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 therapy options. Yet, not all patients have tumor tissue suitable for testing. This side-by-side comparison reveals how true-to-life data from three laboratories across the world shows true shortage of many of these samples. NGS is generally seen as a tissue-saving method given its ability to deliver reliable test 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

- 85% of all samples had less than 25 mm² tumor area
- 46% of all samples had less than 20% tumor content
- 48% of all samples had less than 25 mm² tumor area
- 39% of all samples had less than 20% tumor content

Cancer Genetics, Inc., New Jersey

- 79% of all samples had less than 25 mm² tumor area
- 57.3% of all samples had less than 25 mm² tumor area
- 42.7% of all samples had less than 20% tumor content
- 46% of all samples had less than 20% tumor content

Life Lab, California

- 75% of all samples had less than 25 mm² tumor area
- 78% of all samples had less than 25 mm² tumor area
- 29% of all samples had less than 20% tumor content
- 32% of all samples had less than 20% tumor content

Sarah Cannon Molecular Diagnostic Laboratory, London

- 88% of all samples had less than 25 mm² tumor area
- 82% of all samples had less than 25 mm² tumor area
- 25% of all samples had less than 20% tumor content
- 77% of all samples had less than 20% tumor content

Sample requirements can differ greatly from one test to the next

\[
n = 21,722
\]

<table>
<thead>
<tr>
<th>Test</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor area</td>
<td>≥5 mm²</td>
<td>&gt;5 mm² and ≤25 mm²</td>
</tr>
<tr>
<td>Tumor content</td>
<td>≤10% and ≤20%</td>
<td>&gt;10% and ≤30%</td>
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NGS-based testing input requirements are typically expressed as a quantity of nucleic acid (in nanograms) and can differ significantly between methods. The following side-by-side comparison highlights 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 tissue required are significantly higher for Method 1, in order for testing to be successful.

Potential impact of different sample requirements on patients

<table>
<thead>
<tr>
<th>Method 1</th>
<th>Method 2</th>
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<tbody>
<tr>
<td>42.7% of samples can be tested</td>
<td>98% of samples can be tested</td>
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</tbody>
</table>

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.