Poster ID: 5164

Deployment of ASPYRE-Lung targeted variant panel across three sites and testing with FFPE tissue and cytology-derived nucleic acid samples

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ASPYRE

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ABSTRACT

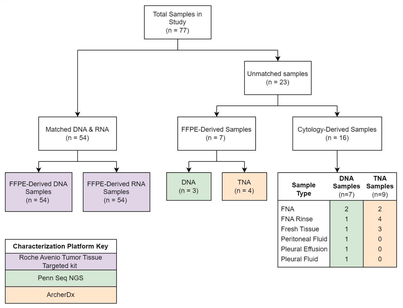
Many patients are unable to access targeted therapies due to challenges in current testing methods including:

- Assessment of multiple variants in both DNA and RNA
- Cost of running multiple assays per sample
- High sample quality control (QC) failure rates
- Clinical need for rapid turn-around time (TAT) to initiate therapy
- Insufficient sample for assay requirements

The ASPYRE-Lung assay addresses the clinical gaps in multiplex testing by simultaneously analyzing DNA and RNA, detecting 114 actionable genomic variants across 11 genes, consistent with current Non-Small Cell Lung Cancer (NSCLC) treatment guidelines. ASPYRE-Lung reagents (for research use only) were utilized for testing at three sites including two academic medical centers and Biofidelity's CLIA Laboratory, with 2-4 trained operators per site. Concordance was evaluated across the three independent sites. In total, 77 patient samples were tested, including 61 derived from Formalin-Fixed Paraffine-Embedded (FFPE) tissue and 16 from cytology specimens. Reproducibility for all 77 samples yielded a positive percent agreement (PPA) of 100% and a negative percent agreement (NPA) of 99.99%. Concordance with NGS was high across all three sites with PPA of 97.2% and NPA of 99.963%. ASPYRE-Lung is a cost-effective, easy to adopt testing method requiring no specialized expertise or complicated bioinformatics, with the potential to rapidly inform genomic data on small tissue samples, thus enabling all patients with NSCLC to initiate appropriate treatment in a timely manner.

INTRODUCTION

Comprehensive genomic testing of *EGFR*, *BRAF*, *ALK*, *RET*, *ROS1*, *ERBB2*, *KRAS*, *NTRK1, NTRK2, NTRK3* and *MET* is indicated in patients with NSCLC. The detection of variants in these genes informs the use of targeted therapeutic agents. We have previously described development of a novel method, Allele-Specific PYrophosphorolysis REaction (ASPYRE®), for rapid and low-cost detection of single nucleotide variants, insertions, deletions and complex events from DNA[1] and fusions and exon skipping events from RNA[2]. Here, we describe a concordance study between two academic centers and Biofidelity. Each site tested 77 NSCLC patient samples, including samples derived from FFPE lung tissue (resections), Fine-Needle Aspiration (FNA), FNA rinses, pleural effusions, peritoneal fluid and fresh tissue. Assay-wide PPA and NPA across sites and a comparison to NGS were assessed.



MATERIALS & METHODS

Clinical samples. FFPE lung tissue or cytology samples were derived from commercial biobanks or residual patient material. Samples had sufficient nucleic acid for ASPYRE at three sites and orthogonal testing.

Nucleic acid extraction. DNA and RNA or TNA was manually extracted using standard commercial kits and aliquoted to use at all three sites.

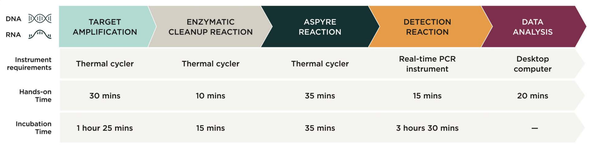
ASPYRE. The ASPYRE® reaction was performed at a DNA input of 20 ng per reaction and RNA input of 6 ng per RT-PCR reaction as previously described[1,2] using the ASPYRE-Lung reagents with the following equipment: T100 (BioRad), Applied Biosystems Veriti, ProFlex, SimpliAmp and QuantStudio 5 qPCR System (ThermoFisher).

Operator training. A Field Application Scientist from Biofidelity visited external sites for between 3-5 days. The trainer ran the assay while trainees observed. Trainees ran the assay with contrived samples once or twice under supervision. Patient samples described in this study were run unsupervised.

