

# Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies

## Guideline From the College of American Pathologists in Collaboration With the American College of Chest Physicians, Association for Molecular Pathology, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology

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• **Context.**—The need for appropriate specimen use for ancillary testing has become more commonplace in the practice of pathology. This, coupled with improvements in technology, often provides less invasive methods of testing, but presents new challenges to appropriate specimen collection and handling of these small specimens, including thoracic small biopsy and cytology samples.

**Objective.**—To develop a clinical practice guideline including recommendations on how to obtain, handle, and process thoracic small biopsy and cytology tissue specimens for diagnostic testing and ancillary studies.

**Methods.**—The College of American Pathologists convened an expert panel to perform a systematic review of the literature and develop recommendations. Core needle

biopsy, touch preparation, fine-needle aspiration, and effusion specimens with thoracic diseases including malignancy, granulomatous process/sarcoidosis, and infection (eg, tuberculosis) were deemed within scope. Ancillary studies included immunohistochemistry and immunocytochemistry, fluorescence in situ hybridization, mutational analysis, flow cytometry, cytogenetics, and microbiologic studies routinely performed in the clinical pathology laboratory. The use of rapid on-site evaluation was also covered.

**Results.**—Sixteen guideline statements were developed to assist clinicians and pathologists in collecting and processing thoracic small biopsy and cytology tissue samples.

**Conclusions.**—Based on the systematic review and expert panel consensus, thoracic small specimens can be

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Authors' disclosures of potential conflicts of interest and author contributions are found in the Appendix at the end of this article.

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handled and processed to perform downstream testing (eg, molecular markers, immunohistochemical biomarkers), core needle and fine-needle techniques can provide appropriate cytologic and histologic specimens for ancillary studies, and rapid on-site cytologic evaluation remains

With the advances in minimally invasive sampling techniques, the ability to provide a diagnosis and perform multiple ancillary studies on limited-volume tissue samples, including small biopsy and cytology specimens, has been a paradigm shift in the management of pulmonary pathology. Advances in molecular diagnostic techniques, including high-sensitivity and high-throughput assays being available clinically, enable pathology laboratories to provide treating physicians with valuable diagnostic, predictive, and prognostic biomarker testing from these thoracic small specimens.

Currently, the 2 most commonly used minimally invasive approaches for collecting diagnostic tissue include fine-needle aspiration (FNA) and core needle biopsy (CNB). Fine-needle aspiration specimens are usually acquired using a fine needle such as 20 to 25 gauge, primarily through the cutting action of the needle during excursion within a lesion and capillary action that extracts cellular material into the needle core, with or without application of negative pressure. Aspirated material is typically either used to prepare direct smears or collected in some type of media solution for further processing. Core needle biopsy, on the other hand, generally uses a hollow-core needle that is slightly larger, usually ranging from 14 to 20 gauge, and uses the physical cutting action of the needle to collect an architecturally preserved, single, tissue core. The tissue core that is acquired is then processed in fixative (for instance, formalin) and embedded in paraffin to create a tissue block for histologic assessment. In general, FNA and CNB are considered complementary diagnostic techniques with specific advantages and limitations that are often used in conjunction for establishing a diagnosis and performing ancillary testing. However, the decision to select one diagnostic modality over the other is largely determined by site and size of the lesion, clinical status of the patient, feasibility and/or availability of resources, experience and expertise of the proceduralist, and institutional preference.

The need for a minimally invasive diagnostic approach is especially true in lung cancer patients, who frequently present with advanced-stage disease. Acquisition of tissue using a minimally invasive sampling technique for diagnosis, tumor subtyping, and performing ancillary biomarker studies is often the approach to guide medical management in these patients. This typically involves using either an endobronchial/transbronchial approach or a percutaneous transthoracic approach to collect FNA and/or CNB samples, bronchial brushings/washings/lavages, or effusion samples by thoracentesis. Given the limited volume of tissue available on these small specimens and the critical need to provide valuable genomic information for a growing number of biomarkers, it is of utmost importance to establish evidence-based recommendations for the appropriate collection and handling of these thoracic small specimens for judicious triage for relevant ancillary tests. The work presented in this manuscript offers 16 guideline statements (recommendations based on a systematic review and 1 expert consensus opinion) to address this topic.

helpful in appropriate triage, handling, and processing of specimens.

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## METHODS

This evidence-based guideline has been developed following the standards developed by the National Academy of Medicine, formerly the Institute of Medicine.<sup>1</sup> A detailed description of the methods and the systematic review used to create this guideline can be found in the supplemental digital content at [www.archivesofpathology.org](http://www.archivesofpathology.org) in the August 2020 table of contents.

### Panel Composition

The College of American Pathologists (CAP), in collaboration with the American College of Chest Physicians, Association for Molecular Pathology, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology, convened an expert panel consisting of 10 practicing pathologists, 2 interventional pulmonologists, 4 interventional radiologists, 1 cytotechnologist, and a research methodologist consultant to develop this guideline. The expert panel members also have expertise in pulmonary pathology, molecular pathology, cytopathology, cytotechnology, microbiology, radiology, and pulmonology. An advisory panel consisting of 11 pathologists, 3 pulmonologists, 2 radiologists, and 1 cytotechnologist assisted the expert panel at specific key stages in the development of the guideline. All panel members, except for the methodologist consultant, volunteered their time and were not compensated for their involvement.

### Conflict of Interest Policy

The collaborators agreed upon a conflict of interest policy (effective March 2016) and members of the expert panel disclosed all financial interests from 3 years prior to appointment through the development of the guideline. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Complete disclosures of the expert panel members are listed in the Appendix. Disclosures of interest judged by the oversight group to be manageable conflicts are as follows: J.N.: stock options/bonds, OmniSeq, LLC (Buffalo, New York); J.V.: research grants, Abbott Molecular Inc (Des Plaines, Illinois); N.P.: consultancy, Cook Medical LLC (Bloomington, Indiana), research grants and honoraria, Olympus Corporation (Tokyo, Japan); P.I.: consultancies, AstraZeneca (Cambridge, England) and Genentech USA, Inc (South San Francisco, California), honoraria, Ventana Medical Systems, Inc (Oro Valley, Arizona); S.D.: advisory board, Bayer AG (Leverkusen, Germany) and Bristol-Myers Squibb Company (New York, New York), consultancies, AstraZeneca and Genentech USA, Inc. The majority of the EP (13 of 18 members) were assessed as having no relevant conflicts of interest. The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. See the supplemental digital content for complete information about the conflict of interest policy.

### Objective

The scope of the expert panel was to develop a clinical practice guideline that would (1) provide recommendations to clinicians obtaining samples within the thorax on how to obtain and handle adequate material for diagnostic testing, and (2) provide recommendations to pathologists for the prioritization of testing and the appropriate processing of thoracic small biopsy and cytology specimens. Core needle biopsy, touch preparation, FNA, and effusion specimens with thoracic diseases including lung carcino-

ma, granulomatous process/sarcoidosis, and infection were deemed within scope. Ancillary studies covered in this review included immunoperoxidase studies (immunohistochemistry [IHC] and immunocytochemistry), fluorescence in situ hybridization (FISH), mutational analysis, flow cytometry, and microbiologic studies routinely performed in the clinical pathology laboratory. The use of rapid on-site evaluation (ROSE) was also covered.

The expert panel formulated and considered the following key questions:

1. During the collection, triage, and processing/handling of minimally invasive pathology specimens from patients with suspected or undiagnosed thoracic abnormalities, what procedural or methodologic variables have been shown to optimize testing outcomes so that pathologists can provide an evaluation and accurate diagnosis?
2. With regard to each of the specimen types of interest, what evidence is available to determine the most effective protocols for sample collection, including the immediate handling of the specimen (ie, how the needle biopsy is expelled from the needle and the selection of the appropriate media), the minimum and maximum number of passes needed to ensure that the laboratory can obtain adequate materials for diagnostic testing, and the impact of ROSE on adequacy, quality, and triage of specimens?
3. With regard to each of the specimen types of interest, the preparations created, and the tests to be performed, what evidence is available to determine the most effective methods for the handling and processing of specimens, including the selection of appropriate media, the priority by disease, and the optimal ischemic time (ie, time between the removal of tissue from the patient and the initiation of fixation)?
4. What evidence is available to support an algorithm(s) for selection of specimens and sequence of testing, under defined circumstances?

The target audience for this guideline is anatomic pathologists, clinical pathologists specializing in microbiology, molecular pathologists, general and thoracic surgeons, pulmonologists, interventional radiologists, laboratory professionals in anatomic pathology, and other health care professions involved in collecting and handling thoracic small biopsy and cytology specimens.

### Literature Search and Collection

Literature search strategies were developed in collaboration with a medical librarian for the concepts of thoracic abnormalities, specimen procurement methods, laboratory tests, and methodologic, analytical, and procedural variables. In consultation with the expert panel, the search strategies were created using standardized database terms and keywords. Databases searched included PubMed and Embase.com. Additional searches for gray literature were conducted in ClinicalTrials.gov, Cochrane Library, Guidelines International Network, National Guideline Clearinghouse, Trip search engine, University of York Centre for Reviews and Dissemination—PROSPERO, and applicable US and international organizational websites. Initial searches were completed on March 30, 2017, and refreshed in PubMed and Embase on May 15, 2018, and April 30, 2019.

All searches were limited to English and from January 1, 2007 to the date of search. Case reports, commentaries, editorials, and letters were excluded. The Cochrane search filter for humans was applied in PubMed and Embase.com. MEDLINE and conference abstract records were excluded in the Embase searches.

The detailed search strategy for PubMed and Embase as well as the PRISMA chart are provided in the supplemental digital content Supplemental Figures 1 and 2.

### Quality Assessment

A risk of bias assessment was performed for all fully published studies meeting inclusion criteria by the research methodologist. The methodologist assessed key indicators based on study design

and methodologic rigor. Following the risk of bias assessment, each guideline statement was given a grade for quality of evidence. Refer to the supplemental digital content for further details.

### Assessing the Strength of Recommendations

Development of recommendations required that the expert panel review the identified evidence, assess the quality of evidence (Supplemental Table 1), and make a series of key judgments. The terminology describing the strength of recommendation grades (Supplemental Table 2) was developed by the CAP Pathology and Laboratory Quality Center for Evidence-Based Guidelines. The Grading of Recommendations Assessment, Development and Evaluation Evidence to Decision framework<sup>2</sup> was used in order to frame, discuss, and document key judgements around the balance of benefits and harms, as well as feasibility and acceptability for each statement. In 2018, the CAP adopted the Grading of Recommendations Assessment, Development and Evaluation methodology,<sup>2</sup> and future updates will reflect this change.

## RESULTS

Of the 4256 unique studies identified in the systematic review, 218 met the inclusion criteria and formed the evidentiary base. All 218 included studies were published, peer-reviewed publications and underwent data extraction and qualitative assessment.

The expert panel met 15 times using Web-based meeting forums from January 3, 2017, through May 23, 2019. Additional work was completed via electronic mail and in 3 in-person meetings. A public comment period was held from May 23 through June 15, 2018, during which 17 guideline statements (recommendations and expert consensus opinions) were posted on the CAP website. The expert panel agreed on the final guideline statements via a formal vote. Ultimately, the process resulted in a total of 16 guideline statements.

An independent review panel masked to the expert panel and vetted through the conflict of interest process provided final approval of the guideline on behalf of the CAP Council on Scientific Affairs. The final guideline statements are summarized in Table 1. In addition to the rationale for the guideline statements below, the discussion of the benefits and harms of the guideline statements is included in the supplemental digital content.

## GUIDELINE STATEMENTS

### Endobronchial Ultrasound-Guided Transbronchial Procedures

The use of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS TBNA) has become more prevalent in the investigation of intrathoracic abnormalities. Initially described to help with noninvasive mediastinal staging of lung carcinoma, EBUS TBNA has now been used in multiple other clinical situations, including the diagnosis of recurrent malignancy and other respiratory diseases. The increasing use of EBUS TBNA during the past decade has substantially improved the ability of the bronchoscopist to obtain material for both diagnostic and ancillary testing purposes in a minimally invasive fashion.<sup>3</sup> A number of specimen acquisition variables may impact the adequacy of specimens obtained by EBUS TBNA, including the needle gauge, number of needle passes, the use of suction when obtaining a specimen, and the use of ROSE. These variables have the potential for impacting the amount of specimen collected that is available for diagnosis and subsequent ancillary testing. Rapid on-site evaluation allows for real-

