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Techniques in
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Endoscopic Ultrasound-Guided Fine Needle Aspiration and Tru-Cut Biopsy

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Endoscopic ultrasound-guided (EUS) biopsy, including both fine needle aspiration (FNA) biopsy and histological tru-cut needle biopsy, have now matured into highly valuable methods for acquisition of cytologic and histological specimens. However, a great deal of practice is required before these procedures are mastered. A number of important steps have to be fulfilled, including real time monitoring of the needle during the entire procedure to obtain sufficient material for evaluation. The present review describes the technique of EUS-FNA and tru-cut biopsy, based on a literature review and the authors' extensive experience with these methods. The endoscopes and needle systems available on the market are presented in detail. The biopsy procedure is carefully detailed, including tips and tricks of the trade. Finally, the limitations and complications of the procedure are reviewed in brief, stressing the low rate of complications (below 1-2%), most of which are minor and self limiting.

KEYWORDS EUS-guided biopsy, EUS-FNA, EUS-TCB, Endosonography guided biopsy, technique, EUS guided intervention

The development of endoscopic ultrasound scanning (EUS) began in the early 1980s with mechanical radial scanning transducers.[1] Despite excellent imaging resolution, it did not become popular until the advent of EUS-guided fine needle aspiration biopsy (EUS-FNA).[2] EUS-FNA has now matured into a highly valuable method for acquisition of cytologic specimens. EUS-FNA is not only limited to gastroenterology since the gastrointestinal tract traverses through anatomical regions related to other specialties, such as pulmonology, thoracic surgery, internal medicine, oncology, urology, gynecology, and endocrinology.[3] There is also considerable evidence that, in experienced hands and in combination with an expert cytopathologist, EUS-FNA is able to provide a cytologic diagnosis in between 80% and 95% of malignant lesions depending on the type of lesion as well as the location, with an overall sensitivity and specificity of 90% and 100%, respectively, and with a complication rate not different from CT or ultrasound-guided needle aspirations.[4-10] In addition, many of the lesions targeted by EUS-FNA are either not reachable or visible by other imaging methods due to small size or overlying bony or air-filled structures. Minute lesions down to a size of 5 mm may be imaged and subsequently biopsied by EUS-FNA.[11] It is at present evident that specimens obtained by EUS-FNA have a high likelihood of providing representative tissue for diagnosis when other techniques have failed or are not applicable. EUS-FNA, aside from establishing primary diagnosis of malignancy, can also accurately stage patients preoperatively and influence the decision-making process, thereby reducing the morbidity and mortality of noncurative surgical interventions. Thus, EUS-FNA can replace many other far more invasive and risky diagnostic procedures. However, there are some limitations of the FNA method and, in some cases, more tissue is needed to further classify a lesion such as lymphoma and gastrointestinal stromal tumor.

Endoscopic ultrasound-guided tru-cut biopsy (EUS-TCB) has recently emerged as a method that tries to overcome the limitations of EUS-FNA by providing a core-tissue specimen needed to increase the yield and accuracy of certain diagnoses.

The aim of the present review is to describe the equipment and the technique of EUS-FNA and EUS-TCB in detail, based on a literature review and our extensive experience with these methods.

Endoscopes for EUS-FNA and EUS-TCB

Different electronic linear scanning endoscopes are commercially available for EUS-FNA and EUS-TCB. Most endoscopes are at present equipped with an elevator to facilitate the po-

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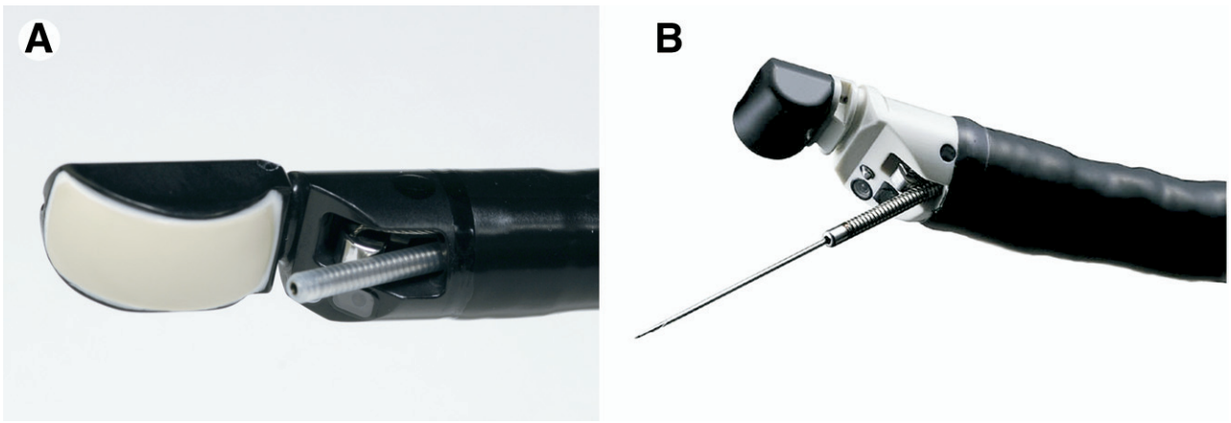


Figure 1 (A) The distal end of a Pentax EUS endoscope with an elevator. A needle sheath is extending beyond the biopsy channel outlet. (B) The distal end of an Olympus EUS endoscope with an elevator. A needle with sheath is extending beyond the biopsy channel outlet. (Color version of figure is available online.)

sitioning of the needle (Fig. 1A and B). However, an elevator was not standard a few years ago, and some of these endoscopes are still in use and functionable (Fig. 2A and B). There are both pros and cons related to the use of an elevator. The drawback is that the addition of an elevator adds increased length to the stiff part of the distal end of the endoscope, therefore reducing the maneuverability. Additionally, the advancement of the needle is more difficult when the elevator is activated due to the resistance against the needle. When deciding which endoscope to choose, several factors must be considered, eg, elevator, stiffness or flexibility and length of the distal part of the endoscope, diameter of the distal end of the endoscope, diameter of the working channel, and the quality of the ultrasonic image.

The endoscope must also be carefully selected depending on the lesion or the place of the biopsy as well as the needle type available. It is evident that EUS-FNA with a small-channel endoscope, although more easily accepted by the patients due to its limited diameter, is more difficult in specific situations compared with a large-channel endoscope. This is especially evident when the endoscope is bent, such as in biopsies of pancreatic head lesions. In biopies of mediastinal lesions, where the endoscope is straight, the choice of endoscope is of less importance.

Regarding EUS-TCB, it is our experience that a large-channel endoscope should always be chosen due to the size and stiffness of the needle.

Needles

During early needle developments, sclerotherapy or transbronchial biopsy needles were used for EUS-FNA but were later abandoned due to their lack of stability and stiffness. In the early 1990s, a special biopsy instrument (GIP-Medizintechnik/Medi-Globe GmbH), was developed by one of the authors (P.V.) and Dr. Søren Hancke (Fig. 3).[2,4] At present, several needles and needle types are commercially available (both reusable and disposable) (Table 1). Disposable needles are most frequently used, and reusable needles are mainly used in Europe.

FNA devices have three main interlocking components. The handle assembly is composed of handle, needle piston, and sheath (Fig. 4A and B). The handle connects the piston, sheath, and needle to the echoendoscope (Fig. 5A–C). A stiff steel needle for FNA is the "core" of this biopsy instrument. The needle is manipulated by a handle piston in the biopsy handle. The handle piston can be locked and unlocked by means of a button (or screw) to avoid advancement of the

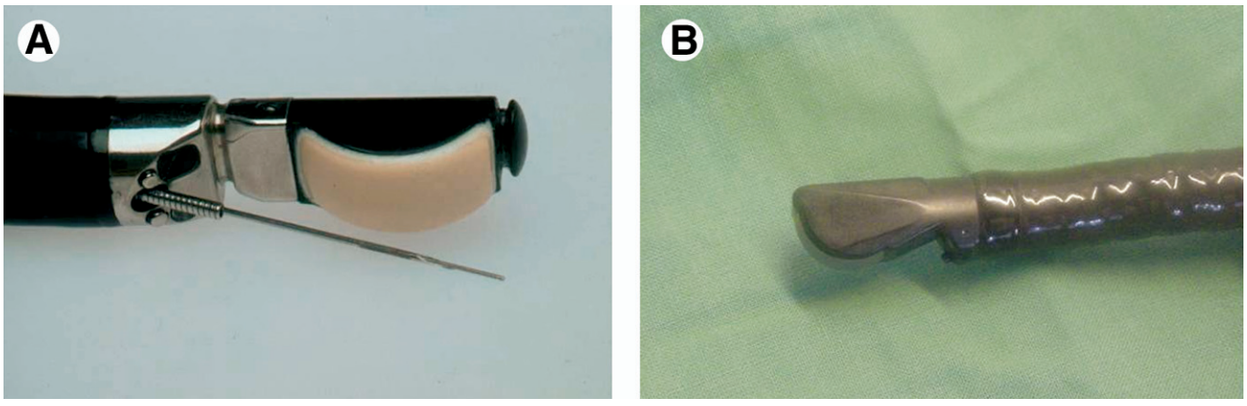


Figure 2 (A) The distal end of a Pentax endoscope without elevator. A needle is extending beyond the biopsy channel outlet. (B) The distal end of a Toshiba/Fujinon endoscope without an elevator. (Color version of figure is available online.)

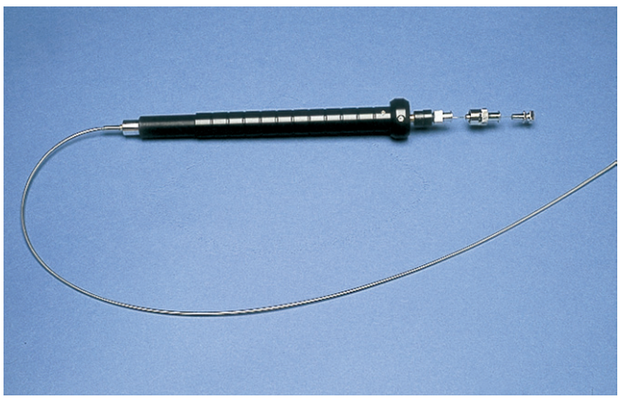


Figure 3 The first dedicated biopsy instrument for EUS guided fine needle biopsy was developed by one of the authors (P.V.) and Dr. Søren Hancke in Denmark (GIP-Medizintechnik/ Medi-Globe GmbH, 1993). (Color version of figure is available online.)

needle during introduction and withdrawal of the biopsy assembly (Fig. 6A and B). The needle is supported by a stable metal spiral sheath which is firmly connected to the handle. The handle can be firmly connected to the endoscope using a Luer-Lock (Fig. 5A). When the handle is screwed on the Luer-Lock connection of the endoscope, the metal spiral should extend 5 to 8 mm out of the distal outlet of the working channel, so that the needle cannot damage the instrument channel of the endoscope (Fig. 7).

