

The Art and Science of Cytopathology: Past, Present and Future

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Abstract

This overview is intended to give a general outline about the basics of Cytopathology. A field that is gaining a tremendous momentum all over the world due to its speed, accuracy and cost effectiveness. This review will include a brief description about the history of cytology from its inception followed by recent developments. Discussion about the different types of specimens, whether exfoliative or aspiration will be presented with explanation of its rule as a screening and diagnostic test. A brief description of the indications, utilization, sensitivity, specificity, cost effectiveness, speed and accuracy will be carried out. The role that cytopathology play in early detection of cancer will be emphasized. The ability to provide all types of ancillary studies that is necessary to make specific diagnosis that will dictate treatment protocols will be demonstrated. A brief description of the general rules of cytomorphology differentiating benign from malignant will be presented. Emphasis on communication between clinicians and pathologist will be underscored. The limitations and potential problems in the form of false positive and false negative will be briefly discussed. Few representative examples will be shown. A brief description of the different techniques in performing Fine Needle Aspirations will be presented. General recommendation for the safest methods and hints to enhance the sensitivity of different sample procurement will be given. It is hoped that this review will benefit all practicing clinicians that may face certain diagnostic challenges requiring the use of cytological material.

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Introduction

The art and science of cytology and cytopathology has been implemented and recognized as early as the 18th and 19th centuries.^{1- 5} However the progress and the standardization of this branch of pathology were not founded completely until the late years of the 20th century. The first American Board of Examination in cytopathology was undertaken in 1989 for the first time.

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Europeans, especially north Scandinavian countries, were able to utilize this technique even before World War II.^{1, 4} The science of cytopathology is currently well standardized with two major branches, exfoliative and aspiration biopsy.

George Papanicolaou, after which the famous Papanicolaou (Pap) smear and Pap stain was named, was one of the initial pioneers who drove

the attention to the science of the ability to make a diagnosis looking at slides with a smear of cells in the period between 1917 and 1928.¹ The initial North American scientific papers describing tumor diagnosis by cytological examination was published in 1930 from New York Memorial Hospital by Drs. Martin and Ellis followed by a publication by Dr. Stewart in 1933.^{6, 7} After which the scientific and the medical community started paying attention and aggressively pursuing this sub specialized field of pathology.

The first examination of American Board of Cytopathology was held in 1989 after standardization of this branch of pathology.⁸ Currently, one-year fellowship of Cytopathology in an accredited program is needed to be eligible for this exam. In addition, the Accreditation Council for Graduate Medical Education (ACGME), the agency that accredits residency training programs in pathology in the United States of America (USA) currently mandated documentation of fine needle aspiration (FNA) performance training for both residents and cytopathology fellows.⁹

Tissue biopsy versus cytological material

Although there are still few limitations for making the initial diagnosis merely on the basis of cytological material, these limitations are shrinking day by day and the role that cytopathology play as an initial diagnostic tool is currently a standard procedure.¹⁰⁻¹⁶

The difference between surgical biopsy and cytopathology material, including fine needle aspiration biopsy is shown in the table 1. Now, it is well recognized that using cytology including fine needle aspiration is cost effective, simple, accurate and a safe procedure for making a specific diagnosis that dictates management decisions by the treating clinicians.¹⁷⁻²⁶

Utilization of cytopathology:

The following are the ultimate objectives from utilization of most cytological specimen and the wide range of diagnostic categories are illustrated in Figures 1-3:

- A. The optimum goal is to reach a definitive diagnosis: this objective is the ultimate goal. Clinicians, patients and pathologists are all interested to reach a definitive specific diagnosis utilizing single diagnostic test. It is well recognized now that the utilization of different cytological examinations from different organs provides sufficient diagnostic information that drives treatment decisions.²⁷⁻³⁸
- B. As a screening tool: the success story of utilizing Papanicolaou smears in detecting early precursor lesions of cervical cancer is well known in the developed world. Rates of cancer death due to cervical cancer dropped tremendously after the 1960s when the the Papanicolaou smears screening programs had started.³⁹⁻⁴³
- C. As a follow-up for different diseases: cytological examinations of specimens taken from different sites as a follow up after establishing the initial diagnosis is a routine procedure. Sputum, bronchoalveolar lavage and bronchial brushings are frequent samples that are used as follow up for patients with a previous diagnosis of pulmonary carcinoma. Additional common samples that can be used include: pleural fluid, peritoneal fluid, discharge samples, cerebrospinal fluid, and fine needle aspiration from any palpable or non-palpable deep-seated new lesions that appear during the follow up period.
- D. For determination of different prognostic factors in neoplasia diagnosis: this is usually achieved as part of staging or using the cytological samples to perform ancillary studies, such as Her-2Neu analysis on breast mass aspirates.