**Table 1. Summary of the Guideline Statements**

Guideline Statement	Strength of Recommendation
1. EBUS TBNA may be used, if available, for initial evaluation (diagnosis, staging, identification of recurrence/metastasis) of mediastinal and hilar lymph nodes, as well as centrally located parenchymal lesions visible with endobronchial ultrasound	Strong recommendation
2. When performing EBUS TBNA, 19-, 21-, or 22-gauge needles may be used	Recommendation
3. When performing EBUS TBNA, ROSE should be used, if available	Recommendation
4. To achieve optimal diagnostic yield, when performing EBUS TBNA without ROSE, the bronchoscopist should perform at a minimum 3 and up to 5 passes, if technically and clinically feasible. When performing with ROSE, clinical judgment should be used to assess the number of passes needed. Additional passes may be required for ancillary studies	Recommendation
5. When performing transthoracic needle procedures, ROSE should be used for adequacy assessment, if available and clinically feasible. If performing CNB without concurrent FNA, touch preparations may be used for adequacy assessment, if available	Strong recommendation for the use of ROSE for adequacy assessment; recommendation for the use of touch preparations without concurrent FNA
6. When performing transthoracic needle procedures, needle size should be determined by the operator and technique. For transthoracic FNAs, needles as small as 25 gauge may be used. For CNBs, needles as small as 20 gauge may be used	Recommendation
7. When performing transthoracic FNA without CNB, the proceduralist should obtain multiple passes, if technically and clinically feasible, and should attempt to collect sufficient material for a tissue block (ie, cell block, tissue clot)	Recommendation
8. To achieve optimal diagnostic yield when performing transthoracic CNBs, the proceduralist should attempt to obtain a minimum of 3 core samples, if technically and clinically feasible. Additional samples may be required for ancillary studies	Recommendation
9. If performing bronchoscopy for the investigation of peripheral pulmonary lesions that are difficult to reach with conventional bronchoscopy, image-guidance adjuncts may be used, if local expertise and equipment are available	Recommendation
10. When performing transbronchial needle aspirates, ROSE should be used for adequacy assessment, if available. If performing transbronchial forceps biopsies without concurrent transbronchial needle aspirates, touch preparations may be used for adequacy assessment, if available	Recommendation for the use of ROSE for adequacy assessment; expert consensus opinion for the use of touch preparations
11. When collecting pleural fluid for a suspected diagnosis of malignancy, the proceduralist should send as much fluid volume as reasonably attainable for cytologic evaluation and ancillary studies	Expert consensus opinion
12. Cytology specimens (smears, cell blocks, liquid-based cytology), may be used for ancillary studies if supported by adequate validation studies	Strong recommendation
13. CNB specimens collected for ancillary studies should be fixed in 10% neutral buffered formalin	Recommendation
14. When performing bronchoscopy for the investigation of tuberculosis, endobronchial ultrasonography may be used to increase the diagnostic yield of bronchoalveolar lavage and transbronchial biopsy	Recommendation
15. When performing EBUS TBNA for the evaluation of intrathoracic granulomatous lymphadenopathy with the suspicion of tuberculosis, specimens should be collected for cytology, microbiology (mycobacterial smear and culture), and TB-PCR evaluation, if available	Recommendation
16. When collecting pleural fluid for diagnosis of extrapulmonary tuberculosis, specimens should be submitted for microbiology culture studies for mycobacteria using liquid media protocol	Recommendation

Abbreviations: CNB, core needle biopsy; EBUS TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; FNA, fine-needle aspiration; ROSE, rapid on-site evaluation; TB-PCR, *Mycobacterium tuberculosis* polymerase chain reaction.

time evaluation and assessment of procured material during a sampling procedure. Although not always used, ROSE can provide adequacy assessment to EBUS TBNA procedures (ie, determining if material representative of the targeted lesion is being sampled), allows for preliminary diagnostic information to be given to the proceduralist, and helps facilitate appropriate specimen triage (eg, for potential/anticipated molecular analysis, flow cytometric studies, microbiology studies, immunohistochemical studies).<sup>4-10</sup> The basic workflow for ROSE involves the preparation of

direct smear slides with rapid staining followed by evaluation by cytopathology personnel, either using on-site microscopy or off-site via telepathology with real-time communication to the proceduralist. The cytopathology personnel involved in ROSE vary across different practice settings and include an on-site cytotechnologist with cytopathologist interpretation, a cytotechnologist or cytopathologist alone, a cytopathology trainee with cytopathologist interpretation, or a cytopathology trainee alone. Based on a recent survey by the American Society of Cytopathol-

ogy,<sup>11</sup> most practice settings offer ROSE as a cytopathology service; however, ROSE may not be readily available or practical in all situations at every center performing EBUS TBNA. Therefore, the following recommendations takes into consideration the published evidence to date on EBUS TBNA procedures to maximize the chances of obtaining adequate diagnostic material for ancillary studies.

**1. Strong Recommendation.—Endobronchial ultrasound guided transbronchial needle aspiration may be used, if available, for initial evaluation (diagnosis, staging, identification of recurrence/metastasis) of mediastinal and hilar lymph nodes, as well as centrally located parenchymal lesions visible with endobronchial ultrasound.**

The quality of evidence is *moderate* to support this guideline statement.

The evidence base supporting this recommendation comprises 15 studies,<sup>12–26</sup> 12 of which assessed initial evaluation,<sup>12–15,17–22,24,25</sup> 2 that assessed disease staging,<sup>17,18</sup> 2 that evaluated the use of EBUS TBNA for identification of recurrence or metastases,<sup>16,19</sup> and 1 that focused on adequacy of samples for ancillary testing.<sup>23</sup> The study that reported on ancillary testing was a high-quality meta-analysis at low risk of bias that focused on adequacy for *EGFR* and *ALK* testing in lung cancer patients after sampling with EBUS TBNA.<sup>23</sup> The remaining 14 studies carried an aggregate serious risk of bias and included 11 retrospective studies<sup>12–18,20–22,26</sup> and 3 prospective studies.<sup>19,24,25</sup> As a consequence of their retrospective design, all retrospective studies suffered from selection bias<sup>12–18,20–22,26</sup>; additional individual studies carried a risk of bias in performance domains,<sup>18</sup> reporting domains,<sup>13,16–18,20–22,26</sup> or detection domains<sup>12–18,20–22,26</sup>; and 5 studies<sup>13,14,18,20,21</sup> did not report funding source. The prospective cohort studies were limited by risk of selection bias in all,<sup>19,24,25</sup> reporting bias in 2 studies,<sup>24,25</sup> and detection bias in 1 study.<sup>19</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 3, a and b, in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 1.

The studies identified in our systematic review evaluated the use of EBUS TBNA for sampling of pulmonary lesions and lymph nodes to obtain tissue for diagnosis and ancillary studies. The overall diagnostic yield of EBUS TBNA remains high (sensitivity of 89% and specificity of 100%) in numerous studies.<sup>12,16–18,20,24–26</sup> Two studies identified in our systematic review indicate that EBUS TBNA is useful in obtaining appropriate tissue for patients that also have parenchymal lung lesions located adjacent to central and/or large airways that will accommodate the size of the convex EBUS puncturescope.<sup>14,21</sup> In addition, EBUS TBNA is also useful in obtaining specimens for staging of carcinoma<sup>17,18</sup> as well as identifying recurrent disease.<sup>16,19</sup> Diagnostic yields have been reported in various collection media, including both alcohol-based and formalin-based media,<sup>12,13,15,20</sup> when sampling mediastinal and hilar lymphadenopathy for an initial diagnosis. Additionally, a meta-analysis, which pooled 33 studies, reported a pooled probability of adequate material for molecular analysis from EBUS TBNA sampling in almost 95% of cases (*EGFR*, 94.48%; 95% CI, 93.2%–96.4%; *ALK*, 94.9%; 95% CI, 89.4%–98.8%).<sup>23</sup>

During the open comment period, there were 242 respondents, of whom 83.47% (n = 202) agreed, 6.2% (n = 15) disagreed, and 10.33% (n = 25) indicated the statement

did not pertain to their area of expertise or practice. There were a total of 21 written comments, including several suggesting that proceduralist competency remains important to the outcomes related to both diagnosis and the ability to obtain appropriate tissue for molecular biomarker testing. Other comments mentioned that other procedures might be used initially, such as endoscopic ultrasound. These comments were taken into consideration in the drafting of this recommendation, and the statement was modified slightly to the current form presented in this document.

**2. Recommendation.—When performing EBUS TBNAs, 19-, 21-, or 22-gauge needles may be used.**

The quality of evidence is *low*.

One systematic review from a clinical practice guideline produced on behalf of the World Association of Bronchology and Interventional Pulmonology Task Force,<sup>27</sup> 2 randomized controlled trials (RCTs),<sup>28,29</sup> 2 prospective cohort studies,<sup>30,31</sup> and 4 retrospective cohort studies<sup>32–35</sup> comprise the evidence base for statement 2. The systematic review<sup>27</sup> carried a low risk of bias, with the only limitation being unclear reporting of whether the study used dual study selection and data extraction. Both RCTs,<sup>28,29</sup> 1 prospective cohort study,<sup>31</sup> and all the retrospective cohort studies<sup>32–35</sup> were published after the search date of the systematic review. The aggregate risk of bias across these studies was very serious.<sup>28,29,31–35</sup> An additional prospective cohort study,<sup>30</sup> which did not evaluate needle gauge in relation to sufficiency for ancillary testing but did report on the outcome, was included as indirect evidence for this statement and was limited by a serious risk of bias. None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 4, a and b, in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 2.

A limited number of studies investigating the impact of needle gauge on specimen cellularity were identified in our systematic review. The needle gauges used in the EBUS TBNA studies identified in our systematic review included 19, 21, and 22 gauge.<sup>27,30,32,33</sup> The most recent and comprehensive of these is a study by Jeyabalan et al<sup>32</sup> in which the authors performed a retrospective analysis of 303 patients referred for EBUS TBNA. The needle gauge used (21 or 22 gauge) was selected at the discretion of the operator. The pathologists reviewing the specimens were blinded to the needle gauge used. No significant difference in diagnostic accuracy was seen for malignancy between the specimens obtained via 21- versus 22-gauge needles (96.6% versus 95.3% accuracy, respectively). However, diagnostic accuracy was greater for 21-gauge than 22-gauge needles when evaluating benign lesions and when subclassifying non-small cell lung carcinomas (NSCLCs) (88% versus 65%).

Four other studies from the systematic review, but not meeting the final inclusion criteria, demonstrate variable results.<sup>36–39</sup> Three of these 4 studies<sup>36,37,39</sup> demonstrated no significant difference in diagnostic accuracy when either 21- or 22-gauge needles were used. A fourth study by Nakajima et al<sup>38</sup> demonstrated that diagnostic accuracy was somewhat superior when 21-gauge needles were used, but both appear to yield sufficient specimen cellularity for diagnosis.

Although a majority of the studies identified in our systematic review focused on the diagnostic yield, a study by

Jeyabalan et al<sup>30</sup> demonstrated that sufficiently cellular specimens for molecular analysis were obtained by EBUS TBNA when either 21- or 22-gauge needles were used. These authors demonstrate that both 22- and 21-gauge needles achieved high success rates for epidermal growth factor receptor (*EGFR*) mutation testing, without significant differences in testing success. The only specimen insufficient for *EGFR* mutation analysis was obtained by a 21-gauge needle. The data available from the studies identified in our systematic review and the 4 not meeting final inclusion criteria indicate that either 21- or 22-gauge needles may be used for EBUS TBNA when collecting specimens for ancillary studies.

A limited number of recent studies have assessed the utility of 19-gauge needles in comparison with 22-gauge needles in terms of diagnostic yield, adverse effects, and adequacy for ancillary testing. One randomized controlled study<sup>29</sup> noted that samples collected with 19-gauge needles had similar diagnostic yield with tumor content, DNA yield, and molecular success rates when compared with 22-gauge needles; however, the 19-gauge needle samples had significantly higher blood content. Similar conclusions also appear within another RCT<sup>28</sup> that noted similar diagnostic sensitivity in samples collected using 19- and 22-gauge needles, but again, significantly higher blood content with the former. The one prospective study<sup>31</sup> included in our evidence base compared 19-gauge with 21-gauge needles and concluded that diagnostic concordance and adequacy for ancillary testing were not significantly different. Two additional retrospective studies<sup>33,34</sup> were used to provide additional evidence base for 19-gauge needles. In one study, although the diagnostic yield for samples collected using 19-gauge needles were slightly better than that of samples collected with a 22-gauge needle, the difference did not achieve statistical significance.<sup>34</sup>

When examining all studies with the evidence base, it appears that 22-, 21-, and 19-gauge needles are relatively equivalent in overall diagnostic yield and adequacy for ancillary testing. There remains some suggestion that cellularity may be improved with the use of larger-gauge needles, but often at the cost of increasing blood contamination within specimens. The overall clinical impact this tradeoff offers currently remains undetermined and a focus of ongoing study.

During the open comment period, a total of 240 individuals responded to this recommendation. Of the respondents, 67.9% (n = 163) agreed with the recommendation whereas 3.75% (n = 9) disagreed and 28.33% (n = 68) indicated that the statement did not pertain to their area of expertise or practice. There were 20 written comments: 6 comments suggested the use of a smaller gauge needle (25 or 23 gauge) as these were thought to be safer and associated with less bleeding; 3 respondents suggested that 19-gauge needles could or should be used as 19-gauge needles produced suitable or perhaps superior specimens; and a single respondent stated larger needles (eg, 21 gauge) were preferable to get higher tumor volume content. These comments were taken into consideration when drafting the recommendation and the statement was maintained with the original language.

### **3. Recommendation.—When performing EBUS TBNA, ROSE should be used, if available.**

The quality of evidence is *moderate*.

The evidence base informing this statement comprises 1 systematic review,<sup>27</sup> 1 RCT,<sup>6</sup> 6 prospective cohort stud-

ies,<sup>7–9,25,40,41</sup> and 13 retrospective cohort studies.\* Of the included 21 studies, 19<sup>†</sup> reported on triage and adequacy outcomes, 2 studies<sup>41,47</sup> reported on adverse events, and 1 study<sup>27</sup> reported on triage and adequacy outcomes as well as adverse events. The aggregate risk of bias across the 21 studies was serious based on poor methodology reporting<sup>7</sup> and risk of selection bias,<sup>7–9,25,41</sup> detection bias,<sup>7–9,25,41</sup> performance bias,<sup>40</sup> and reporting bias<sup>7,9,25,41</sup> in the prospective cohort studies. The retrospective cohort studies carried risk of selection bias inherent in retrospective studies, in addition to detection bias,<sup>‡</sup> performance bias,<sup>18,48</sup> reporting bias,<sup>4,10,18,20,43–45,48</sup> and no reporting of funding source.<sup>4,10,18,20,43–45,47</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 5, a and b, in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 3.