Inside the hollow needle, there is a stylet that has, in its original version, a rounded tip to avoid perforation of the spiral sheath and damage of the working channel (Fig. 8A and B). Needle assemblies with a beveled stylet are also available. All other FNA needles are constructed based on the same principle as described, but several of these have added a fourth component to the needle assembly, ie, a component for adjustment of the lengths of the sheath to fit different types of endoscopes, since there are variations in the length of the biopsy channels between different manufacturers and even between similar types of endoscopes (Fig. 5C).

Disposable FNA needles

The introduction of disposable FNA needles was a major advance in EUS. These FNA needles are user-friendly, single-use, and most importantly, the handles and pistons operate nearly flawlessly and the disposability provides increased convenience. There are at present three companies that offer these devices. There are no major differences in the basic handling of these devices. The major difference is mainly how the handle piston is positioned on the instrument, with the Vizeon Needle/Sonotip II Needle System placed *inside* the handle portion (Fig. 8B) and the Echotip and Ezshot needle placed *over* the handle portion (Fig. 9A and B).

Vizeon Needle/Sonotip II Needle

A disposable, two-stage device was introduced by Medi-Globe on the European market several years ago, and an improved version was recently introduced on the US market (Vizeon Needle System, Conmed). The needle assembly was constructed in collaboration with one of the authors (P.V.) and based on principles and experiences obtained with the first reusable needle system mentioned (Fig. 3). The design is similar to previous models, except that the handle and piston are made of plastic. However, the Luer-Lock connection is still made of metal, which is preferable to obtain a more stable connection with the endoscope compared with plastic connections as in most other reusable needle devices. The piston inserts into the handle assembly and can extend the needle 8 cm beyond the sheath. The length of the sheath can be adjusted to the lengths of the working channel of the endoscope by manipulating of the sheath length's adjuster on the handle (Fig. 7). Adjustment of the length of the sheath of as much as 3 to 4 cm can be made. Once the desired length of the sheath has been selected, the handle position is locked by means of a screw on the handle control (Fig. 7). The sheath is a metal spiral sheath. A version with an extended sheath size at the distal end is available, constructed to better fit into large channel endoscopes (Fig. 10A and B).

The needle is a continuous stainless steel hollow needle and is provided in a 22- or 19-gauge size. The beveled tip of the needle is sandblasted, or in the latest version, laser-treated, to improve the visibility of the needle under ultra-

Table 1 Characteristics of Different EUS-FNA Needles Used for EUS-Guided FNA

Company/Model	Needle	Adjustment of Sheath Length	Disposable/ Reusable	Material
Conmed/Medi-Globe				
Hancke / Vilmann	19-22G	–	Reusable	Metal spiral sheath
Vizeon/Sonotip II	19-22G	+	Disposable	Metal sheath
Olympus				
NA-10J-1	22G	–	Reusable	Metal spiral sheath
Power shot (NA-11J-KB)	22G	+	Reusable	Metal spiral sheath
Ez-shot (NA-00H-8022)	22G	–	Disposable	Hard plastic sheath
Wilson-Cook				
EUSN-1,19,25	19-25G	+	Disposable	Different materials
ECHO-1-22	22G	+	Disposable	Plastic sheath
ECHO-19	19G	+	Disposable	Metal spiral sheath
ECHO-25	25G	+	Disposable	Thin, very flexible
EUSN-19-QC Quick-Core	19G	(+)	Disposable	Plastic sheath, trucut

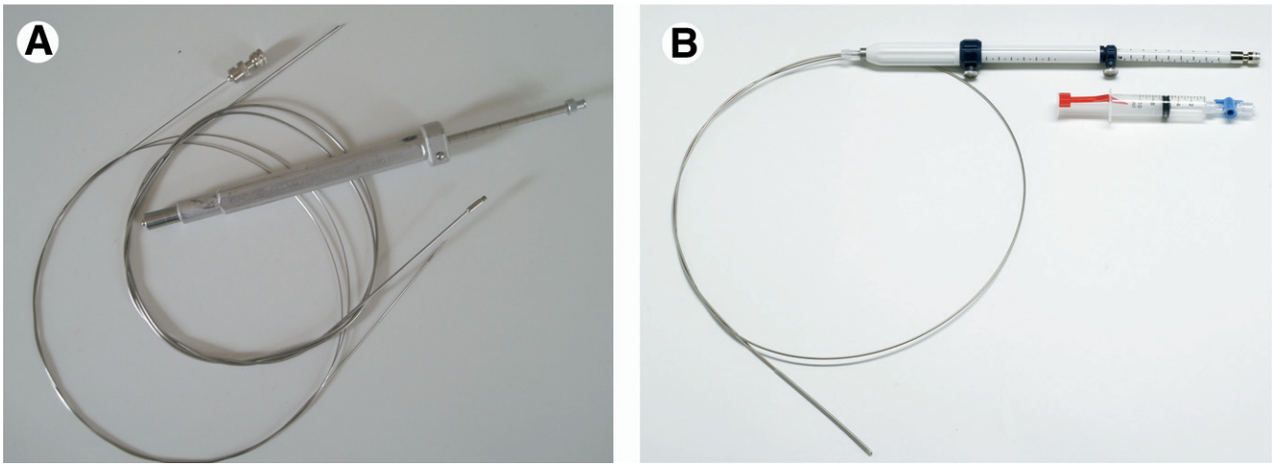


Figure 4 FNA devices have three main interlocking components. The handle assembly is composed of handle, needle piston, and sheath. (A) A multiple-use needle system with handle, sheath, and needle separated (MediGlobe, Type Hancke/Vilmann). (B) A single-use system (Vizeon Needle/Sonotip II, Conmed/MediGlobe). (Color version of figure is available online.)

sound imaging (Fig. 11). The sandblasted marking covers about 5 to 7 mm close to the end of the needle tip. The needle is provided with a nitinol stylet that has either a round tip or a beveled tip.

Echotip Needle

The Echotip needle was made by Wilson-Cook (Fig. 12). The sheath (length: 140 cm) and handle are permanently connected and therefore cannot be exchanged. The plastic handle is attached to the endoscope using a plastic Luer-Lock connector. Wilson-Cook recently released the Echotip Ultra ultrasound needle which has a two-stage plastic handle with an ergonomic handle design (Fig. 9A). The Wilson-Cook FNA devices come in three different needle gauges: 19-, 22- and 25-gauge. All needles are beveled and a segment of the needle tip is dimpled to enhance the visibility of the needle under ultrasound imaging. There are three different types of the sheaths, but all are flexible, covered, metal designs.

Although the disposable one-stage FNA devices have proven extremely successful, the fixed length of the sheath has limited its widespread use in echoendoscopes of different channel lengths. In some echoendoscopes, the FNA sheath extends out of the echoendoscopes for more than 2 cm, whereas in other scopes, the sheath may be too short and the needle is not fully covered. The Echotip Ultra addresses this issue with the ability to adjust the sheath length on the two-stage handle. The handle piston is advanced over the main body of the needle assembly. The maneuvering is basically similar to the above-mentioned device.

Ezshot Needle

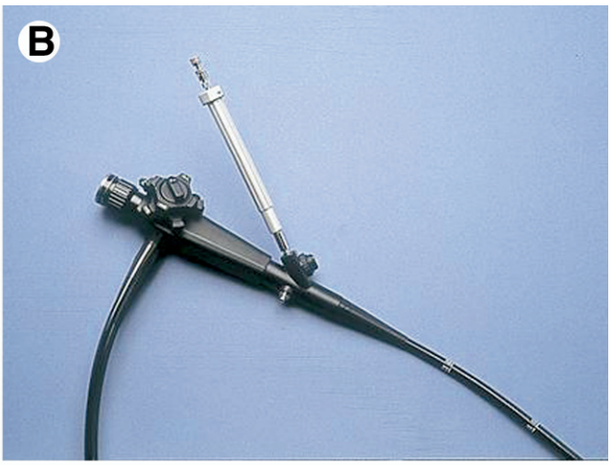
The Ezshot needle is made by Olympus (Fig. 9B). The handle is made of plastic, the handle piston being moved over the handle body similar to the Echotip needle design. The Luer-Lock connection is made of hard plastic, which may be less stable during hard punctures compared with metal connections. The system has no part for adjustment of the sheath lengths. This means that one has to confirm that the needle sheath does not extend too far beyond the biopsy channel when the handle is mounted on the endoscope. The consequence of this would be that insufficient contact between the transducer and the GI wall will result. However, if the length is acceptable, the device itself has the advantage that it is shorter than other disposable needle assemblies. The sheath is made of hard Teflon.

Reusable FNA Needles

The FNA device by Medi-Globe has been in use for many years. The handle assembly (piston, shaft, and sheath) is made of metal and can be used for a larger number of procedures (Fig. 4). The needle and stylet are designed to be used for a few passes of the needle in a single patient and should not be re-used in different patients. Initially, the stylet was made of stainless steel but was easily bent during re-entry into the needle. Newer stylets are made of nitinol and resist kinking because of their great flexibility.

A detachable, flexible, spiral, metal sheath is attached to the handle and is designed to accommodate a 22- or 19-gauge needle (Fig. 4). The sheath, after it is attached to the handle by a screw mechanism, can be readily passed through the instrumental channel of the scope. The entire handle assembly attaches to the scope using the Luer-Lock overlying the accessory channel (Fig. 5B). The needle device is compatible with both Olympus and Pentax echoendoscopes.

