

Introduction to Endoscopic Ultrasound Fine Needle Aspiration (EUS-FNA) Sample Preparation

SAMPLING TECHNIQUES, CYTOLOGY VS HISTOLOGY & CASE STUDIES



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Introduction

The introduction of the curved linear array echoendoscope in the early 1990s created the opportunity for endoscopic ultrasound fine needle aspiration (EUS-FNA). Since that time, FNA has been the main driving force behind the growth of EUS and the vast expansion of its applications. This includes sampling of a variety of pathological conditions, including gastrointestinal and extra gastrointestinal masses, cysts, and lymphadenopathy. The availability of FNA devices of various sizes, and the more recent introduction of core biopsy devices helped improve tissue yield and allowed sampling of most lesions within the reach of any echoendoscope. The high diagnostic accuracy of EUS-FNA and its excellent safety profile facilitated its widespread adoption in most hospitals and cancer centers. This paper aims at introducing the proper technique for EUS guided tissue sampling and highlights the related aspects of cytological and histological processing.

SAMPLING TECHNIQUES

Basic EUS-guided Sampling Technique

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is a valuable and safe tool to obtain cellular material for cytological examination.^{1,2} The advent of EUS accessories like core biopsy devices facilitated obtaining histological materials from solid lesions. In experienced hands, EUS-FNA or core biopsy of lesions and lymph nodes above and below the diaphragm has been demonstrated to be extremely safe when compared with other tissue sampling modalities, with a risk profile similar to that of conventional endoscopy.³

EUS guided tissue acquisition starts by appropriately identifying the lesion of interest and its characteristics including shape, size, and relationship to surrounding structures. The lesion is then interrogated using the linear array echoendoscope to determine an appropriate puncture site. The ultrasound transducer on the distal tip of the echoendoscope permits needle advancement into the lesion under real-time guidance. EUS-FNA and core biopsies are performed after Doppler assessment to avoid puncturing intervening blood vessels.⁴

Once the gut wall is punctured and the needle enters the target lesion, the stylet is withdrawn. Whether or not to apply suction during FNA remains controversial but should be tailored to the specimen's volume and the amount of blood noted on the first pass performed with suction. The needle is then moved back and forth through the lesion for at least 30 seconds or until deemed adequate by the endosonographer. The needle is then withdrawn back into the sheath, the assembly is removed, and the sample is processed per institutional protocol.

Cytology

This refers to the science of cell interpretation, and is the basic and most common pathology preparation technique used to process the material obtained in the vast majority of EUS procedures. Cytological specimens obtained during EUS-FNA consist mainly of "loose" cells or small groups of cohesive cells rather than "cores" of tissue. Cytology interpretation has evolved as an independent sub-specialty in pathology serving EUS and other image-guided tissue acquisition. Cytology preparation starts in the GI lab soon after the FNA is performed.

The target lesion is first identified under EUS guidance and then punctured using the standard 19, 22 or 25 gauge* FNA needle as described above.⁴ The material retrieved from aspiration is then expressed onto a set of two glass slides (referred to as smears). The first slide is air-dried (or placed on a heated plate) for immediate staining and on-site review. The air dried specimens are then dipped in the Diff Quick stain which is typically available in the GI lab (*Figure 1*) and are ready for onsite cytological examination to determine adequacy of the specimen. In most referral institutions, rapid onsite evaluation is available and has been shown to improve the diagnostic yield and provides feedback to the endosonographer about quality and adequacy of the specimen and the need of further passes to reach a diagnosis.⁵⁻⁷

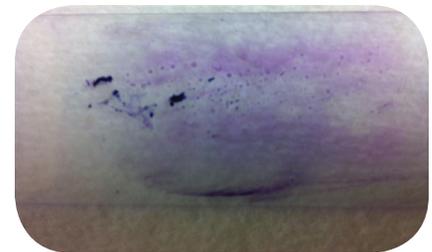


Figure 1

Diff-quick stained slide from a lymph node prepared in the GI lab and ready for rapid on-site cytological review

The other slide is sprayed with 95% ethyl alcohol and polyethylene glycol (Figure 2), followed by Papanicolaou (Pap) staining later in the lab for more detailed cytologic exam (Figure 3). While in the majority of cases, the final diagnosis (e.g. malignancy) is made based on the Diff Quick slides, the Pap smears bring out nuclear details clearly, allowing better identification of malignant cells when the quality of the Diff Quick slides is suboptimal. Therefore, the 2 preparation methods complement each other and are routinely obtained on every pass. It is important to keep in mind that the ability of cytologists and endosonographers to provide a final diagnosis on FNA specimens also takes into account the clinical history of the patient and findings from EUS or imaging studies.

