

The underlying principle of this technique is that the physical damage caused by air-drying to RBCs is more than that caused to the epithelial cells (other cells) and the following rehydration leads to the rupture of the RBCs and simultaneous retention of the epithelial cells (other cells).

Method

- An extra smear from the remaining sediment is prepared.
- The smear is dried in an incubator at 37 $^{\circ}$C or at room temperature for 5 minutes.
- The smear should be kept in a horizontal position (Preferably on a staining bar).
- The smear should be rehydrated with normal saline for 30 seconds.
- Saline is carefully and gently poured through one side of the smear to prevent the washing off.
- The saline is blotted by holding the slides vertically.
- The smears are fixed immediately.

Important factors for optimal results

- Time duration for drying of the smear should not exceed the optimal time (5 minutes).
- The smears should not be re-hydrated for more than optimal time (30 seconds) to avoid blurring of cellular features.
- The time lag between complete drying and re-hydration of smears should be less than 10 minutes.

2.4.2 Fluid with No/Sparse Sediment

After centrifugation, if no sediment or very scant sediment is obtained, the fluid has to be further processed by cytocentrifugation.

Optional:

2.4.3 Liquid-based cytology(LBC) preparation: Following centrifugation and preparation of 1 air-dried smear from the sediment, the remaining sediment is fixed in the manufacturer recommended fixative (methanol based for Thin-Prep and ethanol based for SurePath). Minimum 1 LBC smear is prepared as per manufacturer's protocol, stained with Papanicolaou stain.

2.5 STORAGE OF SAMPLE

ESSENTIAL

After processing, the remaining sample should be stored in refrigerator at 2-8□ till the final report is generated. This stored sample is useful for making repeat smear if the cellularity is inadequate, for making cell block or ancillary techniques if required. After the reporting, the stored sample should be discarded as per the bio-waste management policy.

3. CELL BLOCK

OPTIONAL – Highly Desirable

Cell block preparation is a technique (in addition to smears) of cell concentration without compromising the cellular content and preserving the tissue architecture

3.1 Preparation of Cell Block from the Effusion Sediment is desirable and is being frequently and increasingly requested by the clinician.

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

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

3.2 Methods for preparing Cell block

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

3.2.1 Direct Sedimentation

This method is best used when a large quantity (> 1 ml) of sediment is obtained.

Equipment & Reagents

1. Fixative: 10% buffered formalin
   Formaldehyde solution (40%) 100 ml
   Tap water 900 ml
2. Whatman's No.1 filter paper / lens paper
3. Tissue cassette
4. Applicator sticks
5. Screw capped disposable plastic transparent centrifuge tube.

- **Procedure**
  - The sample should be centrifuged.
  - The supernatant is discarded.
  - An equal volume of fixative is added to the sediment and these are thoroughly mixed.
  - The tube IS CAPPED and kept overnight in refrigerator.
  - The fixed sediment is poured onto filter paper/lens paper
  - The sediment is wrapped securely in the filter paper and placed it into a labeled tissue cassette.
  - Subsequently, the cassette is kept in a jar containing fixative.
  - Finally, this is processed similar to a tissue specimen

3.2.2 Agar Embedding

This method is cost effective and useful when a reasonably good quantity of the sediment is obtained (0.5-1 ml).

Equipment & Reagents

1. 4% Agar solution
   Dissolve 4 gm of bacterial agar in 100 ml of boiling water.
   Stock should be stored in 10 ml aliquots in screw capped test tubes in the refrigerator. Shelf life is best for 2 months.
2. 10% buffered formalin
3. Whatman's No.1 filter paper / lens paper
4. Tissue cassette
5. Applicator sticks
6. Screw capped disposable plastic transparent centrifuge tube.
6. Plastic lid of injection vials to use as mould
7. Pasteur pipette
8. Scalpel
9. Water bath

Procedure

- The sample is centrifuged and the supernatant is discarded.
- The agar is melted in a water bath.
- With the help of a Pasteur pipette, the molten agar is dropped into the vial lid.

