



Review

Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies: Guidelines from the College of American Pathologists (CAP)

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Abstract: With a growing number of clinically relevant biomarkers needed to guide the management of patients with non-small cell lung cancer (NSCLC), pathologists are keenly aware of the need to collect adequate tissue not only for a diagnosis, but also for ancillary studies to provide predictive and prognostic information. Small specimens collected by minimally invasive techniques such as fine needle aspiration and core needle biopsy often fall short in meeting adequacy requirements for lung cancer molecular biomarkers. The College of American Pathologists (CAP) recently published an evidence-based clinical practice guideline, “Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies”, to help direct clinicians and pathology laboratory personnel to optimally collect and handle thoracic small specimens for ancillary testing. This review summarizes the published guideline statements and provides a brief overview of the recommendations and how they impact the practice of pathology.



Citation: Roy-Chowdhuri, S. Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies: Guidelines from the College of American Pathologists (CAP). *J. Mol. Pathol.* **2021**, *2*, 23–28. <https://doi.org/10.3390/jmp2010003>

Academic Editor: Philippe Vielh

Received: 21 January 2021

Accepted: 26 February 2021

Published: 3 March 2021

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Keywords: thoracic guidelines; small specimens; small biopsy; cytology; collection and handling

1. Introduction

In an era of predictive oncology and precision medicine, pathologists are keenly aware of the need to collect adequate tissue not only to arrive at a diagnosis but also to provide predictive and prognostic information that would guide patient care [1–5]. This is especially true in the realm of non-small cell lung cancer (NSCLC), where biomarker testing is routinely used for the clinical management of patients with advanced stage disease [5–8]. The list of clinically relevant biomarkers in NSCLC is constantly expanding. The most recent version of the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology lists eight “must test” genes, including *MET* exon 14 skipping mutations, *RET* and *NTRK* fusions, along with *EGFR* and *BRAF* mutations, *ALK* and *ROS1* rearrangements, and PD-L1 expression [9]. Testing modalities for these biomarkers is quite extensive, ranging from PCR-based methods, to fluorescence in situ hybridization (FISH) assays, to immunohistochemistry (IHC), although recent guidelines have strongly encouraged the use of expanded molecular profiling assays to simultaneously test multiple genes in an effort to conserve limited volume specimens [1,10]. Small specimens collected by minimally invasive techniques such as fine needle aspiration (FNA) and core needle biopsy (CNB) often fall short in meeting adequacy requirements for a growing list of biomarkers, especially when tested using single gene testing methodologies [11–14].

The College of American Pathologists (CAP) recently published an evidence-based clinical practice guideline, “Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies”, in an effort to help direct clinicians and pathology laboratory personnel to optimally collect and handle thoracic small specimens for ancillary testing [15]. The guideline was developed in collaboration with eight other professional medical societies, including the American College of Chest Physicians, American Society for Cytopathology, American Thoracic Society, Association for Molecular Pathology, Papan-

icolaou Society of Cytopathology, Pulmonary Pathology Society, Society of Interventional Radiology, and Society for Thoracic Radiology. A systematic review of the literature was performed by a multidisciplinary expert panel, including the author who co-chaired the guideline development committee, with input from a separate advisory panel. Sixteen guideline statements on the best practices for the acquisition and handling of thoracic small specimens for ancillary studies were developed as part of this effort (Table 1).

Table 1. CAP Guideline Statements for Collection and Handling of Thoracic Small Specimens.

	Guideline Statement	Strength of Recommendation
Statement 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
Statement 2	When performing EBUS TBNAs, 19-, 21-, or 22-gauge needles may be used.	Recommendation
Statement 3	When performing EBUS TBNA, ROSE should be used, if available.	Recommendation
Statement 4	To achieve optimal diagnostic yield, when performing EBUS TBNA without ROSE, the bronchoscopist should perform at minimum three and up to five 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
Statement 5	When performing transthoracic needle procedures, ROSE should be used for adequacy assessment, if available and clinically feasible.	Strong Recommendation
	If performing core needle biopsy (CNB), without concurrent fine-needle aspiration (FNA), touch preparations may be used for adequacy assessment, if available.	Recommendation
Statement 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
Statement 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 (i.e., cell block, tissue clot).	Recommendation
Statement 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
Statement 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
Statement 10	When performing transbronchial needle aspirates, ROSE should be used for adequacy assessment, if available.	Recommendation
	If performing transbronchial forceps biopsies without concurrent transbronchial needle aspirates, touch preparations may be used for adequacy assessment, if available.	Expert Consensus Opinion
Statement 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
Statement 12	Cytology specimens (smears, cell blocks, liquid based cytology), may be used for ancillary studies if supported by adequate validation studies.	Strong Recommendation
Statement 13	CNB specimens collected for ancillary studies should be fixed in 10% neutral buffered formalin.	Recommendation