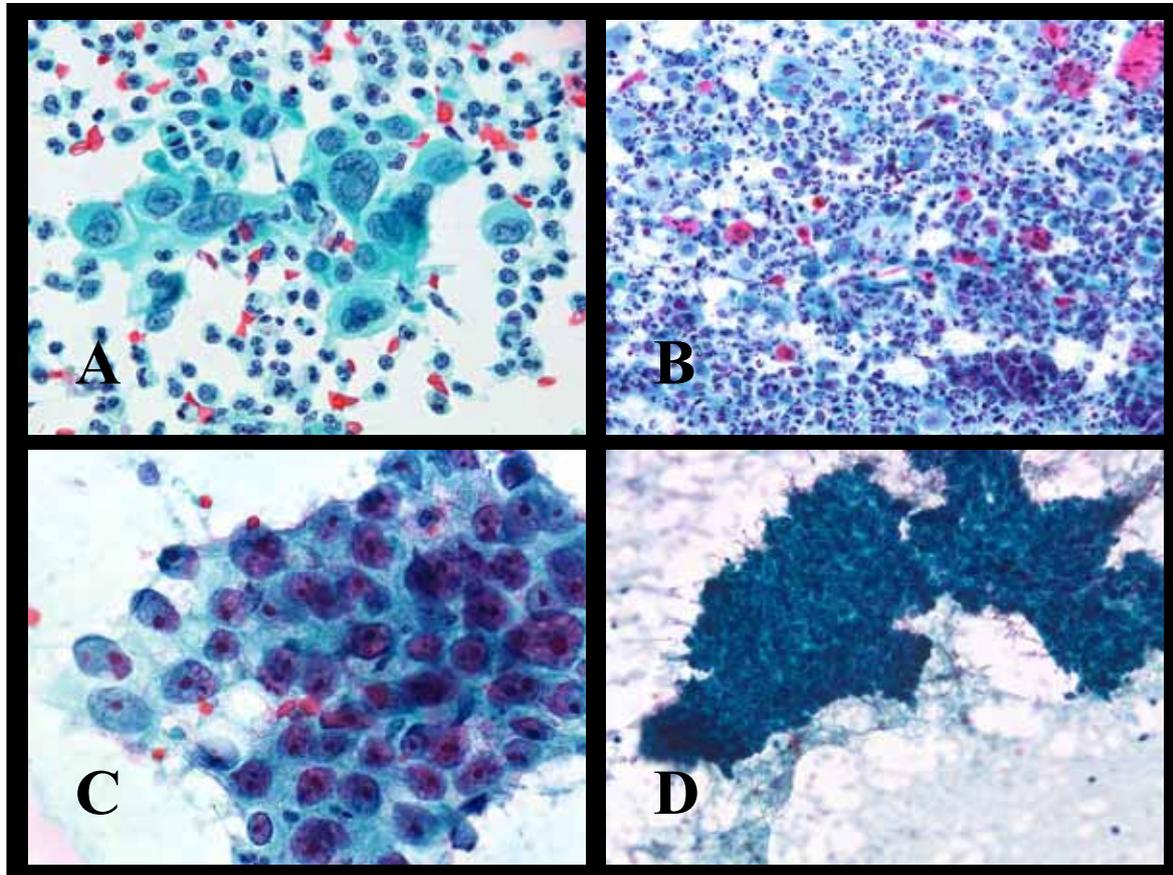


Figure 1:

- A. Papanicolaou smear with cervical adenocarcinoma (Papanicolaou stain 400X).**
- B. Papanicolaou smear with cervical squamous cell carcinoma (Papanicolaou stain 400X).**
- C. FNA ductal adenocarcinoma of breast (FNA, Papanicolaou stain 400X).**
- D. FNA of breast fibroadenoma (, Papanicolaou stain 200X).**

