CHEST

Acquisition and Handling of Endobronchial Ultrasound Transbronchial Needle Samples

An American College of Chest Physicians Clinical Practice Guideline

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BACKGROUND: Endobronchial ultrasound-guided transbronchial aspiration (EBUS-TBNA) has become the standard for initial lung cancer diagnosis and staging. Previous guidelines have generally focused on the "when" and "how" of EBUS-TBNA; however, little guidance is available on handling and processing specimens during and after acquisition to help optimize both diagnostic yield and tissue integrity for ancillary studies. This document examines the available literature on EBUS-TBNA specimen processing and handling.

STUDY DESIGN AND METHODS: Rigorous methodology was applied to provide a trustworthy evidence-based guideline and expert panel report. Panelists developed key clinical questions using the Population, Intervention, Comparator, and Outcome format, addressing specific topics in EBUS-TBNA specimen processing. MEDLINE (via PubMed) and the Cochrane Library were systematically searched to identify relevant literature, supplemented by manual searches. References were screened for inclusion with document evaluation tools to assess the quality of included studies, extract meaningful data, and grade the level of evidence to support each recommendation or suggestion.

RESULTS: Our systematic review and critical analysis of the literature of the nine Population, Intervention, Comparator, and Outcome questions related to handling and processing EBUS-TBNA specimens resulted in nine evidence-based statements.

INTERPRETATION: Evidence of the handling and processing of EBUS-TBNA specimens varies in strength but is satisfactory in some areas to guide clinicians in certain aspects of specimen handling. Additional research in many aspects of specimen handling and processing is needed to help improve our knowledge base. CHEST 2025; 167(3):899-909

KEY WORDS: EBUS-TBNA; endobronchial ultrasound; specimen acquisition; specimen handling

ABBREVIATIONS: EBUS-TBNA = endobronchial ultrasound transbronchial needle aspiration; GRADE = Grading of Recommendations, Assessment, Development, and Evaluation; NSCLC = non-small cell lung cancer; PICO = Population, Intervention, Comparator, and Outcome; ROSE = rapid on-site evaluation; RPMI = Roswell Park Memorial Institute media

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Summary of Recommendations

1. In patients undergoing endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA), we suggest using either standard clinical practice (stylet, air) or alternative methods (needle rinse) for specimen sample expulsion (Conditional Recommendation, very low certainty of evidence).

2. In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest the use of either alternative collection media (formalin, Roswell Park Memorial Institute media [RPMI], saline, phosphate buffered saline) or current clinical practice (standard alcohol-based preparations) (Conditional Recommendation, very low certainty of evidence).

3. In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest using rapid on-site evaluation (ROSE) over usual care (Conditional Recommendation, very low certainty of evidence).

4. In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest using a smaller needle (21 gauge or 22 gauge) over a larger needle (19 gauge) (Conditional Recommendation, very low certainty of evidence).

5. In patients with suspected malignant disease undergoing EBUS-TBNA, we recommend performing four or more needle passes over three or less needle passes (Strong Recommendation, very low certainty of evidence).

6. In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using either alternative collection media (formalin, RPMI, sterile saline, phosphate buffered saline) or current clinical practice (standard alcohol-based preparations) (Conditional Recommendation, very low certainty of evidence).

7. In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using ROSE over usual care (Conditional Recommendation, very low certainty of evidence).

8. In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using either a smaller (21 gauge or 22 gauge) or a larger (19 gauge) needle (Conditional Recommendation, very low certainty of evidence).

9. In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using four or more needle passes over three or less needle passes (Conditional Recommendation, very low certainty of evidence).