Many factors can determine whether ROSE can be used for EBUS TBNA procedures in different practice settings. Based on the studies identified from the systematic literature review, there appears to be a slight trend in increased diagnostic yield when ROSE is used versus when ROSE is not used (absolute percentage increase of 2.9%–8%)<sup>5,8–10,27</sup>; however, only one study<sup>25</sup> reported a statistically significant difference. In studies describing the performance of ROSE with EBUS TBNA without a comparator non-ROSE arm, the reported sensitivities are high, ranging from 85.4% to 89.5%.<sup>7,15,40,42</sup> For other outcome measures, a study by Trisolini and colleagues,<sup>49</sup> which was included via an identified systematic review,<sup>27</sup> demonstrated that ROSE for conventional TBNA led to a statistically significant decrease in the number of sites biopsied (1 versus 2,  $P = .005$ ) and bronchoscopy complication rates (6% versus 20%,  $P = .01$ ), though it is not clear if this would also be the case for EBUS TBNA procedures. Two additional studies demonstrated that using ROSE for EBUS TBNA led to a significant decrease in the number of needle attempts compared with when ROSE was not used.<sup>5,25</sup> A relatively limited number of studies were identified that specifically addressed molecular analysis/ancillary testing in EBUS TBNA procedures using ROSE.<sup>6,43,44</sup> These studies appear to suggest that the use of ROSE may minimize molecular analysis failures. Molecular profiling of NSCLC diagnosed by EBUS TBNA has demonstrated improved success when ROSE was used (90%) as opposed to when ROSE was not used (80%),<sup>6</sup> although the numbers were not statistically significant. When combined with ROSE, EBUS TBNA has also demonstrated a high success rate (98%) when attempting *EGFR* molecular analysis.<sup>43</sup>

Other studies, including recommendations issued by other professional societies, have weighed in on the issue of ROSE for EBUS TBNA specimens in specific situations. In a guideline statement issued by the World Association for Bronchology and Interventional Pulmonology Task Force on Specimen Guidelines, it is stated that “while ROSE offers the possibility of immediate and accurate feedback on the diagnosis and quality of the obtained specimen with the potential to influence the operator’s plan (ie, obtain additional samples for molecular testing, samples for culture,

\* References 4, 5, 10, 15, 18, 20, 42–48.

† References 4–10, 15, 18, 20, 25, 40, 42–48.

‡ References 4, 5, 10, 15, 18, 20, 44–48.

or samples for flow cytometry), its use is not supported by firm evidence but still highly recommended by our expert consensus.<sup>27</sup> They go on to issue this formal recommendation: "In patients with suspected lung cancer and enlarged mediastinal or hilar LN and/or centrally located tumors: Evidence is insufficient to recommend that ROSE be used in every procedure. Grade 1b."<sup>27</sup> A recently published perspective from the Pulmonary Pathology Society<sup>50</sup> on the topic of ROSE for EBUS TBNA specimens for the diagnosis of lung cancer (not included in our systematic review) did recommend the use of ROSE to ensure that the targeted lesion is being sampled, enable appropriate specimen triage, minimize repeat procedures for additional ancillary testing, and provide a preliminary diagnosis. Finally, a recent meta-analysis on the topic came to the conclusion that the addition of ROSE did not lead to a statistically significant increase in diagnostic yield or decreases in procedure time for EBUS TBNA or conventional TBNA procedures; however, there might be a possibility that ROSE can decrease the number of passes or need for repeat bronchoscopic procedures.<sup>51</sup> It is worth noting that most published studies on the topic have focused on endpoints of diagnostic yield or procedure complications; however, there is a paucity of direct evidence with respect to use of ROSE on downstream ancillary testing outcomes.

In the open comment period, there were 238 respondents for this recommendation, of whom 75.21% (n = 179) agreed, 13.45% (n = 32) disagreed, and 11.34% (n = 27) neither agreed nor disagreed as this question did not pertain to their area of expertise or practice. There were 35 written comments. Many were in strong support of always having ROSE present for EBUS TBNA procedures. However, a number of comments raised the issue of when to use ROSE, including multiple comments arguing against having ROSE present at every procedure. The expert panel acknowledges that experienced bronchoscopists may not necessarily benefit from having ROSE for every procedure, because the needs may vary depending upon the clinical situation (eg, routine mediastinal lymph node sampling for lung cancer staging purposes versus sampling of a suspicious lung mass). Some respondents commented on aspects of ROSE that can act as a barrier for its use. This includes a lack of having adequate personnel or staffing to cover ROSE in all situations, the substantial time commitment that ROSE entails for the cytopathology personnel, and comments raising concerns for the inadequate pathologist reimbursement for time spent doing ROSE. Comments were made raising concern that slides prepared for ROSE may waste cellular material that could have been used for downstream molecular testing (because currently many commercial testing laboratories will only accept cytology cell block preparations and not direct smears such as those generated during ROSE). Finally, a few comments were made questioning the necessity or utility of ROSE under any circumstance.

These comments all raised valid concerns and reflect the complexity surrounding the use of ROSE for EBUS TBNA procedures. The use of ROSE in EBUS TBNA procedures for the collection of adequate tissue specifically for potential ancillary testing was taken into consideration for the wording and strength of recommendation in the final draft of statement 3 presented in this document. The statement recommends the use of ROSE for EBUS TBNA procedures, if available, to help ensure adequate material is collected and triaged appropriately for ancillary studies. However, it is the obligation of each individual practice setting to determine the most appropriate EBUS TBNA procedures for using ROSE.

Refer to Table 2 for data on ROSE and EBUS TBNA.

**4. Recommendation.—To achieve optimal diagnostic yield, when performing EBUS TBNA without ROSE, the bronchoscopist should perform at a minimum 3 and up to 5 passes, if technically and clinically feasible. When performing with ROSE, clinical judgment should be used to assess the number of passes needed. Additional passes may be required for ancillary studies.**

The quality of evidence is *moderate*.

Six studies<sup>6,27,52–55</sup> comprise the evidence base for this statement, including 1 systematic review,<sup>27</sup> 1 RCT,<sup>6</sup> 3 prospective cohort studies,<sup>52,54,55</sup> and 1 retrospective cohort study.<sup>53</sup> The aggregate risk of bias for the studies was serious. The RCT<sup>6</sup> suffered from selection bias, performance bias, and detection bias, and the prospective cohort studies were limited by reporting bias,<sup>52</sup> selection bias,<sup>54</sup> or performance bias.<sup>55</sup> In addition to high risk of selection bias, the retrospective cohort study<sup>53</sup> also suffered from risk of detection bias and did not report on funding sources. None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 6 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 4.

A limited number of studies reporting on EBUS TBNA without ROSE were identified in our review of the literature. A systematic review sponsored by the World Association for Bronchology and Interventional Pulmonology cited a prospective study of EBUS TBNA for mediastinal staging of patients with NSCLC in which 100% adequacy and 95% sensitivity were achieved with 3 passes per lymph node and no further improvement in diagnostic yield was achieved with a fourth pass.<sup>27</sup> For evaluation of enlarged mediastinal or hilar lymph nodes in nonmalignant conditions for patients with suspected sarcoidosis, a prospective EBUS TBNA study without ROSE<sup>52</sup> reported that the diagnostic yield of 3 needle passes reached a plateau, and after 5 passes the diagnostic yield decreased. Therefore, based on these studies, in situations where EBUS TBNA is being performed without ROSE, for both malignant and benign disease, a minimum of 3 passes and up to 5 passes is recommended to achieve an optimal diagnostic yield.

When a qualified cytopathology personnel is present at the EBUS procedure to provide immediate feedback on the quantitative and qualitative adequacy of material obtained by the proceduralist, the 3- to 5-pass recommendation is no longer applicable, because ROSE may establish the presence of adequate diagnostic material in fewer than 3 passes, or in some cases may require more than 5 passes to obtain adequate diagnostic material.<sup>6</sup> Therefore, when performing EBUS TBNA with ROSE, clinical judgment informed by close communication between the cytopathology personnel and proceduralist should determine the optimal number of passes in each individual patient.

It should be emphasized that the studies serving as a basis for the 3- to 5-pass recommendation pertained to diagnostic yield and did not specifically address the number of passes needed for ancillary testing. Therefore, the expert panel was unable to recommend a specific number of passes that would maximize material specifically for ancillary testing. Nonetheless, it would stand to reason that after adequate material has been obtained to render a cytologic diagnosis, a few additional passes dedicated to collecting material in appropriate media should be considered to increase the chance of successful ancillary testing.

**Table 2. Rapid On-Site Evaluation (ROSE) and Endobronchial Ultrasound-Guided Transbronchial Needle Aspirations**

Study	Study Design	Sample Size, No.	Diagnostic Yield, %	Diagnostic Sensitivity, % (95% CI) <sup>a</sup>	Molecular Analysis Success Rate
Cardoso et al, <sup>8</sup> 2015	PCS	81	ROSE: 91 Non-ROSE: 83 <i>P</i> = .08		
Madan et al, <sup>9</sup> 2014	PCS	102	ROSE: 96.8 Non-ROSE: 85.7		
Chaiyakul, <sup>25</sup> 2018	PCS	175	ROSE: 100 Non-ROSE: 86.2 <i>P</i> = .005		
Hopkins et al, <sup>44</sup> 2016	RCS	234	ROSE, malignant cases: 95 <sup>a</sup> ROSE, benign cases: 96 <sup>a</sup>		ROSE, adequate material for molecular analysis: 79.3 <sup>a</sup>
Guo et al, <sup>5</sup> 2016	RCS	236	ROSE: 92.1 Non-ROSE: 89.2 <i>P</i> = .27		
Griffin et al, <sup>10</sup> 2011	RCS	149	ROSE: 94 Non-ROSE: 90 <i>P</i> NR		
Gilbert et al, <sup>18</sup> 2009	RCS	172	ROSE: 68.8 <sup>a</sup>	ROSE: 85.4	
Mallya et al, <sup>7</sup> 2015	PCS	80		ROSE: 87.8 (76–95)	
Plit et al, <sup>40</sup> 2013	PCS	60		ROSE: 88.6	
Izumo et al, <sup>42</sup> 2016	RCS	718		ROSE: 89.5 (80.3–95.3)	
Joseph et al, <sup>15</sup> 2013	RCS	170			ROSE: 90.8 Non-ROSE: 80.3 <i>P</i> = .09
Trisolini et al, <sup>6</sup> 2015	RCT	197			ROSE: 98 <sup>a</sup> ROSE, adequate material for molecular analysis: 76.6 <sup>a</sup>
Thiryayi et al, <sup>43</sup> 2016	RCS	68			
Rooper et al, <sup>46</sup> 2016	RCS	107			

Abbreviations: NR, not reported; PCS, prospective cohort study; RCS, retrospective cohort study; RCT, randomized controlled trial.

<sup>a</sup> No non-ROSE comparison.

At the open comment period, there were 240 respondents, of whom 83.75% (*n* = 201) agreed, 4.17% (*n* = 10) disagreed, and 12.08% (*n* = 29) indicated that the statement did not pertain to their area of expertise or practice. There were 24 written comments, most of which sought clarification of whether this statement pertained to diagnostic yield or ancillary testing. This feedback was taken into consideration in rewording statement 4 with additional clarification in the form presented in this document.

Refer to Table 3 for data on number of passes needed for EBUS TBNA with and without ROSE.

### Transthoracic Procedures

With the recommendation by the US Preventive Task Force<sup>56</sup> for lung cancer screening with computed tomography for high-risk patients, the need for minimally invasive tests to diagnose peripheral pulmonary lesions (PPLs) has increased. Several image-guided technologies are available to reach these PPLs, of which computed tomography-guided transthoracic percutaneous biopsy procedures are commonly used. The technique of transthoracic needle biopsy, or percutaneous thoracic needle biopsy, can be obtained with a variety of devices and sizes. Radiologists typically perform transthoracic needle biopsies 1 of 3 ways: FNA, CNB, or FNA with concurrent CNB. Similar to the EBUS procedures described above, a variety of specimen acquisition variables may impact the adequacy of specimens obtained by transthoracic procedures, including the needle gauge, number of needle passes, and the use of ROSE. All

these variables have the potential for impacting the amount of specimen collected that is available for diagnosis and subsequent ancillary testing. The combination of FNA and CNB is particularly helpful when multiple tests are performed. Rapid on-site evaluation allows for real-time evaluation and assessment of procured material during a sampling procedure, and the use of ROSE by cytopathology personnel during transthoracic FNA procedures can potentially help maximize the diagnostic yield, appropriately triage the specimen for ancillary (flow cytometry or microbiology) studies, and ensure adequate material is collected for all anticipated molecular testing. However, ROSE may not be readily available or practical in all situations at every practice center. The following recommendations address specimen collection and handling for transthoracic procedures, including PPLs.

**5. Strong Recommendation.—When performing transthoracic needle procedures, ROSE should be used for adequacy assessment, if available and clinically feasible.**

**Recommendation.—If performing CNB, without concurrent FNA, touch preparations may be used for adequacy assessment, if available.**

The quality of evidence for the use of ROSE is *moderate*. The quality of evidence for the use of a touch preparation is *low*.

The evidence base for this statement comprises 8 studies.<sup>27,41,47,53,57–60</sup> In relation to the use of ROSE, 1 prospective cohort study<sup>57</sup> and 4 retrospective cohort

**Table 3. Number of Passes for Endobronchial Ultrasound-Guided Transbronchial Needle Aspirations**

Study	Study Design	Sample Size, No.	No. of Passes	Diagnostic Yield, %	Adequate Material for Ancillary Testing, %
ROSE Studies					
Trisolini et al, <sup>6</sup> 2015	RCT	197	Median, 4 Range, 3–5		ROSE: 100
Ecka and Sharma, <sup>53</sup> 2013	RCS	646	Mean, 3.12	ROSE: 97.7	
Leong et al, <sup>54</sup> 2017	PCS	40	1		ROSE: 100
Non-ROSE Studies					
Sun et al, <sup>52</sup> 2015	PCS	120	1–7	1 pass: 45.00 2 passes: 79.09 3 passes: 85.87 4 passes: 85.37 5 passes: 92.86 6 passes: 66.67 7 passes: 50.00 P NR	
Oki et al, <sup>55</sup> 2018	PCS	109	1–6	1 pass: 63 2 passes: 75 3 passes: 82 4 passes: 85 5 passes: 86 6 passes: 88 1 versus 2 passes OR, 1.73; 95% CI, 1.27–2.36; P = .007	

Abbreviations: NR, not reported; OR, odds ratio; PCS, prospective cohort study; RCS, retrospective cohort study; RCT, randomized controlled trial; ROSE, rapid on-site evaluation.

studies<sup>47,53,58,60</sup> reported on diagnostic yields using ROSE, and 1 systematic review,<sup>27</sup> 1 prospective cohort study,<sup>41</sup> and 1 retrospective cohort study<sup>47</sup> reported on adverse events. Included studies carried a serious aggregate risk of bias and were limited by their risk in relation to patient selection,<sup>41,47,53,58,60</sup> performance,<sup>60</sup> detection,<sup>41,53,60</sup> and reporting,<sup>41,60</sup> as well as a lack of reported funding.<sup>41,47,53</sup> Evidence supporting the use of touch preparations for adequacy assessment comprises 1 prospective cohort study<sup>59</sup> and 1 retrospective cohort study<sup>58</sup> with an aggregate very serious risk of bias. None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 7 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 5.