What is a Cell-Block?

Cell blocks are prepared from residual material obtained from FNA after smears are prepared and are useful adjuncts for establishing a more definitive cytopathological diagnosis. Although cell blocks are almost routinely obtained during EUS-FNA using excess material after smear preparation, they can be particularly useful for the diagnosis and classification of lesions that otherwise may not be possible from smears only. In such cases, it is important to obtain at least 2 additional passes dedicated for cell block (i.e., no smears are prepared from these passes) to ensure adequate material is provided. The specimens are typically submitted in a special preservative solution, a commonly used one being RPMI-1640 (Roswell Park Memorial Institute medium). The solution undergoes centrifugation in the lab to provide a pellet of solid material (Figure 4). The pellet is then fixed in 10% Formalin, and subsequently embedded in paraffin, sectioned in thin slices, and stained with H&E (*hematoxylin and eosin* – Figure 5) for further review by the pathologist.

Additional studies (like immunocytochemistry-ICC) can be performed easily on cell block. ICC employs a wide battery of antibodies that bind to specific antigens on certain cells (like cancer cells) and therefore help confirm the diagnosis if such cells are present within the cell block. ICC on metastatic liver lesions and lymph nodes can also help further define what type of primary cancer is suspected. If lymphoma is suspected, 2-3 dedicated passes from the lymph node(s) are usually submitted in RPMI for flowcytometry, a specialized test that may allow the identification of monoclonal lymphocytes (lymphoma cells) based on specific surface antigens.

Histology

This concept refers to a different method of pathological processing and is the basic technique used to examine the vast majority of specimens obtained during surgical resection (like removing tumors) or endoscopy (e.g., removing polyps from the colon, or taking mucosal biopsies using forceps). Also referred to as “Trucut” biopsies in EUS guided sampling, this method was developed to process “pieces” or “cores” of tissue for histological examination, which are often difficult to obtain during routine EUS-FNA, due to the very small caliber of the needles used, and the dependence of the FNA needles on suction to acquire tissue.⁸⁻¹³ Therefore, specialized needle core biopsy devices had to be developed for use in EUS to reliably provide cores that depend on mechanical shearing to obtain tissue.

The performance of an older generation spring-loaded EUS Trucut biopsy device was suboptimal due to its stiffness, and difficulty to advance through the scope in angulated positions (like the duodenum), leading to frequent mechanical failures. The tissue cores are typically visible to the

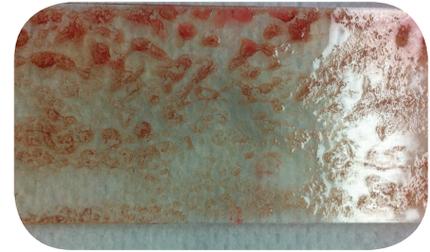


Figure 2

Alcohol-fixed slide from a lymph node prior to further processing in the lab for Pap staining.

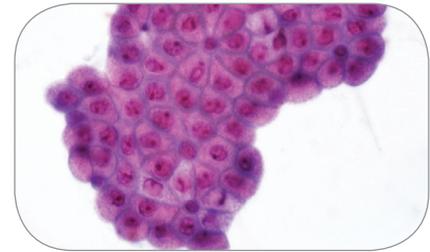


Figure 3

A Pap smear from a pancreatic cyst FNA demonstrating mucinous epithelium. The degree of nuclear detailing provided by Pap smears facilitates the diagnosis.

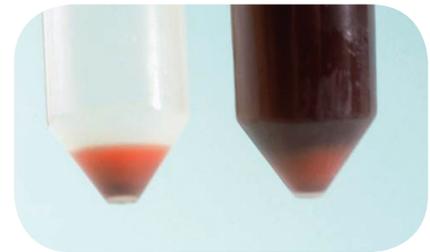


Figure 4

Cell block precipitates after centrifuging the tube. The supernatant (red fluid on the right tube) is discarded and the cell pellet (left tube) is retrieved for further cytological processing.