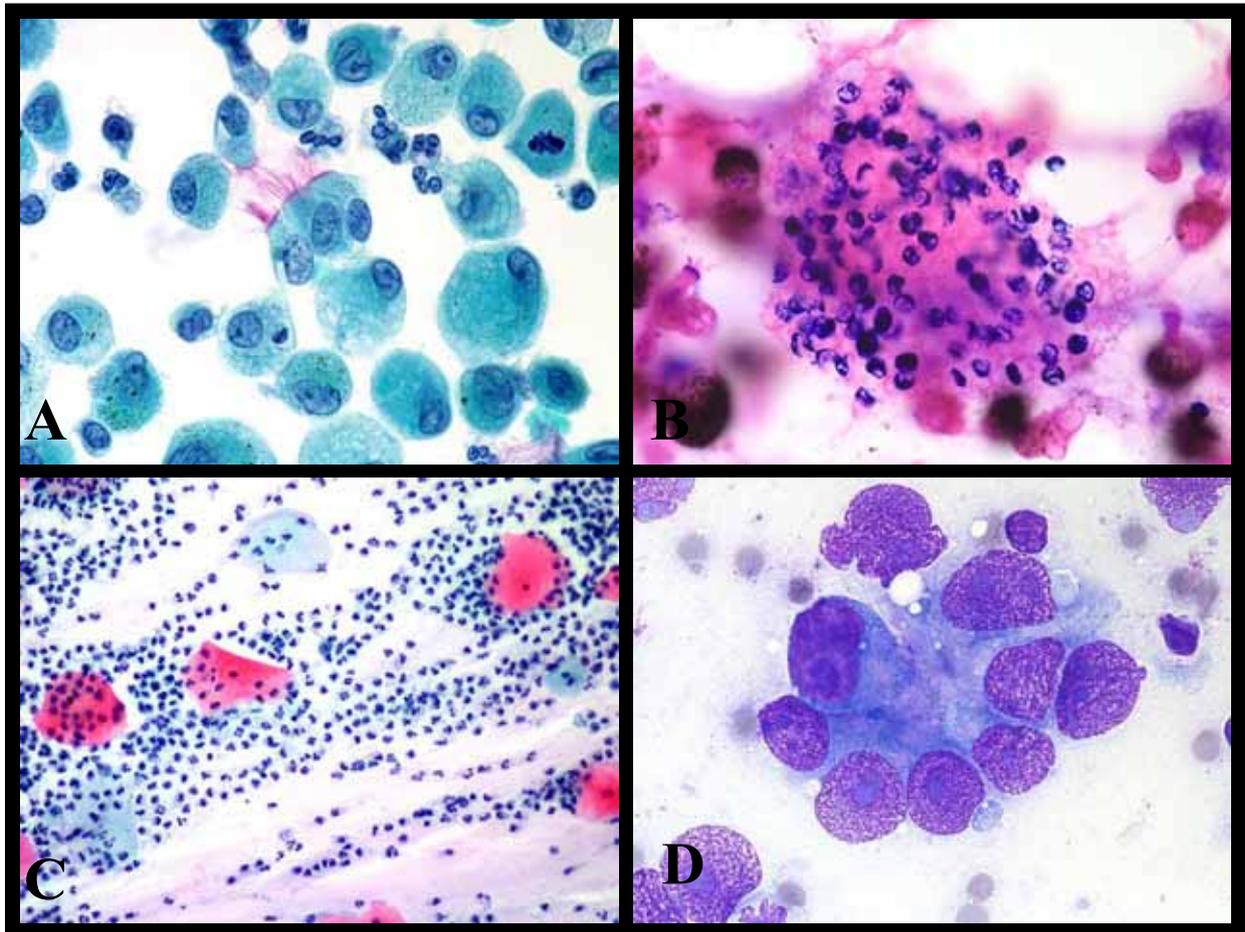


Figure 2:

- A.** *Deep sputum cytology smear with ciliated reactive bronchial epithelial cells and surrounding macrophages (Papanicolaou stain 400X).*
- B.** *Broncho-alveolar lavage with characteristic "fluffy Pneumocystis carinii" colonies in an immunocompromised patient (Papanicolaou stain 400X).*
- C.** *Acute pneumonia on sputum cytology showing numerous neutrophils (Papanicolaou stain 200X).*
- D.** *Pulmonary adenocarcinoma diagnosed by FNA of lung nodule under CT scan guidance (Diff Quik stain 400X).*