Background

The introduction of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in the early 2000s[1] has led to revolutionary changes in evaluating lung cancer and intrathoracic disorders. The American College of Chest Physicians (CHEST) lung cancer guidelines report an overall median sensitivity of 89% and a negative predictive value of 91% using EBUS-TBNA.[2] As a result, the subsequent recommendation suggests that EBUS-TBNA be used over surgical staging techniques as the initial step in mediastinal staging during lung cancer evaluation. As the use of EBUS-TBNA has become more widespread, numerous questions have begun to arise about specimen handling to help maximize patient benefit and outcomes. Current guidelines describe *when* to use EBUS-TBNA[2] and *how* to use EBUS-TBNA[3]; however, limited information exists on EBUS-TBNA specimen management after retrieval.[4]

The objective of the present CHEST organization report was to examine the current knowledge on acquiring, handling, and processing EBUS-TBNA specimens in both malignant and nonmalignant diseases. We elected not to examine the decisions of when or how to perform EBUS-TBNA, but rather the major focus of this report was review of EBUS-TBNA retrieval and specimen handling.

Methods

Full methods can be found in e-Appendix 1.

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TABLE 1] PICO Questions

Question 1	In patients undergoing EBUS-TBNA, should alternative methods of specimen expulsion from the EBUS-TBNA needle be used compared to clinical practice?
Question 2	In patients being evaluated for malignancy undergoing EBUS-TBNA, should alternative collection media be used compared to clinical practice?
Question 3	In patients being evaluated for malignancy undergoing EBUS-TBNA, should rapid on-site evaluation be used?
Question 4	In patients being evaluated for malignancy undergoing EBUS-TBNA, should a larger or smaller needle be used?
Question 5	In patients being evaluated for malignancy undergoing EBUS-TBNA, should biopsy include four or more needle passes or three or less needle passes?
Question 6	In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should alternative collection media be used compared to clinical practice?
Question 7	In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should rapid on-site evaluation be used?
Question 8	In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should a larger or smaller needle be used?
Question 9	In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should biopsy include four or more needle passes or three or less needle passes?

EBUS-TBNA = endobronchial ultrasound transbronchial needle aspiration; PICO = Population, Intervention, Comparator, and Outcome.

The guideline panel consisted of two guideline chairs, 12 panelists, two methodologists, and an additional panelist serving as a liaison to CHEST's Guidelines Oversight Committee. The chairs and each panel member were reviewed for potential conflicts of interest and approved by CHEST's Professional Standards Committee. Panelists' conflicts of interest are detailed in e-Appendix 2.

The guideline panel developed nine key clinical questions using the Population, Intervention, Comparator, and Outcome (PICO) format (Table 1). A comprehensive search using MEDLINE via PubMed, Embase, and the Cochrane Library was performed to identify evidence that could inform clinical decision-making. The search (e-Appendix 3) was conducted in July 2021, and a pragmatic update was conducted in November 2023 to identify relevant studies published after the original literature search.

Results from the completed literature search were reviewed for relevance over two rounds of study selection (e-Fig 1). Structured data tables were used to extract relevant data from all studies included after the second round of screening. Relevant studies were summarized to inform recommendations for each PICO question.

The methodologist assessed the risk of bias in all included studies using various assessment tools, as appropriate, based on study design. After the quality assessment and data extraction were completed, the computer program Review Manager version 5.4 was used to perform meta-analyses when data were homogeneous and pool-able using a random effects model.

The overall certainty (quality) of the evidence was assessed for each outcome of interest using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach. Evidence profiles (e-Tables 1-19) were created using the GRADEPro Guideline Development Tool. The panel drafted recommendations based on the evidence that addressed the key clinical questions. Recommendations were graded based on the GRADE approach.

The panel voted on the direction and strength of the recommendation through a modified Delphi survey. As per CHEST policy, consensus was achieved through up to three rounds of anonymous voting or until 80% agreement in directionality was reached for each recommendation with at least 75% of the panel participating.

Results

Question 1: In patients undergoing EBUS-TBNA, should alternative methods of specimen expulsion from the EBUS-TBNA needle be used compared to clinical practice?

CHEST Recommendation: In patients undergoing EBUS-TBNA, we suggest using either standard clinical practice (stylet, air) or alternative methods (needle rinse) for specimen sample expulsion

(Conditional Recommendation, very low certainty of evidence).

