

# How to prepare, handle, read, and improve EUS-FNA and fine-needle biopsy for solid pancreatic lesions: The pathologist's role

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## INTRODUCTION

Endoscopic ultrasound (EUS)-guided tissue sampling is a well-established method to accurately diagnose pancreatic lesions. The accuracy of fine-needle aspiration (FNA) and fine-needle biopsy (FNB) is influenced by several factors, including the operator's skill, target lesion characteristics, sampling equipment and technique, tissue handling and processing, and the experience of the pathologist. Importantly, EUS-guided tissue sampling practices vary substantially within the international endosonographic community, and there are no common guidelines for handling samples. A recent international survey showed that the approach to sample handling is highly variable, including tissue processing and rapid on-site pathological evaluation (ROSE).<sup>[1]</sup> As a result, the diagnostic accuracy among institutions is still highly variable; most highly experienced

institutions report a sensitivity of 80% or more.<sup>[2-4]</sup> It must be noted that technological research efforts in this field primarily focus on improvement of the material, design, and size of sampling devices to provide material for histological rather than cytological analysis. However, cytological tissue handling procedures have recently undergone limited developments for thin preparations (liquid-based cytology [LBC]). Importantly, the cellblock techniques are proposed as an adjunct or an alternative to conventional smears. The implementation of these methods requires an additional, although limited, investment in laboratory infrastructure and specific training for cytotechnologists and pathologists for accurate interpretation. Adequate preparation of FNA and FNB samples and dedicated training of cytotechnologists and pathologists are the prerequisites for achieving optimal results.

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## ENDOSCOPIC ULTRASOUND-FINE-NEEDLE ASPIRATION TISSUE PREPARATION

### *Smear preparation and rapid on-site evaluation*

In current EUS-FNA practice, pancreatic cancer diagnosis is typically accomplished through cytological smear preparation. Unfortunately, smear samples often show pronounced artifacts such as cell degeneration, obscuring material, and drying effect. More importantly, diagnostic accuracy might be hampered by the technique of making the smear if preparation of an optimal smear is not possible, even if the quantity of the material is sufficient. Therefore, if a trained cytotechnician cannot attend the endoscopy session, specific training of the endoscopy personnel responsible for properly preparing smear samples is strongly advised. Ideally, the aspirated material should be dispersed evenly onto the glass slide by an experienced operator. The next steps in the preparation of the smears are either air drying for Romanowsky or May–Grunwald–Giemsa staining, submersion of the smear in ethanol, or spraying of the smear with alcohol-based fixatives such as Cytofix®.

The purpose of ROSE is to improve the diagnostic performance of EUS-FNA.<sup>[5-7]</sup> This is supported by a recent meta-analysis of 34 distinct studies that included 3644 patients and showed that ROSE significantly increases the diagnostic yield of EUS-FNA.<sup>[8]</sup> However, according to the recent international survey, only 50% of endoscopists from Europe and Asia use ROSE, while it is routinely performed by 98% of US endoscopists. Reasons for omitting ROSE included “limited pathology staffing” (74%), “disbelief in its additive value” (32%), “high cost” (24%), and “additional procedure time” (24%).<sup>[1]</sup> This evidence reflects the fact that ROSE frequently refers to the amount of material but not to its diagnostic quality,<sup>[9]</sup> as in most European institutions, cytotechnicians and not pathologists are available for ROSE. The “ideal” ROSE should meet adequacy as well as diagnostic requirements: the ability of the operator to make an on-site diagnosis. Therefore, the ROSE criteria should be further standardized. Important questions for standardization are: (1) who is performing the ROSE (pathologists, cytotechnicians, endoscopists); (2) what is the optimal technique for on-site sample preparation; (3) which expertise is required for the ROSE assessment; and (4) is there a role for digital pathology?