The newest reusable FNA device is marketed by Olympus (Fig. 13). This device, like the Medi-Globe needle, employs a reusable handle and sheath with a disposable needle and stylet. The 22-gauge needle is available with a maximum insertion portion diameter of 2.35 mm and a working length of 145 cm. The sliding sheath design allows some flexibility to accommodate different scope lengths and varying scope positions to facilitate FNA. The handle assembly, needle, and stylet are made of stainless steel, and the outer sheath is a stainless steel spiral. The surface of the distal end of the needle is processed with ring-shaped dimples to increase echogenicity. The stylet is fashioned into a sharpened conical shape to enhance tissue penetration while minimizing con-



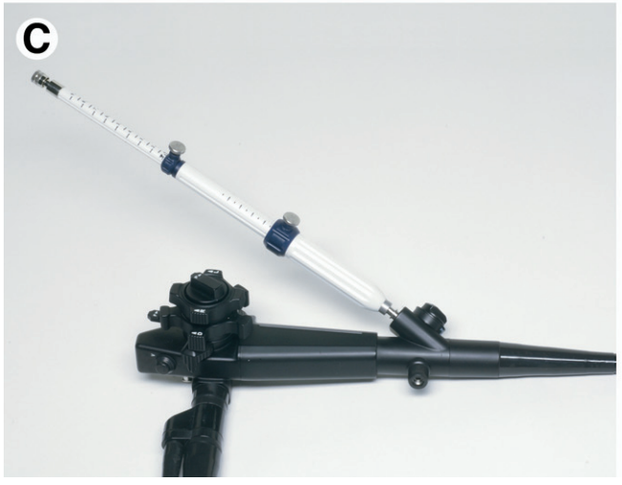


Figure 5 The handle connects the piston, sheath, and needle to the echoendoscope. (A) The handle is Luer-Locked to the endoscope channel inlet. (B) A single-use (disposable) needle system (Vizeon Needle/Sonotip II, Conmed/MediGlobe) connected to the endoscope. (C) A reusable needle system connected to the endoscope (MediGlobe, Type Hancke/Vilmann). (Color version of figure is available online.)

tamination of specimens with gastrointestinal mucosa. The needle can be extended from the sheath for a distance up to 65 mm to maximize successful targeting.

Spring-Loaded FNA Devices

The first spring-loaded FNA device was originally described by Dr. Binmoeller in 1997.[12] In the original configuration, it was designed to obtain cytology material using aspiration as well as histologic material using core tissue biopsies. The spring-loaded feature was designed to improve the ability to penetrate dense pancreatic tissue. The device required the use of a 2.8-mm channel echoendoscope and was never manufactured nor made commercially available, but provided a prototype for manufacturers.

Recently introduced, the Power Shot Needle from Olympus is a spring-loaded needle device that is reusable (Fig. 14). Designed to aid endosonographers in accessing hard lesions, the Power Shot Needle provides a method for the rapid and forceful placement of needles into firm tissues, such as gastrointestinal stromal tumors, pancreatic malignancies, and thick pseudocyst walls. The needle and stylet are composed of stainless steel, and the protective sheath is a stainless steel spiral. The tip of the needle is marked for enhanced ultrasound visibility by a process which creates ring-shaped dim-

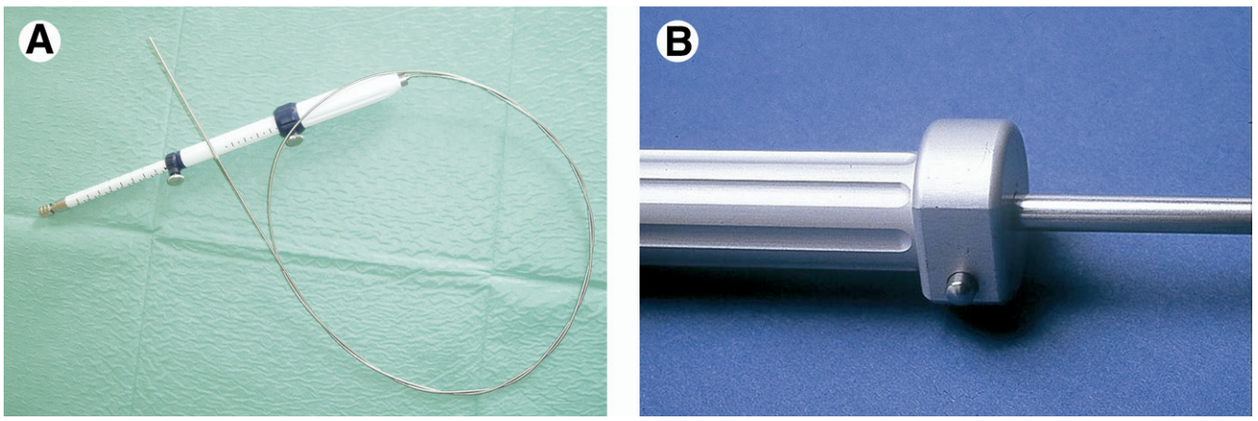


Figure 6 The handle piston can be locked and unlocked by means of a button (or screw) to avoid advancement of the needle during introduction and withdrawal of the biopsy assembly. (Color version of figure is available online.)

ples on the distal portion of the needle similar to those found on a golf ball. The maximum insertion portion diameter of the 22-gauge needle is 2.35 mm with a working length of 1450 mm. The needle offers a maximum automatic piercing stroke of 30 mm and a maximum manual piercing stroke of 60 mm for a total of 90 mm.

Tissue Core Needles

The first core-needle device designed for EUS was a tru-cut needle designed by Caletti.[13] A special guillotine biopsy device was designed to provide histologic material from submucosal lesions of the stomach. Binmoeller reported the use of an 18-gauge needle that provided cytologic and histologic material.[14] However, neither of these needles are commercially available any longer. Recently, a TCB needle (QuickCore; Wilson-Cook Medical Inc, Winston-Salem, NC) has been introduced (Fig. 15). Initial experience in swine[15] and later in humans[16] demonstrated the ability of this device to acquire histologic tissue representative of the target organ sampled. These initial studies suggested greater diagnostic accuracy when obtaining biopsy specimens with the EUS-guided TCB (EUS-TCB) device compared with EUS-FNA needles for submucosal mass lesions and lymphoma, and potentially the need for fewer needle passes for the diagnosis of solid pancreatic neoplasms. Recently, indications of EUS-TCB have expanded, including the diagnosis of autoimmune pancreatitis and cystic pancreatic tumors, disease processes that, until now, required surgical intervention to conclusively establish the diagnosis.

The EUS-TBC device contains a disposable 19-gauge needle and an 18-mm specimen tray that is large enough to allow collection of a tissue core sufficient for histologic examination. A standard spring-loaded mechanism is used within the handle to permit automated procurement of biopsy specimens (Fig. 16A and B). As with EUS-FNA needles, an adjustable "screw-stop lock" is incorporated into the handle and when unlocked allows advancement of the needle up to 8 cm. The handle also incorporates an "adjustment wheel," which rotates the device into the proper orientation and slightly varies the length. In addition, a "spacer" can be added to allow for the variation in echoendoscope length from different manufacturers (Fig. 17). The outer "catheter sheath" is made of hard Teflon. The needle consists of two components: an outer 19-gauge "hollow cutting needle" and a 18-mm-long "inner specimen tray" (Figs. 18 and 19).

Needle Selection

The technique of FNA is very similar with all needles and handle assemblies. A 22-gauge needle is used routinely for all lesions, pancreatic masses, and lymph nodes. Recently, a 25-gauge needle was introduced, and preliminary data suggest that the results with this thin needle may be comparable to the 22-gauge standard needle; however, firm data are still missing. The use of the 19-gauge needle is usually reserved for a thick-walled pseudocyst, mucinous cystic neoplasms, or very firm pancreatic masses. The use of properly oriented, beveled stylets will minimize the contamination of cytology specimens with gastrointestinal mucosa. However, rounded stylets have the advantage of minimizing the risk of damage to the channel of the echoendoscope.

A consideration regarding the needle assembly should be mentioned when biopsies with large-channel endoscopes are performed. The sheath covering the needle should have a size not too small to fit the size of the biopsy channel because the ultrasonic monitoring of the needle during biopsy may be more difficult if the size difference between the needle sheath and the working channel is too large. There is a tendency, especially in difficult biopsies, that a thin needle sheath becomes "floppy" in a large channel, resulting in a difficult needle monitoring due to a lack of stable support. This tendency may be reduced by using the elevator. However, there are needles with an extended sheath size especially designed for large-channel endoscopes (Conmed/Medi-Globe; Fig. 10A and B).

Preparation of the Patient

EUS-FNA can safely be performed as an outpatient procedure. Laboratory tests are only necessary in patients on anticoagulants or with known or potential bleeding disorders. As with all endoscopic procedures, the patient should be fasting for at least 4 to 6 hours. Under normal conditions, conscious

Figure 7 When the handle is screwed on the Luer-Lock connection of the endoscope, the metal spiral should extend no more than 5 to 8 mm out of the distal outlet of the working channel, so that the needle cannot damage the instrument channel of the endoscope. The length of the sheath can be adjusted to the lengths of the working channel of the endoscope by manipulating of the sheath length's adjuster on the handle. Adjustment of the length of the sheath of as much as 3 to 4 cm can be made. Once the desired length of the sheath has been selected, the handle position is locked by means of a screw on the handle control (below). (Color version of figure is available online.)

sedation used in EUS-FNA is similar to that used in conventional endoscopy.

Biopsy Procedure

EUS-FNA

Before an EUS-FNA procedure is started, several issues need to be taken into consideration (Table 2). (1) Primarily, the procedure should be indicated with due regard to patient safety. (2) A cytologic diagnosis of a disorder and/or its stage should potentially affect the management of patients undergoing EUS-FNA. (3) Operator experience with the procedure needs to be factored in, before embarking on more difficult procedures. Contrary to what is expected, the stomach wall is often the most difficult to biopsy since it tends to move together with the needle, and most lesions that need EUS-FNA, ie, GIST tumors and leiomyomas, are submucosal and difficult to diagnose by FNA alone.[17] Based on our experience, a gradual escalation in the difficulty as familiarity with the procedure grows is advisable (Table 3). A large mediastinal tumor is a good case for a beginner to start with as in most cases it is easy to target.[18] (4) Patient cooperation is pivotal to reduce procedure-related complications. Sudden movements have to be avoided to prevent injuries. (5) Presence of a vessel at the needle tumor interface is a contraindication to EUS-FNA, and this possibility can be effectively excluded using color or power Doppler (Fig. 20). (6) The transducer has to be first brought into a stable position in front of the targeted lesion. Because the angle between the needle and the transducer is quite small, it is advantageous to position the lesion cranial in the image, close to the transducer, by using the up–down wheel of the scope before introducing the needle (Fig. 21A and B). The metal spiral is then introduced into the biopsy channel observing carefully that the needle piston is securely locked and the needle is completely retracted. The spiral is inserted entirely, and the handle with the Luer-Lock is firmly screwed onto the biopsy channel (Fig. 5A–C).

To ensure that the sheath is protecting the entire length of the working channel of the endoscope, two methods can be used: (1) By using the optic of the endoscope, it can be observed that the sheath is extending from the distal end in a secure distance of 3 to 5 mm, or (2) alternatively by controlling the position of the sheath guided by ultrasound (Fig. 21A). The needle should only be moved forward when the handle is firmly screwed onto the biopsy channel and the sheath is visible at the distal end. This avoids damage to the working channel by unintended disconnection of the Luer-lock connection during biopsy.

While monitoring the needle ultrasonically, the examiner must try to keep a very firm and stable contact between the transducer and the inner surface of the gastro-intestinal tract. This is done by deflection of the endoscope tip by means of the large wheel on the control body of the endoscope (Fig. 20A). Straightening of the endoscope should be done, especially when puncturing the pancreatic head (like the ERCP position), because these tumors are often very hard to biopsy.