There are minimal data on the optimal method to expel EBUS-TBNA samples for diagnostic yield and ancillary testing. As a result, the choice of an expulsion method for EBUS-TBNA should be based on local expertise and cytopathology requirements. Historically, most bronchoscopy needles have not possessed a stylet. Therefore, air has been used to expel the specimen onto cytopathology slides. The EBUS-TBNA needle system added an inner stylet to provide needle stability and clearance but can also be used to expel specimens onto slides.

Our literature search did not return studies addressing the impact of different methods of specimen expulsion. Stylet use has been investigated in a randomized controlled trial comparing EBUS-TBNA with or without the stylet.[5] No difference in diagnostic outcomes and/or quality of tissue specimens was identified, but stylet use was performed in both arms, therefore not allowing comparison. Additional literature exists in which specimens are expelled using the stylet or air to facilitate expulsion of the specimen; these studies, however, were designed to compare the media or preparation method (formalin vs alcohol, slides vs liquid media, etc.). Therefore, no available literature is available to compare these different methods of expulsion.

When EBUS-TBNA specimens are being prepared onto slides, the stylet and/or air can be used to expel the sample. When EBUS-TBNA specimens are being prepared directly into liquid collection media (ie, formalin, alcohol-based, etc.), any method of expulsion such as stylet, air, needle rinse, etc can be used. These decisions as well as any local laboratory requirements should be actively discussed with the cytopathology team to help define the best local practice.

Question 2: In patients being evaluated for malignancy undergoing EBUS-TBNA, should alternative collection media be used compared to clinical practice?

CHEST Recommendation: In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest using either alternative collection media (formalin, RPMI, saline, phosphate buffered saline) or current clinical practice (standard alcohol-based preparations) (Conditional Recommendation, very low certainty of evidence).

Limited data exist to suggest one specific collection media over the other. Alcohol-based preparations are generally considered standard for most cytological specimen preparation. However, numerous studies have used alternative collection media with acceptable results. Certain specimens may require specific media to allow for specialized testing (eg, suspicion of lymphoma and preservation in RPMI), so we recommend individualized discussion at the local level with cytopathology to determine the preferred media for each specific disease entity.

We did not find prospective studies directly comparing one medium to another. Additionally, local practice may dictate that alternative media may allow for additional sample analysis that may not be available with standard alcohol-based preparations (ie, some next-generation sequencing platforms or research trials may require formalin-fixed paraffin-embedded blocks).

A pooled analysis of nine studies examining diagnostic yield of different media collection methods[6-14] identified a diagnostic yield of 67.7% (1,280/1,860) for alternative collection media vs 74.1% (1,396/1,883) for current clinical practice, resulting in an OR of 0.51 (CI, 0.26-1.01). A pooled analysis of six studies comparing the diagnostic yield of formalin (65.2%) vs cytology smears (72.6%) resulted in an OR of 0.33 (CI, 0.11-0.98). Pooled analysis of eight studies comparing diagnostic yield of cell blocks (65.4%) vs cytology smears (73.3%) resulted in an OR of 0.41 (CI, 0.19-0.89). A pooled analysis of four studies examining diagnostic yield of paraffin-embedded vs cytology preparations revealed no significant difference in OR (0.37; CI, 0.11-1.23). Additional analysis of mutational analysis adequacy studies revealed minimal differences in media collection. One trial reported an increase in yield when using cytology smears (80.6%) compared to formalin-fixed paraffin-embedded cell blocks (61.2%).[15] However, two additional trials noted no significant difference when comparing cytology smears vs liquid-based cytology[16] or cytology vs histopathology samples.[17]

Ultimately, decisions on which medium and collection method are used should be discussed with the proceduralist and pathology department to help define the best local practice. There are likely benefits to discussions regarding the utilization of alcohol-based preparations and/or 10% neutral buffered formalin depending on the clinical needs (ancillary testing, etc.). Local laboratory processing may require certain methods and should be defined and disseminated.