Rapid on-site evaluation can be used during transthoracic image-guided biopsy procedures to assess for specimen adequacy, help appropriately triage the specimen for ancillary testing (such as for flow cytometry or microbiologic cultures), and reduce the rate of repeat procedures for discordant or nondiagnostic biopsies. In cases where only CNB is performed without a concurrent FNA, adequacy assessment may be performed using touch preparation using a technique that does not compromise the integrity of the CNB sample.<sup>58</sup> Many factors can determine whether ROSE/touch preparation may be used for transthoracic procedures in different practice settings, and this recommendation takes into consideration the published evidence to date that either supports or refutes its use. The use of ROSE<sup>47,53,57,58</sup> and touch preparation<sup>58,59</sup> at the time of transthoracic biopsy procedure significantly improved diagnostic yield across all modalities. There is, however, a concern that using ROSE adds to procedure time and

therefore increases the risk of complications. This was not substantiated in the literature review, as studies showed no significant increase in the rate of complications, including hemoptysis and pneumothorax, when using ROSE.<sup>27,41,47</sup> One study<sup>58</sup> specifically mentions the use of touch preparations on CNB as a way of adequacy assessments leading to higher success rates. Additional studies<sup>61–63</sup> not meeting the final inclusion criteria of at least 30 samples indicate that adequacy assessment of CNB by touch preparation improves diagnostic adequacy, improves proper specimen triage for ancillary studies, and reduces the number of CNBs needed. Two of these studies<sup>61,62</sup> have also demonstrated the utility of touch preparations as an alternate rapid method for tumor enrichment in lung carcinoma that can be used for molecular testing. It is worth noting that vigorous touch preparations should be avoided and appropriate care should be taken not to deplete the cellularity of the CNB, which would render the biopsy insufficient for diagnosis and ancillary studies.<sup>64,65</sup> Some studies have shown light touch imprints and/or limited drags are preferred methods for touch preparation that minimize the loss of cellularity of the CNB.<sup>64</sup> Although the touch preparation slide can be potentially used for molecular testing, the feasibility of a full ancillary workup (including mutational analysis, FISH, and IHC) is limited on a touch preparation slide in cases where the CNB is depleted and inadequate for further ancillary testing.

During the open comment period, 67.89% (n = 148) of the respondents agreed with the proposed statement, 22.48% (n = 49) disagreed, and 9.63% (n = 21) indicated this statement did not pertain to their area of expertise or practice. A total of 44 comments were received, which could be categorized in 3 themes: a modest number of comments highlighted the

**Table 4. Adequacy for Transthoracic Needle Procedures**

Study	Study Design	Sample Size, No.	Diagnostic Yield, %	Adverse Events, %
Mfokazi et al, <sup>57</sup> 2016	PCS	64	CSC: 76.6 CSC + ROSE: 90.6 <i>P</i> < .001	
Ecka and Sharma, <sup>53</sup> 2013	RCS	646	ROSE: 97.7 Non-ROSE: 64.8 <i>P</i> = .001	
Tachibana et al, <sup>47</sup> 2013	RCS	ROSE, n = 172 Non-ROSE, n = 98	ROSE: 94.8 Non-ROSE: 79.6 <i>P</i> < .001	Mild pneumothorax ROSE: 17.4 Non-ROSE: 20.4 <i>P</i> = .546 Moderate to severe pneumothorax ROSE: 2.9 Non-ROSE: 14.3 <i>P</i> < .001
Fassina et al, <sup>41</sup> 2011	PCS	311		Pneumothorax ROSE: 4.3 <sup>a</sup>
Chang et al, <sup>59</sup> 2008	PCS	622		Hemothorax Touch preparation: 1.1 <sup>a</sup> Pneumothorax Touch preparation: 38.0 <sup>a</sup>

Abbreviations: CSC, conventional smear cytology; PCS, prospective cohort study; RCS, retrospective cohort study; ROSE, rapid on-site evaluation.

<sup>a</sup> No comparison group.

inability to generalize situations in which ROSE may not be appropriate based on technical, patient-related, or institution-specific reasons; a small number of comments claimed superiority of FNA over CNB or vice versa but unrelated to ROSE; and a significant number of comments argued against the use of ROSE, stating that it does not improve diagnostic outcomes and increases complications. These comments were taken into consideration at the time of the final wording for this statement. Considering the strong evidence from the systematic literature review demonstrating that ROSE significantly improves diagnostic yield in transthoracic procedures without any significant increase in the rate of complications, only minor changes to the verbiage were made to add clarity to the definition of ROSE/touch preparation in situations where a CNB was performed without a concurrent FNA. Furthermore, the specific nature of ROSE is defined elsewhere in this document to address a lack of clarity in ROSE methodology suggested by the comments.

Refer to Table 4 for study data on adequacy assessment for transthoracic needle procedures.

**6. Recommendation.—When performing transthoracic needle procedures, needle size should be determined by the operator and technique. For transthoracic FNAs, needles as small as 25 gauge may be used. For CNBs, needles as small as 20 gauge may be used.**

The quality of evidence is *low*.

Evidence supporting this statement is based on studies that evaluated adequacy using various needle gauges and studies that reported on adverse events using various needle gauges. The evidence base comprises 12 studies with an aggregate very serious risk of bias. Of the included studies, 1 reported on adequacy outcomes alone,<sup>66</sup> 1 reported on both adequacy and adverse events,<sup>67</sup> and 10 reported only on adverse events.<sup>59,68–76</sup> Of the 2 studies reporting on tissue adequacy following specimen collection, 1 study was an intermediate- to low-quality meta-analysis<sup>66</sup> and the other

study was a low-quality prospective cohort study.<sup>67</sup> Although based on a systematic review, the meta-analysis<sup>66</sup> did not include an a priori design, duplicate study selection or data extraction, a complete list of included and excluded studies, or quality assessment of the included studies. Studies reporting on adverse events included 6 prospective cohort studies<sup>59,70,72–75</sup> and 5 retrospective cohort studies.<sup>67–69,71,76</sup> These studies were limited by their risk of bias, including selection bias,<sup>59,68–76</sup> performance bias,<sup>59,75</sup> detection bias,<sup>59,69,71,72,74,76</sup> and reporting bias,<sup>59,68,69,71–74,76</sup> as well as a lack of reported funding.<sup>59,69,72–76</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 8 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 6.

The technical aspects of the transthoracic procedure, including the choice of needle gauge used, vary among operators, but the principle is to procure the largest tissue sample with minimal complications. Additionally, a coaxial needle can be used, as it creates a channel through which FNA and CNB can be performed without having to puncture the pleura multiple times. In these procedures, the size of the outer sheath of the coaxial needle is independent of the gauge of the FNA and CNB needles passing through the trocar and is partially independent of the number of samples procured, unless pulmonary hemorrhage occurs. Because of the increased risk of complications when puncturing the pleura in transthoracic procedures (see section on complications), when a non-coaxial technique is used, more passes with a smaller needle gauge may be preferable to fewer larger passes, particularly if samples need to be sent to multiple laboratories for analysis.

Based on the studies identified from the systematic literature review, the needle size for transthoracic procedures varies between 10 and 25 gauge, although most

**Table 5. Needle Gauge for Transthoracic Fine-Needle Aspirations and Core Needle Biopsies**

Study	Study Design	Sample Size, No.	Needle Gauge(s)	Adverse Events, %
Uzun et al, <sup>74</sup> 2017	PCS	432	20, 22	Pneumothorax: 19.6 Pulmonary hemorrhage: 20.8 Hemoptysis: 7.9 Mild hemothorax: 0.4
Konjengbam et al, <sup>75</sup> 2014	RCS	61	22	Hemoptysis: 1.6
Khan et al, <sup>71</sup> 2012	RCS	101	22	Pneumothorax: 25.7
D'Alessandro et al, <sup>72</sup> 2007	PCS	583	19, 20, 21	Pneumothorax: 18.0
Konofaos et al, <sup>73</sup> 2006	PCS	80	21	Pneumothorax: 5.0
Aktas et al, <sup>76</sup> 2015	RCS	85	18, 22	Pneumothorax: 28.7 Hemothorax: 4.1
Chang et al, <sup>59</sup> 2008	RCS	622	20	Hemothorax: 1.1 Pneumothorax: 38.0
Tam et al, <sup>70</sup> 2013	RCS	151	20	Pneumothorax: 15.3
Jaconi et al, <sup>68</sup> 2015	RCS	375	18	Pneumothorax: 21.5
Chen et al, <sup>69</sup> 2014	RCS	353	16	Postbiopsy complications: 13.6
Lalji et al, <sup>67</sup> 2015	RCS	35	10	Pneumothorax: 40.0 Hemoptysis: 0

Abbreviations: PCS, prospective cohort study; RCS, retrospective cohort study.

centers use 18 to 20 gauge for CNB and 21 to 22 gauge for FNA.<sup>66</sup> More studies reported the use of a coaxial technique<sup>59,67,68,70,73,76</sup> than a noncoaxial technique.<sup>69,74,75</sup> The coaxial needle sizes reported most frequently were 17 and 19 gauge. Diagnostic accuracy is reported as up to 97% with sensitivity of 71% to 100% and specificity of 97% to 100%.

In addition to the aforementioned studies, a number of studies not meeting the final inclusion criteria and excluded from the evidence base of this systematic review provide some support to this recommendation but suggest the optimum CNB size is 18 gauge. Cheung et al<sup>77</sup> found that ancillary studies in lung cancer were possible with 18- and 20-gauge CNB, although the larger cores provided more viable tissue. Jamshidi et al<sup>78</sup> found that 18-gauge needles with fewer passes yielded better samples than 20-gauge needles for genomic studies.

Although several studies from the systematic review used coaxial needles, no study directly compared coaxial with noncoaxial needles. As such, the expert panel did not have sufficient evidence to make a recommendation for or against the use of a coaxial needle.

During the open comment period there were 214 respondents, of whom 62.15% (n = 133) agreed, 7.94% (n = 17) disagreed, and 29.91% (n = 64) indicated that the statement did not pertain to their area of interest. There were 23 written comments, including a number that suggested that 20 gauge was too small for adequate tissue for CNB and that 25 gauge for FNA was too flimsy to pass through the chest wall. Most comments favored 18 gauge for CNB, although noting that the preference and experience of the operator is important. None of the comments, however, took into account the incidence of complications. Based on the review of the evidence and the expert panel's considered judgment after discussing the comments, the recommended needle gauges remained unchanged following public comment with minor changes to the verbiage for clarity of the needle size.

Refer to Table 5 for study data on needle gauge for transthoracic FNAs and CNBs.

**7. Recommendation.—When performing transthoracic FNA without CNB, the proceduralist should obtain multiple passes, if technically and clinically feasible, and should attempt to collect sufficient material for a tissue block (ie, cell block, tissue clot).**

The quality of evidence is *low*.

The evidence base for this statement comprises 5 studies<sup>41,60,79–81</sup> that evaluated diagnostic yield and adequacy outcomes based on number of passes, 2 studies<sup>41,60</sup> reporting on adverse events of multiple passes, and 8 studies<sup>59,82–88</sup> reporting on diagnostic yield and adequacy when a tissue block was created. The aggregate risk of bias across the 13 studies was very serious. The 5 studies reporting on diagnostic yield and adequacy for multiple passes included 3 prospective cohort studies<sup>41,80,81</sup> and 2 retrospective cohort studies.<sup>60,79</sup> Of the studies reporting on multiple passes, 1 prospective cohort study<sup>41</sup> and 1 retrospective cohort study<sup>81</sup> also reported on adverse events. All studies<sup>41,60,79–81</sup> reporting on diagnostic outcomes suffered from risk of bias in relation to patient selection, 4 studies<sup>41,60,79,80</sup> were limited by detection bias, 4 studies<sup>41,60,79,81</sup> contained missing data, and 2 studies<sup>41,79</sup> did not report on funding source. Of the 8 studies reporting on the creation of tissue blocks, 3 were prospective cohort studies<sup>59,84,87</sup> and 5 were retrospective cohort studies.<sup>82,83,85,86,88</sup> Included studies were limited by their risk of bias in relation to patient selection,<sup>59,82–88</sup> performance,<sup>59,85</sup> detection,<sup>59,83,85–88</sup> and reporting,<sup>59,83–87</sup> as well as a lack of reported funding.<sup>59,85,86</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 9 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 7.

Transthoracic needle biopsies may sometimes be performed solely with FNA technique in specific situations, such as in lesions that are deemed unsafe for CNB (eg, risk of significant hemorrhage because of adjacent blood vessels), when the lesion is too small for CNB, or in situations where the proceduralist does not have the

necessary expertise to perform CNB procedures. In these cases, the transthoracic FNA specimen may be the only tissue available for diagnosis and subsequent ancillary studies.

