# Precision Medicine:

## Sample Requirements and Testing Approaches

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Precision Medicine

# What makes a good biomarker test?

## A good biomarker test should:

### Be scientifically rigorous

- Strong, evidence-based support for accuracy with which a test measures[1,2]:
  - The target biomarker(s) (analytic validity)
  - A patient's clinical status (clinical validity)
  - The risks and benefits (clinical utility)
- Tests should be sensitive, specific, accurate, and precise (<1%–5% limit of VAF detection) to effectively detect genomic alterations[1,2]
- Tests should be performed on samples with documented and optimized collection, fixation, processing, and storage for biomarker testing[1,3,4]

### Provide valuable, actionable, and timely information

- Results should be **actionable** and/or inform clinical decision-making[1]
- **Clear protocols** should exist for interpreting test results, including protocols for negative results[1,4]
- **Turnaround times should be optimized** (≤10 working days*), allowing results to impact treatment decisions[2,4]

# Biomarker testing offers a range of clinically relevant information[5]

## Biomarkers can be diagnostic, prognostic, and/or predictive[5]

| | |
|---|---|
| **Diagnostic biomarker** testing detects the presence of cancer and can be performed on tissue.[5] | Early-stage HCC is frequently detected by high levels of AFP combined with ultrasound.[6] |
| **Prognostic biomarker** testing predicts the prognosis of a particular cancer, independent of any particular treatment.[5] | Patients with prostate cancer with *BRCA1/2* germline mutations have increased risk of progression on local therapy and decreased OS.[7†] |
| **Predictive biomarker** testing may help predict potential response to a specific therapy.[5] | *EGFR* exon 19 deletions and exon 21 L858R mutations are often sensitive to *EGFR* TKIs.[8,9] |

*Per CAP molecular testing guidelines for patients with lung cancer. †NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

# **Precision Medicine:** Sample Requirements and Testing Approaches

## Considerations when determining biopsy method[10]

### Tumor location

**Tumor accessibility and invasiveness of the procedure must be weighed**[10,11]

*NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer recommend the least invasive biopsy with the highest yield as the first diagnostic study*[12]*

### Tumor characteristics

**Tumor characteristics, such as intra- and inter-tumoral heterogeneity,** may impact the biomarker results and success and likelihood of response to treatment[11]

*Up to 53% of brain metastases contain potentially clinically actionable mutations not detected in the matched primary tumor samples*[11]

### Tissue requirements for biomarker testing

**Biopsy methods differ in the amount of tissue they can obtain,** impacting testing options[10]

*FNA produces smaller sample volumes than CNB, limiting the number of molecular tests that can be performed per sample*[10]

## Tissue can be obtained from a number of different procedures[10,13,14]

Skin biopsy

Endoscopic biopsy

Surgical biopsy

Percutaneous biopsy

Bone biopsy

**While tissue biopsy is often the primary sample, liquid biopsy can be used as an alternative in certain cancers**[10,12,14,15]*

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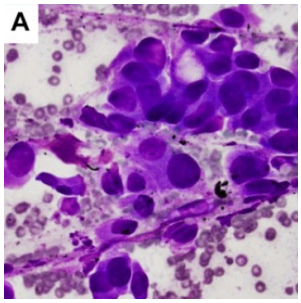
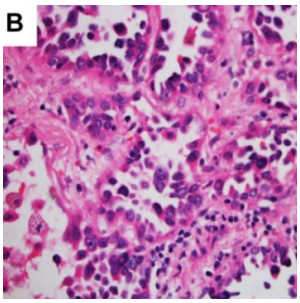
# Fine-needle aspiration (FNA) and core needle biopsy (CNB) are common biopsy methods[16]




| | FNA[16–19] | CNB[16,18,19] |
|---|---|---|
| **Cancer types** | • Lung, thyroid, prostate, liver | • Breast, lung, liver |
| **Needle** | • Fine needle (20–25 gauge) | • Hollow-core needle (14–20 gauge) |
| **Estimated tissue volume ($mm^3$)*** | • 0.5–2.9 | • 2.9–20.1 |
| **Sample and processing** | • Direct smears prepared from aspirate (containing fluid, cancer cells, and surrounding cells) collected in media solution | • Tissue blocks, fixed and paraffin-embedded |
| **Limitations** | • Lower diagnostic sensitivity and specificity than CNB<br>• Tissue architecture is not maintained; cannot determine invasion status | • More invasive than FNA<br>• Longer turnaround time than FNA |

Images adapted from Zhang X, Goldstein DY, Khader SN. Educational case: non-small cell lung cancer: pathologic diagnosis and molecular understanding. *Acad Pathol*. 2019;6:2374289519881951. doi:10.1177/237428951
*Assumes 10-mm length.