Question 3: In patients being evaluated for malignancy undergoing EBUS-TBNA, should ROSE be used?

CHEST Recommendation: In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest using ROSE over usual care (Conditional Recommendation, very low certainty of evidence).

ROSE can be defined and may be practiced in different fashions; however, the general goals are to provide immediate feedback for sampling adequacy, triage sample material, and provide preliminary diagnostic information. Common practice environments involve cytopathology personal available in the near vicinity of procedure performance allowing for specimen preparation, evaluation, and triage at the point of patient contact.[18] The use of ROSE in EBUS has remained a controversial topic in diagnostic bronchoscopy. However, after reviewing the available literature, the panel concluded that ROSE is beneficial when compared to no ROSE.

Five observational studies evaluated diagnostic yield on using ROSE during EBUS-TBNA. A prospective observational trial (n = 81) reported diagnostic accuracy of 91% in the ROSE group and 83% in the non-ROSE group, resulting in a nonsignificant trend ($P = .08$) toward improved diagnostic accuracy in the ROSE group.[19] A prospective observational trial (n = 175) reported a diagnostic accuracy in the ROSE group of 100% and 86% in the non-ROSE group ($P = .005$).[20] Another trial examined 294 lymph nodes, reporting diagnostic specimens in 94% with ROSE, whereas 90% without ROSE.[21] Another trial of suspected lung cancer (n = 236) reported a final diagnostic yield of 92.1% with ROSE and 89.2% without ROSE ($P = 0.272$).[22] A trial of 260 patients undergoing EBUS-TBNA for parenchymal abnormalities reported a diagnostic yield of malignancy in 29.9% with ROSE and 11.1% without ROSE.[23] A pooled analysis of all five studies examining the diagnostic yield of using ROSE (78.0%) vs no-ROSE (71.4%) resulted in an OR of 2.35 (CI, 1.47-3.74) favoring the use of ROSE. Additionally, a single-institution randomized trial (n = 108) demonstrated a diagnostic yield of 85.5% in the ROSE arm compared to 75.5% in the non-ROSE arm.[24] This resulted in a nonsignificant trend toward improved diagnostic yield in ROSE (relative ratio, 1.13; CI, 0.94-1.37).

ROSE offers the potential to immediately identify sample adequacy and preliminary diagnostic information. As the incidence of new lung nodules suspicious for early-stage lung cancer increases, ROSE during EBUS-TBNA to stage multiple lymph nodes in a single setting *before attempts at peripheral nodule diagnostics* provides an invaluable resource to reduce procedural complications and potentially improve procedural efficiency. In cases with ROSE-positive lymph nodes, performance of peripheral bronchoscopy and its affiliated risk of pneumothorax and bleeding can be reduced. The overall complication profile of EBUS-TBNA itself remains low.[25] However, in a large multicenter registry in procedures where ROSE was not used during EBUS-TBNA, more transbronchial biopsies were ultimately performed, which was the strongest risk factor associated with complications (ROSE, 12.6%; no ROSE, 19.1%).[26] Other potential benefits of ROSE may include decreasing the number of passes performed, identified in two trials (3.4 vs 6.1, $P < .001$[20] and 2.7 vs 2.9, $P = .001$),[22] although importantly, these studies assessed the impact of diagnostic yield and not sufficiency rates to determine if samples were adequate for mutational analysis. In one study, ROSE was associated with a reduced percentage of nondiagnostic specimens (0.9% vs 4.4%, $P = .018$).[22] Some studies have also shown the use of ROSE resulting in decreased procedural times (32.3 min vs 50.3 min; $P < .001$).[20] However, other trials have identified no significant difference (37.6 min vs 37.4 min; $P = .883$).[20] A prospective, randomized trial of patients undergoing EBUS-TBNA with or without ROSE, identified a nonsignificant trend in the ability to complete molecular genotyping ($P = .09$) in the ROSE arm.[25] They also identified fewer samples in the ROSE arm with minimal tumor burden.[25]

While the available data on ROSE have remained controversial and debated, the authors do believe that ROSE is often able to offer intraprocedural insight, resulting in significant clinical impacts. The need to perform repeat procedures to obtain appropriate samples that can undergo molecular testing can significantly impact and delay patient care, an outcome we should strive to avoid. This recommendation is made with the knowledge that the current state of pathology staffing and reimbursement likely does not favor this approach given limited reimbursement models for ROSE services. Different models for delivering ROSE have been described, including the use of telecytopathology, trainees, cytotechnologists, artificial intelligence, etc, and may be more appropriate in some resource-limited areas.