- A small depression is made in the centre of the semisolid agar.
- With the tip of a Pasteur pipette, the sediment is carefully taken out little by little and deposited into the depression. Care should be taken not to lose the sediment into the lumen of the pipette.
- The sediment deposit is covered and filled with few more drops of molten agar.
- The agar is allowed to completely solidify.
- With a scalpel, slowly the agar is taken out and mounted (with intact sediment inside) from the vial lid. Care should be taken not to break the pellet while removing.
- The pellet is wrapped securely in the filter paper (pre-moistened with formalin fixative) and placed in a labeled tissue cassette.
- The cassette is kept in a jar containing fixative (at least for 4 hours) and this material is further processed as a tissue specimen

3.2.3 A. Plasma –Thrombin Clot Method
B. Plasma-Thromboplastin Clot Method [2]

This method is applicable for any quantity of sediment and is even more useful when the quantity of the sediment obtained (<0.5 ml) is scant.

A and B. Equipment & Reagents

1. 10% buffered formalin
2. Whatman's No.1 filter paper / lens paper
3. Tissue cassette
4. Applicator sticks (optional)
5. Screw capped disposable plastic transparent centrifuge tube.
6. Pasteur pipette
7. Thrombin / Thromboplastin
8. Pooled plasma

A. Procedure – Suitable when effusion sample is received with anticoagulant

- Sample is centrifuged and supernatant discarded.
- To the cell sediment, 5ml of 10% buffered formalin is added and fixed at room temperature for at least 6 hrs. Overnight fixation may be done.
- Sample is centrifuged and supernatant is discarded.
- Two washes with Phosphate buffered saline are given and supernatant removed as much as possible to leave only cell sediment behind.
- Two drops (100µl) of pooled plasma is added to the sediment and mixed well.
- Single drop (50µl) of thrombin / thromboplastin is added and mixed well again.
- The tube is kept in standing position for five minutes to form the clot.
- The clot in the test tube is slid onto the pre-moistened filter paper and placed in a labeled tissue cassette.

- This is processed further as a small biopsy in the tissue processor.

B. Procedure – Suitable when effusion sample is received without anticoagulant

- Sample is centrifuged and supernatant discarded.
- Two drops (100µl) of pooled plasma is added to the sediment and mixed well.
- drops (50µl) of thromboplastin is added and mixed well again.
- The tube is allowed to stand for five minutes to form the clot.
- The clot in the test tube is slid onto the filter paper pre-moistened with formalin fixative.
- The sediment is wrapped securely in the filter paper (pre-moistened with formalin fixative) and place it into a labeled tissue cassette.
- The cassette is placed in a jar containing neutral buffered formalin (fixative) for at least for 4 hours or left overnight.
- This is processed further as a small biopsy in the tissue processor.

Technical Consideration

1. Pooled plasma can be preserved in a freezer up to 1 month in 1 ml aliquots. Plasma may be extracted from blood collected in EDTA vial.
2. Both thrombin and thromboplastin can be used interchangeadbly with 'A' and 'B' procedures with similar results.
3. Store Thrombin / thromboplastin in the refrigerator at 2 - 8^0 C.
4. Reagents should be brought to the room temperature before processing.
5. Care should be taken to use different pipettes to take out plasma and thrombin / thromboplastin.
6. If clot is not formed within 5 minutes, extra PBS washings are given and procedure is repeated using fresh plasma.
7. Check the expiry date of thrombin/thromboplastin vial before use.

Optional

3.2.4 Automated Cell Block Systems

Various methods for preparing cell blocks (CB) have been reported and the techniques are in a state of continuous improvement. Different methods include usage of various adjuvants such as agar, thrombin, gelatine, and egg albumin. The ideal method should be simple, faster, reproducible and able to concentrate cells in a limited field without loss of cellular material and is cost effective.

