Cytology

DIAGNOSTIC CYTOLOGY

Preservation and processing of Smears

There are two fundamental methods of processing smears obtained by FNA. Smears are prepared and fixed according to the requirements of the stain to be used.

1. Air-drying followed by hematological stains like May – Grunwald –Giemsa (MGG), Diff Quik, Giemsa etc.: In this method, smears are intentionally air dried, but if smears are not correctly made and dried quickly artifacts will result. One advantage is the speed with which smears can be stained especially with use of rapid stains like Diff Quik (2-3 minutes). Rapid stains are particularly useful in preliminary assessment of adequacy of the sample before the patient is released. Colloid, mucin, endocrine cytoplasmic granules etc are better brought out in air-dried preparations. It is also useful in patients with hematological malignancies like lymphoma or leukemia.

FIXATION & STAINING

- 2 fundamentally different methods of fixation & staining are used in FNAC:

1. **Air drying** followed by staining with a haematological stain such as MAY GRUNWALD-GIEMSA STAIN , Jenner-Giesma, Diff-Quik
2. **Alcohol fixation** and staining according to PAP or with H&E.

Papanicolaou stain

Contents:

- Harris's hematoxylin
- Orange G6
- EA 50

Results:

- Nuclei- blue/black
- Cytoplasm (non-keratinizing)- blue/green
- Keratinizing cells- pink/orange

Romanowsky stain

Contents:

- Methylene blue/azure B and eosin, dissoved in acetone-free methanol, include jenner, Giesma, May Grunwald and Leishman stain

Results:

- Nuclei- purple/blue
- Cytoplasm- pink/blue
- Eosinophils- pink/red

Romanowsky dye: (a) unstained slides because of contact with the formalin vapours;
(b) the same sample not exposed to formalin fumes

- **Diff-Quik** is a commercial Romanowsky stain variant, commonly used in histological staining to rapidly stain and differentiate a variety of smears, commonly blood and non-gynecological smears, including those of fine needle aspirates.

MAY GRUNWALD- GIEMSA STAIN

- commonly used staining of blood smears

Contents:

- methylene blue (a basic dye)
- Azures (also basic dyes)
- Eosin (an acid dye)

Results:

- Nuclei of white blood cells and the granules of basophil granulocytes- blue
- Red blood cells and eosinophil granules – red
- cytoplasm of white blood cells - light blue

SPECIAL STAINS

1. PAS or Alcian blue - mucins, glycogen
2. Prussian blue - iron
3. Masson-Fontana - melanin
4. Congo red - amyloid
5. Ziehl-neelson - acid fast bacilli
6. Bile pigment- Fauchet's reagent counterstained with sirus red.
7. Gram, PAS or Gomori's silver stain for microorganism

Periodic acid–Schiff staining: fungal bodies stained magenta

Ziehl–Neelsen staining: mycobacteria are detected as bright red bacilli in the cytoplasm of macrophages

Preservation and processing of Smears

2. Alcohol fixation followed by Papanicolaou (pap) or hematoxylin and eosin (H&E) staining: Rapid fixation in alcohol (wet fixation) is essential for pap staining, which brings out nuclear details clearly, allowing better identification of malignant cells. It also allows better comparison with histology and hence is favored by majority of pathologists. But if the smears are not quickly made and fixed, drying artifact can occur in which case, the cytoplasm takes up more eosin (red color) and nuclear details are less clear. A cellular sample can be unfit for diagnosis if there is significant drying.

Hence with pap staining, air-drying is avoided as much as possible especially by dropping the slides into the fixative immediately after the smears are made. Poor quality of preparation, fixation or staining can all make a cellular sample unsatisfactory for evaluation. Hence great care must be taken in preparation and fixation of smears.

systematic inclusion of clinical and lab data should be considered as part of the procedure. The technique (aspirator), morphological interpretation (pathologist) and clinical information (clinician) constitute a diagnostic triad on which the FNA diagnosis rests.

It is preferable not to report on technically poor slides or give a definite diagnosis without adequate clinical information and correlation. Clinical data serves as a safeguard in avoiding errors.

Other Quality control Measures

In addition to details of technique (procedure, preparation, quality of materials used) and clinical correlation; other routine quality control practices regarding specimen reception (checking patient details, identification of slides, number of slides from each patient, labeling the slides), preparation and maintenance of stains, staining procedure, mounting, record keeping etc. are applicable to FNA also for optimal quality of diagnosis.

Imprint Cytology Smears

This is indicated in the case of tumours especially of lymph nodes. Soon after an excision biopsy of lymph node, the specimen is cut using a sharp scalpel blade. If there is blood oozing from the outer surface, touch the surface with a cotton ball soaked in normal saline.

Then take imprint smears by touching the cut surface with a clean microslide and fix, immediately.

3-Body fluids

- Peritoneal, pericardial and pleural fluids
- Cerebrospinal fluid (CSF)
- Nipple discharge
- Bronchial brushing/washings
- Sputum
- Gastric washings
- Urine sediment
- Prostatic secretions
- Cervicovaginal smears (PAP)

Body fluids

Urine: For cytological evaluation of bladder, three morning samples of urine (each of 50 - 100 ml) obtained on consecutive days are recommended. Centrifuge the urine for 10 minutes and place one or two drops of sediment on a glass slide, spread the material and fix immediately. Catheterised samples are also acceptable.

Cerebrospinal Fluid (CSF): CSF and other fluids of small volume have considerable bearing on diagnostic accuracy, the larger the sample the better the results. If several samples are obtained the second or third should be used for cytology. The addition of an equal amount of ethyl alcohol to the CSF is recommended if a delay in processing is anticipated.

Considering the low volume and cellularity, CSF specimen should be processed by cytocentrifugation.

Cytocentrifugation: The fluid samples with low cell content such as CSF and urine are centrifuged in Cytospin where the cells are sedimented directly on the microslides.