Figure 5

A paraffin block (middle) is used to embed histology specimens (including cell pellets) prior to being sectioned in thin slices, stained with H&E (hematoxylin and eosin-slide on right) for further review by the pathologist.

naked eye and can range in size from a few to several millimeters in size (*Figures 6 and 7*). Recent early clinical data using a 19 gauge needle (Expect 19 gauge Flex™, Boston Scientific) show promise in providing adequate cores from solid lesions (*Figures 6 and 7*), but large scale studies are needed to confirm this observation.¹⁴

Tissue obtained for histopathology is submitted to the lab in Formalin for standard histology processing that later involves paraffin-embedding and staining with hematoxylin and eosin (H&E), the most commonly used stain. If immediate cytology review is desired, touch preps can be prepared by rolling the tissue core(s) onto a glass slide (prior to dropping it in Formalin), followed by air drying it and staining it as routinely done. However, in most cases, no immediate cytology review is performed, and no special preparation of the specimen is needed prior to sending it to the lab.

Lacking the ability to perform on site review to assess sample adequacy, core biopsy sampling can sometimes be inadequate on its own to render a diagnosis. Indeed, many experts advocate combining FNA with core biopsies to increase sample adequacy.^{15,16} Once core biopsies are processed and deemed adequate, special staining using immunohistochemistry (IHC, which is similar in concept to the immunocytochemistry discussed above) can be readily performed, if needed, to confirm the diagnosis of certain tumors. The turnaround time for IHC on biopsies is typically 7-10 days and varies from one laboratory to another.



Figure 6

Several visible cores obtained using the Expect™ 19-gauge Flex Needle from a gastric subepithelial lesion and placed in Formalin.



Figure 7

Two visible cores from a gastric subepithelial lesion obtained using the Expect 19-gauge Flex Needle displayed temporarily on a glass slide prior to placing in Formalin for histological processing.

CYTOLOGY VS. HISTOLOGY

Cytology vs. Histology

During EUS, samples could be obtained for both cytology and histology if needed. The acquisition of histology samples in addition to cytology is particularly helpful if rapid onsite interpretation is not available, or when preliminary onsite review indicates inadequate or non-diagnostic specimen. In cytology, the presence of large amounts of blood, necrotic material or inflammatory cells can mask tumor cells leading to non-diagnostic specimen. In addition, when certain conditions or diagnoses are suspected, the presence of a “core” of tissue could be important to provide a definitive diagnosis. For example, histology, if obtainable, is favorable to cytology in sampling enlarged lymph nodes (to rule out lymphoma) or the liver (when cirrhosis is suspected), where tissue architecture is important to study. In submucosal lesions^{17,18} (like gastrointestinal stromal tumors) and autoimmune pancreatitis,¹⁹ histology allows for special staining (immunohistochemistry) that provides accurate diagnosis. Nevertheless, obtaining core biopsies during EUS is not always feasible due to the large size of the biopsy device (typically 19-gauge needle), location and size of the lesion, and the need for operator expertise. Therefore, a core biopsy device that is consistently able to access lesions in various locations and provide adequate histological samples is much needed.

Molecular (DNA) Analysis

This is available in many referral hospitals as well as a central commercial lab (RedPath®, Pittsburgh, PA). This test is commonly used in pancreatic cysts, but could be used in other pancreatic and biliary cancers.²⁰ In the case of pancreatic cysts, a small portion of the pancreatic cyst fluid (usually 200 mcg) is analyzed for common mutations (like K-ras point mutations) that could accurately categorize the pancreatic cyst as mucinous or non-mucinous, and potentially identify the malignant cysts in an early stage. Mucinous cysts are considered premalignant and, therefore, should be surveyed periodically by imaging or surgically removed when appropriate. Material to be sent for this analysis is submitted separately but, typically, in addition to routine cytology and other tumor markers from the cyst fluid. The same test recently became commercially available to help differentiate benign from malignant pancreatic masses when standard cytology is non-diagnostic.