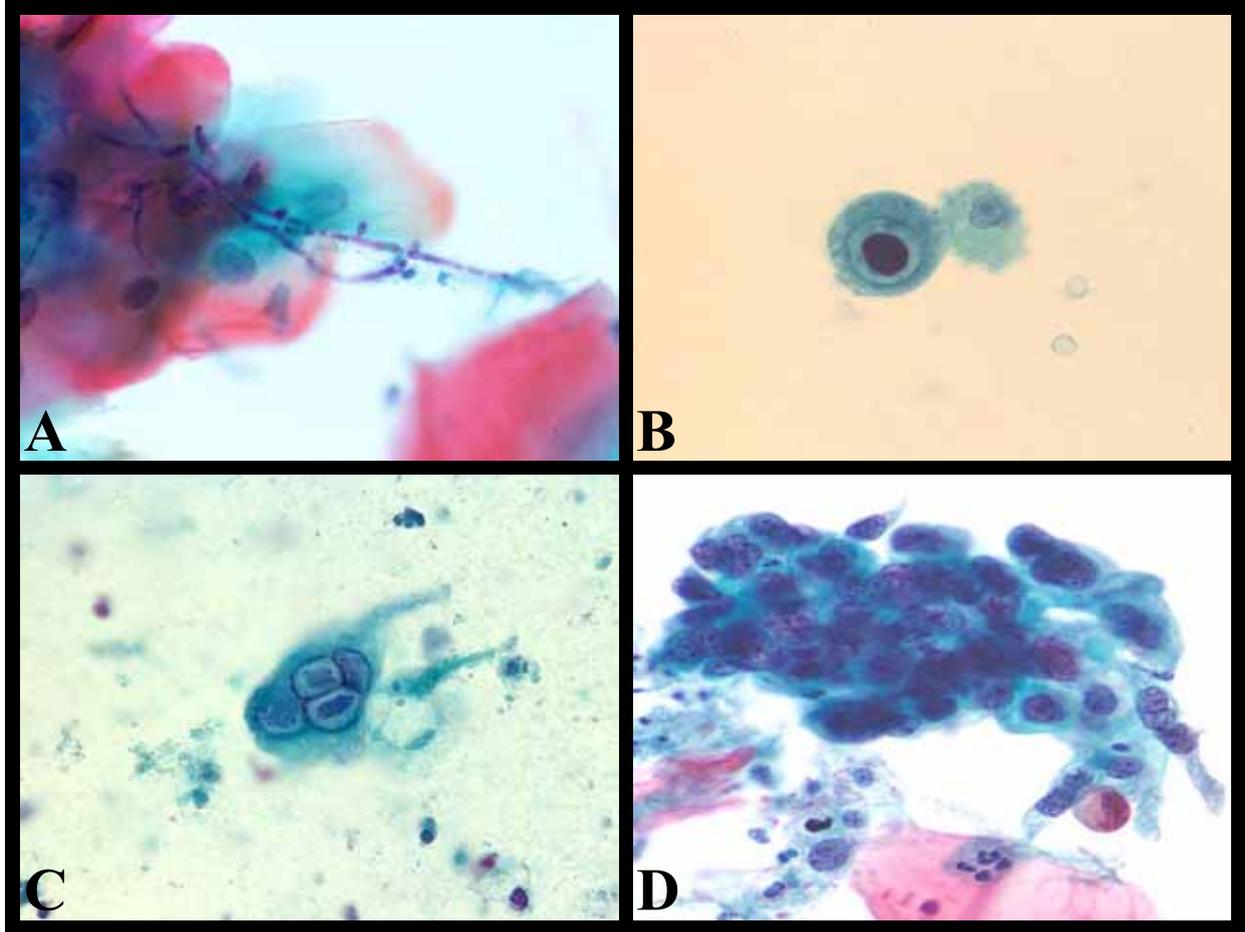


Figure 3: Esophageal brushing specimens.

- A. Candida hyphae seen in a case of Candida esophagitis (Papanicolaou stain 400X).**
- B. Cytomegalovirus esophagitis with cell enlargement and nuclear inclusions (Papanicolaou stain 400X).**
- C. Herpes simplex virus esophagitis with multinucleation and nuclear inclusions (Papanicolaou stain 400X).**
- D. Esophageal adenocarcinoma cells (Papanicolaou stain 400X).**

Advantages of using cytology

The advantages of utilizing cytological examination over traditional tissue are well known, the most important of which are:

- A. Safe: the procedures that are used to get cytological samples are extremely safe. Complications are very rare and when they occur they are relatively mild. Hematomas and pneumothoraces are among those. The most serious complication that may occur and had been reported is the development of pneumothorax during FNA of lung lesions. However, less than 5% of those are serious and require insertion of chest tube.⁴⁴⁻⁴⁷ In addition, if the procedure is done under image guidance, immediate evacuation using the same needle is now recommended and had been successfully achieved.⁴⁵ Paying attention to the risk factors for the development of pneumothorax may decrease their rate. Hematomas are observed more frequently in patients who have coagulopathies.⁴⁸⁻⁵¹ Prevention of such complications is easily achieved by applying gentle pressure for longer periods after the procedure. It is also recommended to consult with the hematologist in the institution to prepare those patients who suffer from bleeding disorders or are on anticoagulation therapy. Pain and patient discomfort are relatively mild and can be prevented by appropriate preparation of patients and by applying local anesthesia if necessary. Infections are extremely rare and can be avoided by following the international safety guidelines and sterile techniques.⁵²⁻⁵⁴
- B. Simple: it is well known getting most cytological samples is simple. With increasing familiarity of different sampling techniques, currently almost all institutions and health care providers are aware of the technology and it is part of routine investigative and diagnostic patient work up. Description of different types of samples will follow.

- C. Quick: the procedure is very quick and diagnostic answers can be provided immediately at the time of procedure if needed or within the next 24-48 hours.⁵⁵⁻⁶¹
- D. Cost effective: the cost effectiveness of cytological examination is well documented in the literature, a feature that is becoming very critical given the current high health care costs. Compared to surgical biopsies, the amount of savings is substantial.⁶²⁻⁶⁸

What is needed to have an effective cytopathology service?

The most important principal is to have a simple clear communication between pathologists and clinicians with the basic understanding of teamwork. In addition, using a common clear language of communication is absolutely critical to avoid mismanagement. This includes a clear understanding of the terminology which is used in the cytopathology final report. It is always desirable to communicate with clinicians at any time. Pathologists who are trained and familiar with cytopathology are obliged to establish bridges of communications with radiologists and clinicians. This can be achieved by one on one personal contact or through tumor board settings and clinicopathological correlation conferences. This, in many times, will have a positive impact in patient care since a diagnosis based on cytological examination will not be made in vacuum. The technical aspects of establishing a cytology and aspiration services are described below.

