



COLLEGE of AMERICAN  
PATHOLOGISTS

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

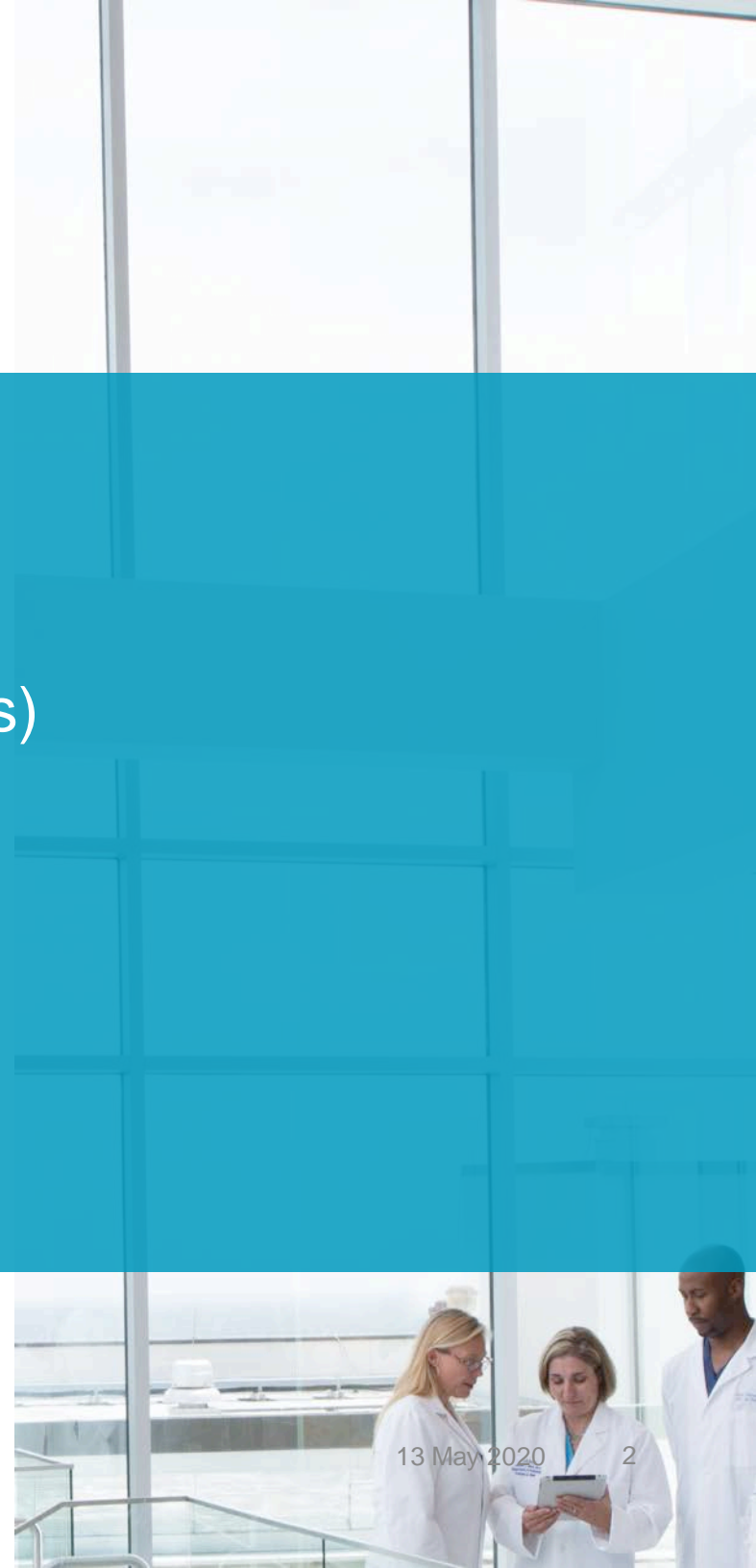
**Guideline from the College of American Pathologists (CAP)  
in collaboration with the American College of Chest  
Physicians, Association for Molecular Pathology, American  
Society for Cytopathology, American Thoracic Society,  
Pulmonary Pathology Society, Papanicolaou Society of  
Cytopathology, Society of Interventional Radiology, and  
Society for Thoracic Radiology**