EUS-FNA SAMPLE PREPARATION SUMMARY

Cytology

What is it?	How to Perform?	When to use it ?
Cytology obtained during EUS-FNA consist mainly of “loose” cells or small groups of cells	Obtained during EUS-FNA using a standard 19, 22 and 25 gauge FNA needles. Specimens can be air dried for immediate on-site review and/or alcohol fixed for later Pap staining and interpretation. Cell block can be performed by submitting additional passes in cell preservative for later processing and special staining. It is possible that larger size (like 19 gauge) needles could provide maximal amounts of tissue in fewer passes.	The majority of aspirates obtained during EUS are processed as cytological specimens and are important for the diagnosis of tumors, cysts, lymph nodes and other lesions examined by EUS

Histology

What is it?	How to Perform?	When to use it ?
Cores of tissue obtained provide more abundant material than cytology but requires larger caliber and more specialized needles	Tissue is acquired similar to the FNA process. Specimens are submitted in Formalin and sent directly to the pathology lab	Adjunct to cytology to establish diagnosis when: <ol style="list-style-type: none">1. FNA is non-diagnostic or inadequate2. Tissue architecture is needed (e.g. lymphoma, chronic liver disease, auto-immune pancreatitis – AIP)3. Special stains (immunohistochemistry) are needed (e.g. gastrointestinal stromal tumors – GIST)

Molecular Analysis

What is it?	How to Perform?	When to use it ?
DNA analysis of common genetic mutations in the specimen	Standard FNA needles are used to sample pancreatic cysts or masses	Helps accurately identify malignant and premalignant pancreas cysts, and assists in differentiate benign from malignant pancreatic masses

Introduction

A 46-year-old female patient with no known history of gastrointestinal illness presented to the local emergency room with one week history of melena and generalized weakness. On physical exam she was found to have conjunctival pallor and epigastric tenderness. On laboratory tests her hemoglobin was 6.5 mg/dl. She admitted to the hospital for blood transfusion and investigation of her suspected gastrointestinal bleeding.

Evaluation and Management

The patient was initiated on intravenous proton pump inhibitor therapy and transfused with 3 units of packed red blood cells. Upper endoscopy was performed within 12 hours of admission and revealed a 3.5 cm ulcerated subepithelial mass in the proximal gastric body. Two distinct ulcers were noted on the surface of the lesion, one of which exhibited a visible vessel, seen after flushing an adherent clot (*Figure 1*). This ulcer was deemed to be the source of the recent gastrointestinal bleeding and was injected with epinephrine and treated with bipolar cautery. The patient showed no signs of continued bleeding and was discharged home in 48 hours in a stable condition on oral proton pump inhibitor agent.

Endoscopic ultrasound (EUS) was performed one week after discharge from the hospital and confirmed the presence of the gastric mass. After complete submersion under water, the lesion was carefully interrogated and was found to be hypoechoic and homogenous, originating from the fourth wall layer (muscularis propria). Fine needle aspiration was performed using the 19 gauge Expect™ Flex Needle (*Figure 2*). Rapid on-site cytological review of the first pass revealed clusters of spindle cells, and 2 subsequent passes were then directly submitted for cell block for immunocytochemistry. To ensure specimen adequacy for special staining, 2 additional passes were performed and submitted in formalin for histological exam and immunohistochemistry. Several small cores were visible, ranging in size from 2-4 mm. Final cytological and histological examination confirmed the diagnosis of GIST (*Figure 3 - core biopsy, hematoxylin and eosin stain*), where both immunocytochemistry and immunohistochemistry demonstrated positive staining for c-kit (*CD 117 - Figure 4*). The patient was referred for surgical resection.

Learning Points and Discussion

Obtaining samples with adequate cellularity from intramural lesions can be difficult during EUS-FNA due to the extreme mobility of those lesions. It is important to keep the echoendoscope in a stable position and perform quick but precise movements while the lesion is punctured initially and during the back and fro cycles. In this case, the use of large caliber needle (Expect 19-gauge Flex Needle) facilitated the diagnosis by providing highly cellular passes with a relatively limited number of passes. When a stromal tumor is suspected, it is helpful to perform a core biopsy which supplements the cytological diagnosis from FNA and help confirm the diagnosis by allowing immunohistochemical stains. In this case, the Expect 19-gauge Flex Needle was able to provide adequate cytological material and good quality core biopsies.