# Sample sufficiency considerations

## Sufficient sample sizes are required for accurate results on necessary tests[4,16]

Different **tests have different limits of detection**, requiring a minimum number or percentage of cancer cells per sample for testing[4]

**Opportunity to optimize**

To avoid QNS, ensure that ≥20% of cancer cells are present in the tissue biopsy sample[4]

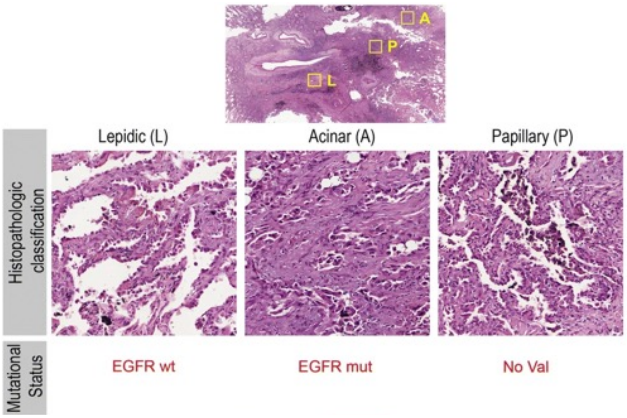
**False negatives may result from tests with insufficient tissue[4]**

**Opportunity to optimize**

An anatomic pathologist should determine sample adequacy[4]

# Additional tissue biopsy considerations

## Intra- and inter-tumoral heterogeneity can impact biomarker testing[16,20]




**Intra-tumor heterogeneity** may result in different biomarkers being present in different tumor regions[20,21]

**Genomic alterations in primary tumors and metastatic tumors may vary due to increased clonality in metastatic sites[22–24]**

Images adapted from: Ramón y Cajal S, Sesé M, Capdevila C, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. *J Mol Med (Berl)*. 2020;98:161–177. https://creativecommons.org/licenses/by/4.0/

**Opportunity to optimize**

When feasible, consider biopsy methods that account for intra- and inter-tumoral heterogenicity[20]

# Rapid on-site evaluation (ROSE) may help improve sample quality for biomarker testing[25–27]

**ROSE**

- Involves an on-site cytopathologist or cytotechnologist performing a rapid stain in the suite or operating room to confirm the presence of tumor cells and estimate neoplastic cell content in the biopsy[28]

**ROSE can help**

**Improve the adequacy rate of biopsy materials for biomarker testing** and diagnosis. In individual studies evaluating ROSE in multiple cancer types and biopsy methods[25,26]

- **Less than half** of bronchoscopic biopsies **had adequate material on first pass**[26]
- Cytological **adequacy** in thyroid FNA was **14.3% higher with ROSE**[25]

**Minimize the need for repeat biopsies** due to inadequate sample or need for additional tissue for ancillary studies[26]

- **Without ROSE,** up to
  - 53.6% of bronchoscopic
  - 15% of head and neck
  - 60.3% of thyroid

  **cases in biopsy studies did not produce adequate samples on the first pass**[25–27]

**Decrease required procedure time** following confirmation that adequate tissue has been collected, or **improve sensitivity** by re-directing to alternate sites following negative ROSE findings[26]

# ROSE can be performed live or virtually[29]

Samples can be evaluated on-site or remotely:

**On-site method: pathologist supports on-site**[29]

- Does not require remote setup or remote coordination with cytotechnologists
- Greater time and cost investment

**Telecytology method: cytotechnologists on-site prepare the sample for a remote pathologist**[29]

- Requires remote coordination with a trained cytotechnologist
- Allows pathologist to support multiple sites simultaneously

**Opportunity to optimize**

## ROSE can help with obtaining an adequate biopsy tissue sample for biomarker testing needs[25]

# Liquid biopsy can complement tissue biopsy[30]

**Liquid biopsy** biomarker testing utilizes bodily fluids, typically blood,* to detect ctDNA, ctRNA, CTC, and exosomes for genomic testing[30–32]

**Liquid biopsy can provide a more complete picture of the molecular heterogeneity** of a patient's cancer than biopsy of a single site[30,32]

**Limits of detection: up to a 30% false-negative rate[33]**
- Negative results should be confirmed with complementary testing[33]

**Larger tumors and advanced or metastatic disease** are more likely to have detectable levels of circulating markers[30,32,34]