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# Outline

- Introduction
- Key questions and results
- Guideline statements (recommendations)
- Guideline development process
- Conclusion



# Introduction

- **With the advances in minimally invasive sampling techniques, thoracic pathology often hinges on the diagnosis of small specimens.**
- **Advances in molecular diagnostic techniques enable pathology laboratories to provide treating physicians with valuable diagnostic, predictive, and prognostic biomarker testing from thoracic “small specimens.”**
- **Currently, the two most commonly used minimally invasive approaches for collecting diagnostic tissue include fine needle aspiration (FNA) and core needle biopsy (CNB).**

# Introduction, continued

- **Given the limited volume and the need to provide valuable genomic information for a growing number of biomarkers, establishing evidence-based recommendations for the collection and handling of these thoracic small specimens for judicious triage for relevant ancillary tests is of great importance.**

# Key Questions

The expert panel formulated and considered four key questions\*:

- 1. What procedural or methodological variables have been shown to optimize testing outcomes so that pathologists can provide an evaluation and accurate diagnosis?**
- 2. What evidence is available to determine the most effective protocols for sample collection?**
- 3. What evidence is available to determine the most effective methods for the handling and processing of specimens?**
- 4. What evidence is available to support an algorithm(s) for selection of specimens and sequence of testing, under defined circumstances?**

\*Key questions were shortened for this presentation. Refer to the guideline manuscript and supplement for complete questions, included abnormalities, specimen types, and collection parameters.

# Results

- **16 guideline statements were developed to assist clinicians and pathologists collect and process thoracic small biopsy and cytology tissue samples.**

# Guideline Statements

## **Endobronchial Ultrasound Guided Transbronchial Procedures**

# Statement 1

**1. *Strong Recommendation.* – Endobronchial ultrasound guided transbronchial needle aspiration (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.**



# Rationale

- **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.**
  - Overall diagnostic yield remains high (sensitivity of 89% and specificity of 100%) in numerous studies.
  - Useful in obtaining appropriate tissue for patients that have parenchymal lung lesions located adjacent to central and/or large airways that will accommodate the size of the convex EBUS puncturescope.
  - Useful in obtaining specimens for both staging of carcinoma as well as identifying recurrent disease

## Statement 2

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

# Rationale

- **The needle gauge used in the EBUS TBNA studies identified in our systematic review included 19G, 21G, and 22G.**
- **In a study of 303 EBUS TBNA patients, the authors found:**
  - **No significant difference in diagnostic accuracy was seen for malignancy between the specimens obtained via 21G versus 22G needles.**
  - **However, diagnostic accuracy was greater for 21G needles than 22G, when evaluating benign lesions and when sub-classifying non-small cell lung carcinomas (NSCLC).**

# Statement 3

**3. *Recommendation.* – When performing EBUS TBNA, ROSE should be utilized, if available.**

## Rationale

- **There appears to be a slight trend in increased diagnostic yield when ROSE is employed versus when ROSE is not used, however, only one study from our systematic review reported a statistical significance difference.**

## Statement 4

**4. *Recommendation.* – To achieve optimal diagnostic yield, when performing EBUS TBNA without ROSE, the bronchoscopist should perform at a 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.**

# Rationale

- **Based on studies from our systematic review, in situations where EBUS TBNA is being performed without ROSE, for both malignant and benign disease, at least 3 and up to 5 passes is recommended to achieve an optimal diagnostic yield.**
- **When cytopathology personnel is present at the EBUS procedure to provide immediate feedback, the 3 to 5 pass recommendation is no longer applicable, since ROSE may establish the presence of adequate diagnostic material in fewer than three passes, or in some cases may require more than 5 passes.**