Additionally, we acknowledge that interpretation of small biopsy specimens is a unique skill that not every cytopathologist has and may not be available at all centers. Given the benefits of ROSE for EBUS procedures described here, future advocacy efforts should focus on pathways for improved ROSE reimbursement to allow for needed resources and clinical growth of ROSE service.

Question 4: In patients being evaluated for malignancy undergoing EBUS-TBNA, should a larger or smaller needle be used?

CHEST Recommendation: In patients with suspected malignant disease undergoing EBUS-TBNA, we suggest using a smaller needle (21 gauge or 22 gauge) over a larger needle (19 gauge) (Conditional Recommendation, very low certainty of evidence).

Current EBUS-TBNA needles are available in multiple needle gauges (25, 22, 21, 19). The appropriate needle size remains a relevant topic of discussion as proceduralists are asked to provide more and/or higher quality amounts of tissue for diagnostic information and testing capabilities. Numerous trials have evaluated EBUS-TBNA needle gauges and their impact on diagnostic yield, including a large comparison of 21G to 22G needles that reported no significant difference in specimen adequacy or diagnostic yield.[27] The subsequent introduction of a 19G and 25G needle has stirred additional debate. After reviewing the current literature base, and given concerns with tissue integrity and/or specimen contamination with higher amounts of blood, the panel suggests that the use of smaller-sized needles (21/22 and/or 25 gauge) is preferred over the larger-sized (19 gauge) needle in known or suspected malignant disease. Additional studies are needed to determine differences between needle size and mutational testing adequacy.

Five observational studies evaluated diagnostic yield comparing needle gauge size during EBUS-TBNA. Two small trials reported no significant improvement in diagnostic yield with the 19G needle (22G [95%] vs 19G [93%] needle, $P = .62$[28]; 19G [89.4%] and 21G [88.7%] needle, $P = .71$[29]). A larger observational trial (n = 300) reported sensitivities for 19G, 21G, and 22G (95.7% vs 94.7% vs 87.5%) and accuracy (96.0% vs 96.0% vs 93.0%), which were not significantly different ($P = .76$).[30] Another trial (n = 411) reported diagnostic yields of 78.1% with 19G needles and 70.8% with 21G/22G needles.[31] Pooled analysis of these 1,813 patients notes an overall improved diagnostic yield with the 19G needle (89.5%) when compared to 21G/22G needles (83.2%) with an OR of 1.52 (CI, 1.14-2.03).

Two randomized trials comparing 19G to 22G needles were available to review. The first trial randomized (n = 78) to 19G vs 22G, reporting no significant difference in diagnostic yield (100% vs 100%; $P = .1$).[32] The median amount of DNA extracted trended higher in the 19G needle group (1,150 ng vs 818 ng; $P = .09$); however, no difference in next-generation sequencing testing was identified ($P = 0.59$).[32] A second randomized trial (n = 40) reported no significant difference in diagnostic yield, cytologic examination, histopathologic examination, or subsequent molecular analysis comparing 19G with 22G needles.[33] Pooled analysis from these two trials results in a nonsignificant relative ratio of 1.01 (95% CI, 0.94-1.09).