Ancillary testing in lung carcinoma, including molecular analysis, FISH, and IHC, has traditionally been validated and performed on formalin-fixed, paraffin-embedded (FFPE) tissue (ie, tissue blocks from surgical pathology biopsies/resections or cytology cell block preparations). Although several studies have demonstrated the feasibility and utility of molecular testing using cytology smears and liquid-based preparations, most commercial laboratories have not validated testing on non-FFPE-based specimen sources. Of note, the updated CAP/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP) lung carcinoma molecular testing guideline now recommends that any cytology sample, and not just cell blocks, can be used for testing.<sup>89</sup> However, until most molecular laboratories validate and accept non-FFPE cytology specimens for testing, cell blocks remain the cytologic substrate of choice for lung carcinoma-related biomarker testing in most laboratories. Further, the ability to perform multiple IHC markers on cell block sections, some of which may be used for lung carcinoma predictive/prognostic testing, also makes a strong case for the acquisition of a tissue block. Therefore, if a transthoracic FNA is performed without CNB, the proceduralist should perform multiple FNA passes, if possible, and attempt to obtain sufficient material for a tissue block (ie, cell block, tissue clot) to perform all the necessary ancillary studies. The role of transthoracic FNA in diagnosing pulmonary malignancies is well established, with most of the supportive studies published prior to the limits defined by the systematic literature review. Performing multiple FNA passes has been shown to confer incremental increases in diagnostic yield.<sup>41</sup> Despite the performance of multiple passes to create a tissue block, there does not appear to be an association between increased number of FNA passes and pneumothorax rates.<sup>79</sup> As previously mentioned, the use of a coaxial needle can minimize the number of pleural punctures and may reduce the complication rates of pneumothorax. Combining cytology with a cell block preparation increases diagnostic yield as well as the ability to perform ancillary testing.<sup>84</sup>

Based on the systematic literature review, there were several studies that evaluated ancillary testing on cytology cell blocks from transthoracic procedures. A few studies compared success of ancillary testing on cell blocks with surgical pathology specimens, some of which note similar performance rates between cell blocks and surgical core biopsies,<sup>82,84,88</sup> whereas others report lower performance on cell blocks when compared with CNBs.<sup>85,86</sup> One study reported that adding dedicated passes to the collection media for cell block preparation led to an apparent increase in tissue collected.<sup>86</sup>

During the open comment period, there were 220 respondents, of whom 89.09% (n = 196) agreed, 3.64% (n = 8) disagreed, and 7.27% (n = 16) indicated the statement did not pertain to their area of expertise or practice. There were 28 written comments. Several comments emphasized how important the recommendation is in order to obtain adequate material for molecular testing and other ancillary studies. Several comments stated that multiple passes may not be possible depending on the lesion and that additional passes should be performed only if they do not compromise

patient safety. There were a few comments regarding laboratory or personal preferences that did not warrant changes to the recommendation. All comments were taken into consideration in the final draft of statement 7, which remains largely unchanged as presented in this document.

**8. Recommendation.—To achieve optimal diagnostic yield when performing transthoracic CNBs, the proceduralist should attempt to obtain a minimum of 3 core samples, if technically and clinically feasible. Additional samples may be required for ancillary studies.**

The quality of evidence is *low*.

One low-quality retrospective study<sup>90</sup> supported this statement. This study reported on both diagnostic accuracy of 1 through 5 passes and adverse events. The study was limited by risk of study selection bias, as well as detection bias. Refer to Supplemental Table 10 in the supplemental digital content for complete quality assessment results for statement 8.

The decision regarding the optimal number of CNB samples collected for adequacy is frequently operator dependent and balanced by a number of factors, including the potential for complications by repeating passes of a larger core biopsy needle or device, prolonging the procedure and any concurrent anesthesia or sedation, and the need for more tissue for ancillary studies. The existing literature was reviewed for evidence guiding the delineation of this balance. It is worth noting that the amount of tissue/tumor can vary in CNB samples depending on the length of the core and the fragmentation of CNB samples leading to a loss of tissue in processing; however, the literature review did not provide any insight into a specific volume of tissue that would correlate with an adequate amount of tumor for ancillary studies.

Evidence supporting this recommendation comes from a retrospective cohort study<sup>90</sup> that showed that accuracy significantly increased as the number of CNB samples increased to 3. However, although this was not explicitly stated in the study manuscript, accuracy did not increase with further passes beyond 3, and may have declined by 5 passes. Complication rates reported by that study were not different from those of other studies reviewed during the entire literature review, and therefore the study lends support to the above statement.

Further studies, which did not report on diagnostic accuracy or yield in relation to number of specimens collected and ultimately were not included in our evidence base, indirectly support the above statement. One prospective study, which evaluated the use of CNB for biomarker trials, collected at least 2 CNB samples when the specimens were small or fragmented and reported 82.9% adequate material for biomarker analysis.<sup>70</sup> Another study reporting an average of 2 to 3 samples collected demonstrated a diagnostic yield of 94.6% with molecular adequacy of 96.8% to 98.6%.<sup>91</sup> Finally, a systematic review of 11 studies reported minimal complications in studies that collected 2 CNB samples on average.<sup>92</sup>

During the open comment period, 77.38% (n = 171 of 221) of the commenters agreed with the proposed statement, 12.67% (n = 28) disagreed, and 9.95% (n = 22) indicated that the statement did not pertain to their area of expertise or practice. Most comments purported that as the sample number increased beyond 3, diagnostic yield should increase as well. Although this was not explicitly found during the literature review, the sole study<sup>90</sup> included in the review may have found decreasing accuracy after 3 samples,

**Table 6. Adequacy for Transbronchial Needle Aspirates**

Study	Study Design	Sample Size, No.	Diagnostic Yield, % (95% CI)	Diagnostic Sensitivity, %
Mondoni et al, <sup>102</sup> 2016	SR	1687 (18 studies)	ROSE: 62 (40–80) <sup>a</sup>	
Sindhvani et al, <sup>103</sup> 2013	PCS	40	ROSE: 75 <sup>a</sup>	
Conti et al, <sup>98</sup> 2016	PCS	101		TP or CS + ROSE: 85 CS: 51 TP: 54 P NR
Diacon et al, <sup>99</sup> 2010	PCS	126		ROSE, Giemsa stain: 93 <sup>a</sup> ROSE, Papanicolaou stain: 100 <sup>a</sup>
Madan et al, <sup>101</sup> 2016	RCS	41	ROSE: 78 <sup>a</sup>	
Chen et al, <sup>97</sup> 2015	RCS	815	ROSE: 86.7 Non-ROSE: 71.8 P < .001	ROSE: 98.2 <sup>a</sup>
Loo et al, <sup>100</sup> 2014	RCS	40		ROSE: 85 <sup>a</sup>

Abbreviations: CS, conventional smear; NR, not reported; PCS, prospective cohort study; RCS, retrospective cohort study; ROSE, rapid on-site evaluation; SR, systematic review; TP, ThinPrep.

<sup>a</sup> No non-ROSE comparison.

which may be related to technical, lesion-dependent, or other factors. Furthermore, a minority of comments were concerned with procedural feasibility for numerous samples in the original language of the recommendation. These comments were taken into consideration, and minor changes to the statement were made with regard to attempted biopsies, “if technically and clinically feasible.”

### Bronchoscopic Procedures

As noted in the previous section, the prevalence of intrathoracic abnormalities and PPLs is likely to increase in the setting of increased chest imaging. Although computed tomography–guided transthoracic needle biopsy remains a commonly used modality for sampling these lesions, several image-guided bronchoscopic technologies are available, including computed tomography–guided bronchoscopy, virtual bronchoscopy with ultrathin bronchoscope, radial endobronchial ultrasound, and electromagnetic navigation bronchoscopy (ENB). However, these image-guided technologies may not be available in all practice centers. Transbronchial sampling methods—transbronchial biopsy (TBB) and transbronchial needle aspiration—are commonly performed during bronchoscopic investigations of peripheral nodules, and, as with endobronchial ultrasound–guided and transthoracic procedures, specimen adequacy depends on multiple factors. The following recommendations address specimen collection and handling for bronchoscopic procedures.

**9. Recommendation.—If performing bronchoscopy for the investigation of PPLs that are difficult to reach with conventional bronchoscopy, image-guidance adjuncts may be used, if local expertise and equipment are available.**

The quality of evidence is *low*.

One prospective cohort study<sup>93</sup> and 1 retrospective cohort study<sup>94</sup> comprise the evidence base for statement 9. The aggregate risk of bias was very serious based on risk of selection bias and detection bias in both studies.<sup>93,94</sup> Neither study was found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 11 in the supplemental digital content for the quality

assessment results of all studies included in the evidentiary base of statement 9.

The evidentiary base supporting this recommendation comprised 2 studies.<sup>93,94</sup> When compared with using fluoroscopy alone for peripheral bronchoscopy, radial-probe endobronchial ultrasound along with fluoroscopy improved the diagnostic accuracy for PPLs.<sup>93</sup> The difference in yield was statistically significant only when the nodule size was less than 30 mm. Balbo et al<sup>94</sup> showed that ROSE combined with ENB provides a diagnostic yield of 70.7% when patients had had a prior nondiagnostic bronchoscopy. Therefore, based on low-level evidence, the use of adjunct image guidance techniques may help improve the ability to accurately sample and obtain smaller tissue nodules during bronchoscopy.

In addition to the aforementioned studies, studies not included in our systematic review support this recommendation. A meta-analysis by Wang Memoli et al<sup>95</sup> of single-center cohort studies by university-based bronchoscopists reported diagnostic yields of approximately 70% for either radial probe endobronchial ultrasound, virtual bronchoscopy, or ENB. However, a large recently published multicenter registry<sup>96</sup> of 581 bronchoscopies (included community and university based) reported yields of 57.0% for radial probe endobronchial ultrasound alone, 38.5% for ENB alone, and 47.1% with ENB combined with radial probe endobronchial ultrasound. For that reason, multicenter RCTs are still needed to further evaluate these technologies.

The use of ROSE during image-guided bronchoscopy of PPLs has been less studied. Although ROSE may identify the correct sampling of a lesion during bronchoscopy, only a few heterogeneous single-center studies have shown yields of 67% to 84% for pulmonary nodules when ROSE is added. Additionally, there remains a paucity of data on techniques for processing tissue from these procedures.

During the open comment period, of the 215 respondents, 70.69% (n = 152) agreed with the recommendation, 4.19% (n = 9) disagreed, and 25.12% (n = 54) indicated that the statement did not pertain to their area of expertise or practice. There were 11 written comments, many of which implied that the success of guided bronchoscopic techniques is highly dependent on the available local expertise and the

lesion characteristics (size, location, and risk for malignancy). Transthoracic needle aspiration was considered the most accurate nonsurgical technique, so the rationale for image-guided bronchoscopic techniques should be justified on a case by case basis using criteria such as a higher pneumothorax risk of transthoracic needle aspiration, technical difficulties of performing transthoracic needle aspiration, or combining mediastinal staging and nodule biopsy in one procedure. These responses were taken into account in the recommendation with minor change to the original draft, to consider this procedure in cases that are difficult to reach with conventional bronchoscopy, if “local expertise and equipment are available.”

**10. Recommendation.—When performing transbronchial needle aspirates, ROSE should be used for adequacy assessment, if available.**

**Expert Consensus Opinion.—If performing transbronchial forceps biopsies without concurrent transbronchial needle aspirates, touch preparations may be used for adequacy assessment, if available.**

The quality of evidence supporting the use of ROSE is *moderate*. The quality of evidence supporting the use of touch preparations is *very low*.

The evidence base for the use of ROSE during bronchoscopic procedures comprises 7 studies,<sup>97–103</sup> whereas evidence supporting the use of touch preparations comprises 1 study.<sup>59</sup> Studies evaluating ROSE carried a serious aggregate risk of bias and included 1 systematic review,<sup>102</sup> 3 prospective cohort studies,<sup>98,99,103</sup> and 3 retrospective cohort studies.<sup>97,100,101</sup> Included cohort studies were limited by their risk of bias in relation to patient selection,<sup>97,98,100,101,103</sup> performance,<sup>97,98,100</sup> detection,<sup>97,98,100,103</sup> and reporting,<sup>97–100,103</sup> as well as a lack of reported funding.<sup>97,98</sup> Evidence supporting the use of touch preparations for adequacy assessment comprises 1 prospective cohort study,<sup>59</sup> which was limited by risk of bias related to patient selection, performance, detection, and reporting domains. None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 12 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 10.

TBNA and TBB, with or without bronchial brushings/washings, are often used as a minimally invasive bronchoscopic approach to establishing a diagnosis for PPLs.<sup>102</sup> Although transbronchial sampling via bronchoscopy is a safe and useful diagnostic technique for PPLs, the sensitivity and accuracy show wide variance in terms of the diagnostic yield and has been well documented in several studies to be directly related to, among other clinical and radiologic factors, the availability and use of ROSE for adequacy assessment.<sup>98,99,101–103</sup>

Evidence from the systematic review<sup>98,99,101–103</sup> supports the use of ROSE for TBNA of PPLs to enhance diagnostic yield, thus improving the sensitivity of the technique and reducing the number of biopsy sites and adverse events, by allowing the bronchoscopist to stop sampling once adequate material for diagnosis and ancillary studies has been collected. Studies having control arms without ROSE<sup>98,103</sup> show lower diagnostic yield and the need for potential repeat procedures, potentially impacting the efficacy of transbronchial samplings and the overall burden and cost. Although TBBs are also frequently performed for the diagnosis of PPLs, a limited number of studies comparing TBNA and TBB performed in the same patients<sup>102</sup> have

established a significant superiority of the former, and therefore indicating, if an adequate sampling for diagnosis and ancillary studies can be acquired via TBNA, unnecessary procedures such as TBB and brushings can be potentially avoided. Further, ROSE has shown significantly improved diagnostic yield in PPLs with a size less than 3 cm and greater than 7 cm, when using radial probe EBUS-guided TBB and brushing).<sup>97</sup> In procedures using ENB FNA, diagnostic yields for PPLs are significantly higher when using ROSE, irrespective of lesion size, with no additional benefit noted with concurrent TBB and bronchial brushing.<sup>100</sup>

During the open comment period, of the 217 respondents, 64.98% (n = 141) agreed with the recommendation, 23.96% (n = 52) disagreed, and 11.06% (n = 24) indicated the statement did not pertain to their area of expertise or practice. There were 23 written comments. Most of these comments indicated confusion over the wording “transbronchial samples” that was used in the original draft recommendations, and were accordingly taken into consideration in the final drafting of statement 10 presented in this document, by making a clear distinction between TBNA and TBB. Other comments were directed at the utility of ROSE and/or the ability to perform ROSE in a TBB, and consequently, a brief description of the studies including the key evidence that contributed to the final draft of statement 10 has been provided.