Once the position is carefully adjusted, the needle with the attached stylet should be advanced until the biopsy direction can be estimated and the target can be reached easily. There is no doubt that an elevator is an advantage when very deep lesions have to be reached. However, it has to be taken into account that the needle with stylet is stiff and the activation of the elevator causes considerable strain to the sheath, the biopsy needle, and the stylet. Additionally, the flexion makes it more difficult to move the needle forward and backward during the biopsy procedure. In most cases, it is possible to adjust the direction of the biopsy needle insertion very precisely with the conventional endoscopic handle control buttons (Fig. 20A and B).

The next step is determined by the nature of the stylet, either round or sharp (beveled). The round type leads to significantly less damages of the instrument channel and should be preferred by the inexperienced. When performing a biopsy using a beveled stylet, advancement of the needle

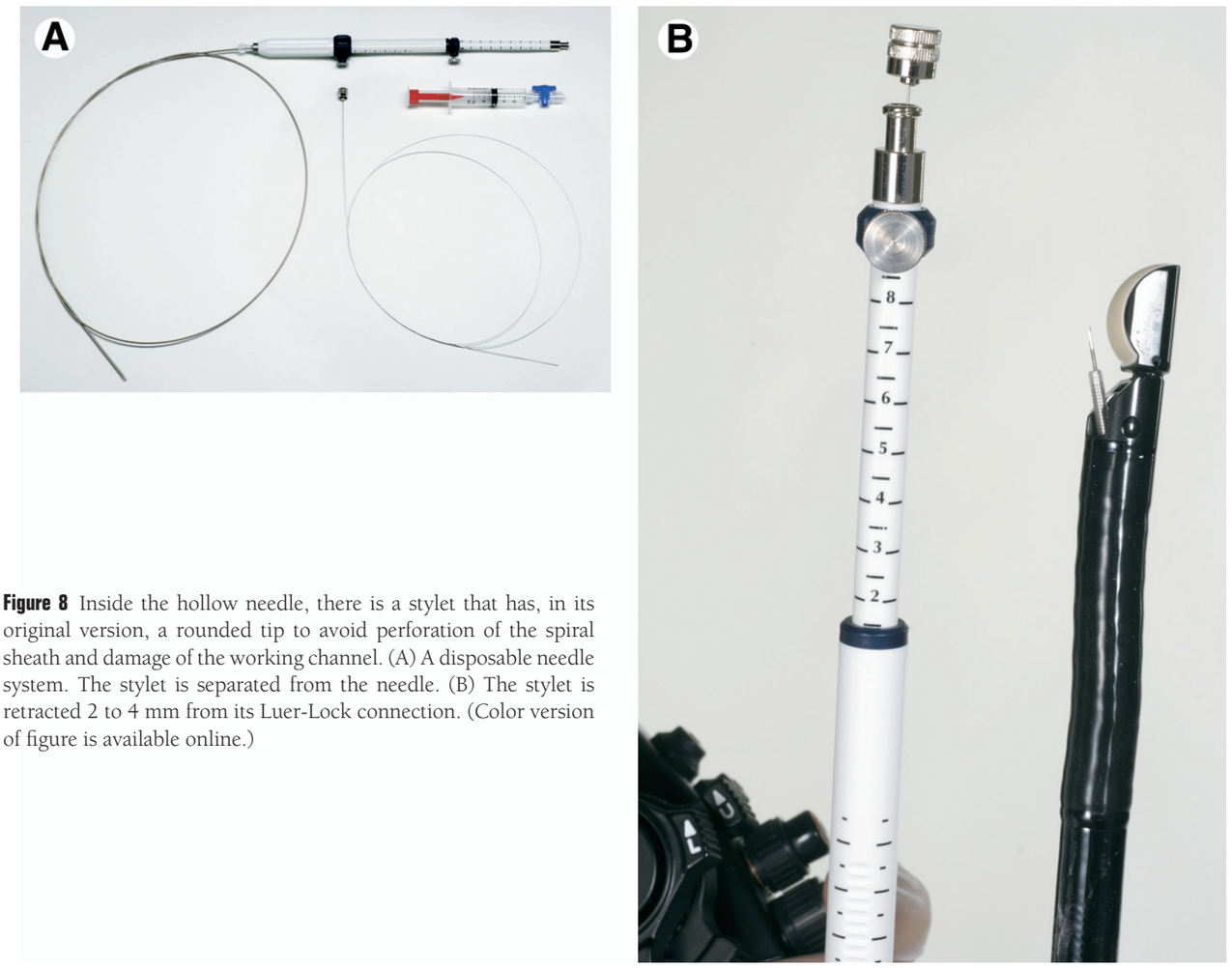


Figure 8 Inside the hollow needle, there is a stylet that has, in its original version, a rounded tip to avoid perforation of the spiral sheath and damage of the working channel. (A) A disposable needle system. The stylet is separated from the needle. (B) The stylet is retracted 2 to 4 mm from its Luer-Lock connection. (Color version of figure is available online.)

into a lesion can be performed directly after advancing the needle. When choosing a round type of stylet, it has to be retracted a few millimeters (as it normally extends beyond the needle tip by 2-3 mm) before performing the actual penetration through the gut wall (Fig. 8B). On retracting the stylet, the needle tip is exposed and can freely penetrate the tissue (Fig. 21B). It is often unavoidable that a few cells from the gut wall (esophageal, gastric, or duodenal mucosa) appear in the cytological material. Consequently, it is very important to inform the cytologist precisely about the method and the route of the biopsy.

With the stylet retracted but still inside the needle, the biopsy needle is now moved forward into the lesion under full real-time ultrasound control. Complete monitoring of the

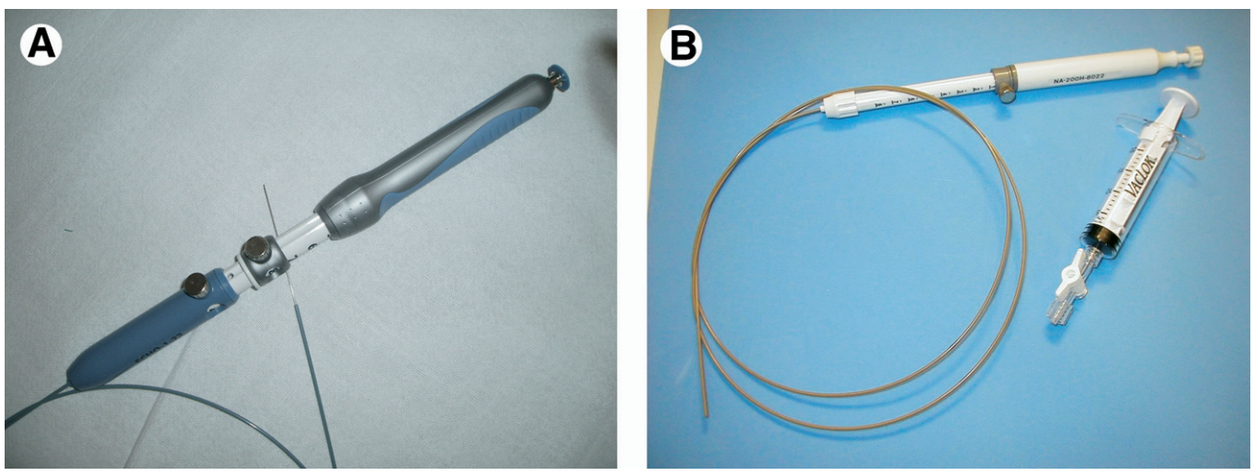


Figure 9 (A) The Echotip Ultra ultrasound needle system (Wilson-Cook) which has a two-stage plastic handle with an ergonomic handle design. (B) The Ezshot needle made by Olympus. (Color version of figure is available online.)

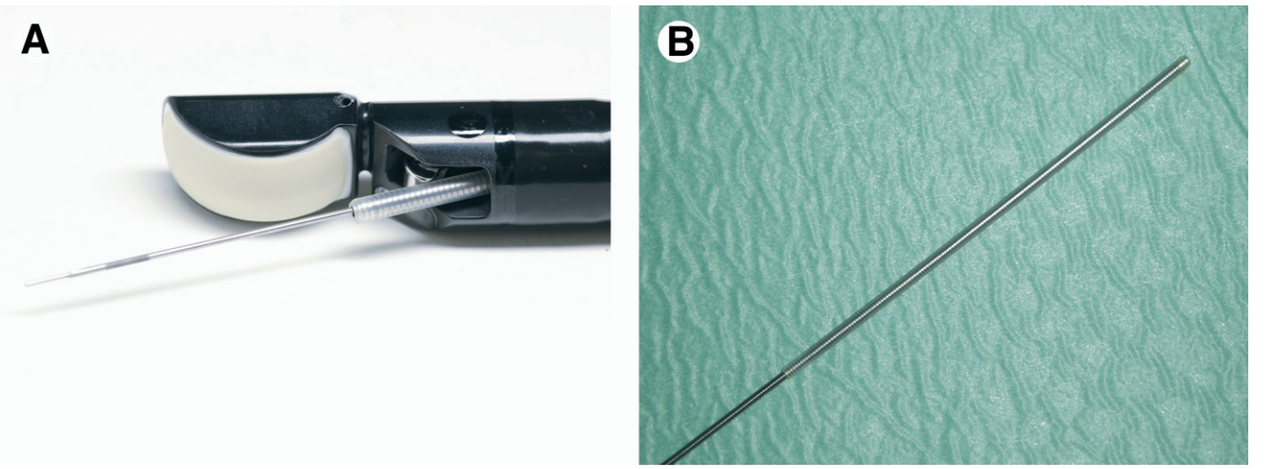


Figure 10 The sheath is a metal spiral sheath. The present version is shown with an extended sheath size at the distal end (2.7 mm). This is constructed to better fit into large channel endoscopes. (A) The needle is extending beyond the sheath. The stylet is still in place. (B) The distal part of the sheath with a plastic extention to better fit the size of a large channel endoscope. (Color version of figure is available online.)

needle tip is important whenever possible (Fig. 21B). If monitoring of the lesion fails, the needle should be retracted completely and a new biopsy should be started after smearing of the material. There are a few cases where a "punch" technique is the only way to penetrate a hard lesion. When puncturing hard tumors, a bending of the needle is sometimes observed; this may create problems with the monitoring of the needle tip and may potentially cause injury to surrounding structures and especially vessels. If severe bending of the needle is experienced, a new needle should be chosen for a repeat biopsy. There is also a tendency created by the needle advancement to push the transducer apart from the mucosa, thereby losing the ultrasonic image. The loss of ultrasonic needle monitoring may be avoided by inflating the balloon immediately after mucosal contact with the needle initially.