All traditional methods of cell block require overnight formalin fixation and processing and subsequent manual embedding similar to histological techniques. This would cause the delay in diagnosis.

Shandon Cytoblock (Thermo) and Cellient Automated Cell Block System (Hologic) are the two automated cellblock preparation system available. For these systems, manufacturer's recommendations should be followed.

4. Reporting of Effusion Cytology –

4.1 ESSENTIAL components of report

Volume:

Appearance:

Specimen Cellularity: Low / Moderate / High

Description (Optional)

Background: Clear / Proteinaceous / Granular / Hemorrhagic

Interpretation: After evaluation of the smears prepared, the case may be placed in any of the categories

Report Category	Cytopathology Diagnosis	Remarks
1	**Unsatisfactory for evaluation**	No cells seen / Obscuration by blood
2 A **B**	**No Malignant Cells Detected** **Benign Changes seen** i. Reactive mesothelial cells ii. Inflammatory cells seen iii. Lymphocyte-rich effusion iv. Specific infections Microfilaria, Tuberculosis, fungal infection, Hydatid cyst , any other	Correlate clinically and with imaging studies, microbiological studies
3	**Atypical cells, Not Otherwise Specified**	Repeat Cytology and collect sample after rolling the patient in bed. Correlate clinically and with imaging studies
4	**Atypical cells, Suspicious for malignancy**	Repeat Cytology evaluation Ancillary techniques-Optimal
5	**Malignant cells seen**	Subtype the malignancy wherever possible on cytomorphology and ancillary techniques of immunocytochemistry

Explanatory Note for Categories

Category 1: Unsatisfactory for evaluation: Smears with no cells for evaluation or those which shown contamination by artifacts, bacterial colony or those which show cells that are poorly preserved and show cellular degenerative changes and therefore not suitable for interpretation.

Category 2: No malignant cells Detected / Benign Cellular changes

This category represents a wide spectrum of cases of effusions in the absence of cancer. Hence, besides reporting that there are No malignant cells, the report can also describe the cellular changes which can include presence of mesothelial cells and inflammatory cells in variable numbers. The specific diagnoses in this category include i. Reactive mesothelial proliferation. ii. Acute inflammation. iii. Chronic inflammation. iv. Lymphocytic effusion. v. Specific infections with organism identified such as cocci, bacilli, M.tuberculosis, Nocardia, fungus, parasites such as microfilaria, hydatid cyst, or any other infectious agent.

Category 3: Atypical cells, Not Otherwise Specified

This category represents smears that show cells with cytological atypia that quantitatively or qualitatively do not favour malignancy. The cytopathologists may favour benign cellular changes mostly reactive mesothelial hyperplasia or may leave it not otherwise specified.

Category 4: Atypical cells, Suspicious for malignancy

This category represents smears that show cells with cytological atypia that quantitatively or qualitatively fall short of a diagnosis of frank malignancy but are enough to warrant suspicion of malignancy. Ancillary techniques of immunocytochemistry are useful in this setting and are recommended.

Category 5: Malignant cells seen

This category represents smears that show unequivocal malignant cells. Further categorization into carcinoma and non-carcinomatous malignancy is warranted. The different types of malignancy and the optimal use of immunocytochemistry is detailed below.

4.2 Immunocytochemistry (ICC): -OPTIMAL

ICC may be performed on Cell blocks or direct smear preparations or on LBC smears

For smear preparations – Fixatives may be 95% ethanol, formalin-alcohol, acetone-alcohol

4.2.1 Adenocarcinoma vs Reactive mesothelial cells:

Any 2 mesothelial markers and any 2 markers for adenocarcinoma may be chosen from the following list:

(a)Mesothelial markers: Calretinin, CK5/6, desmin, D2-40

(b) Adenocarcinoma markers: MOC31, EMA / BerEP4

4.2.2 Typing of Malignant tumours:

(a) Mesothelioma: Diagnosis of mesothelioma requires integration of clinico-radiological features with cytopathological findings, including immunostaining results.

