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INSPYRATIONS | VOLUME 1

ASPYRE®-Lung addresses critical gaps in NGS-based biomarker testing: robust variant calling from NGS QC fails

ASPYRE®
LUNG

Summary

A significant challenge with next-generation sequencing (NGS)-based testing of patients with non-small cell lung cancer (NSCLC) is that **up to 25% of tissue samples fail sequencing due to quality control (QC) parameters,** a major contributing factor to inadequate patient care in more than half of lung cancer patients in the US.[1,2]

In this study, we investigated an NSCLC patient sample set composed of commercially biobanked clinical specimens. These specimens had previously failed NGS QC—despite having sufficient clinical material—and genomic biomarker data was unavailable to guide patient treatment decisions.

Key findings:

Of the 94 patient samples that failed NGS QC, **98% of samples (92/94) passed** ASPYRE-Lung and were able to inform patient care.

In the 92 patient samples that passed ASPYRE-Lung, **47% (43/92) had an actionable variant** identified by ASPYRE-Lung.

Of the 5 NGS QC-failure samples with inadequate DNA amounts for ASPYRE-Lung, ASPYRE-Lung was able to **generate data on 4/5 (80%) samples and 2/4 had an actionable variant.**

All samples were processed with next-day results.

ASPYRE-Lung can be utilized as a rapid clinical testing solution for the significant number of patient samples that fail NGS and has the potential to provide actionable clinical information on samples deemed to be of insufficient quantity (QNS) for NGS testing.

Results

Matched DNA and RNA from 120 biobanked NSCLC FFPE patient samples previously assessed by Illumina TruSight Oncology 500 (TSO 500) were tested using the ASPYRE-Lung Laboratory Developed Test in Biofidelity's CLIA laboratory. These included 94 samples which failed NGS QC parameters for DNA, RNA, or both, and an additional 26 control samples which passed NGS QC. Samples were tested by ASPYRE-Lung using an input of 20 ng DNA and 6 ng RNA, with the exception of 5 DNA patient samples which had insufficient quantity and were run at lower inputs, ranging from 4.25 ng-14 ng DNA input.

Results are shown in **Table 1.** Of the 26 control samples that passed NGS QC, all 26 also passed ASPYRE-Lung. Of the 94 samples that failed NGS QC, 98% of samples (92/94) passed ASPYRE-Lung. In the 92 samples that passed ASPYRE-Lung, 47% (43/92) had a detectable variant (see **Table 2**). In one sample with RNA sequencing results that passed TSO 500 QC, ASPYRE-Lung identified a *ROS1* fusion that was not identified by NGS.

Table 1: Cumulative QC pass/fail results*

Number of samples	% passing NGS TSO 500	% passing ASYPRE-Lung
26	100% (26/26)	100% (26/26)
94	0% (0/94)	98% (92/94)

*Assay fails include samples that failed either DNA or RNA analysis, or both DNA and RNA analysis.

Table 2: Variants identified by ASPYRE-Lung in samples which failed NGS

Variant	# of Occurrences
DNA *BRAF* exon 15 p.V600E	1
DNA *EGFR* exon 18 p.G719C	1
DNA *EGFR* exon 18 p.G719S	1
DNA *EGFR* exon 19 p.E746_A750del	3
DNA *EGFR* exon 19 p.L747_K754del	1
DNA *EGFR* exon 20 p.S768I	3
DNA *EGFR* exon 20 p.T790M	2
DNA *EGFR* exon 21 p.L858R	7
DNA *EGFR* exon 21 p.L861Q	1
DNA *ERBB2* exon 20 p.Y772_A775dup	1

Variant	# of Occurrences
DNA *KRAS* exon 2 p.G12A	1
DNA *KRAS* exon 2 p.G12C	10
DNA *KRAS* exon 2 p.G12D	5
DNA *KRAS* exon 2 p.G12V	6
DNA *KRAS* exon 2 p.G12R	1
DNA *KRAS* exon 2 p.G13C	1
DNA *KRAS* exon 3 p.Q61H	1
RNA *ALK* Fusion	1
RNA *MET* exon 14 skipping	1
RNA *ROS1* Fusion	2

Conclusion

Advanced non-small cell lung cancer treatment guidelines recommend testing of all patients for genomic actionable mutations associated with over 30 highly active FDA-approved targeted therapies, which can prolong survival in biomarker-selected patient populations.

Importantly, this opportunity is only available to the ~50% of patients who successfully undergo biomarker testing. Multiple gaps in current multiplexed or NGS genomic testing options collectively hinder patients from receiving more effective targeted therapies, including:

QC test failures:
Multi-step workflows, complicated library preparation and enrichment steps, resulting in high rates of QC failures.

"Quantity not sufficient" (QNS) rates:
Excessive assay tissue requirements require rebiopsy of patients, delaying initiation of therapy.

DNA-only NGS based assays:
May not fully identify actionable RNA fusion mutations, resulting in missed opportunities for effective targeted treatments in a significant number of patients.

Cost:
Tests are prohibitively expensive.