After penetration into the middle of a lesion, the stylet is re-advanced to the tip of the needle to push out any potentially needle-clogging tissue or body fluids before its removal. Then, a 10-mL syringe with a locking device is firmly screwed on the needle, pulling the syringe piston to create a low pressure (Fig. 22A and B). The syringe piston is locked in this position for permanent suction. It is important to remember that EUS-FNA needles have a beveled, "cutting-edge" design, which means that these needles obtain tissue by cutting or scrapping cells out of the lesion instead of removing them through applied suction.[19] Because the needle shaft is filling with tissue by cutting and scraping through the lesion, it is the inward thrusts of the needle that need to be rapid, whereas the pulling back motion can be slower. Just letting the needle sit motionless in the lesion with suction applied will only draw up a blood sample. The needle is now moved to and fro 5 to 10 times inside the lesion under complete ultrasonic control (Fig. 23). The number of back and forth motions needed has not been specifically studied, but some 10 or more motions commonly are used.[20] If considerable resistance is experienced when using the elevator during the biopsy, try to neutralize the elevator as soon as the needle tip is in the middle of the lesion. With the needle tip still in the lesion, suction is slowly released and the needle safely

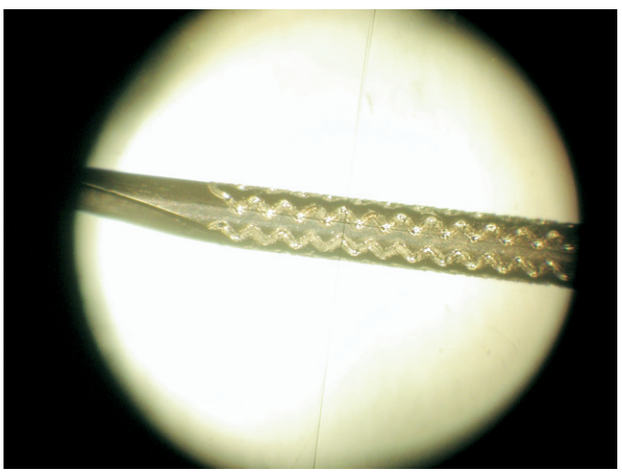


Figure 11 A laser-treated needle tip, to improve the visibility of the needle under ultrasound imaging. (Color version of figure is available online.)

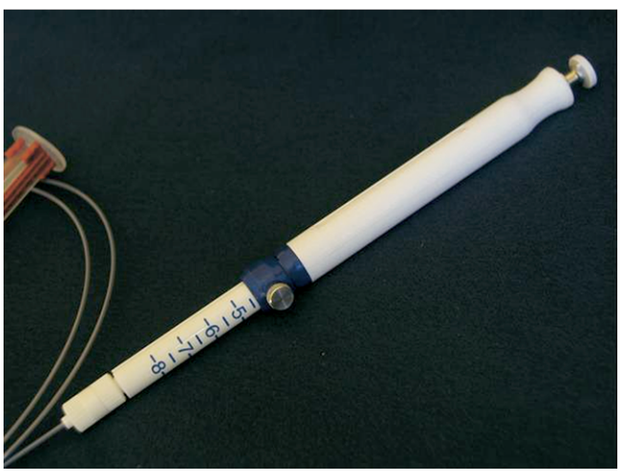


Figure 12 The Echotip disposable needle system (Wilson-Cook). (Color version of figure is available online.)

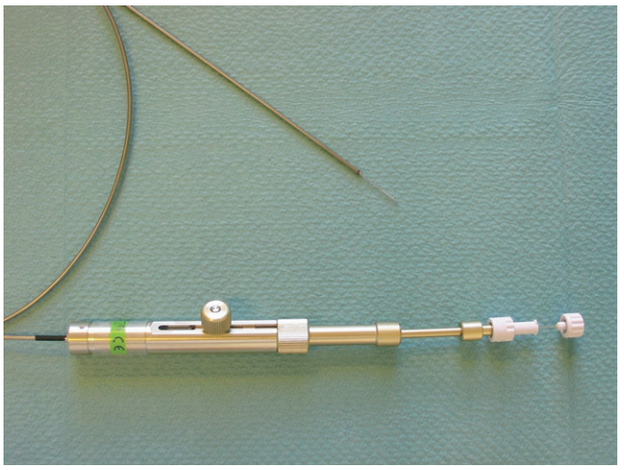


Figure 13 A reusable needle system with sheath, disposable needle, and stylet (Olympus). The length of the sheath can be adjusted to the lengths of the working channel of the endoscope by manipulating of the sheath length's adjuster on the handle to accommodate different scope lengths. (Color version of figure is available online.)

retracted inside the needle sheath and locked in a secure position. The complete needle assembly is now removed by disconnection from the inlet of the endoscope.

The number of needle passes needed to provide a high likelihood of a cytologically adequate sample is debated. Most endosonographers agree that pancreatic neoplasms are the most difficult to biopsy, so most of the data on the number of passes required for successful EUS-FNA of mass lesions come from series on pancreatic EUS-FNA. Interestingly, these series uniformly report that, on average, it takes 3 to 4 passes to provide a definitive cytologic diagnosis of a pancreatic malignancy.[21-24] There are no clinical or EUS features of pancreatic mass lesions that predict when a patient's lesion may need more FNA passes to make a diagnosis. The major determinant of FNA pass number appears to be the differentiation of the tumor,[25] with some lesions taking up to 10 passes or more to make a definitive diagnosis in well-differentiated tumors.[26] Malignant lymph nodes[27,28] and liver

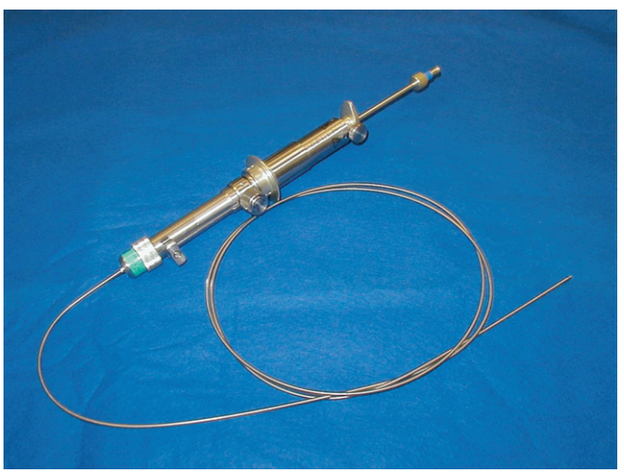


Figure 14 The Power Shot Needle from Olympus is a spring-loaded needle device that is reusable. The system is designed to aid endosonographers in accessing hard lesions. (Color version of figure is available online.)

Figure 15 A tissue core needle system (Quick-Core; Wilson-Cook Medical Inc, Winston-Salem, NC). Once the device is properly prepared, it is advanced through the echoendoscope and screwed securely onto the biopsy-channel Luer-Lock adapter. (Color version of figure is available online.)

metastases[29-33] usually require only 1 or 2 EUS-FNA passes for a definitive diagnosis with a range of only 1 to 4 passes. If a cytopathologist is not immediately available, generally 4 to 6 "good" passes (ie, not just blood or a scant sample) into a mass lesion, or 3 or 4 into a node or liver lesion, are recommended, realizing that this approach may still result in a nondiagnostic specimen in up to 15% to 20% of the time[34] or more.[35]

Only a few EUS-FNA methodologies have actually been subjected to formal studies. Bhutani and coworkers[36] reported that lymph nodes are best aspirated by using a 10-mL syringe under continuous suction than by intermittent suction with larger syringes. On the other hand, Wallace and coworkers[27] performed a randomized, controlled clinical study of lymph node aspiration techniques that suggested that applying suction actually worsened the results of node aspiration by increasing the risk of a nondiagnostic bloody aspirate. Sampling the node with no suction at all provided adequate cellularity with less blood contamination. Aspiration needle design probably makes little difference in the hands of an experience endosonographer. Larger needles (18- or 19-gauge) and tru-cut designs have been used to obtain actual core tissue samples[14,16,37-39]; however, use of these larger needles has failed to demonstrate that they significantly improve diagnostic accuracy for malignancies,[14,16,39,40] except perhaps in the case of unusual histology.[41] Spring-loaded needle designs also are available to help penetrate difficult-to-pierce lesions, such as extremely desmoplastic pancreatic masses or small, hard submucosal tumors; but, formal studies of the advantages of this design in specific lesions are lacking.

If several lesions are present during EUS examination, a decision should be made on how many of these lesions, which lesion, and in which order these lesions should be biopsied. A careful consideration incorporating knowledge of the TNM stage grouping of each disease is mandatory. It is obvious that a lesion suspected to be a distant metastasis should be biopsied before local lymph nodes and last the primary lesion, in successive order, if the same needle is to be used. If this is not done, a potential false-positive upstaging of

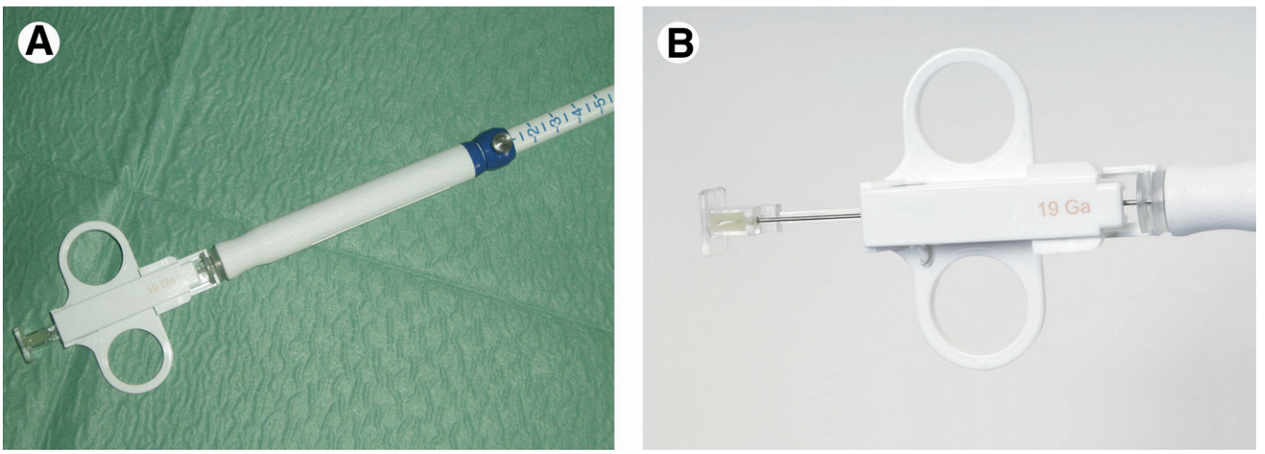


Figure 16 A standard spring-loaded mechanism is used within the handle to permit automated procurement of biopsy specimens. (A) The spring handle before it is retracted in the "firing" position. (B) The spring handle is retracted in the "firing" position, which retracts both the cutting sheath and the specimen tray. (Color version of figure is available online.)

the disease may occur. If another distant lesion is detected after an initial biopsy, a complete new needle should be chosen.