# Guideline Statements

## Transthoracic Procedures

## Statement 5

**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.



# Rationale

- **ROSE can be used during transthoracic image-guided biopsy procedures to assess for specimen adequacy, help appropriately triage the specimen for ancillary testing, and reduce the rate of repeat procedures for discordant or non-diagnostic biopsies.**
- **In cases where only CNB is performed without a concurrent FNA, adequacy assessment may be performed using touch preparation employing a technique that does not compromise the integrity of the CNB sample.**
- **Our systematic review found that the use of ROSE and touch preparation at the time of transthoracic biopsy procedure significantly improved diagnostic yield across all modalities.**

## Statement 6

**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.

# Rationale

- **The technical aspects of the transthoracic procedure, including the choice of needle gauge used, vary between operators but the principle is to procure the largest tissue sample with minimal complications.**
- **Studies from our systematic literature review for transthoracic procedures included needle gauges between 10G-25G, although most centers use 18-20G for CNB and 21-22G for FNA.**
  - **More studies used coaxial needles than not.**

# Statement 7

**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).**

# Rationale

- **The ability to perform ancillary tests, including multiple immunohistochemistry (IHC) markers on cell block sections, makes a strong case for the acquisition for 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.**

## Statement 8

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

# Rationale

- **The systematic review identified a study that showed accuracy significantly increased as the number of CNB samples increased to three but did not increase beyond three.**
- **Other studies indirectly informed the recommendation.**

# Guideline Statements

## Bronchoscopic Procedures



## Statement 9

**9. Recommendation - 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.**

### **Rationale**

**Supported by two studies which both showed better diagnostic yield for smaller nodules. Other studies outside the systematic review support this recommendation.**

# Statement 10

**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.

# Rationale

- **Studies from the systematic review support the use of ROSE for TBNA of peripheral pulmonary lesions to enhance diagnostic yield.**
  - Particularly in those less than three cm and greater than seven cm when using radial probe EBUS guided transbronchial biopsy and brushing.
- **Touch preparations may also be used if available.**
  - Vigorous touch preparations should be avoided and appropriate care should be taken not to deplete the cellularity of the CNB.

# Guideline Statements

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

# Statement 11

**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.**

# Rationale

- **Due to heterogeneity of the studies, the panel could not recommend a specific quantity and no studies reported an upper limit.**
- **The larger the volume submitted, the more likely it will be adequate for ancillary studies and final diagnosis.**  
**Therefore, the panel recommends sending as much fluid as reasonable attainable.**

# Guideline Statements

## **Considerations for Ancillary Studies during Malignant Investigations**

# Statement 12

**12. Strong *Recommendation*.** – Cytology specimens (smears, cell blocks, liquid-based cytology), may be used for ancillary studies, if supported by adequate validation studies.



# Rationale

- **Cytology is sometimes the only option for molecular biomarker testing/ancillary studies, so as not to require a repeat tissue biopsy for patient management.**
- **Evidence from the systematic review demonstrates the feasibility of mutational analysis, fluorescence in situ hybridization (FISH), and IHC for lung carcinoma using common cytology specimens.**

# Statement 13

**13. Recommendation – CNB specimens collected for ancillary studies should be fixed in 10% neutral buffered formalin.**

# Rationale

- **Ten percent neutral-buffered formalin (NBF) is the most commonly used fixative for CNB specimens and NBF 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.**
- **Similarly, harsh acids should be avoided for decalcification of bone metastases, as they hamper the quality of the molecular analytes to be tested.**

# Guideline Statements

## **Considerations for Ancillary Studies During Non-malignant Investigations**

# Statement 14

**14. *Recommendation.* – When performing bronchoscopy for the investigation of tuberculosis, endobronchial ultrasonography may be used to increase the diagnostic yield of bronchoalveolar lavage and transbronchial biopsy.**

# Rationale

- Evidence from the systematic review identified a study comparing the diagnostic yield of acid fast bacilli (AFB) smears and mycobacteria cultures in bronchoalveolar lavage (BAL) fluid as well as histopathologic specimen of transbronchial lung biopsies (TBLB) between conventional and EBUS bronchoscopic sampling techniques. In this study, the addition of EBUS improved the overall diagnostic yield from 58.3% to 80.8% ( $p = .04$ ).