Table 1. Cont.

	Guideline Statement	Strength of Recommendation
Statement 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
Statement 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
Statement 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

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The guideline covers six main aspects of thoracic small specimen acquisition including, "Endobronchial Ultrasound-Guided Transbronchial Procedures", "Transthoracic Procedures", "Bronchoscopic Procedures", "Pleural Effusions: Considerations for Malignancy", "Considerations for Ancillary Studies during Malignant Investigations", and "Considerations for Ancillary Studies during Nonmalignant Investigations" and include ancillary studies used for patients with lung cancer to those used in the diagnosis of infectious processes such as pulmonary and extra-pulmonary tuberculosis. A brief overview of the guideline statements is discussed below.

2. Endobronchial Ultrasound-Guided Transbronchial Procedures

The guideline strongly recommends the use of endobronchial ultrasound-guided transbronchial needle aspirations (EBUS TBNA), if available, for the initial diagnosis, staging, and identification of recurrence/metastasis of mediastinal and hilar lymph nodes, as well as parenchymal lesions that are visible with endobronchial ultrasound. Further EBUS procedural recommendations include the use of 19-, 21-, or 22-gauge needles for collecting an adequate sample and a minimum of 3 and up to 5 passes when performing EBUS TBNA without rapid on-site evaluation (ROSE). The use of ROSE is recommended to guide adequacy of the specimen, if available, and should be used to determine the number of passes needed to procure an adequate specimen for ancillary studies.

3. Transthoracic Procedures

For transthoracic needle aspirations, the guideline strongly recommends the use of ROSE for adequacy, if available and clinically feasible. In situations where a CNB is procured without a concurrent FNA, touch preparations (TP) may be used to evaluate adequacy. The guideline recommends that the needle gauge for transthoracic procedures be determined by the proceduralist; however, they note that needles as small as 25-gauge for FNA and 20-gauge for CNB may be used for procuring adequate samples. To ensure optimal adequacy, the guideline recommends attempting to procure a minimum of 3 core samples, if feasible. And in situations where only an FNA is performed without a concurrent CNB, multiple passes are recommended to collect adequate material for a tissue block.

4. Bronchoscopic Procedures

While peripheral pulmonary lesions are generally sampled via a percutaneous modality, sometimes bronchoscopic procedures can be used to sample these lesions. If bronchoscopic techniques are used to procure tissue from peripheral pulmonary lesions, the guideline recommends the use of image guidance, if available, to ensure collection of an

adequate sample. The guideline also recommends the use of ROSE, for adequacy assessment of transbronchial needle aspirates. In the event that a transbronchial forceps biopsy is performed without a concurrent needle aspirates, touch preparations may be used for adequacy assessment, if available.

5. Pleural Effusions: Considerations for Malignancy

For pleural effusions that are collected with a clinical suspicion of malignancy the expert panel suggests sending as much fluid volume as reasonably attainable to the cytopathology laboratory. This guideline statement comes as an expert consensus opinion as there was insufficient evidence in the systematic literature review to recommend a specific effusion volume that would be considered adequate for ancillary studies. The general assumption is that the adequacy of a pleural effusion depends on the cellularity of the fluid and therefore, the larger the volume submitted to the laboratory, the greater the chances that the specimen would be adequate for ancillary studies.