**Liquid biopsy can be considered[35]:**
- As a complement to tissue testing
- Where tissue biopsy is not possible or sufficient
- When only archival tissue DNA is available

**Tumor load is directly proportional to ctDNA levels, which may impact detectability[32,33]**

## Although tissue biopsy is recommended, liquid biopsy is emerging as a useful option for predictive testing of certain heterogeneous and metastatic cancers[12,15,30,32,35†]

**Opportunity to optimize**

## Both liquid and tissue biopsy may be considered when determining the optimal method for obtaining biomarker information for your patients[12,15,30,32,35†]

*Urine, saliva, CSF, and other bodily fluids may also be used for diagnosis, disease monitoring, or assessing acquired resistance.[30,32]

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# Tissue vs liquid biopsy in molecular testing

| | Tissue biopsy | Liquid biopsy |
|---|---|---|
| **Pros** | ✓ Is the primary sample type for biomarker testing[10,30,36]<br>✓ Provides a snapshot of the histology and molecular makeup of the tumor[36]<br>✓ Does not require shedding of circulating tumor markers[36] | ✓ Is minimally invasive[10,30]<br>✓ Can provide an overview of molecular heterogeneity from all sites (primary and metastatic)[32,36]<br>✓ Serial biopsies can monitor sub-clonal evolution/acquired resistance[30] |
| **Cons** | ☒ Is invasive[36]<br>☒ Cannot be used on inaccessible tumors or in patients who are not stable enough for biopsy[36]<br>☒ Serial tissue biopsies are not recommended[36]<br>☒ Will not detect biomarkers not present in the sample[36]<br>☒ Provides information from only 1 site[36] | ☒ Samples from patients with a low tumor burden may be below the limit of detection, depending on the tumor type and stage[30,32]<br>☒ May be associated with false negatives or may identify VUS[32,36]<br>☒ May be associated with false positives due to CHIP-associated mutations[36,37] |
| **Tissue handling considerations** | **Proper sample handling and processing may vary by tissue type**<br>Ensure proper fixation of samples for IHC[4]<br>• Avoid destructive protocols, such as acidic decalcification protocols for bone, that damage nucleic acids and inhibit genomic testing[4] | To prevent ctDNA dilution with gDNA[4,38]:<br>• Use EDTA or leukocyte-stabilizing tubes<br>• Centrifuge tubes immediately and store at 4°C for up to 24 hours |

**Opportunity to optimize**

## Planning ahead helps align biopsy handling with planned testing

# Optimize sample adequacy for biomarker testing

An estimated
**60%–70%**
of laboratory-associated errors are due to preanalytical factors[3]

## Pre-procedural evaluation[4,19]

- Identify the reason for biopsy
- Choose the biopsy method
- Optimize pre-procedural imaging to maximize yield

## Specimen collection[3,19,28]

- Image guidance
- Utilize ROSE
- Use correct needle gauge and number of passes

## Specimen handling and diagnostic testing[4,19,28]

- Collect specimen in right media/fixative
- Communicate case details with pathologist
- Use minimum number of slides for diagnosis

## Appropriate molecular tests[4,19]

- Select appropriate biopsy type for molecular test at diagnosis and progression

**Opportunity to optimize**

Optimizing preanalytical factors may help improve the odds of successful biomarker testing and help ensure patients receive appropriate and timely care[3,4]

# Biomarker testing is a multi-factorial process that requires all stakeholders to conserve tissue, to allow fully informed treatment decisions[3,4,19]

Collaboration and precision are important elements of the biomarker testing process[4,19,39]

Considering sample adequacy and testing requirements may help address specific patient needs[4,10]

Optimizing the testing process can help ensure treatment decisions are biomarker-informed[4,19,39]

AFP, alpha-fetoprotein; *BRCA1/2*, breast cancer gene 1/2; CAP, College of American Pathologists; CHIP, clonal hematopoiesis of indeterminate potential; CNB, core needle biopsy; CSF, cerebrospinal fluid; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; ctRNA, circulating tumor ribonucleic acid; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetra-acetic acid; *EGFR*, epidermal growth factor receptor; FNA, fine-needle aspiration; gDNA, genomic deoxyribonucleic acid; HCC, hepatocellular cancer; IHC, immunohistochemistry; NCCN, National Comprehensive Cancer Network; OS, overall survival; QNS, quantity not sufficient; ROSE, rapid on-site evaluation; TKI, tyrosine kinase inhibitor; VAF, variant allele frequency; VUS, variants of unknown significance.

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