Data analysis. Variant calls for samples and controls were generated by processing the raw data file generated by the Design and Analysis2 software (v2.6.0, Thermo Scientific) through the ASPYRELab software (v1.0.0).

Orthogonal testing. Nucleic acid was sequenced through targeted enrichment NGS (Roche Avenio Tumor Tissue Targeted kit) or the PennSeq™ and Archer Custom Fusion Transcript panel.

ASPYRE-Lung ASSAY



Panel A – General overview of the ASPYRE®-Lung workflow. Targets are amplified by multiplex (RT-)PCR and variants detected using ASPYRE detection chemistry with read-out on a real-time PCR instrument. Targets include single nucleotide variants (SNVs), insertions and deletions (indels), RNA fusions and splice variants in nucleic acid derived from formalin fixed paraffin embedded tissue (FFPE).

DNA & RNA

ASPYRE-Lung simultaneously analyzes DNA and RNA in a single assay, maximizing the opportunity to identify mutations and fusions, while avoiding the additional time and expense of running separate assays.

Fast time to results

ASPYRE-Lung testing requires only four reagent transfer steps and enables fast analysis of comprehensive panels of genomic biomarkers.

Reduced sample requirement

ASPYRE's high sensitivity enables testing from tissue samples with as little as 10% tumor content.

Runs on existing instruments

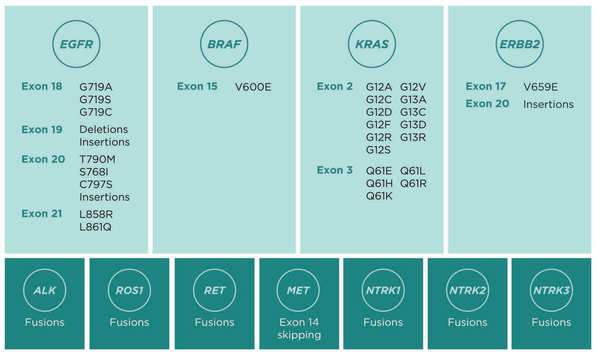
ASPYRE-Lung does not require any new expensive instrumentation, but it runs on existing real time PCR instruments already available in many laboratories around the world.

Ease of implementation

ASPYRE-Lung testing can be rapidly implemented on existing PCR instrumentation without the need for highly trained laboratory staff or complex bioinformatics.

Actionable results

ASPYRE-Lung comprehensive lung panel includes NCCN guideline recommended genomic biomarkers associated with targeted therapies for NSCLC.



Panel B – ASPYRE-Lung's comprehensive lung panel identifies variants across all guideline-recommended genes for NSCLC; 11 genes, 114 variants (77 DNA Mutations, 36 RNA fusions and one exon skipping event).

RESULTS

Variant ID	Sample Type	ASPYRE-Lung Count			Targeted enrichment NGS Counts
		UPenn	MCW	Biofidelity	
EGFR p.E746_A750del	FFPE lung tissue, DNA	4	4	4	4
EGFR p.L747_S752del	FFPE lung tissue, DNA	1	1	1	1
EGFR p.L747_P753delinsS	FFPE lung tissue, DNA	1	1	1	1
EGFR p.A767_V769dup	FFPE lung tissue, DNA	0	1	0	0
EGFR p.S768_D770dup	FFPE lung tissue, DNA	1	1	1	1
EGFR p.T790M	FFPE lung tissue, DNA	1	1	1	1
EGFR p.L858R	FFPE lung tissue, DNA	2	2	2	2
EGFR p.L861Q	FFPE lung tissue, DNA	1	1	1	1
KRAS p.G12C	FFPE lung tissue, DNA	3	3	3	3
KRAS p.G12V	FFPE lung tissue, DNA	1	1	1	1
KRAS p.G12A	FFPE lung tissue, DNA	1	1	1	1
KRAS p.G12A	FFPE lung tissue, DNA	1	1	1	1
KRAS p.Q61L	FFPE lung tissue, DNA	1	1	1	1
BRAF V600E	FFPE lung tissue, DNA	3	3	3	3
ALK fusion	FFPE lung tissue, RNA/TNA	1 (RNA) 1 (TNA)	1 (RNA) 1 (TNA)	1 (RNA) 1 (TNA)	2 (RNA) 1 (TNA)
MET exon 14 skipping	FFPE lung tissue, RNA/TNA	1 (RNA) 1 (TNA)	1 (RNA) 1 (TNA)	1 (RNA) 1 (TNA)	2 (RNA) 1 (TNA)
ROS1 fusion	FFPE lung tissue, RNA	1	1	1	0
No variant detected in sample	FFPE lung tissue, DNA/RNA, DNA and TNA	36	36	36	35
Total positive calls:	FFPE lung tissue	26	27	26	27

