<table>
<thead>
<tr>
<th>Pre-Procedural Evaluation</th>
<th>Specimen Collection</th>
<th>Specimen Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Choose the best biopsy method to optimize yield (EBUS-TBNA for large mediastinal adenopathy, TTNA for peripheral nodule, etc.)</td>
<td>• Image guidance to improve sample acquisition</td>
<td>• Utilizing ROSE to triage specimen</td>
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</table>
| • Identify reason for biopsy  
  • Initial diagnosis  
  • Known diagnosis but need additional tissue for molecular testing  
  • Optimize pre-procedural imaging to maximize procedural yield | • Utilize ROSE to confirm adequate tissue for testing needs  
  • Needle gauge (procedure dependent)  
  • Number of passes  
  • Operator skill and technique | • 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 |

**Request for molecular studies and follow up on the biopsy results**

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](http://www.amp.org/PocketGuides)

See Reverse
Biopsy/FNA Procedure

- Additional NFC Specifically for Molecular

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

Non-FFPE Cytology (NFC)
- Smears
- Cytospin preparations
- Touch preparations (TP)
- Liquid based cytology (LBC)

Molecular Testing

Diagnostic Workup

Transfer directly to molecular

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

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

**Assay specific validation required

Molecular In My Pocket™

ONCOLOGY: Molecular Testing in NSCLC – Laboratory Aspects in Small Specimen Processing

See Reverse

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