Refer to Table 6 for data on adequacy assessment of transbronchial needle aspirates.

#### **Pleural Effusions: Considerations for Malignancy**

Pleural effusions related to malignancy are fairly common in advanced malignancies and may be the first manifestation of disease in many cases. The identification of positive cytology results from pleural fluid frequently fulfills both a diagnostic and staging need and may often be the only specimen available to perform ancillary studies. Pleural fluid processing in the laboratory generally involves centrifugation of an aliquot of the specimen to produce a sediment cell pellet. Depending on the preference of the laboratory, direct smears, cytospins, or liquid-based cytology (LBC) slides are prepared from a representative aliquot and a paraffin embedded cell block is prepared from the remaining pellet. In some laboratories, additional unstained cytospins are prepared, if needed for further morphologic evaluation or performing immunocytochemistry. A diagnosis of malignancy can be issued in the majority of cases through morphologic evaluation of the smear/cytospin/LBC preparation and hematoxylin-eosin-stained section of the cell block. Although in the past IHC was only performed in select cases, currently, with the increasing need for further classification of NSCLC, assessment of primary origin of malignancy, and biomarkers for patient management, IHC is required in most cases. Additionally, once a diagnosis of adenocarcinoma is established, molecular studies are required for patient management.

**11. Expert Consensus Opinion.—When collecting pleural fluid for a suspected diagnosis of malignancy, the proceduralist should send as much fluid volume as reasonably attainable for cytologic evaluation and ancillary studies.**

The quality of evidence is *insufficient*.

Although the systematic review identified 3 prospective cohort studies<sup>104–106</sup> and 4 retrospective cohort studies<sup>107–110</sup> that all reported on minimum volume of fluid

required for a diagnosis, the expert panel believed there was too much heterogeneity within the identified studies upon which to base an evidence-based statement. Additionally, no study reported on an upper limit of fluid. None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 13 in the supplemental digital content for the quality assessment results of studies informing this statement.

Currently, no standardized volume requirements exist for cytologic evaluation of effusions. As a result, laboratories may be receiving pleural fluid volumes ranging from a few milliliters to several liters. The evidence base from the systematic review identified adequate or minimum volume of pleural fluid required for the cytologic diagnosis of malignancy but was unable to provide specific data reflecting pleural fluid volumes needed for ancillary testing. Buckley et al<sup>105</sup> prospectively evaluated fluids from 50 patients, comparing aliquots of 10 mL with 50 mL. Carcinoma was identified in 7 patients with 100% agreement between the 2 volumes and the authors determined that 10 mL is a sufficient volume. Swiderek et al<sup>106</sup> prospectively reviewed aliquots of 10 mL, 60 mL, and greater than or equal to 150 mL from 121 thoracenteses with a total of 90 patients diagnosed with malignancy and noted that the sensitivity of cancer diagnosis appeared volume dependent. When both smears and cell blocks were evaluated, a significant difference in cancer detection between only 10 mL and greater than or equal to 150 mL was noticed, but when only smears were evaluated, 60 mL was adequate for the diagnosis of malignancy; therefore, the authors recommended a minimum of 50 to 60 mL. Abouzgheib et al<sup>104</sup> prospectively evaluated fluids from 44 patients using 2 aliquots, the initial 50 mL and greater than or equal to 50 mL, noting a 100% concordance in diagnosis. Thomas et al<sup>109</sup> evaluated 2155 pleural effusions attempting to define the minimum required volume by examining the plateau phase of a graph of threshold volumes. They concluded that the sensitivity did not improve with volumes above 50 mL and that the minimum required volume was between 25 and 50 mL of fluid. Wu et al<sup>110</sup> evaluated 74 samples divided into 3 volumes: 25 mL, 50 mL, and 150 mL noting an increase in sensitivity as the volume increased, but the difference was never statistically significant. Rooper et al<sup>108</sup> retrospectively evaluated 2540 specimens of both benign and malignant effusions with a malignant fraction of 20.1%, received during 9 years, ranging from 5 mL to greater than 900 mL and divided into 9 groups of roughly equal sample size. The malignant fraction improved with the increase in volume; however, it was independent of the volume once it exceeded 75 mL. The authors also underscored the fact that there is no minimum volume in which malignant cells could not be detected, and therefore any volume retrieved should be sent for morphologic evaluation; however, it is equally important to realize that sending low volumes “carries a risk of producing nondiagnostic, atypical or falsely benign diagnosis.”<sup>108</sup>

It is noteworthy that all the above studies focused solely on the detection of cancer and did not correlate the successful preparation of an adequate cell block with the volume evaluated or the success of relevant ancillary studies that may be performed on a malignant lung carcinoma specimen. However, examining the data, one may deduce that far fewer cell blocks were prepared in low-volume

specimens. Also, the influence of immunohistochemical stains on arriving to a definitive diagnosis was not addressed (eg, differential of reactive mesothelium versus adenocarcinoma in the presence of borderline atypia) or in further confirming the primary site of the malignancy (eg, lung adenocarcinoma versus metastatic breast carcinoma or subclassifying a poorly differentiated squamous versus adenocarcinoma). These are all important factors in arriving at a definitive diagnosis that will trigger the appropriate patient management plan especially in the era of personalized management.

Another important factor to consider is that patients with positive fluids are classified as advanced-stage disease. Molecular profiling performed on those fluids may obviate the need for additional procedures and expedite patient management decisions. It is therefore logical that although a volume as low as 10 mL of a truly cellular sample can be adequate for morphologic evaluation, it could potentially be insufficient for further testing.

In summary, adequacy of a fluid for a diagnosis can vary widely depending on the cellularity of the fluid and the needed workup to reach a diagnosis and direct further patient management. Therefore, the larger the volume submitted to the laboratory the more adequate the specimen would be for the additional workup and final diagnosis. On the other hand, it is not always possible to send large volumes to the laboratory for a variety of reasons that may involve carrier restrictions or available containers, among other reasons. Consequently, it is not possible to recommend a minimum or maximum volume, and instead, the required volume should be based on communication with the laboratory who can appropriately triage the specimen (even for large ones) as needed.

During the open comment period there were 215 respondents, of whom 74.88% (n = 161) agreed, 15.35% (n = 33) respondents disagreed, and 9.77% (n = 21) indicated the statement did not pertain to their area of expertise or practice. There were 41 written comments. The majority commented that the volume of fluid submitted should be adequate to allow proper diagnosis and additional ancillary testing including IHC and molecular profiling. Many suggested a minimum volume of 50–100 mL. Although some suggested that the laboratory should encourage submission of all aspirated fluid at least for the initial workup, many expressed concern about the difficulty of discarding large volumes and asked to limit the volume submitted. These comments were taken in consideration in the final draft presented in this document.

### Considerations for Ancillary Studies During Malignant Investigations

**12. Strong Recommendation.**—Cytology specimens (smears, cell blocks, LBC), may be used for ancillary studies, if supported by adequate validation studies.

The quality of evidence is *low*.

The evidence base for this statement comprises 10 studies,<sup>43,46,82,83,111–116</sup> carrying a very serious aggregate risk of bias. Of the 10 studies, 2 reported on the use of cytology specimens for IHC,<sup>112,116</sup> 2 for FISH analysis on cytology specimens,<sup>113,116</sup> and 7 conducted mutational analysis on cytology specimens.<sup>5</sup> Studies reporting on the use of

<sup>5</sup> References 43, 46, 82, 83, 111, 114, 115.

cytology specimens for IHC were both retrospective cohort studies<sup>112,116</sup> limited by risk of bias in selection,<sup>112,116</sup> detection,<sup>112</sup> performance,<sup>116</sup> and reporting domains.<sup>112</sup> One prospective cohort study<sup>113</sup> and 1 retrospective cohort study<sup>116</sup> comprise the studies reporting on the use of cytology specimens for FISH. Finally, all 7 studies that reported on cytology specimens when conducting molecular analysis were retrospective in design and carried the inherent risk to selection bias found in retrospective studies,<sup>43,46,82,83,111,115</sup> plus risk of bias in performance,<sup>111,114,115</sup> detection,<sup>46,83</sup> and reporting domains.<sup>43,83,111,115</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 14 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 12. Although the evidence to support this statement is limited, the expert panel proposes a strong recommendation for the use of cytology specimens when supported by adequate validation studies. It is believed that, in addition to the benefits of using cytology specimens outweighing any perceived harms when conducting ancillary testing on these specimens, the use of cytology specimens will result in resource savings. The expert panel believes this guidance to be both feasible to implement and acceptable to key stakeholders.

As many patients with malignancy, particularly lung cancer, often present in advanced stages of disease, surgical resection of the primary lesion with curative intent is usually not a feasible option for testing. For a large fraction of these patients diagnosed by minimally invasive procedures, a cytology specimen may be the only specimen available for molecular biomarker testing/ancillary studies, without requiring a repeat tissue biopsy for patient management. In most molecular testing laboratories, the use of mutational analysis, FISH and IHC are typically validated for FFPE tissue; therefore, good laboratory practice and governmental regulations should continue to dictate that nontraditional specimen types should be specifically validated for the protocols used in individual laboratories.<sup>117,118</sup>

Data from the systematic review reveal ample evidence demonstrating the feasibility of mutational analysis, FISH, and IHC for lung carcinoma using common cytology specimens. Multiple studies have identified the adequacy of appropriately tested/validated techniques for use of cytologic specimens; including the use smears for IHC,<sup>116</sup> smears for next-generation sequencing,<sup>82,83</sup> and LBC preparations.<sup>113</sup> The use of small biopsies and cell block preparations for nucleic acid–based testing has been fairly well established, following routine protocols for processing FFPE tissue. The use of stained archival smears (or LBC slides) as sources for DNA analysis has been more challenging. Nevertheless, a handful of laboratories have effectively demonstrated the feasibility of doing so,<sup>82,83,114</sup> with some noting a significantly better DNA quality obtained from direct smears than from biopsies or cell block preparations<sup>83</sup> and higher tumor cell content and DNA concentrations from cytology direct smears than from cell block specimens.<sup>114</sup> These studies highlight the potential of using cytologic specimens for even the most sophisticated molecular evaluations. The extensive preanalytical requirements for next-generation sequencing–based testing, in terms of tumor cell fraction and DNA quantity and quality, complement certain characteristics of the cytologic specimen. Tumor cell content and fraction can be readily

assessed and documented for smears. DNA quantity and quality are routinely addressed for these molecular tests, as is the reliability of any results through depth of coverage considerations. Merging these 2 techniques, however, demands close communication between the cytopathologist and the molecular laboratory, as is demonstrated by the cited studies. Furthermore, most studies demonstrating feasibility are performed using laboratory-developed tests and laboratory-specific protocols, and direct comparisons of outcomes is at best difficult and often impossible. Hence, rigorous intralaboratory validation is critical not only for mutational assays but for all lung cancer biomarker studies, including FISH and IHC. Understanding the significance that many current FISH and IHC biomarkers assays have for determining potential therapeutic options, the need to perform adequate validation studies to establish necessary positive and negative predictive values on cytology specimens is particularly important, and laboratories using such specimens for these purposes should not presume performance characteristics for FFPE are valid for cytology specimens. Goldsmith et al<sup>117</sup> recently reviewed the essential principles for validating immunohistochemical assays. They specifically note that for cytologic preparations (smears, cell blocks, and LBC slides) “reasonable efforts should be made to assure that these assays perform adequately before they are used on patient samples.”<sup>117</sup>

During the open comment period, there were a total of 212 respondents: 87.73% (n = 186) agreed with the guideline statement, 4.72% (n = 10) disagreed, and 7.55% (n = 16) indicated that the statement did not pertain to their area of expertise or practice. Thirty-five participants offered comments. Twelve comments expressly noted the importance of validation and a further 6 indicated concerns about various elements of validation (eg, fixative differences, processing differences). Consequently, the recommendation was modified to state the need for “adequate validation studies.”

### **13. Recommendation.—Core needle biopsy specimens collected for ancillary studies should be fixed in 10% neutral buffered formalin.**

The quality of evidence is *very low*.

Although no study directly compared the use of one fixative with another, 4 studies<sup>70,91,119,120</sup> reporting on adequacy for ancillary testing or successful ancillary testing following fixation with 10% neutral buffered formalin comprise the indirect evidence supporting this statement. Studies reporting on mutational analysis comprise 2 prospective cohort studies,<sup>70,119</sup> and 2 retrospective cohort studies.<sup>91,120</sup> One of the prospective cohort studies<sup>70</sup> also reported on IHC and FISH, and 1 retrospective cohort study<sup>91</sup> reported on FISH in addition to mutational analysis. Included studies carried an aggregate very serious risk of bias and were limited by their risk of bias in relation to patient selection,<sup>70,91,119,120</sup> detection,<sup>91,120</sup> and reporting,<sup>91,120</sup> as well as a lack of reported funding.<sup>119,120</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 15 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 13.