EUS-TCB

Before an EUS-TCB procedure, all requirements should be fulfilled as in EUS-FNA. The spring handle is retracted in the "firing" position, which retracts both the cutting sheath and the specimen tray (Fig. 16A and B, and Fig. 24). The inner needle remains in the withdrawn position until obtaining a biopsy specimen. Next, the needle is advanced by means of the handle piston until the tip is nearly flush with the catheter sheath. Under-advancement of the needle tip may result in inadvertent puncture of the catheter sheath and the echoendoscope, whereas over-advancement risks damage to the echoendoscope accessory channel. Once the device is properly prepared, it is advanced through the echoendoscope and screwed securely onto the biopsy-channel Luer-Lock adapter (Fig. 15). The needle is then carefully advanced under real-time EUS visualization (Fig. 25A–C).

Complete monitoring of the needle tip is important whenever possible. Once the needle is in the lesion, the spring handle is pressed forward until resistance is felt. Doing so advances the open specimen tray into the target lesion (Fig. 26). When targeting the lesion, one should realize that the specimen tray will extend approximately 20 mm beyond the hollow cutting needle tip. All controls (up–down and left–right) and the elevator should be released into a "relaxed" position. Further pressure on the spring handle will fire the device and obtain a biopsy specimen. Firing requires full depression of the plunger in the direction of the accessory channel. This action serves to spring fire the cutting sheath over the specimen tray. Distinct echo features are discernible for the cutting sheath and the specimen tray (Fig. 25C and D). This allows imaging of the specimen tray within the target tissue, thereby allowing monitoring of each step under continuous EUS imaging. The needle assembly is withdrawn into the catheter, the screw-stop is locked, and the entire appara-

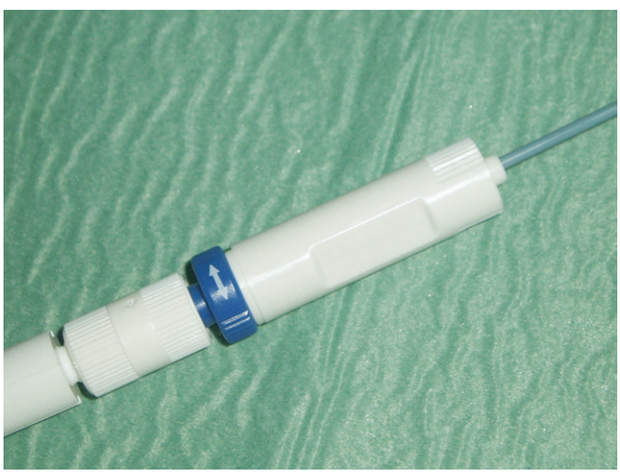


Figure 17 The handle also incorporates an "adjustment wheel," which rotates the device into the proper orientation and slightly varies the length and a "spacer" that adjust the sheath lengths to different lengths of the endoscopes. (Color version of figure is available online.)

Figure 18 The needle consists of two components: an outer 19-gauge "hollow cutting needle," and an 18-mm-long "inner specimen tray." The hollow cutting needle covers the inner specimen tray. (Color version of figure is available online.)

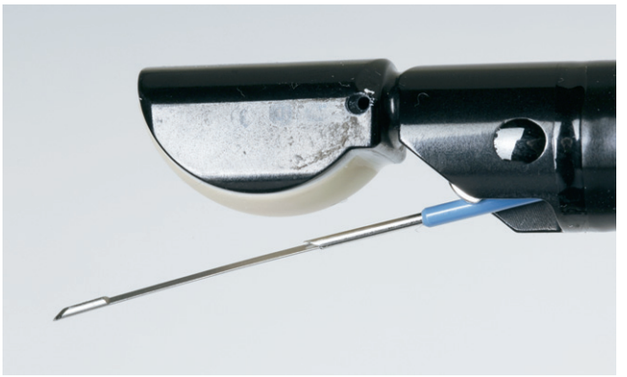


Figure 19 The 18-mm-long "inner specimen tray" is extended beyond the hollow cutting needle. (Color version of figure is available online.)

tus is removed from the endoscope. A similar technique is used for both solid and cystic lesions.

To increase EUS-TCB success rates, certain points need to be emphasized: (1) site of TCB, (2) proper device rotational orientation, (3) targeting, and (4) straightening of the echoendoscope and the needle.

1. The EUS-TCB device functions well in the esophagus, the rectum, and most of the stomach. Use is difficult and less often yields an adequate specimen when used in the fundus, the antrum, and the duodenal bulb, and is not recommended beyond the apex of the bulb. In all of these locations, echoendoscope angulation produces sluggish advancement of the cutting sheath over the specimen tray.
2. Proper device orientation can improve tissue collection, which is performed by rotating the needle so that the 19-gauge marker on the handle is aligned with the model number on the echoendoscope handle. Correct orientation of the handle results in alignment of the specimen tray so that it directly faces the transducer.
3. When initially advancing the needle, the 18-mm-long specimen tray and 1 mm of the distal stylet are retracted and contained within the cutting sheath. Therefore, as the spring handle is pressed, the device will advance another 19 mm, potentially leading to traversal of the lesion and puncture of adjacent structures. This is of particular concern for small lesions and those juxtaposed to a vascular structure. For such lesions, tissue can often be obtained only by partially advancing the specimen tray into the lesion so that some of the specimen tray is within more superficial tissues and/or the luminal mucosa. Caution is advised with this approach because of the potential for echoendoscope damage. Another approach for obtaining a biopsy specimen of smaller lesions and/or superficial lesions is to advance the specimen tray deeper than usual so that the specimen tray overlaps both the target tissue and deeper tissues. The safety of this approach depends on the tissue underlying the target lesion.
4. Keeping the echoendoscope and the needle straightened as much as possible, both within and outside of the patient, facilitates tissue acquisition. Elevator and tip deflection should be minimal. Once the target lesion is entered, the controls should be relaxed. Failing to do so results in sluggish advancement of the cutting sheath over the specimen tray. Attention to each of these technical aspects enhances tissue

Table 2 The Biopsy Procedure: Tips and Tricks

Preparation
Before introducing the needle check biopsy equipment with checklist:
• Are needle and stylet firmly screwed and connected?
• Make sure that the needle does not extend distally from the metal spiral.
• Is the needle piston locked firmly in the handle?
After introducing the needle into the instrument channel:
• Is the handle firmly screwed and connected with the Luer-Lock of the channel inlet of the endoscope?
Before advancing the needle:
• Is the metal spiral sheath visible in the endoscopic image?
Before the basic biopsy procedure:
• Check exact positioning of the transducer in front of the biopsy target, which should be in the needle's potential pointing direction.
• Exclude significant interposed vessels by using color or power Doppler techniques.
The biopsy procedure
• Advance the needle together with the stylet up to the inner surface of the gastro-intestinal tract. If insufficient acoustic coupling is experienced in the duodenum, fill the balloon shortly after needle contact with the duodenal wall.
• Try to keep the endoscope in a short position (like ERCP); bending of the endoscope in the duodenum might put significant strain on the needle and make the biopsy more difficult.
• Release the elevator after the EUS-guided tumor penetration, to reduce the resistance against the needle.
• When using a round stylet, retract it about 5 mm; a beveled stylet can be advanced together with the needle.
• The needle is advanced into the lesion, carefully monitored under ultrasonic control; the stylet is reintroduced to exclude obstructing tissue plugs inside the needle tip and then completely removed.
• Using a 10-ml syringe, a low pressure is created while the needle is moved about 5-10 times to and fro in the lesion.
• Low pressure is released while the needle is still in the lesion.
• The entire needle assembly can only be removed from the endoscope after complete retraction of the needle into the sheath. If the needle is not retracted completely. it may damage the entire instrument channel!
• The specimen is either expelled by air with a syringe onto glass slides or distributed evenly by re-introduction of the stylet into the needle. A beveled stylet can be advanced together with the needle.

Table 3 Difficulty Level of EUS-FNA in Increasing Order

9. Stomach wall (submucosal tumors)
8. Pancreatic head tumors
7. Peripancreatic lymph nodes
6. Pancreatic body and tail tumors
5. Perigastric lymph nodes
4. Adrenals
3. Liver lesions
2. Mediastinal lymph nodes
1. Large mediastinal tumors

acquisition and may improve patient safety and minimize instrument damage.

Preparing the Biopsy Material

The Smear

Under normal conditions after FNA biopsy, it is recommended to expel the biopsy material by air with the syringe immediately onto the prepared specimen slides. Another method that can be recommended at EUS-FNA is to re-introduce the stylet into the needle and move it slowly forward. This creates a high pressure in the needle, and the material can be expelled carefully and controlled droplet by droplet onto the specimen slides. The decisive advantage of this method is that numerous same-quality specimens can be prepared, whereas, when using the expelling by air method, a risk of uncontrollable expelling of the material may occur. This latter technique represents an important advantage, especially regarding the fact that immunohistochemical stains are often necessary and become more and more important. Using this method, it is namely easy to identify cylinders that can be processed with histological examinations. The technique of smearing should be discussed with the cytopathologist. For optimal smears, after the aspirate has been expelled onto the glass slide, another glass slide is placed upside down on top of the first slide (Fig. 27). Without applying pressure, the glass slides are moved in opposite directions. As a result, the material is spread in a thin layer. If the material is very thick with clumps, some pressure with the second glass slide should be applied. The material can also be spread with a small cover-glass with rounded edges.

There is some evidence in the literature that it is preferable to have an attending cytopathologist in the examination room for an immediate evaluation of the specimen.[35,42] A recent study demonstrated that conclusive cytologic diagnosis were achieved more frequently in the presence of an on-site cytopathologist compared with no cytopathologist at the endoscopy room (78% and 52%, respectively). Moreover, there were significantly lower insufficient material obtained (9% versus 20%).[35] Providing an on-site cytopathologist helps to ensure that the cytologic sample aspirated by the endoscopist is both representative of the target organ and adequate for a diagnosis.[43,44] Preliminary assessment of the specimen also allows the cytopathologist to prospectively identify cases that would benefit from additional aspirate for performing confirmatory special immunocytochemical stains, as in the cases of suspected GI stromal tumors (GIST).[45,46] On applying a ready-to-use staining liquid, it is possible to assess and evaluate the sufficiency of the specimen already after a few minutes. However, according to the experience of the authors, this is not absolutely necessary if just observing that the ultrasonic monitoring of the needle during the biopsy is optimal and that the macroscopic appearance of the material seems to contain minute tissue fragments. Thin bloody aspirates often do not contain material.