## Statement 15

**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 (TB-PCR) evaluation, if available.

# Rationale

- **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.**



# Statement 16

**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.**

# Rationale

- **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.**

# Guideline Development Process

# Collaboration

- The CAP collaborated with eight organizations to develop the guideline. Each organization provided members to participate on the expert and advisory panels and approved the guideline prior to submission to publication.

**AMP**

**ASC**

**ATS**

**CHEST**

**PPS**

**PSC**

**SIR**

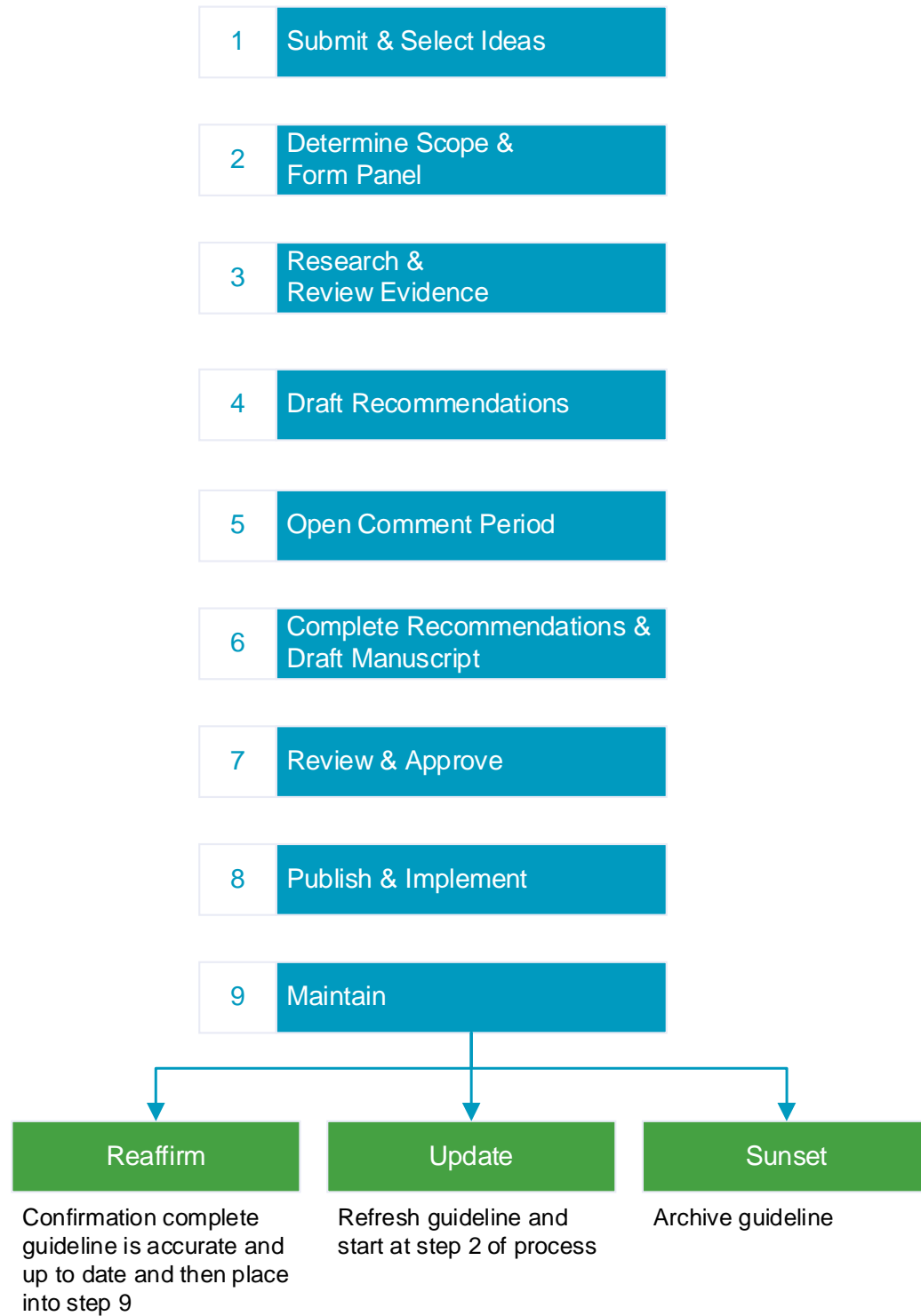
**STR**

# Expert Panel Members

- **Sinchita Roy-Chowdhuri, MD, PhD, Co-chair, CAP**
  - **Christopher R. Gilbert, DO, Co-chair, ATS**
  - **Sanja Dacic, MD, PhD, AMP**
  - **Mohiedean Ghofrani, MD, MBA, CAP**
  - **Peter Illei, MD, ASC**
  - **Lester Layfield, MD, PSC**
  - **Christopher Lee, MD, STR**
  - **Claire Michael, MD, PSC**
  - **Ross A. Miller, MD, PPS**
  - **Jason W. Mitchell, MD, MPH, MBA, SIR**
  - **Boris Nikolic, MD, MBA, SIR**
  - **Jan Nowak, MD, PhD, AMP**
  - **Nicholas J. Pastis, Jr., MD, CHEST**
  - **Carol Ann Rauch, MD, PhD, CAP**
  - **Amita Sharma, MD, STR**
  - **Lesley Souter, PhD, methodology consultant**
  - **Paul VanderLaan, MD, PhD, ASC**
  - **Jesse Voss, CT(ASCP), ASC**
- Staff**  
**Nicole E Thomas, MPH, CT(ASCP)cm**  
**Carol Colasacco, MLIS, SCT(ASCP)**

# Advisory Panel Members

- Tim Allen, MD, JD, PPS
- Dara Aisner, MD, AMP
- Olga R Brook, MD, SIR
- Michael Dugan, MD, CAP
- Tarik Elsheikh, MD, PSC
- Kazunori Kanehira, MD, CAP
- Elliott B. Levy, MD, NCI
- Irina A. Lubensky, MD, NCI
- Hala R. Makhlouf, MBBCH, MD, PhD, NCI
- Dina Mody, MD, CAP
- Robert Najarian, MD, CAP
- Lynnette S. Pineault, MBA, SCT(ASCP), ASC
- Nichole Tanner, MD, ATS
- Patricia Tiscornia-Wasserman, MD, ASC
- Renu Virk, MD, ASC
- Momen M. Wahidi, MD, CHEST