Barring the need for microbiologic studies for an infectious process, all CNB samples need to be fixed in a timely manner to minimize the impact of cold ischemic time on the tissue. Evidence from the systematic review indicates that a high percentage (>95%) of formalin-fixed CNB

specimens are suitable for IHC, FISH, and biomarker testing.<sup>91,120</sup> However, results from a prospective trial suggest the percentage may be a more modest 83% of specimens,<sup>70</sup> although it is unclear whether the failures were due to formalin fixation or other preanalytic variables that affect sample adequacy. Most molecular assays used in clinical practice and in clinical trials are routinely validated on formalin-fixed specimens. In addition, most commercially available Food and Drug Administration assays have been validated on formalin-fixed tissues only (eg, HER2, programmed death ligand-1 [PD-L1]). Additional evidence outside our systematic review and commonly known good laboratory practice indicate that preanalytic variables, including fixative, time of fixation, processing temperature, and storage conditions, affect the quality and quantity of nucleic acids and proteins that appear to have an impact on downstream ancillary testing.<sup>121</sup> Although fresh or snap frozen CNB specimens can be used for molecular assays, they remain suboptimal for histologic assessment and therefore are often not used in clinical practice when tissue is obtained for diagnosis and potential ancillary studies. Ten percent neutral buffered formalin is the most commonly used fixative for CNB specimens,<sup>121</sup> and neutral buffered formalin is favored over unbuffered formalin solutions as the latter spontaneously oxidizes to formic acid over time and may inhibit molecular assays. Acidic or heavy metal fixatives (eg, Zenker, B5, B plus) are also not recommended for use in specimens as they inhibit most of the molecular assays.<sup>122</sup> Similarly, harsh acids should be avoided for decalcification of bone metastases, as they hamper the quality of the molecular analytes to be tested.<sup>123</sup> Nonacidic chelating decalcifying solutions may be used as an alternative.

Nevertheless, there are potential technical issues caused by formalin fixation that may affect the assay interpretation.<sup>124–128</sup> Therefore, it is essential for laboratories to perform a rigorous clinical validation of IHC, FISH, and molecular assays in order to prevent false-positive or false-negative results caused by fixation artifact.

During the open comment period, of the 209 respondents, 80.86% (n = 169) agreed with the recommendation, 9.09% (n = 19) disagreed, and 10.05% (n = 21) indicated that this recommendation does not pertain to their area of expertise or practice. There were 26 written comments. Most of the comments reflected confusion with the specimen type with respondents commenting on alcohol fixation of FNAs rather than fixation of CNB. A few comments suggested that a portion of a sample should be frozen for molecular testing and that each molecular laboratory should decide about preferred method of specimen fixation. These comments were taken into consideration; however, the draft recommendation was maintained with the original language.

### Considerations for Ancillary Studies During Nonmalignant Investigations

Thoracic small biopsy and cytology specimens are also often procured for the determination of infectious processes. For microbiologic culture studies, a separate sterile aliquot of the specimen should be collected for microbiologic studies. In patients with suspected pulmonary tuberculosis, the evaluation often begins with an attempt to collect sputum, followed by induced sputum for analysis. If these efforts do not yield diagnostic specimens, patients may be considered for a potentially more invasive technique such as bronchoscopy. In the clinical setting of suspected tuberculosis without parenchymal lesions, sputum and/or lavage

cultures may not be able to provide a diagnosis; therefore, the use of EBUS TBNA has emerged as a potentially viable modality for the collection of diagnostic specimens. The systematic review identified only limited literature pertaining to ancillary studies for infectious processes, and most of the studies that were identified were related to the investigation of pulmonary tuberculosis. Therefore, the following recommendations take into consideration the published evidence on ancillary studies for pulmonary tuberculosis as identified in the systematic review.

### 14. Recommendation.—When performing bronchoscopy for the investigation of tuberculosis, endobronchial ultrasonography may be used to increase the diagnostic yield of bronchoalveolar lavage and TBB.

The quality of evidence is *low*.

This statement is supported by 1 retrospective cohort study,<sup>129</sup> which was limited by a risk to selection bias, performance bias, reporting bias, and detection bias. The study did not contain methodologic flaws that would raise concerns about its findings. Refer to Supplemental Table 16 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 14.

Multiple methods of bronchoscopic sampling exist, including bronchoalveolar lavage, brushings, and TBB. Although standard bronchoscopy techniques are fairly safe and well tolerated, the performance of TBB can increase the risks of complications (mainly bleeding and pneumothorax). The use of endobronchial ultrasound allows for direct imaging lesion visualization and potentially a more targeted biopsy, oftentimes with a potentially lower complication rate.

Evidence from the systematic review identified a study<sup>129</sup> comparing the diagnostic yield of acid-fast bacilli (AFB) smears and mycobacteria cultures in bronchoalveolar lavage fluid as well as histopathologic specimen of transbronchial lung biopsies between conventional and EBUS bronchoscopic sampling techniques. The study evaluated 121 patients undergoing bronchoscopy who previously had either negative sputum AFB smears or a lack of sputum production. In this study, the addition of EBUS improved the overall diagnostic yield from 58.3% to 80.8% ( $P = .04$ ).<sup>129</sup>

During the open comment period, of the 209 respondents 60.29% (n = 126) agreed with the guideline statement, 13.88% (n = 29) disagreed, and 25.84% (n = 54) indicated that the statement did not pertain to their area of expertise or practice. The draft recommendation at the time of the open comment period stated that EBUS TBNA “should be performed” and several respondents stated that clinical judgment is required (ie, the decision should be based on clinical context). This was reassessed by the expert panel, and the panel decided to remove TBNA from the recommendation. The expert panel also decided to replace “should be performed” with “may be performed” to allow for flexibility. A few comments reflected concern regarding potential additional exposure of medical personnel and equipment to AFB with associated risk of resulting infection and contamination when performing EBUS TBNA in addition to bronchoalveolar lavage and TBB. The expert panel appreciates such secondary considerations and refers to corresponding documents that have been issued by the appropriate government institutions.<sup>130,131</sup> Proceduralists should follow local institutional policies, which ideally are aligned with such guidance from public health authorities.

**15. Recommendation.—When performing EBUS TBNA for the evaluation of intrathoracic granulomatous lymphadenopathy with the suspicion of tuberculosis, specimens should be collected for cytology, microbiology (mycobacterial smear and culture), and *Mycobacterium tuberculosis* polymerase chain reaction (PCR) evaluation, if available.**

The quality of evidence is *low*.

The evidence base for this statement comprises 3 retrospective cohort studies limited by a very serious aggregate risk of bias.<sup>132–134</sup> Studies carried risk of bias in patient selection,<sup>132–134</sup> performance,<sup>132</sup> detection,<sup>132–134</sup> and reporting domains,<sup>132–134</sup> as well as a lack of reported funding.<sup>132–134</sup> None of the studies were found to have methodologic flaws that would raise concerns about the findings. Refer to Supplemental Table 17 in the supplemental digital content for the quality assessment results of all studies included in the evidentiary base of statement 15.

In patients with isolated intrathoracic granulomatous lymphadenopathy, primary considerations in the differential diagnosis are often sarcoidosis, tuberculosis, and malignancy. As it appears that no single technique for testing EBUS TBNA specimens has yet been shown to perform with sufficient diagnostic accuracy to serve as a standalone method, multiple testing methods can be combined to provide increased yield and offer complementary information. Cytology identifies a characteristic host inflammatory response; microbiological smear and staining for AFB may more specifically identify the presence of mycobacteria, whereas culture serves as the gold standard for identification of *M tuberculosis* as well as the source of isolates needed for drug susceptibility testing. However, culture-based methods often take weeks until results are available. Molecular tests (PCR, nucleic acid amplification tests) have the potential to provide rapid results; however, these also incur significant additional expense, and may not be justified and/or available in low-resource settings.

Evidence from the systematic review reveals that use of EBUS TBNA in countries with high-risk tuberculosis exposure has demonstrated promising results. In Turkey, 42 patients (95.4%) were diagnosed as having tuberculous lymphadenopathy using cytology and/or microbiology. Adding culture to cytology improved the diagnostic value of EBUS TBNA from 72.7% to 95.4%, providing an additional 22.7%.<sup>133</sup> In South Korea, using *Mycobacterium tuberculosis* PCR (nested PCR using FFPE specimens, or real-time PCR using specimens in sterile saline) from EBUS TBNA specimens was determined to have overall diagnostic accuracy of 85% for patients with isolated granulomatous lymphadenopathy, with no difference demonstrated between the PCR methods.<sup>132</sup> Using a combination of *Mycobacterium tuberculosis* PCR, histology and microbiology methods together increased the diagnostic yield to 94%. The use of EBUS TBNA allowed the avoidance of more invasive procedures such as mediastinoscopy with general anesthesia. In India, the use of EBUS in evaluation of patients with mediastinal lymphadenopathy was diagnostic in 77% (106 of 138 patients) with 46% (63 patients) demonstrating granulomatous inflammation. Ultimately 35 patients were diagnosed with tuberculosis using multiple methods of evaluation including AFB smear, mycobacterial culture, PCR, cytology, and histology. Authors recommended that EBUS specimens should be analyzed by mycobacterial smear, culture, and PCR.<sup>134</sup>

During the open comment period, of the 209 respondents, 80.86% agreed (n = 169) with the guideline statement, 3.35% disagreed (n = 7), and 15.79% (n = 33) indicated that the statement did not pertain to their area of expertise or practice. Among the 9 comments received, some mentioned restrictions from institutional infection prevention policies whereas others recommended testing strategies determined by the ROSE pathologist or according to specific method preferences, especially if there is limited sample volume. These comments were taken into consideration at the time of the final wording for this statement.

**16. Recommendation.—When collecting pleural fluid for diagnosis of extrapulmonary tuberculosis, specimens should be submitted for microbiology culture studies for mycobacteria using liquid media protocol.**

The quality of evidence is *low*.

One prospective cohort study,<sup>135</sup> which suffered from risk of reporting and detection bias, supported this statement. The study contained no methodologic flaws that would raise concerns about its findings. Refer to Supplemental Table 18 in the supplemental digital content for the complete quality assessment results for the evidentiary base of statement 16.

Of the estimated 9.6 million worldwide incident cases of tuberculosis reported for 2014, approximately 22% are thought to represent extrapulmonary tuberculosis.<sup>136</sup> Challenges in making the diagnosis of extrapulmonary tuberculosis include nonspecific symptoms, paucibacillary nature of infection as compared with pulmonary tuberculosis, and the need for invasive specimen collection techniques.<sup>135</sup> In mycobacterial laboratory diagnostics, automated liquid culture systems have increased the recovery rates and decreased the time to detection of mycobacteria. Timely identification of patients with tuberculosis supports both the medical management of the patient as well as infection prevention measures designed to protect public health. In addition to recognizing the presence of mycobacteria, the clinical laboratory must identify the strain as *M tuberculosis* and further determine susceptibility to various antimycobacterial agents by testing in vitro. Liquid media for mycobacterial culture is preferred because of the improvement in time to result, enabling earlier therapy and therefore minimizing the risk of transmission to others. Earlier identification of positive cultures enables earlier identification of isolates and susceptibility testing, important for tailoring individualized therapy to improve outcomes. To mitigate against development of drug resistance and possible transmission of resistant strains, timely results should be sought to support the application of infection prevention measures (eg, isolation of infected patients) and use of drugs to which the mycobacteria have been demonstrated to be susceptible.

Evidence from the systematic review reveals that the combination of solid and liquid media is recommended for mycobacterial cultures to enhance sensitivity and minimize the time to positive results. A comparison of the recovery rate, time to positivity, and contamination rate for 2 types of liquid media (Myco/F lytic system and BACTEC Mycobacteria Growth Indicator Tube or MGIT 960 system, Becton Dickinson Diagnostic Instrument Systems, Sparks, Maryland) was compared with 1 type of solid media (Lowenstein-Jensen) in 214 sterile body fluids (including 114 pleural effusions) from patients with suspected extrapulmonary tuberculosis. Liquid media demonstrated both significant improvement in recovery rate as compared with Lowenstein-Jensen, as well as significantly faster time to

detection. The authors did not attribute these differences in performance to differences in specimen volumes, and they note that there is increased cost associated with liquid media systems, which may be an important factor in choices of methods for laboratories.<sup>135</sup>

Although not included in this evidence-based review, further support for the use of at least liquid media and ideally multiple types of media can be found in multiple important guidance documents. First, an evidence-based review and recommendations were recently published from the combined effort of experts from the American Thoracic Society, Infectious Diseases Society of America, and the Centers for Disease Control and Prevention published in 2016.<sup>137</sup> The guideline recommends that both liquid and solid media be used for mycobacterial culture for every specimen obtained from a patient with suspected pulmonary tuberculosis disease. A meta-analysis cited in this article<sup>138</sup> described that liquid culture methods were more sensitive than solid culture (88% and 90% as compared with 76%) when a combination of methods was used as a reference standard, and that liquid cultures yielded results in a shorter time frame than solid culture (13.2 and 15.2 days as compared with 25.8 days). The guideline goes on to include a comment that solid cultures alone have insufficient sensitivity to reliably identify *M tuberculosis*, and take longer than liquid cultures, although some isolates are only detected using solid medium. For the diagnosis of extrapulmonary tuberculosis, the guideline suggests that AFB smear microscopy, mycobacterial culture, and nucleic acid amplification tests be performed, although the sensitivity is lower for all methods than for pulmonary tuberculosis.<sup>137</sup>

Note: In the section on diagnosis of extrapulmonary tuberculosis, specific guidance on choices of media is not discussed. Second, as stated in another guidance document, *Clinical and Laboratory Standards Institute M48-A*, it is common practice to inoculate at least 2 types of media for mycobacterial cultures to maximize recovery.<sup>139</sup> Solid media allows detection of mixed cultures and serves as a backup for when there is a contaminated liquid culture (more likely with sputum specimens that contain oropharyngeal flora, collected from patients suspected of pulmonary tuberculosis). In general, using both solid and liquid media is preferable to use of either type alone to increase the rates of recovery of mycobacteria. Third, this guidance to prefer liquid media and to use multiple types of media is carried as well from the *American Society for Microbiology's Manual of Clinical Microbiology*.<sup>140</sup> For extrapulmonary tuberculosis infections, the role of liquid media is especially important because of the lower numbers of organisms typically found in specimens including pleural fluid.