Outcomes reporting sample adequacy for molecular testing remain mixed; some suggest improvements with 19G needles,[29,34] some no difference,[30,32,33,35] and some with decreased adequacy.[28] One trial noted no significant difference in ROSE adequacy (41.1% vs 36.2%, $P = .27$), smear cellularity (19.9% vs 20.6%, $P = 0.89$), or cell block cellularity (26.2% vs 19.2%, $P = .21$) when comparing 19G to 21G needles.[29] A randomized trial of 19G vs 22G needles reported similar abilities to procure tissue cores for cell blocks (67% vs 72%; $P = .81$) and similar median tissue surface areas (6.0 mm^2 vs 4.6 mm^2; $P = .16$).[32]

There appears to be no significant difference in overall procedural complication rates of EBUS-TBNA needle sizes.[34,35] Some trials have also reported the "bloodiness" during sample acquisition. One prospective trial noted significantly more bloody samples using the 19G needle when compared to the 22G needle (58.9% vs 18.5%, $P < .001$).[28] Two randomized trials have reported significantly increased bloodiness/content when using 19G vs 22G needles.[32,34]

Current literature supports that 19G EBUS-TBNA needles obtain larger samples with more DNA. However, these surrogate outcomes should be validated against more patient-centered outcomes (ie, appropriate tissue testing to start treatment, need for additional procedures, etc) and balanced against what appears to be higher specimen contamination rates (increased blood contamination during 19G needle aspiration). This is not related to significant bleeding complications; however, excessive blood during ROSE may alter interpretation or may be responsible for the decreased yields noted in some studies. This potential, along with

the known increased cost related to the 19G needle, led the panel to suggest against the 19G needle in cases of suspected malignancy.

The panelists acknowledge that the 19G needle may be preferable in some populations/situations. The current 19G needle offers increased flexibility, particularly when compared to the 21G needle. In cases where larger samples with the potential for definition of some tissue architecture may be of benefit, the 19G needle may be preferable. The panelists also noted that while many have described using the 19G needle to deliver a "core" sample, this is not true. Inherent to its standard needle design, it is a larger aspiration needle; however, it does not reliably deliver a "core" tissue sample akin to the common acquisition of tissue with a core biopsy needle designed for such purpose. Additionally, many oncology clinical trials have often required "core" biopsies for eligibility which may be a somewhat arbitrary inclusion/exclusion criteria as smaller needles can give adequate (and even more cellular) samples. We would suggest that future clinical trials eliminate this terminology requiring "core" biopsies and rather base these criteria on the ability to obtain adequate tissue for appropriate (ie, molecular markers, etc.) testing.

Question 5: In patients being evaluated for malignancy undergoing EBUS-TBNA, should biopsy include four or more needle passes or three or less needle passes?

CHEST Recommendation: In patients with suspected malignant disease undergoing EBUS-TBNA, we recommend using four or more needle passes over three or less needle passes (Strong Recommendation, very low certainty of evidence).

The need for adequate tissue collection is paramount for patients with suspected underlying malignancy, including non-small cell lung cancer (NSCLC). As many patients with NSCLC present with advanced disease, the majority of patients undergo minimally invasive sampling to maximize risk/benefit. These procedures provide small histology and cytology samples instead of large resection specimens. Therefore, proceduralists must be aware and able to provide samples adequate for diagnosis and subsequent ancillary testing.

One trial (n = 1,700) reported specimen adequacy rates for one needle pass (66.9%), three passes (85.8%), four passes (88.6%), and six passes (100%)[36]; however, the overall population undergoing more than three passes was significantly less than those undergoing ≤ 3 passes, somewhat limiting the interpretation. Another trial of lung cancer patients (n = 453) identified that number of passes per lymph node was independently associated with successful molecular testing. Successful molecular testing was observed with needle pass 1 (52.4%), pass 2 (77.3%), pass 3 (82.9%), and reached 100% success at pass 4.[37] Another trial (n = 85) reported that a median of 4 (interquartile range, 3-5) passes being performed resulted in successful NSCLC molecular testing.[38]