The further procedure, especially the fixation, should also be discussed with the cytopathologist. Generally, the specimen can be air-dried and fixed later, but some cytopathologists recommend immediately fixating and coloring the smear. Different stains are used for air-dried and alcohol-fixed smears, the most common being modified Giemsa, Diff-Quik, and Papanicolau stains, respectively. In addition liquid preservation of the aspirated material (PreservCyt) followed by ThinPrep slides can be used, although the accuracy of this split sample technique may be inferior to that of conventional smears.[47] When additional material is available, a micro-histological cylinder (cell block) can be prepared, formalin-fixed, and used for immunohistochemical studies, with a possible increase in diagnostic accuracy.[38,45,48,49] However, despite the excellent immunohistochemical possibilities, the

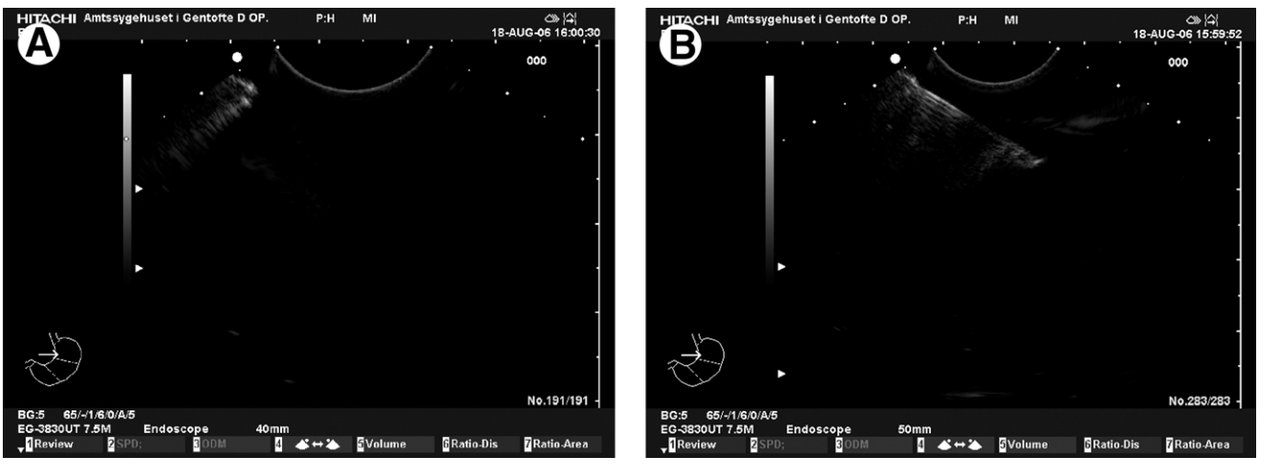


Figure 20 The sheath length is adjusted to the endoscope channel length guided by ultrasound before the needle is advanced. (A) An EUS image demonstrating the reflexions created by the metal sheath. (B) The needle is advanced after the sheath lengths has been adjusted. The needle is monitored during the entire biopsy procedure.

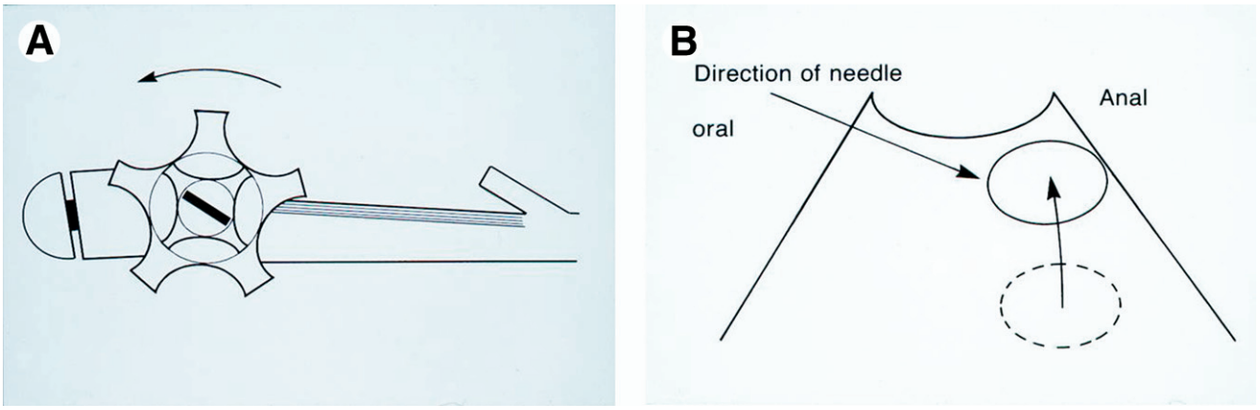


Figure 21 (A, B) The position of the lesion and the direction of the needle can be adjusted by using the up–down wheel of the scope. (Color version of figure is available online.)

diagnostic benefit of a histological specimen should not be over-rated. Flow cytometry and immunocytochemistry may increase the diagnostic yield of EUS-guided FNA used for the diagnosis of lymphoma. Molecular and/or genetic techniques are increasingly used for the analysis of the material aspirated during EUS-FNA. Although not ready for daily clinical practice, they may provide supplemental information in addition to conventional cytology.[50] Quantitative analysis of mutant K-ras gene may also enhance the diagnostic accuracy of conventional cytology, for the differential diagnosis of pancreatic cancer and focal chronic pancreatitis.[51,52] Comprehensive genetic analysis through PCR was reported to be possible for EUS-FNA biopsy specimens, enabling an assessment of the biological characteristics of pancreatic cancer before treatment.[53]

The TCB Specimen

To retrieve the tissue from the specimen tray, the screw-stop is unlocked and the needle assembly (cutting sheath and internal specimen tray) is fully advanced. The spring handle is then retracted until it clicks into the "firing" position, which retracts both the outer cutting sheath and inner needle specimen tray. The spring handle is advanced until resistance is felt. This action advances the inner specimen tray to expose the biopsy tissue. Once resistance is felt, stop pressing the plunger to avoid inadvertent firing. A needle is used to carefully remove the specimen from the specimen tray or simply place a tissue pad against the specimen to remove it. If needed, touch preps are made from the histologic core. The touch prep is prepared by gently touching a glass slide against the core of tissue. An onsite cytopathologist thereby can provide an adequacy assessment. The tissue cores are fixed in formalin, embedded in paraffin, and stained with H&E before histologic examination.

Microscopic Evaluation

There are some pitfalls which should be taken into consideration before interpretation of EUS-FNA results. A close cooperation between clinician and cytopathologist is essential.

Contamination of the aspirate with cells from the esophagus is very common and such squamous cells may invoke a false diagnosis of a metastatic lesion. Another problem is the interpretation of an EUS-FNA comprised of malignant and lymphatic cells. In aspirates from mesenchymal tumors, cellularity may be scanty and material may be present in clumps and clusters. The morphology of the cell on the periphery of these clumps is usually characteristic.

Complications

A multicenter study by Wiersema and Vilmann[21] showed that the complication rate was about 2%, with the majority being minor complications. Thus, nonfatal complications occurred for a rate of 0.5% (95% confidence interval, 0.1-0.8%) in solid lesions as compared with 14% (95% confidence interval, 6-21%) in cystic lesions. Other reports, as well as a recent ASGE guideline, reported the same complication rate of 1% to 2% for EUS-FNA, mostly with no severe or fatal incidents.[24,54]

The infection rate is also remarkably low, and antibiotic prophylaxis is usually not necessary after biopsy of solid lesions. The frequency of bacteremia as a complication of EUS-FNA has been prospectively studied in three separate trials, none of which included rectal EUS. These studies could not find any statistically significant increase in the rate of bacteremia after the biopsy.[55-57] EUS-FNA of mediastinal cysts, however, is associated with an increased risk of bacterial or fungal infection, which can lead to life-threatening mediastinitis.[58-60] Biopsy of pancreatic pseudocysts or cystic tumors is also an exception, and antibiotic prophylaxis is preferable to use due to a relatively high infection risk.[9,21,54] However, data concerning EUS-guided pancreatic cyst aspiration are conflicting, as two recent studies, including a large number of patients, found a low complication rate (1.2% and 2.2%, respectively) similar to that reported for solid pancreatic lesions.[24,61] Other studies have noted febrile episodes after EUS-FNA at a rate of 0.4% to 1%.[62,63] Based on these data, it can be argued that the risk of bacteremia after EUS-FNA is low and is comparable with that of diagnostic endoscopy.[64] Prophylactic antibiotics are not recommended for FNA of solid masses and lymph nodes. Some experts recommend prophylactic antibiotics as well as 48 hours of antibiotics after the procedure for EUS-FNA of the perirectal space.[65] EUS-FNA of cystic lesions appears to carry an increased risk of febrile episodes and possibly sepsis and, therefore, warrants

Figure 22 A 10-mL syringe with a locking device is firmly screwed on the needle, pulling the syringe piston to create a low pressure. The syringe piston is locked in this position for permanent suction. (A) The needle system with a 10-mL syringe. (B) The syringe is used for aspiration of cells during the EUS-guided biopsy (Dr. R.P.) (Color version of figure is available online.)

prophylactic antibiotics, as well as a short postprocedure course.