6. Considerations for Ancillary Studies during Malignant Investigations

One statement from the guideline that is of importance to the cytopathology community is the strong recommendation for the use of all cytology specimens for ancillary studies. This is of special relevance as historically most ancillary studies in cytology, including molecular/PCR-based testing, FISH, and IHC, have primarily been limited to formalin-fixed paraffin-embedded (FFPE) cell block preparations due to their similarity to histologic tissue blocks [5,16–18]. The use of non-FFPE cytologic substrates such as smears and liquid-based require additional validation studies that pose a major limitation to their widespread use, especially in reference laboratory and commercial settings [5,17,19,20]. However, with the growing number of ancillary biomarker studies required for NSCLC, it is important for the cytology and molecular pathology community to recognize the potential advantage of using the various cytologic preparations to be able to provide adequate testing needed for patient care. This has also been highlighted in the most recent iteration of the CAP/International Association for the Study of Lung Cancer/Association for Molecular Pathology (CAP/IASLC/AMP) molecular testing guidelines for lung cancer, where the use of all cytologic preparations was recommended in contrast to the prior preference for cell blocks [10,21]. The guideline notes that cytology specimen processing varies across laboratories and the lack of standardization precludes specific recommendation for the choice of a specific collection medium, fixative, or stain in specimens that will be used for ancillary studies. In contrast, CNB specimens, which are typically processed as FFPE blocks across most histology laboratories, are recommended to be fixed in 10% neutral buffered formalin when collected for ancillary studies.

7. Considerations for Ancillary Studies during Nonmalignant Investigations

While most of the guideline statements focus on the collection of small specimens for malignant conditions, the expert panel made some recommendations for specimens collected for infectious processes, such as pulmonary and extra-pulmonary tuberculosis. For bronchoscopic procedures performed for the investigation of tuberculosis, the guideline recommends the use of endobronchial ultrasonography to increase the diagnostic yield. EBUS TBNA specimens procured for the investigation of intrathoracic granulomatous lymphadenopathy with a suspicion of tuberculosis are recommended to be collected for cytology, microbiology (mycobacterial smear and culture), and TB-PCR evaluation, if available. Pleural fluid specimens collected for a diagnosis of extrapulmonary tuberculosis are recommended to be submitted for microbiology culture studies using liquid media protocol.

These guideline statements from CAP and the collaborating professional medical societies aim at providing direction to clinicians and proceduralists for collecting a small specimen that is adequate for downstream ancillary testing. Several of the guideline statements pertain to optimal laboratory processing to ensure adequacy of the specimen

for ancillary studies. Specific guidelines are provided to the proceduralist pertaining to the choice of needle gauge (Statements 2 and 6), the number of passes (Statements 4 and 7), the utility of ROSE (Statements 3, 4, 5, and 10), and to the optimal volume and triage of pleural effusion specimens (Statement 11). The multiple guideline statements pertaining to the use of ROSE, when available and clinically feasible, highlight the role of the cytologist during minimally invasive procedures, that can ensure adequate sampling of the targeted lesion as well as appropriate specimen triage for necessary ancillary studies. While the overall use of ROSE is recommended whenever possible, the expert panel acknowledges the potential complexity of different practice settings and clinical scenarios where ROSE may not be needed or even practical, leaving room for clinical judgment to be used for determining the need for ROSE in all procedures [22,23].

In conclusion, this new guideline provides some direction to pathologists, laboratory personnel, and our clinical colleagues to better collect and handle thoracic small specimens that are adequate for ancillary studies to help guide therapeutic decisions. However, one of the major limitations highlighted in the guideline is the paucity of high quality, well-designed published studies in cytology that specifically address some of the pre-analytic variables such as the choice of an optimal collection medium, fixative, or stain in cytology specimens, a recommended cold ischemic time, or the duration of fixation for optimal ancillary testing success. This underscores a need for high quality published studies in cytology that provide comparisons of pre-analytic variables between specimen preparations, testing methods, and practice settings to help guide future guideline efforts. The CAP and the eight collaborating medical societies that approved the guideline are encouraging their members to adopt the guideline recommendations and coordinate efforts to determine how best to implement these recommendations into their clinical practice.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author is a member of the expert panel and co-chaired the committee that developed the CAP guidelines discussed in this review. All guideline statements are reproduced from "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." *Arch Pathol Lab Med.* May 13 2020, doi:10.5858/arpa.2020-0119-CP with permission from Archives of Pathology & Laboratory Medicine. Copyright 2020 College of American Pathologists.

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