Figure 1

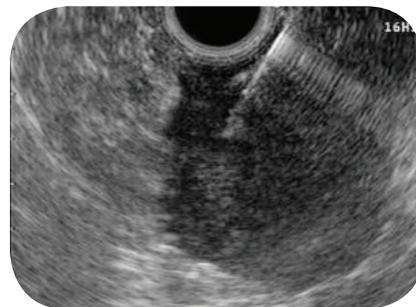


Figure 2

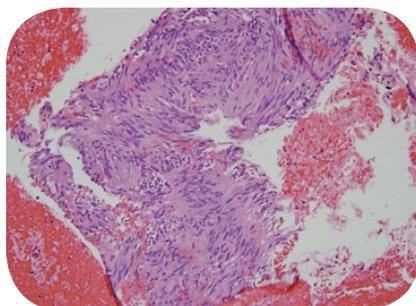


Figure 3

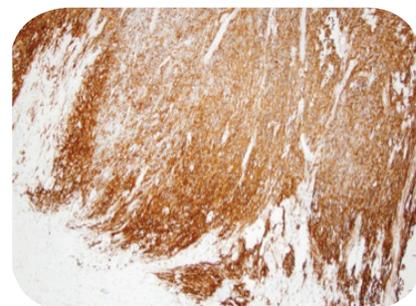


Figure 4

Introduction

A 62-year-old male patient presented with a 2-month history of unexplained weight loss of 20 lbs. and a 1 week history of pruritus. He was admitted to the hospital after his primary care physician detected jaundice on physical exam. Routine laboratory work revealed total bilirubin of 5.8 mg/dl. A CT scan of the abdomen with intravenous contrast revealed a solid pancreatic head mass (*Figure 1*) with dilation of both the bile duct and pancreatic duct upstream from the mass. The patient was referred to endoscopic ultrasound (EUS) for biopsy and staging of the pancreatic mass.

Evaluation and Management

Endoscopic ultrasound was performed and confirmed the presence of a 3.3 cm solid hypoechoic pancreatic head mass partially encasing the superior mesenteric vein (*Figure 2*). The mass was visible from the duodenal bulb. Fine needle aspiration (FNA) was performed using the Expect™ 19-gauge Flex Needle. On-site cytological review of the first pass revealed pleomorphic, overlapping cells with an increased nuclear-to-cytoplasmic ratio consistent with adenocarcinoma (*Figure 3, Diff Quick stain*). Two additional passes were subsequently submitted directly for cell block analysis. Final cytological interpretation confirmed the diagnosis of pancreatic adenocarcinoma. The patient was referred to a pancreatic surgeon.

Learning Points and Discussion

FNA of pancreatic head and uncinate lesions has traditionally been performed using either 22 or 25 gauge needles. The use of traditional 19 gauge needles for sampling of such lesions was technically challenging. This was related to the angulation of the tip of the echoendoscope, typically located in the first or second parts of the duodenum, prohibiting the advancement of 19 gauge needles through the scope and into the lesion of interest. The advent of the Expect 19-gauge Flex Needle expanded the potential applications of 19 gauge needles to include sampling pancreatic head lesions. Additional advantages of the needle include adequate cytological sampling in potentially fewer passes, and ability to provide histological specimens (core biopsies) if needed. This needle can be considered as a first line needle or when smaller needles fail to provide the diagnosis when sampling pancreatic head lesions.



Figure 1



Figure 2

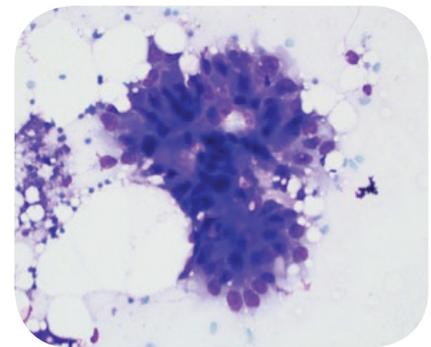


Figure 3

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*SI unit conversion: 19 gauge needle = .040"; 22 gauge needle = .028"; 25 gauge needle = .020"
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