Adequate tissue acquisition for both a diagnosis and subsequent ancillary testing has now become the standard of care for many malignancies, including NSCLC. The majority of data available regarding the number of passes and use of ROSE often references diagnostic yield. The ultimate goal of ROSE use is to assure that tissue sampling is adequate for all diagnostic purposes (cytopathologic diagnosis, molecular testing, etc.). Additional literature not included in the review also suggests that three to six needle passes provide adequate specimens for next-generation sequence testing in 80% to 90% of the samples.[39,40] However, the use of ROSE to dictate the number of passes needed for appropriate tissue acquisition for advanced molecular tissue analysis remains unclear, in particular as there are limited data on the number of passes needed specifically for molecular testing. At the time of ROSE, the ascertainment of specimens with high tumor content can be reviewed, and in collaboration with the cytopathology team, the number of additional needle passes may be decided.

Despite the lack of moderate to high certainty evidence in this area, the panel feels that due to the higher likelihood of successful molecular testing associated with a greater number of needle passes per lymph node, a strong recommendation is warranted considering the large potential for benefit and apparent absence of harm.

Question 6: In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should alternative collection media be used compared to clinical practice?

CHEST Recommendation: In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using either alternative collection media (formalin, RPMI, sterile saline, phosphate buffered saline) or current clinical practice (standard alcohol-based preparations) (Conditional Recommendation, very low certainty of evidence).

Similar to malignant disease, minimal data exist to suggest one specific collection medium over the other, and it is likely best based on local practice and processing. Many alcohol- and formalin-based media

are available and appear adequate for specimen testing. As the need for ancillary testing is generally not an issue in this population, additional validation of these techniques is less relevant.

A pooled analysis of eight observational studies examining diagnostic yield associated with different media collection techniques[6-9,11-14] identified a diagnostic yield of 72.5% (788/1,087) for current clinical practice vs 64.6% (670/1,037) for alternative collection media, resulting in an OR of 0.50 (95% CI, 0.29-0.85). Pooled analysis of four studies comparing diagnostic yield of formalin vs alcohol-based media[9,11-13] identified an OR of 0.60 (95% CI, 0.27-1.35), suggesting no significant difference. Pooled analysis of six studies examining diagnostic yield of cytology smears vs cell block[6,7,9,12-14] identified an OR of 0.37 (95% CI, 0.20-0.68) favoring cytology smears.

The ultimate decision as to which medium and collection method is used should be discussed with the proceduralist and pathology department to help define best local practice. Often, the diagnosis may be unclear at the time of initial needle puncture. ROSE may help triage specimens to different media if required/desired; however, if ROSE is unavailable, a medium that performs well in both malignant and nonmalignant disease should be used. Additionally, local laboratory requirements for processing should be broadly disseminated.

Question 7: In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should ROSE be used?

CHEST Recommendation: In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using ROSE over usual care (Conditional Recommendation, very low certainty of evidence).

The use of ROSE in EBUS has remained a controversial topic in diagnostic bronchoscopy, both in suspected malignant and nonmalignant disease. After reviewing the available literature, the panel concluded that ROSE is beneficial when compared to no ROSE in patients with suspected nonmalignant disease. A pooled analysis of 550 patients notes a diagnostic yield of 95.3% in the ROSE arm and 87.1% in the non-ROSE arm (OR, 2.76; 95% CI, 1.12-6.79).

This recommendation is made with awareness that limitations related to staffing, availability of ROSE resources, and reimbursement may not readily support such an approach. Different models for delivering ROSE may allow delivery of high-quality cytopathology without more traditional restraints. The use of ROSE for bronchoscopy procedures, including EBUS-TBNA, has also been proposed by the College of American Pathologists in prior guideline statements.[4]

Question 8: In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should a larger or smaller needle be used?

CHEST Recommendation: In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using either a smaller (21 gauge or 22 gauge) or a larger (19 gauge) needle (Conditional Recommendation, very low certainty of evidence).

The appropriate needle size for EBUS-TBNA (25G, 22G, 21G, 19G) remains a relevant topic of debate in both the malignant and nonmalignant arena. The introduction of a 19G and subsequent 25G needle has increased interest in improved diagnostics. At the time of the literature review, there were no available data on the 25G needle, and therefore we cannot provide a literature-based comment.