During the open comment period there were 210 respondents, of whom 72.38% agreed (n = 152) with the guideline statement, 2.86% disagreed (n = 6), and 24.76% (n = 52) indicated that the statement did not pertain to their area of expertise or practice. Among the 6 comments received, 1 respondent asked about the specification for use of liquid media for culture, deferring to individual microbiology laboratories to develop their own protocols for processing and testing specimens. The preference for liquid media was considered relevant for inclusion in this venue in part to raise awareness, and because 1 paper<sup>135</sup> discussed inoculation of specimens at the patient's bedside. Another respondent described a lack of experience with tuberculosis diagnosis using pleural fluid specimens, and the comment about procedural restrictions from institutional infection

prevention policies was reiterated. All comments were taken into consideration in the final draft of this statement, which remains largely unchanged as presented in this document.

### Additional Discussion

In addition to the guideline statements presented in this manuscript, the expert panel reviewed the literature to develop recommendations for a few items for which the data were either sparse or showed no differences between the items being compared. As such, recommendations are not offered, but the findings are discussed below.

**No recommendation can be made for or against the use of one collection medium, one fixative, or one stain over another for ancillary studies in cytology specimens.**

Cytology laboratories use a wide variety of collection media, fixatives, and stains for specimen preparation. A wide range of cytology specimen processing techniques were identified in the systematic review, including CytoLyt (Hologic Inc, Marlborough, Massachusetts),<sup>141–143</sup> Hanks,<sup>44</sup> RPMI,<sup>144</sup> saline,<sup>111,132,145–155</sup> and CytoRich (Becton, Dickinson and Company, Franklin Lakes, New Jersey)<sup>43</sup> as collection media; alcohol,<sup>6,46,115,156</sup> formaldehyde,<sup>112</sup> Mount-Quick,<sup>119</sup> and formalin<sup>¶</sup> as fixatives; and Diff-Quik,<sup>6,44,46,166</sup> Giemsa,<sup>43</sup> and Papanicolaou for staining.<sup>46,167</sup> However, none of the listed studies performed direct comparisons between the different processing techniques that enable making a specific recommendation for the use of one method over another. There was reasonable success for all methods reported in the studies reviewed. Papanicolaou, Diff-Quik, and Giemsa stains were used in these studies, and all methods had acceptable rates of adequacy (73%–100%) and successful ancillary testing (80%–98%).<sup>6,43,44,46,167</sup> Formalin was the most common fixative used,<sup>¶</sup> followed by alcohol,<sup>6,46,115,156</sup> Mount-Quick,<sup>119</sup> and formaldehyde.<sup>112</sup> All fixatives had acceptable rates of adequacy (79%–100%) and successful ancillary testing (65%–100%).<sup>#</sup> When considering only studies that reported on adequacy for ancillary testing or success rate for ancillary testing, identified studies used CytoLyt, CytoRich, TRIZol (Thermo Fisher Scientific, Waltham, Massachusetts), and Hanks solution for collection methods, with all methods resulting in acceptable adequacy (60%–98%) and successful ancillary testing (89%–94%).<sup>43,44,141–143,168</sup> The assortment of collection media, fixatives, and stains found in the systematic review and among comments from the open comment period highlights the utilization variability of these methods throughout clinical practice and that satisfactory results may likely be obtained regardless of method. However, as previously noted, the expert panel suggests that all laboratories perform adequate and rigorous validation studies to ensure that the intended use of collection media, fixatives, and stains provides suitable results for the ancillary test of interest and that these assays perform adequately before they are used on patient samples. The expert panel also suggests caution in the use of media/fixatives that are known to potentially cause aberrant results when used with certain ancillary testing methods and advises that additional

<sup>¶</sup> References 6, 30, 46, 58, 70, 82, 85, 88, 91, 119, 120, 143, 157–165.

<sup>#</sup> References 6, 30, 46, 58, 70, 82, 85, 88, 91, 119, 120, 143, 157–165.

<sup>¶</sup> References 6, 30, 46, 58, 69, 70, 82, 85, 88, 91, 112, 115, 116, 119, 120, 143, 156–165.

consideration should be taken when validating assays that may use specimens treated with these substances.<sup>169–174</sup> Additional studies specifically designed to evaluate the different collection media, fixatives, and stains during the processing of thoracic cytology samples for ancillary testing are needed before issuing any additional formal recommendations.

**No recommendation is made regarding the use of stylet in EBUS TBNA.**

The quality of evidence was *insufficient* to support a recommendation for or against the use of an inner stylet during the aspiration phase of EBUS TBNA.

Endobronchial ultrasound-guided transbronchial needle aspiration is widely used for mediastinal lymph node sampling and is recommended for initial staging of mediastinal lymph nodes in thoracic malignancies.<sup>175</sup> Many studies have been published on needle gauge size, number of passes, and use of suction, but there is only limited publication on the use of stylet. During traditional EBUS TBNA, the lumen of the needle is filled by a metal stylet when entering the targeted area; the stylet is removed prior to aspiration of the targeted lesion. Two studies that evaluated the use of a stylet compared with no stylet were identified by the literature search.<sup>176,177</sup> In the first prospective randomized single-blind controlled clinical trial, the authors studied 194 lymph node biopsies in 120 patients.<sup>176</sup> Each lymph node was sampled with and without a stylet by alternating the 2 methods and randomizing the order. The authors found that stylet use does not affect the diagnostic accuracy or the amount of tissue procured for ancillary studies. The authors also did not find significant difference in the amount of contaminating tissue (eg, bronchial epithelium, cartilage, and soft tissue fragments). The second prospective study compared a group with 2 samples collected with a stylet followed by 2 samples collected without a stylet with a group using the alternate order of collection for the 4 samples.<sup>177</sup> This study indicated no significant sample adequacy differences when stylet was and was not used. However, although the study compared background blood and clot between the collection methods, the study did not compare amount of benign bronchial cells. Based on the limitations of these studies, we find that there is insufficient evidence to make a recommendation for or against the use of a stylet in EBUS TBNA.

**No recommendation is made regarding optimal ischemic time.**

The quality of evidence was *insufficient* to support a recommendation.

The expert panel believed that recommendations regarding optimal ischemic time (time to fixation) would be useful but found insufficient published evidence. Ischemic time and length of fixation are well studied preanalytic factors that are known to affect tissue quality, histologic appearance, and ancillary testing.<sup>121</sup> Many thoracic diseases are sampled using small biopsy or cytology techniques and the samples are either fixed immediately or tissue/cell suspension is placed in a nonfixative solution that prevents cell breakdown. The samples that are not fixed can be used for special studies that require unfixed material, including microbiologic studies, flow cytometry, and cell cultures. Fixation times of 6 to 48 hours for lung CNB specimens are usually recommended,<sup>100,178,179</sup> although guidelines for the processing of breast carcinoma specimens for biomarker testing recommend fixation times of 6 to 72 hours with ischemic time (time to fixative) within an hour.<sup>180</sup>

In summary, the expert panel believes that although there is insufficient evidence to support a specific recommendation regarding optimal ischemic time, every attempt should be made to minimize ischemic time followed by adequate fixation.

**No recommendation is made regarding the sequence algorithm for testing.**

The quality of evidence was *insufficient* to support a recommendation.

The expert panel was interested to determine if there is sufficient evidence to suggest an algorithm or algorithms regarding the sequence of testing. Although this is an important question, it is difficult to study because most relevant studies are from single institutions that did not satisfy our criteria for inclusion in the analysis. Testing sequence is particularly important for small biopsy and cytology samples of lung cancer. The primary objective is to establish a morphologic diagnosis that can be aided by a minimal number of special stains including IHC. The current CAP/IASLC/AMP guideline emphasizes the need to minimize the diagnostic workup in order to preserve tissue for biomarker testing but does not make any recommendation regarding the sequence of testing.<sup>89</sup> Lung cancer biomarker testing is primarily determined by stage and histologic type, and to a lesser degree by some clinical characteristics (smoking status) and tissue type (biopsy versus resection). Advanced-stage NSCLC patients should be tested for predictive and prognostic biomarkers that have been outlined in greater detail in other guidelines.<sup>89</sup> Although the above-mentioned guidelines make no recommendation regarding the method or sequence of testing, it is important to determine all targetable alterations with Food and Drug Administration–approved therapy in a timely manner to ensure best possible personalized therapy for the individual patient.

**Limitations**

We originally set out to develop recommendations that would guide clinicians on how best to collect and handle small thoracic specimens and guide laboratories on how best to prioritize testing. Initially we desired for this guideline to be all-encompassing, focusing on hematologic, primary, and metastatic disease, noncancerous abnormalities, and infection, to try to encompass the wide spectrum of findings in the thorax. Some limitations to this guideline are noted:

1. Although the expert panel included representatives from various stakeholders (clinicians and pathologists alike), the panel decided the recommendations would not inform clinicians how best to perform the procedures to acquire samples nor which procedure (transthoracic biopsy versus bronchoscopy versus pleural drainage), as this work is more readily available in the pulmonology literature and not discussed here.
2. During our initial planning, we noticed that this guideline might overlap with other CAP guidelines, in particular the Lung Cancer Biomarker Testing guideline<sup>89</sup> and the American Society for Clinical Pathology/CAP/American Society of Hematology Requirements for Laboratory Workup of Lymphoma guideline, currently in development. Because those guidelines are more narrowly focused, we decided not to include hematologic specimens and/or individual biomarkers in our literature search, and we chose not to offer recommendations for

specific molecular tests that should be performed. As a result, we were unable to offer an algorithm/workflow that would detail the sequence for which testing should be performed or prioritized. (See key question 3, previously listed in this manuscript.)

Additionally, this guideline focused primarily on small thoracic specimens that require ancillary studies for malignant processes such as lung carcinoma as well as nonmalignant conditions such as infections. However, the literature in the systematic review pertaining to infections was limited. Therefore, no recommendations could be made regarding nontuberculous infections such as fungal, bacterial, and/or nontuberculous mycobacteria. Also, this guideline does not encompass thymomas or spindle cell neoplasms. None of the studies meeting our final inclusion criteria evaluated these entities.

### Guideline Revision

This guideline will be reviewed every 4 years or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the guideline recommendations. If necessary, the entire expert panel will reconvene to discuss potential changes.

### CONCLUSIONS

The use of small tissue specimens has become increasingly popular as techniques for acquisition of tissue have become more advanced and are often less invasive. Although the procedure may be less invasive and have less morbidity for patients, it has induced a paradigm shift in small specimens that are currently available for pathologists to perform an ever-expanding array of testing needed by the treating physician for personalized care. This change in specimen acquisition translates to a change in specimen handling and processing in order to maximize the information that can be made available to treating clinicians.

This expert panel, through a rigorous systematic review, has provided 16 formal recommendations and expert consensus opinions on the handling and processing of small biopsy specimens in order to maximize ancillary testing. Throughout our search we identified a paucity of high-quality studies on this topic pertaining specifically to the collection and handling of thoracic specimens for ancillary testing, something that hopefully will evolve with future research, literature updates, and reviews in the coming years.

### Disclaimer

The CAP developed the Pathology and Laboratory Quality Center as a forum to create and maintain evidence-based practice guidelines and consensus statements. Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision-making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and statements cannot

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**APPENDIX. Disclosed Interests and Activities From April 2016 to February 2020<sup>a</sup>**

Sanja Dacic, MD, PhD	Consultancies	AstraZeneca Bayer Bristol-Myers Squibb Genentech
Christopher R. Gilbert, DO, MS	Consultancies	Olympus Verathon Inc AstraZeneca
Peter B. Illei, MD	Board, advisory boards	Genentech Guardant Health
	Consultancies	AstraZeneca AbbVie Inc Veran Medical Technologies, Inc Roche Diagnostics
	Grants/collaborative agreements	Bristol-Myers Squibb Biomarker Strategies, LLC
	Lecture fees (honoraria)	Ventana Medical Systems, Inc
Christopher Lee, MD	Grants/collaborative agreements	MD Anderson Cancer Center California Community Foundation Canon Inc University of California, Los Angeles ATGen Ltd/Cynvenio Biosystems Inc
Jan A. Nowak, MD, PhD	Ownership or beneficial ownership of stocks	OmniSeq, LLC
Nicholas J. Pastis Jr, MD	Consultancies	Cook Medical Boston Scientific Olympus Olympus Olympus
Sinchita Roy-Chowdhuri, MD, PhD	Grants/collaborative agreements	Canadian Association of Pathology
Amita Sharma, MD	Lecture fees (honoraria)	Hummingbird Diagnostics
	Grants/collaborative agreements	Elsevier
Jesse S. Voss, CT, MB(ASCP) <sup>CM</sup>	Royalties	Abbott Molecular Inc
Nicole E. Thomas, MPH, CT(ASCP) <sup>CM</sup>	Grants/collaborative agreements	Centers for Disease Control and Prevention
Paul A. VanderLaan, MD, PhD	Consultancies	Gala Therapeutics Clearview Healthcare Partners Flatiron Health, Inc Caris Life Sciences, Inc Foundation Medicine
	Board, advisory boards	Boston Scientific Cook Medical NuVaira, Inc Olympus Pulmonx, Inc
Momen M. Wahidi, MD, MBA <sup>b</sup>	Consultancies	CSA Medical Olympus Olympus
	Grants/collaborative agreements	Olympus
	Lecture fees (honoraria)	Olympus
Lonny B. Yarmus, DO, MBA <sup>b</sup>	Consultancies	Boston Scientific Olympus Veran Medical Technologies
	Grants/collaborative agreements	Veran Medical Technologies
	Lecture fees (honoraria)	AstraZeneca

<sup>a</sup> The following individuals have reported no relevant disclosures: Mohiedean Ghofrani, MD, MBA; Lester J. Layfield, MD; Claire W. Michael, MD; Ross A. Miller, MD; Jason W. Mitchell, MD, MPH, MBA; Boris Nikolic, MD, MBA; Carol Ann Rauch, MD, PhD; Brooke L. Billman, MLIS, AHIP; Lesley Souter, PhD.

<sup>b</sup> Manuscript author but not member of the expert panel.