Mild or moderate acute pancreatitis has been described, especially when EUS-FNA biopsy of the pancreas is performed in patients with benign pancreatic diseases.[9,24,66-69] The rate of self-limiting acute pancreatitis was 1% after EUS-guided FNA biopsy performed in patients with suspected pancreatic cancer.[67] A large retrospective multicenter study

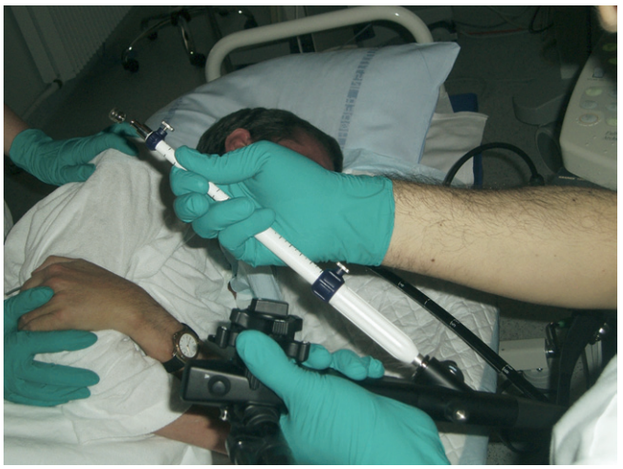


Figure 23 The image is obtained during an EUS-guided procedure. Note the position of the thumb and index finger of the endosonographer during to and fro movements of the needle piston. (Color version of figure is available online.)

also established that EUS-FNA is infrequently associated with acute pancreatitis after biopsy of solid pancreatic masses.[68]

Reported rates of pancreatitis associated with pancreatic EUS-FNA range from 0% to 2%.[9,24,67,69] One study evaluated pancreatitis specifically among 100 patients undergoing EUS-FNA (median 3.4 passes; range 2-9) and found a 2% rate of pancreatitis.[66]

Minor biopsy point bleedings are clinically insignificant, although they occur in as many as 4% of cases.[63] More heavy bleedings are rare, but they may occur due to shearing of the mucosa by the needle and injury of adjacent vessels when examining restless patients. Two episodes of clinically significant bleedings were described after EUS-FNA of pancreatic lesions, with one resulting in death.[69] Acute extraluminal hemorrhage at the site of the EUS-FNA is also rare, with a reported frequency of 1.3%, but no clinically recognizable consequences.[70] Acute intracystic hemorrhage can also rarely occur during EUS-FNA of cystic pancreatic lesions, without

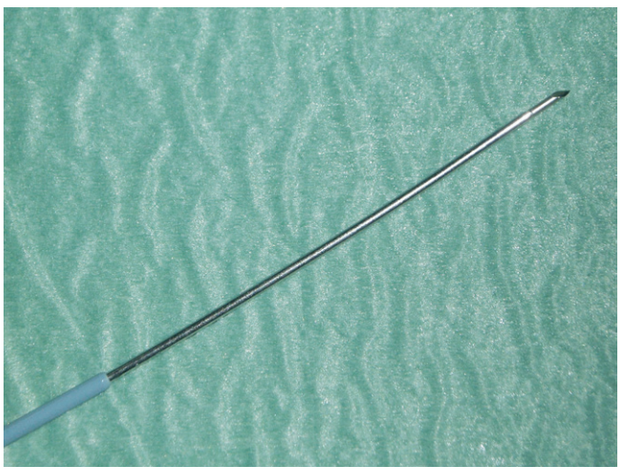


Figure 24 The distal end of the true-cut needle. The inner needle remains in the withdrawn position until a biopsy specimen is obtained. (Color version of figure is available online.)

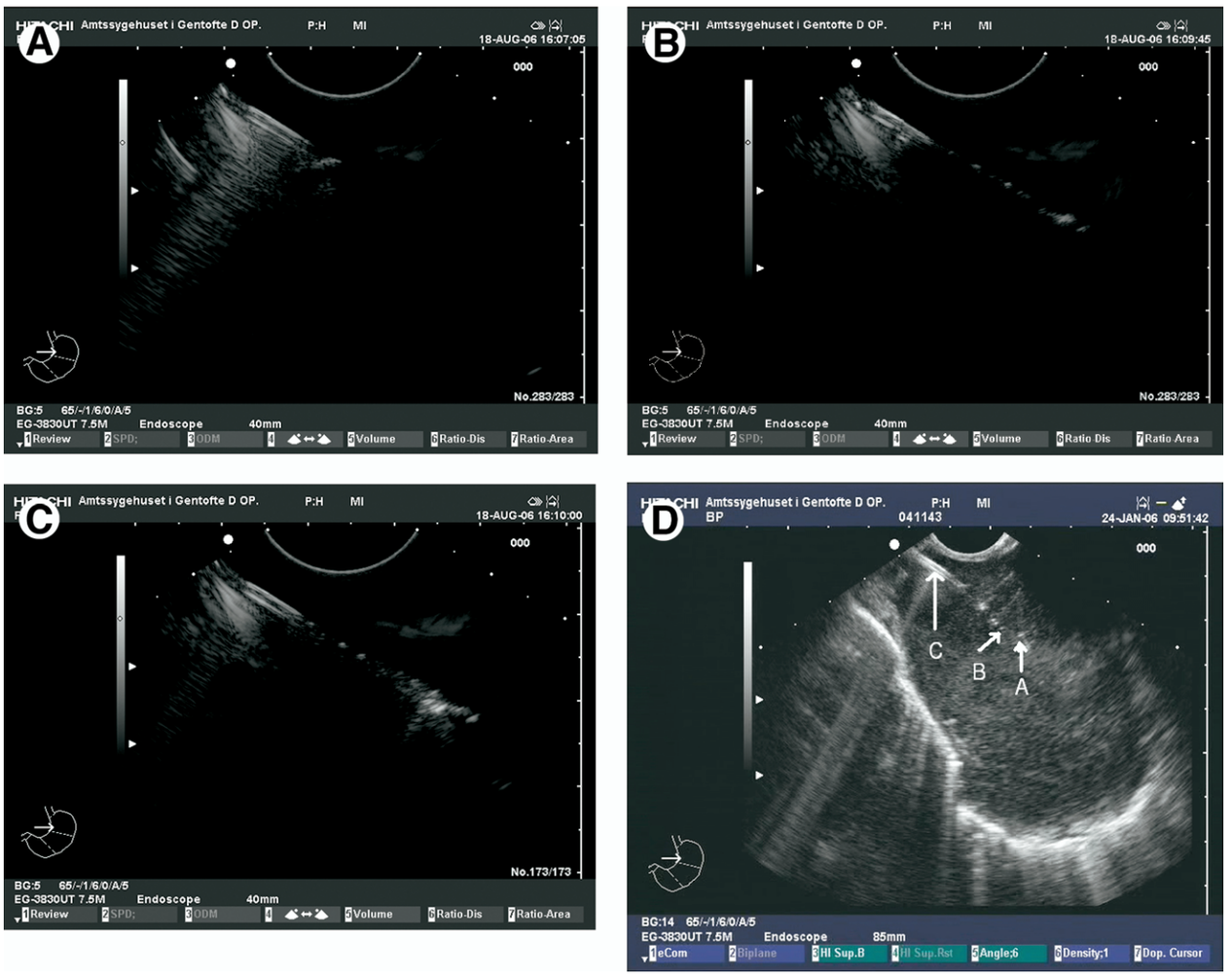


Figure 25 The needle is then carefully advanced under real-time EUS visualization. (A) Complete monitoring of the needle tip is important. Reflexions from the needle tip are seen. (B) Once the needle is in the lesion, the spring handle is pressed forward until resistance is felt. Doing so advances the open specimen tray into the target lesion. Reflexions from the specimen tray are seen. (C) Further pressure on the spring handle will fire the device and obtain a biopsy specimen. Firing requires full depression of the plunger in the direction of the accessory channel. This action serves to spring fire the cutting sheath over the specimen tray. Distinct echo features are discernible for the cutting sheath and the specimen tray. Reflexions from the hollow cutting needle are seen. (D) An EUS guided true-cut biopsy monitored by EUS. The lesion depicted was a mediastinal metastases from a renal cell carcinoma.

serious consequences, except a possible transient increase of abdominal pain.[71]

Bile peritonitis is a rare complication of EUS-FNA. One patient developed bile peritonitis after an EUS-FNA of a pancreatic-head mass that inadvertently perforated the distal common bile duct in a patient with biliary obstruction and ultimately required laparotomy.[72] During a study of the use of EUS-FNA to obtain bile directly from the gallbladder, in an attempt to identify patients with microlithiais, bile peritonitis developed in two of the first three patients enrolled. This resulted in termination of the study.[73] EUS-FNA of solid gallbladder masses has been reported as safe in one small series of six patients.[74]

Tumor cell seeding has always been a matter of discussion and concern; however, "hard facts" supporting this potential risk are not available yet. Only two cases of suspected seeding or dissemination after EUS-guided FNA biopsy have been published.[75,76] Moreover, it seems that EUS-guided FNA has

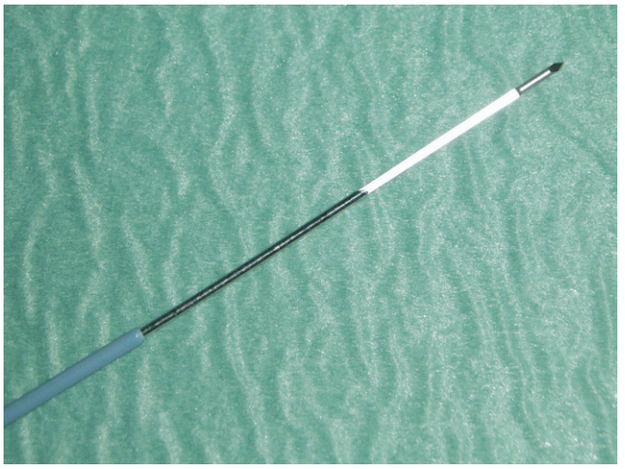


Figure 26 The spring handle is pressed forward until resistance is felt. This is either done to open the specimen tray into the target lesion during the biopsy or to collect the histological specimen after the biopsy. (Color version of figure is available online.)

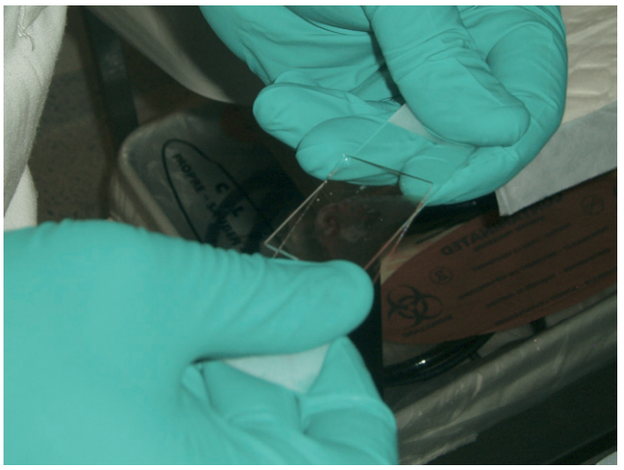


Figure 27 For optimal smears, after the aspirate has been expelled onto the glass slide, another glass slide is placed upside down on top of the first slide and smeared for a cytological evaluation. (Color version of figure is available online.)

a decreased risk of peritoneal contamination with malignancy as compared with CT-guided FNA (2.2% versus 16.3%).[77] Because of its advantages in imaging pancreatic neoplasms, high diagnostic yields, and the concern over needle-tract seeding with transcutaneous aspiration, the American Joint Committee on Cancer has recommended EUS-FNA as the preferred sampling technique in pancreatic masses if it is available.

It is not, in any way, recommended to penetrate through malignant tissue to reach a suspicious lesion. An example of this may be a patient with gastric or esophageal cancer and a suspicious lymph node adjacent to the primary tumor.

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