Branches of cytopathology

- A. Exfoliative Cytology: the samples represent cells that exfoliate from superficial or deep serosal or mucosal surfaces. This includes:
 - 1. Gynecological samples: Papanicolaou smears are the first samples that started the exponential revolution of the cytopathology field. Recently almost all health care providers are moving a way from the conventional Papanicolaou smears and

moving to what is called fluid-based technology that can provide more accurate interpretation and allows for molecular testing for the Human Papilloma Virus (HPV) infection.⁶⁹⁻⁷²

2. Respiratory/exfoliative cytology, which includes bronchial washing, sputum, bronchoalveolar lavage and bronchial brushing cytology. Those are commonly used to detect pulmonary infections and malignancies.
3. Urinary cytology: Urine cytology, bladder washing and brushing cytology. The urinary cytology field is passing through recent tremendous research. So, in addition to cytomorphological examination, the utilization of urine samples for detection of common chromosomal aberrations in urothelial neoplasms has been recently refined. Commercial kits utilizing the Fluorescent In Situ Hybridization (FISH) are already available and in use.⁷³⁻⁷⁷
4. Body fluid cytology: common samples include pleural fluid, pericardial fluid, peritoneal fluid, and cerebrospinal fluid (CSF) cytology. Similar to respiratory samples, those are also used mainly to detect malignancies and infections.
5. Gastrointestinal Tract: sampling the mucosa of the gastrointestinal tract is becoming a routine procedure during endoscopy. Brushing samples are used to detect viral and fungal infections, and neoplasia with its precursor lesions.
6. Discharge cytology: discharge from any anatomic location can be easily examined to investigate infections and malignancies. The most common sample is breast nipple discharge that is used as a screening method for detection of mammary carcinoma.^{78, 79}
7. Scrape cytology: this technique is very simple and can be performed by either clinicians or pathologists at the bedside or in the clinic. Detection of infections and cancer cells at any surface (skin or mucosa) can be quick and accurate.⁸⁰⁻⁸⁴

B. Aspiration Cytology: Different names are used to describe this expanding technique. The most famous ones are FNA (fine needle aspiration), FNAB (fine needle aspiration biopsy), and NABC (needle aspiration biopsy cytology). All of them mean the same thing; aspirating cellular material using a fine needle to make a diagnosis. This technique has been used from any lesion in the body which includes two major areas:

1. Palpable lesions: Palpable lesions can be targeted by a clinician and preferably by an experienced cytopathologist. The advantages of having a cytopathologist performing or at least be available to confirm material adequacy are well studied in the literature (see below).
2. Non-palpable lesions: The non-palpable lesions are usually done with the help of image analysis (CT scan guided, ultrasound guided, fluoroscopy guided, and recently endoscopic Ultrasound guided fine needle aspiration).

The benefits of having a pathologist/cytopathologist performing or available at the time of fine needle aspiration are well documented.^{62, 63, 85, 86} They are summarized as follow:

1. Making sure that the material is adequate for making specific diagnosis. This needs the use of immediate stains on smears with microscopic evaluation.
2. The ability to triage the case at that time. This means that after the initial evaluation of the smears the pathologist/cytopathologist will decide if additional material is needed to do ancillary studies such as cultures, molecular pathology studies, cytogenetic analysis, and immunophenotypic analysis by flow cytometry.⁸⁷⁻⁸⁹
3. The pathologist will be able to see the patient, take history, and perform quick physical examination. This will allow the pathologist to have a feeling and understanding of the clinical condition of the patient and formulate a clinically based differential diagnosis.

Technical aspects of cytology

The initial smears are usually stained by a quick stain (stains which needs approximately one minute to perform) such as Diff Quick (DQ) stain, a modified Romanowsky stain. This type of stain is done on slides with material after air-drying and this is why they are also called air-dried based stains. The other set of slides are fixed in basic ethanol-based solution (preferable 95% ethanol) for different type of stain, the Papanicolaou stain. In addition to the previous smears which are prepared at the time of the fine needle aspiration, the rest of the material is usually flushed in a ethanol or formalin based solution after which the material is centrifuged and a small mini biopsy is created from the concentrated cellular material at the bottom of the tube, this is known as the cell block. The slides from the cell block are usually stained by a regular Hematoxylin and Eosin (H&E) stain. These three types of stains are commonly used in different laboratories. Each one of those has its advantages and disadvantage. For example, Diff Quik stain is good for microorganisms, cytoplasm and background material staining. In the meantime, Papanicolaou stain is more superior for demonstrating the nuclear details, which are the most important and specific in making the diagnosis of malignancy. The H&E stain combines the advantages of both Papanicolaou and DQ stains and gives the pathologist a chance to evaluate tissue-like stains similar to routine biopsies.