A pooled analysis of five observational studies[28,30,31,41,42] suggests an improved diagnostic yield when using a larger 19-gauge needle (219/259 – 84.6%) vs a smaller 21- or 22-gauge needle (316/459; 68.8%) resulting in an OR of 2.95 (CI, 1.01-8.61). There appears to be a moderate to serious risk of bias within these studies with fairly wide CIs. Existing literature suggests that smaller needles provide improved diagnostic yields (22 gauge = 72.8% vs 19 gauge = 46.0%),[28] with others reporting improved smear cellularity (21 gauge = 25.0% vs 19 gauge = 11.9%).[29]

Overall, there appears to be no significant difference in overall procedural complication rates of EBUS-TBNA when using different sized needles[35]; however, the bloodiness of specimens appears different. One observational trial noted significantly more bloody samples using the 19G needle when compared to the 22G needle (58.9% vs 18.5%; $P < .001$).[28] A randomized trial also noted significantly more "severe bloodiness" when using a 19G vs 22G needle (36% vs 8%; $P = .0035$).[32]

The decision as to which needle size to use may be difficult as the diagnosis at the time of bronchoscopy may be unclear. There appears to be no clear diagnostic yield advantage to using larger or smaller needles

from the current data. The panelists acknowledge that the 19G needle may be preferable in certain situations; however, its routine use should likely be tempered against the increased cost.

Question 9: In patients being evaluated for nonmalignant disease undergoing EBUS-TBNA, should biopsy include four or more needle passes or three or less needle passes?

CHEST Recommendation: In patients with suspected nonmalignant disease undergoing EBUS-TBNA, we suggest using four or more needle passes over three or less needle passes (Conditional Recommendation, very low certainty of evidence).

The acquisition of adequate tissue sampling remains important for any diagnostic procedure. Therefore, the number of needle passes performed during EBUS-TBNA should be balanced with the goal of maximizing diagnostic material but minimizing patient risk. Three studies were available for review, all identifying that increasing the number of needle passes was associated with increased yield.[36,43,44]

These limited data suggest that diagnostic yield may plateau around 4 passes for nonmalignant disease, suggesting that continuing to take multiple passes at one site may not be fruitful. This concept may be different in malignancy, where obtaining more tissue to help with ancillary testing may be necessary. Additionally, these trials were performed without the use of ROSE.[36,43,44] In situations where ROSE is available, the utility of performing additional passes should likely be left to the discretion of the bronchoscopist and team interpreting ROSE.

Discussion and Summary

This report provides an evidence-based approach to handling and processing EBUS-TBNA specimens for bronchoscopists. With CHEST methodologist guidance, we applied a rigorous evidence-based process and explored available literature on topics related to EBUS-TBNA specimen processing. Questions with acceptable levels of evidence were answered with recommendations based on the strength and quality of the data. Questions that lacked sufficient and appropriate data were addressed with a consensus-based statement generated from expert knowledge of the panelists.

As with many topics within the field of medicine, there is some evidence base for what we do. However, the supporting literature available is often limited, and this topic appears to be no exception. Our review has identified that many aspects of specimen handling and processing that may impact EBUS-TBNA yield remain unknown. As advanced molecular and ancillary testing continues to expand within the oncology space, the ability to do more with less tissue will continue to impact bronchoscopists and patients. Identifying optimal handling and processing of specimens will remain an important topic for future research.

We hope that this document provides clinicians with guidance for the handling and processing of EBUS-TBNA specimens during clinical care. This document, in conjunction with other prior guidelines, provide the when[2] and how[3] of EBUS-TBNA and what to do with specimens obtained during the procedure. Additionally, these recommendations may provide discussion points for ongoing collaboration with other specialties such as pathology.[4,45-50]

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Panelists' conflicts of interest are detailed in e-Appendix 2.

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Additional information: The e-Appendixes, e-Figure, and e-Tables are available online under "Supplementary Data."

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