Mesothelial markers: Calretinin / WT1/D2-40 (positivity for any 2 markers is desirable)
Strong diffuse EMA positivity is also a feature of mesothelioma

(a) Non-Mesothelial neoplasms: Carcinomas and other malignant neoplasms - See Table in Annexure

(b) Reactive Mesothelial proliferation and distinction from adenocarcinoma
BerEP4 and MOC31 (EMA) positivity favours adenocarcinoma; Calretinin / Desmin positivity favours reactive mesothelial proliferation

4.2.4 Lung Cancer Specimen - Adenocarcinoma Lung: **OPTIONAL**

Malignant pleural effusion sample is a commonly received sample in advanced stage lung adenocarcinoma and after confirming the lung primary, the cytopathologists are expected to do other ancillary tests, which are required for treatment decision making.

4.2.4.1 Samples: Cell sediment from the effusion; Residual sediment after LBC preparation; Direct scrape from an air-dried / H&E / Papanicolaou stained cellular representative smear / Cell blocks are all suitable for molecular testing

Immunohistochemistry – Cell blocks are preferred. If unavailable, smears may be used but only in laboratories that have the method standardized on smears.

Tests done: EGFR mutation testing; testing for ALK and ROS 1 rearrangements and PDL1 testing

4.2.4.2 For molecular testing – Samples that are representative of the tumour and with adequate cellularity may be shipped to the molecular laboratory for further testing.

DNA extraction may be carried out from any of the samples from a commercially available DNA extraction kit.

4.2.4.2.1 *EGFR* mutation testing:

Testing for known *EGFR* mutations in Exon 18, 19, 20 and 21 can be done by conventional polymerase chain reaction (PCR) or real-time PCR using commercially available kits.

4.2.4.3 Testing for ***ALK* rearrangements**: ICC on cell-block can be done for ALK testing using D5F3 antibody-based Ventana automated immunohistochemical assay.

4.2.4.4 Testing for ***ROS 1* rearrangements**: ICC on cell-block can be done for ROS 1 testing using clone D4D6 (Cell Signaling Technology, Danvers, MA).

4.2.4.5 Testing for PD-L1 testing: ICC on cell-block can be done for PDL1 testing using SP263 clone (Ventana), 28-8 (Dako) or 22C3 (Dako) clone.

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Annexures

Reference Table for Immunocytochemical markers useful in Effusion Cytology

Diagnosis	Calret	D2-40	WT-1	TTF-1	CK 7	CK20	PAX 8	SYN/CHR	MOC-31 EMA	GATA 3	P63/p40
Mesothelioma	+	+	+	-	-	-	-	-	-	-	-
Small cell carcinoma	-	-	-	-	-	-	-	+	-	-	-
Squamous cell carcinoma	-	-	-	-	+/-	-	-	-	-	-	+
Adenocarcinoma, lung origin	-	-	-	+	+	-	-	-	+	-	-
Adenocarcinoma, ovary origin	-	-	-	-	+	-	+	-	+	-	-
Adenocarcinoma, breast origin	-	-	-	-	+	-	-		+	+	-
Adenocarcinoma, stomach origin	-	-	-	-	+	-	-	-	+	-	-
Adenocarcinoma, colorectal origin	-	-	-	-	-	+	-	-	+	-	-
Germ cell tumours	**SALL4** +	Negative for all mesothelial and epithelial markers									
Lymphoma	**CD45+**	Negative for all mesothelial and epithelial markers									

Note: ICC marker panel must be chosen based on the cytomorphological diagnosis or differential diagnosis in the clinical context. Any 2 expected positive and expected negative markers may be chosen based on the differential diagnosis

Additional markers:

p53 – useful for confirming mesothelioma and High grade serous carcinoma of ovarian origin.