Different types of smear preparations are utilized in the cytopathology laboratory, which includes:

1. Direct smears as described above.
2. Cyto-centrifuge smears which are prepared using the cyto-centrifuge method. This method concentrates the material and specially advantageous when few cells are present in a large amount of fluid, such as pleural or peritoneal fluids.
3. Centrifuge smears using membrane filters. This method utilizes the use of paper filter with very small pores to trap contaminating material. It is becoming obsolete since the slide cellular morphology is usually compromised.
4. Monolayer liquid-based cytology. This technology is now the standard method that is used to prepare Papanicolaou smears. The smears are superior to their conventional counterparts and also allow testing for Human Papilloma Virus DNA particles.⁶⁹⁻⁷² The cells are laid on the slide in a monolayer fashion and the background is clean
5. The last groups of slides are those prepared using the Cellblock technique, as described above. Those slides are prepared utilizing formalin-fixed paraffin-embedded tissue. The availability of such material provides the pathologist with material that can be used to do special necessary stains. Microorganisms and immunohistochemical stains are the most commonly used.^{90, 91}

Fine needle aspiration technique:

There is still no agreed upon standard for the best aspiration technique in cytopathology. However, all FNA experts agree on one thing, every aspirator have to get comfortable with one method and modify it as more experience is gained. The bottom line is to get enough diagnostic cells from the area of interest. The gauges of the needles used vary, however, 21-25 French gauge needles are the most frequent. Whether to use a gun (syringe holder) or not when negative pressure is utilized, is left for the level of comfort of the aspirator. Some believe that using the gun provides more control and more cells. The most common techniques that are used include:

1. Aspiration using a fine needle (gauge range 21-25) without negative pressure or a syringe. This technique is also known as “the French technique” and clinicians, radiologists and pathologists who use this method believe that it is less traumatic than the others and yield enough diagnostic cells by the mere capillary pressure.
2. Aspiration using a syringe and needle without negative pressure. This method allows the aforementioned capillary pressure to push cells in the hub of the needle avoiding the trauma of negative pressure. It is believed that adding the syringe will allow collection of fluids if the lesion turned to be cystic.
3. Aspiration using a syringe and needle with utilization of negative pressure. The amount of negative pressure varies; however, 2-3 cm. of negative pressure in a 10 ml syringe is commonly used. This is the method that the author uses without a gun and inserting the needle in a circular way to sample the whole area of the lesion (Figure 4).

What are the ancillary studies which could be used on cytological material?

Basically all ancillary studies can be done using cellular material obtained either from exfoliative or fine needle aspiration technique. These include:

1. Simple special stains such as stains for microorganisms.
2. Immunohistochemistry (Figure 5).
3. Flow cytometry (Figure 6).
4. Cytogenetic analysis.
5. Molecular pathology studies.
6. Electron microscopy

The cytopathology report:

To have an informative final cytopathology report after doing the procedure and making the appropriate studies to make a specific diagnosis, it is very important that it expresses few important components.

1. Adequacy: it is recommended that a statement describing if the material was adequate to make an interpretation is inserted in the final report. This becomes critical if the material is inadequate and the final message is to re-evaluate and/or re-investigate. As mentioned before, the presence of a pathologist or performance of the procedure by a pathologist is highly recommended in order to increase the adequacy rate.
2. Diagnosis: a specific diagnosis is always desired when possible. Sometimes the diagnosis is broad, such as “positive for malignant cells” and then this will be followed by descriptive diagnosis and a comment entailing a differential diagnosis to help the clinicians. In some cases, not all the diagnostic criteria are present or the atypical cells are very few; in these circumstances a “suspicious for malignancy” diagnostic category can be used. This has to be interpreted so that a second diagnostic approach is necessary.
3. Descriptive Diagnosis (microscopic description): sometimes descriptive diagnosis and microscopic description of the smears may be helpful for the clinicians to make a therapeutic decision. For example, if a nipple discharge was submitted on two smears from the clinician’s office and sent to pathology department and those smears contained numerous macrophages but no mammary epithelial cells are seen for evaluation. Although no epithelial cells are present in this case, the features are most likely consistent with a benign process since the increased number of macrophages and the lack of epithelial cells. In this circumstance, writing a simple microscopic descriptive diagnosis is of a great help to the clinician.
4. Comment: in certain circumstances a comment is needed to clarify or add some information that may harbor clinical importance.
5. Recommendations: sometime we need to call the clinicians and discuss the case with him either face to face or on the telephone.

