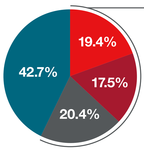
High sample input requirements significantly limit usability of hybrid capture-based NGS tests

Clinical practice guidelines recommend broad genetic profiling by next-generation sequencing (NGS) for advanced non-small cell lung cancer (NSCLC) to guide first-line treatment. Yet, small biopsies and low–tumor content samples pose challenges to testing. The data below, from laboratories across the world, show how limited many of these samples are. While NGS is generally seen as a tissue-saving method given its ability to deliver multiple biomarker results with a single sample, it is important to understand that the sample size and content requirements are not equal for all NGS-based methods. Some NGS-based methods can test smaller samples and deliver results for more patients.

Multicentric feasibility study, US[1]

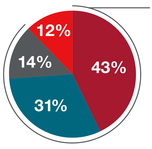


57.3% of all samples had less than 25 mm² tumor area

Tumor area
- ≤4 mm²
- >4 mm² and ≤10 mm²
- >10 mm² and ≤25 mm²
- >25 mm²

21,722
NSCLC samples

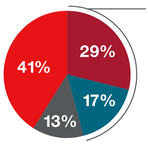
Cancer Genetics, Inc., New Jersey[2]



88% of all samples had less than 25 mm² tumor area

Tumor area
- ≤1 mm²
- >1 mm² and ≤5 mm²
- >5 mm² and ≤25 mm²
- >25 mm²

1,791
lung samples

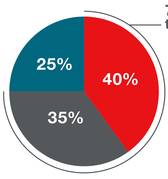


46% of all samples had less than 20% tumor content

Tumor content
- ≤10%
- >10% and ≤20%
- >20% and ≤30%
- >30%

Life Lab, California[3]

627
lung samples

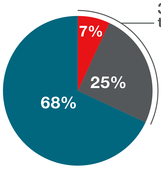


75% of all samples had less than 25 mm² tumor area

Tumor area
- ≤5 mm²
- >5 mm² and ≤25 mm²
- >25 mm²

Sarah Cannon Molecular Diagnostic Laboratory, London[4]

2,796
lung samples



32% of all samples had less than 20% tumor content

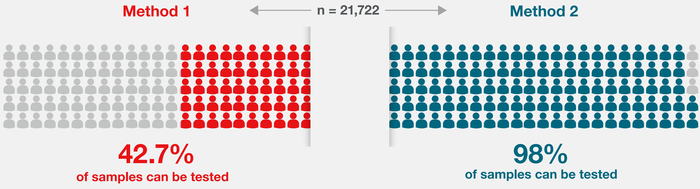
Tumor content
- ≤5%
- >5% and ≤20%
- >20%

Sample requirements can differ greatly from one test to the next

Method 1 (hybrid capture-based)	20% minimum, 30% optimum tumor content	25 mm² minimum sample surface area, entire block or 10 slides required	50 ng–1,000 ng minimum input
Method 2 (PCR amplicon–based)	10% minimum tumor content	No minimum surface area requirement; 2 slides for resection, 9 for CNB required	10 ng minimum input required

NGS-based testing input requirements are typically expressed as a quantity of nucleic acid (in nanograms) and can differ significantly between different NGS-based tests. The figures above explain the practical implications of these different requirements in terms of tissue, tumor area, and content. Even if similar numbers of slides are required for both tests, the tumor area and percentage of tumor content required are significantly higher for Method 1, in order for testing to be successful.

Potential impact of different sample requirements on patients



42.7%
of samples can be tested

98%
of samples can be tested

Only one of two patients would have enough sample to be tested by Method 1 based on hybrid capture NGS, while >98% of samples could be successfully tested with a PCR amplicon–based method.

References
1. Scott, A, et al. (2020) Actionable CR-based comprehensive genomic profiling (PCR-CG P) : Feasibility from >20,000 tissue specimens and predicted impact on actionable biomarker identification vs. hybrid capture (H)-CG P and plasma (P)-CGP. Presented at ASCO 2020.
2. NGS to take top spot as cancer biomarker testing broadens. *CAP TODAY*, June 2018
3. Life Lab Internal Audit data on file
4. Tissue is still the issue, David Moore; *The Pathologist*, May 2018