- Lonny B. Yarmus, DO, ATS



# Literature Search

- **PubMed and the Embase were searched**
  - Six additional databases were searched for grey literature
- **Search dates were January 1, 2007 – March 30, 2017 and refreshed in PubMed and Embase May 15, 2018 and April 30, 2019**
- **The searches identified 4,718 abstracts (from initial search and literature refreshes) and ultimately, 100 papers met the selection criteria.**



# Panel proceedings

- **The expert panel met via conference call/webinar monthly and met twice in-person to review data and draft the recommendations.**
- **The draft recommendations were released to the public for comments May 23 through June 15, 2018.**
- **Comments were reviewed and the panel agreed to revisions. 16 recommendations were made.**
- **All changes were incorporated prior to manuscript approval.**

# Conclusions

# Conclusions

- **Thoracic small specimens can be 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.**
- **Rapid on-site cytologic evaluation remains helpful in appropriate triage, handling, and processing of specimens.**

# References

- **Roy-Chowdhuri S, Dacic S, Ghofrani M, et al. 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 for Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society for Thoracic Radiology. *Arch Pathol Lab Med*. In Press. DOI: [10.5858/arpa.2020-0119-CP](https://doi.org/10.5858/arpa.2020-0119-CP)**

# Disclosure

**Practice guidelines and consensus statements are intended to assist physicians and patients in clinical decision-making. 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 cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for a patient. Refer to the guideline manuscript for complete details about the recommendations. The CAP and its collaborators make no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The CAP and its collaborators assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.**



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