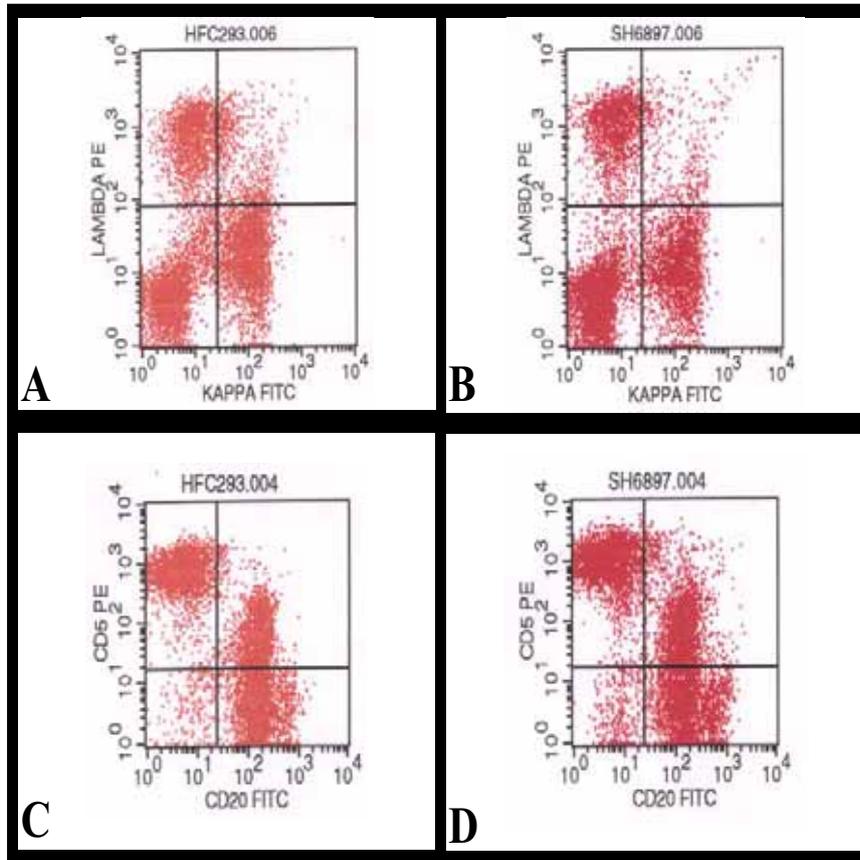


Figure 4:

Flow cytometry dot blot images from a submental lymph node. The panels on the left side are from the FNA material using the CRAT method and the panels on the right are from the same patient after the node was excised surgically. Both panels are identical demonstrating the efficiency of the “CRAT” method.

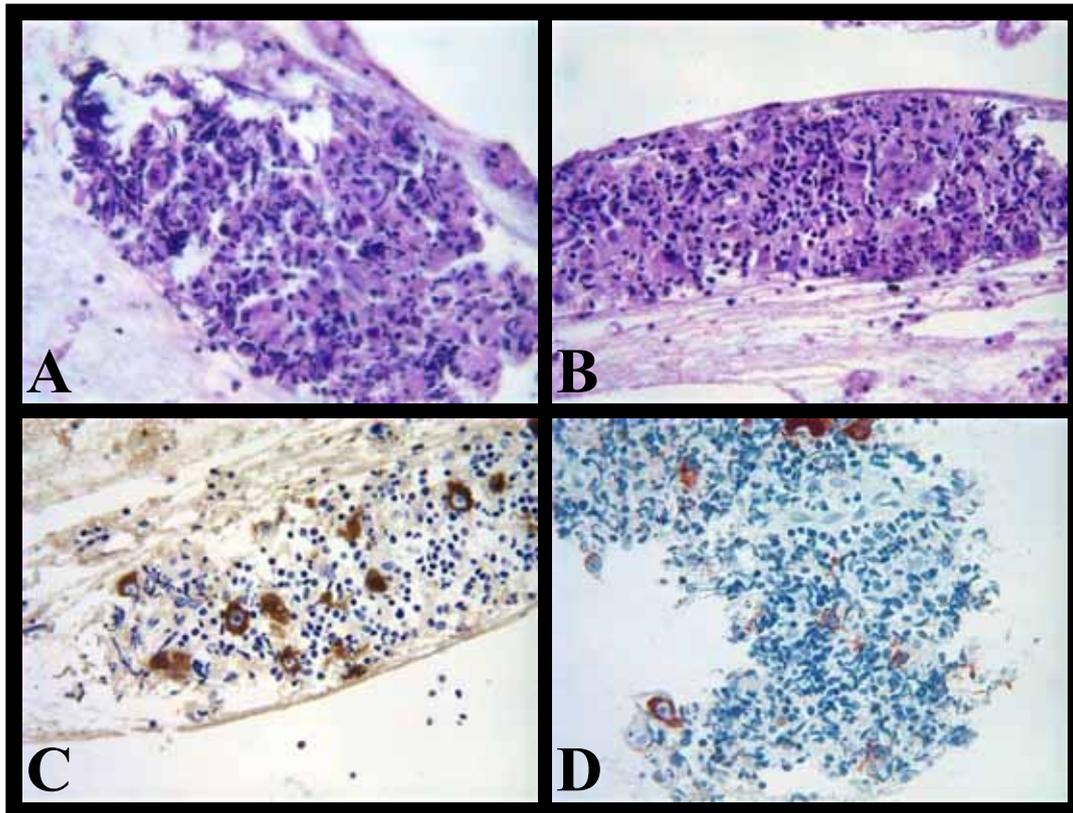


Figure 5: A case of Hodgkin's lymphoma that was diagnosed by FNA. A and B showing the cell block of this case that contains few Reed-Sternberg cells. The cells were immunoreactive for CD 15 (C) and CD 30 (D) and were negative for CD 45 and CD 20, confirming the diagnosis.