### *Partitioning and preservation of the material*

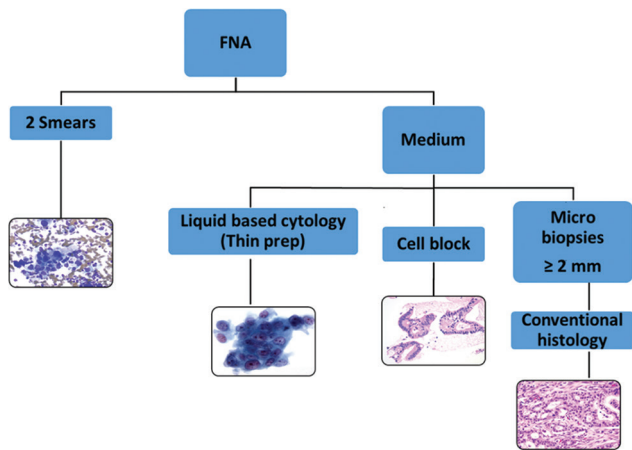
To increase the diagnostic yield, a portion of pancreatic samples obtained by EUS-FNA or FNB could be processed using a fixative for additional evaluation. These include mostly cellblock and thin preparations (LBC). LBC may be a good complement to smear preparation if blood contamination is profound.<sup>[10]</sup> Although cellblock preparation is not cost-effective in the short term and increases the technical workload, many pathologists prefer the evaluation of cellblock-processed samples to that of conventional smears. When experienced operators performed EUS-FNA of pancreatic lesions in the absence of an on-site cytopathologist, rinsing the entire sample in fixative demonstrated an improved diagnostic rate with adequate visualization of the sampled material.<sup>[11]</sup> In addition, direct histological processing of EUS-guided biopsies may permit more rapid molecular biomarker analysis.

In the absence of exact guidelines for material processing, each institution should thoroughly evaluate the optimal preparation method of EUS-FNA samples. Based on (cyto)pathologist's experience, the EUS-FNA material could be divided into smears ( $n = 2$ , per pass) and fixative for additional evaluation. Very tiny (<2 mm) cores should be evaluated using cellblock preparation, while larger cores (>2 mm) might be processed as histological material [Figure 1].

## DIAGNOSIS OF ENDOSCOPIC ULTRASOUND-FINE-NEEDLE ASPIRATION SAMPLES AND ANCILLARY TECHNIQUES

Limits of EUS-FNA include sampling error and interpretation error. One difficulty of EUS-FNA sample evaluation is the lack of uniform criteria for adequacy. Cellblock preparations may allow improvements in the diagnostic accuracy of the samples. In each case, pathologists should be informed of the clinical/biochemical and radiological findings before histological examination. The reporting is preferably done using the standardized terminology proposed by the six-tiered Bethesda classification.<sup>[12]</sup>

Evaluation of smears, small tissue fragments in cellblocks, or small core biopsies often provide sufficient information for correct diagnosis [Figure 2]. Some differential diagnoses might still be difficult, such as a well-differentiated adenocarcinoma *versus* chronic