Complicated and time-consuming bioinformatics:
Need for specialized staff and genomic specialists for variant calling and interpretation of "variants of unknown significance."

Centralized testing:
Tests are only available at large academic centers or centralized laboratories, which delay patient treatment decisions.

Slow turnaround time (TAT) of test results:
Results in the need for many patients to initiate cytotoxic chemotherapy prior to receiving their genomic data.

This study demonstrates that ASPYRE-Lung is able to address critical gaps in NGS-based NSCLC biomarker testing; clinical reports covering guideline-recommended NSCLC genomic biomarkers were generated for 98% of samples that failed NGS QC. Additionally, ASPYRE-Lung has the potential to address tissue-limited (QNS) specimens due to ASPYRE's decreased tumor cell content and input requirements.

Compared to NGS, ASPYRE-Lung has a high success rate, is easily adoptable and cost effective making it suitable as a first-line testing option, or as a salvage test method for clinical samples that are either QNS or fail NGS QC, consistent with current practice guidelines. ASPYRE-Lung is a transformative option in cancer care management, providing more patients with NSCLC actionable biomarker information, enabling all patients the potential to benefit from highly effective and better tolerated targeted therapies.

About ASPYRE®-Lung

Allele-Specific PYrophosphorolysis REaction (ASPYRE) is a novel method for molecular testing of both DNA and RNA biomarkers[3,4] that relies on the highly specific enzymatic degradation of probes hybridized with perfect complementarity to target DNA strands. Built on the innovative ASPYRE technology, ASPYRE-Lung is a targeted multi-gene panel that detects 114 genomic biomarkers in 11 genes (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *RET*, *ROS1*, *MET*, and *NTRK1/2/3*) that have associated FDA-approved targeted therapeutics and well-established clinical utility in NSCLC.

ASPYRE-Lung's simple workflow enables straightforward adoption without new instrumentation, staffing, or IT

- → DNA and RNA are extracted concurrently from formalin-fixed paraffin embedded (FFPE) tissue with **at least 10% tumor cell content.**
- → **RNA is converted to cDNA** by reverse transcription using standard PCR equipment.
- → **Genomic targets are amplified in separate multiplex PCR reactions** including either DNA or cDNA.
- → 114 DNA and RNA biomarkers are **simultaneously detected.**
- → Analysis is via a **user-friendly web application** with no requirement for specialized bioinformatics.

DNA / RNA	TARGET AMPLIFICATION	ENZYMATIC CLEANUP REACTION	ASPYRE REACTION	DETECTION REACTION	DATA ANALYSIS
Instrument requirements	Thermal cycler	Thermal cycler	Thermal cycler	Real-time PCR instrument	Desktop computer
Hands-on Time	30 mins	10 mins	35 mins	15 mins	20 mins
Incubation Time	1 hour 25 mins	15 mins	35 mins	3 hours 30 mins	—

The ASPYRE-Lung workflow is simple and easy to run:

- → **Can be performed manually or straightforwardly automated** on small footprint liquid handlers.
- → **Simultaneous analysis of 77 DNA variants and 37 RNA fusions,** enabling robust fusion detection.
- → **High sensitivity enables testing from tissue samples with as little as 10% tumor content,** compared to 20% to 30% for most competing assays.
- → Workflow involves **only reagent transfer and incubation.**
- → **Requires low inputs** of only 20 ng DNA and 6 ng RNA.
- → **No specialist bioinformatics skills required** for analysis.
- → **Run time of 2 days** from specimen to result.

The ASPYRE-Lung workflow offers the potential for increased sensitivity of both DNA and RNA biomarker detection, less tumor cell content requirements compared to many NGS assays, many fewer steps than complex NGS-based testing, reduced bioinformatics requirements, and next-day TAT. Collectively, ASPYRE-Lung addresses the current multiple critical gaps in patient access to genomic testing.

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Accelerating the genomics revolution

Our revolutionary technology fundamentally changes the way genomic analysis is conducted. By eliminating uninformative DNA, our products massively reduce the time, cost, and complexity of genomic analysis, improving accuracy and enabling faster and simpler decision-making.

Visit **Biofidelity.com** to learn more.

References

1. Hagemann IS, Devarakonda S, Lockwood CM, et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. *Cancer*. 2015;121(4):631-639. doi:10.1002/cncr.29089
2. Sadik H, Pritchard D, Keeling DM, et al. Impact of clinical practice gaps on the implementation of personalized medicine in advanced non-small-cell lung cancer. *JCO Precis Oncol*. 2022; 6:e2200246. doi:10.1200/PO.22.00246
3. Gray ER, Mordaka JM, Christoforou ER, et al. Ultra-sensitive molecular detection of gene fusions from RNA using ASPYRE. *BMC Med Genomics*. 2022;15(1):215. doi:10.1186/s12920-022-01363-0
4. Silva AL, Powalowska PK, Stolarek M, et al. Single-copy detection of somatic variants from solid and liquid biopsy. *Sci Rep*. 2021;11(1):6068. doi:10.1038/s41598-021-85545-3

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