### Pre-Procedural Evaluation

- Choose the best biopsy method to optimize yield (EBUS-TBNA for large mediastinal adenopathy, TTNA for peripheral nodule, etc.)
- Identify reason for biopsy
  - Initial diagnosis
  - Known diagnosis but need additional tissue for molecular testing
- Optimize pre-procedural imaging to maximize procedural yield

### Specimen Collection

- Image guidance to improve sample acquisition
- Utilize ROSE to confirm adequate tissue for testing needs
- Needle gauge (procedure dependent)
- Number of passes
- Operator skill and technique

### Specimen Handling

- Utilizing ROSE to triage specimen
- Collection of specimen within appropriate media (formalin/non-formalin fixatives)
- Perform additional passes for cell block
- Communicate case details with pathology to optimize specimen triage

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**Initial biopsy reveals adenocarcinoma**

 PD-L1 immunohistochemistry  
Test for actionable mutations (NGS panel testing favored over individual tests*).

**Initial biopsy reveals adenocarcinoma, but limited tissue remains after diagnostic workup**

 Communicate presence of limited testing material to the ordering provider and prioritize testing based on discussion  
Consider ordering cell-free DNA test (informative, if positive)  
Consider repeat biopsy, communicate “molecular priority” protocol for known diagnosis

**Patients progressing on initial EGFR TKI**

 Test for actionable mutations such as T790M, MET amplification, ERBB2/Her-2 amplification  
Cell-free DNA test (informative, if positive), otherwise repeat tissue biopsy

**Patient progressing after immunotherapy: biopsies remain experimental in this situation.**

See online supplement for references and abbreviations: www.amp.org/PocketGuides

* if the sample is too small to do mutation testing, reflex to fluorescence in situ hybridization (FISH) for rearrangements of ALK, ROS1, RET, and MET amplification.

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Prepared by the Association for Molecular Pathology Training and Education Committee  
For more educational resources, see: www.amp.org/AMPEducation

Revised 7/2023
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ONCOLOGY: Molecular Testing in NSCLC – Laboratory Aspects in Small Specimen Processing

**Biopsy/FNA Procedure**

- Fine Needle Aspiration
  - EBUS TBNA
  - Transthoracic (lung)
  - Metastatic sites
- Other Cytology
  - Brushing/Washing
  - Bronchial Lavage
  - Effusion
- Needle Biopsy
  - Transthoracic (lung)
  - Metastatic sites
- Forceps Biopsy
  - Transbronchial

**FFPE**
- Biopsy
- Cytology cell block

**FFPE Histology Processing**
- 10% neutral buffered formalin
- Volume of fixative (10:1)
- Fixation Time (6-72 h)
- Avoid acid/heavy metal fixatives
- Avoid decal with harsh acids
- Separate soft tissue before decal
- Use EDTA/formic acid for decal

**Non-FFPE Cytology (NFC)**
- Additional smear/TP for molecular**
- LBC residual needle rinse**

**Molecular Testing**

**Diagnostic Workup**

**Transfer directly to molecular**

**Tissue Preserving Processing**
- Minimize IHC use (TTF-1 & p40 as first line IHC)
- Sectioning protocols with designated upfront sections for potential IHC, FISH, and molecular
- Special tissue preserving techniques for molecular priority cases
- Use paired FNA as non-decal source
- Use paired NFC (smear, TP, LBC) as alternate source for molecular testing

**Tissue Preserving Processing**

**Molecular In My Pocket™**

See Reverse

See Reverse

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