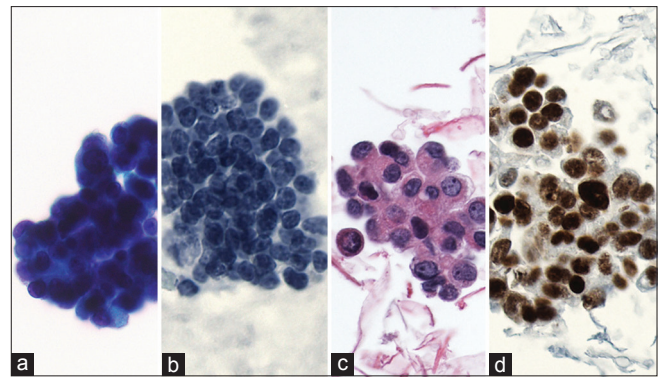


**Figure 1.** Proposed algorithm for tissue processing of endoscopic ultrasound aspirations. FNA: Fine needle aspiration

pancreatitis; some differential diagnosis including serous cystadenoma and autoimmune pancreatitis might be extremely difficult, if not impossible, on cytological smears. In difficult cases, a second opinion from an expert cytopathologist using digital slides might be useful and could enable a rapid diagnosis. Immunohistochemical staining performed on cell blocks using additional liquid-based slides and already stained conventional slides might be helpful. The technique is especially helpful for differentiation of metastasis *versus* primary lesions, as well as for less frequent pancreatic tumors such as solid pseudopapillary neoplasm using beta-catenin immunostaining. If a well-differentiated adenocarcinoma is suspected, additional analysis, such as SMAD4 immunohistochemistry, might be useful. Approximately 50% of pancreatic adenocarcinomas show the loss of tumor suppressor gene SMAD4, while reactive ductal epithelium is SMAD4-positive. Mutation analysis, including TP53, CDKN2A, and SMAD4, may also prove useful for supporting diagnosis when cytology is indeterminate. KRAS mutation analysis must be interpreted with caution, as this genomic alteration is present very early in pancreatic carcinogenesis and cannot be considered indicative of a malignant process.

### FINE-NEEDLE ASPIRATION OR FINE-NEEDLE BIOPSY: THE DEBATE CONTINUES

To increase the yield of sampling and core tissue, many endoscopists utilize needles designed to obtain material for biopsy. Expected advantages of FNB are as follows: (1) greater cellularity, and thus, improved adequacy, (2) histologic sections retaining tissue



**Figure 2.** Example of endoscopic ultrasound-fine-needle aspiration (a) with subsequent smear, (b) thin preparation, and (c) cellblock preparation (H and E) staining, (d) Immunohistochemical staining

architecture that most pathologists consider more familiar, (3) more standardized specimen handling and tissue processing, (4) diminished need for ROSE, (5) fewer passes necessary to obtain sufficient material, and (6) extensive ancillary studies are possible. Currently, randomized trials comparing different FNA and FNB needles are underway, and initial results indicate that the diagnostic efficacy, technical performance, and safety profiles of FNA and FNB needles are comparable.<sup>[13-15]</sup> However, the use of larger needles may have some disadvantages due to increased tissue injury, a more hematic specimen, and increased difficulty for the endoscopist performing the procedure. Therefore, FNB is not yet fully established as a routine technique for sampling of solid pancreatic lesions, and further studies are needed to determine the utilization and performance of different FNB needles.

### FINAL REMARKS AND FUTURE CHALLENGES

The handling procedures of pancreatic samples are highly variable within the international pathology community. They include sample processing, ROSE, and the experience of the operating cytotechnicians and pathologists. Cell block preparation and LBC are becoming common additional methods for the evaluation of pancreas EUS-FNAs. However, standardization of these procedures and clear cytological criteria for optimal categorization of samples are needed to further improve diagnostic accuracy and achieve uniformity in pathological evaluation. Optimally, each pathology laboratory should annually monitor the diagnostic accuracy of pancreatobiliary cytology. New centers beginning diagnostic EUS of pancreatic lesions are strongly advised, at least in the early phases, to use

ROSE to optimize the quality and quantity of material and to establish strict cooperation between endoscopists and pathologists. The utilization of digital images and remote evaluation should be evaluated as a surrogate of ROSE when and where ROSE is not feasible for logistical or economic reasons, and also to improve the diagnostic accuracy of difficult lesions.

We must also be prepared for the next challenges in this diagnostic field; immunophenotypic and genomic characterization are becoming more relevant to select personalized therapeutic strategies.<sup>[16,17]</sup>

For molecular characterization, both the quality and quantity of the material are of key relevance. We must fill the gap between the high technology endosonography apparatus with sophisticated needles and the low/no technology sample preparation tools that pathologists use. It is of strategic relevance and includes the involvement of industrial companies, design of new devices, and development of new protocols to provide optimal diagnostic support to echoendoscopic activities.

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