Factors that affect adequacy:

The presence of a pathologist/cytopathologist at the time obtaining material, especially in fine needle aspiration biopsy procedures, is highly recommended and saves a lot of effort and money in making one procedure diagnostic and cost effective. However, sometimes the material is non-diagnostic or acellular, and this should be conveyed in the final cytopathology report. So careful reading of the final cytopathology report is mandatory so that no misunderstanding or miscommunication can occur. Sometimes the material is sub optimal due to multiple factors, the most frequent are:

1. Air drying artifacts (leaving the smears for too long before staining). This will sometimes give false impressions of enlargement of the cells and nuclei, which in inexperienced hands may increase the incidence of false positive diagnosis.
2. Marked acute and chronic inflammation. The presence of marked inflammation sometime obscure diagnostic cellular details. Paying attention to this is very important. Taking into consideration the history and the clinical scenario of the patient should alert the cytopathologist from making a false/negative diagnosis. A comment in the report indicating that there is marked inflammation obscuring cellular details may be a necessity and this process should always be conveyed to the clinician to make sure that appropriate patient triage is carried out.

3. Sometime the material contains too much blood elements, especially red blood cells. These cells which are usually characteristic of certain organs (such as thyroid and liver) has to be interpreted with caution and thorough screening of the slides is mandatory so that abnormal/malignant cells can be detected and interpreted appropriately.

Cytological features of malignancy

There is no single feature that is diagnostic of malignancy. It is the constellation of multiple factors that vary depending on the tissue aspirated, the collection technique and the smear preparation method. It is very important to be aware of these variables before attempting to make a final cytological diagnosis. The general features of malignancy in cytological slides are high cellularity, cellular enlargement, Increased nuclear/cytoplasmic ratio, nuclear hyperchromasia, discohesiveness of cells, prominent and large nucleoli, abnormal distribution of nuclear chromatin, increased mitotic activity and specially the presence of abnormal ones, nuclear membrane abnormalities, cellular and nuclear pleomorphism, and background tumor necrosis (also known as tumor diathesis). Ultimately, we are all responsible for providing an accurate cytological diagnosis.

Problems can arise anytime anywhere from the time the patient is seen until the time the final report is transcribed and faxed or sent to the clinician. Trouble shooting is very important to identify problems which can arise anytime.

Diagnostic pitfalls

Diagnostic pitfalls can still occur and are usually due to:⁹²⁻⁹⁴

1. Poor collection technique: this can occur when the appropriate slides or containers with appropriate fixatives are not used at the time of the procedure.

This can be resolved by consulting with the pathology/cytopathology department for help.

2. Poor fixation: this is sometimes seen when there is no experience with cytopathology material preparation and collection. Communication with your pathologist is recommended.
3. Inflammatory changes: as described before, sometime extensive inflammation may obscure cellular details and prevent appropriate interpretation. To avoid this problem, treating the patient and repeating the procedure afterwards is recommended.
4. Cellular changes related to radiation and/or chemotherapy: this issue comes up if the patient had already been diagnosed with malignancy and was treated with chemotherapy and/or radiation therapy. Certain changes are induced by these treatment modalities. To decrease the pitfalls from these changes, appropriate and detailed history should be given by clinicians and awareness of the changes by the pathologist should be taken into consideration.
5. Atypical cellular changes related to hemorrhage, infarction or necrosis can be problematic. Awareness of these changes by the cytopathologist is very helpful to prevent both false positive and false negative diagnosis. Having a pathologist/ cytopathologist at the time of the procedure or performance of the procedure by a pathologist will help alert the pathologist to these changes.

False negative and false positive diagnosis:

Despite efforts to be as accurate as possible, both false negative⁹⁵⁻⁹⁹ and positive¹⁰⁰⁻¹⁰³ diagnosis can still occur. False negative diagnoses are most commonly related to:

1. Desmoplasia: this is defined as the presence of fibrosis which is induced by certain tumors due to secretion of fibrogenic materials. Many tumors can cause fibrosis around the malignant cells.

The most notorious are mammary, pancreatic and biliary tree carcinomas in addition to nodular sclerosing Hodgkin's lymphoma. Applying negative pressure and multiple passes during the FNA procedure can help.

2. Well-differentiated tumor cells: certain tumors are extremely well-differentiated and they resemble their original cells. For example, well differentiated thyroid follicular carcinoma and well differentiated hepatocellular carcinoma can be deceiving. Awareness of these tumors and appropriate understanding of limitations of cytology is recommended. In these circumstances, making the final diagnosis on tissue sections probably is more appropriate.
3. Sampling problems: sometimes the needle is not in the appropriate lesion of interest. This can be resolved by having an experienced aspirator and judicious utilizing of image guidance.
4. The presence of inflammation, radiation and chemotherapy changes sometime can be over interpreted. Applying strict cytological criteria in these situations is very helpful.