Table 1. Comparison of variant profiling of 61 FFPE NSCLC. The ASPYRE-Lung assay was run independently at three sites: Biofidelity's in-house laboratories, the Precision Medicine Laboratory of Medical College of Wisconsin and Hospital of University of Pennsylvania. Variable results between ASPYRE-Lung sites and those that yielded discordant results compared to NGS are highlighted in grey. Two samples yielded two concordant variant calls, and one sample yielded one concordant and one discordant variant call, thus total variant call numbers are greater than the sample numbers.

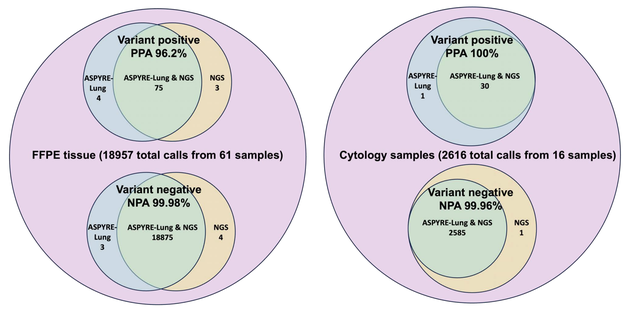
Variant ID	Sample Type	ASPYRE-Lung Count			NGS Counts
		UPenn	MCW	Biofidelity	
EGFR p.E746_S752delinsS	Peritoneal fluid; DNA	1	1	1	1
EGFR p.H773dup	FNA; DNA	0	1	0	0
EGFR p.T790M	Pleural effusion; DNA	1	1	1	1
EGFR p.L858R	Pleural fluid; DNA	1	1	1	1
EGFR p.L858R	Pleural effusion; DNA	1	1	1	1
ERBB2 G776delinsVC	FNA rinse; DNA	1	1	1	1
ROS1 fusion	FNA rinse; TNA	2	2	2	2
RET fusion	FNA rinse; TNA	2	2	2	2
MET exon 14 skipping	Fresh tissue; TNA	1	1	1	1
No variant detected in sample	FNA; DNA, TNA	2 (DNA) 2 (TNA)	1 (DNA) 2 (TNA)	2 (DNA) 2 (TNA)	2 (DNA) 2 (TNA)
No variant detected in sample	Fresh tissue, DNA, TNA	1 (DNA) 2 (TNA)	1 (DNA) 2 (TNA)	1 (DNA) 2 (TNA)	1 (DNA) 2 (TNA)
Total positive calls:		10	11	10	10

Table 2. Comparison of variant profiling of 16 unmatched cytopathology samples (7 DNA and 9 TNA). Samples that gave variable results between ASPYRE-Lung sites and those that yielded discordant results compared to NGS are highlighted in grey. One sample yielded two confirmed variant calls, thus total calls are greater than the number of samples.

	ASPYRE-Lung reproducibility (inter-run precision) between three sites (95% confidence intervals)	ASPYRE-Lung reproducibility AND concordance with NGS (95% confidence intervals)
FFPE lung tissue samples	PPA: 100% (95.4%-100%) NPA: 99.99% (99.95%-100%)	PPA: 96.2% (89.2%-99.2%) NPA: 99.97% (99.92%-99.99%)
Non-FFPE samples	PPA: 100% (88.4%- 100%) NPA: 99.93% (99.6%-100%)	PPA: 100% (88.4%-100%) NPA: 99.93% (99.63%-100%)

Table 3. Summary comparison of results of variant profiling between sites. The denominator result was taken from the ASPYRE-Lung assay variants that are also captured by the NGS assay.

SUMMARY



- ASPYRE® is a novel technology based on pyrophosphorolysis of oligonucleotide probes perfectly hybridized to sample-derived target DNA or cDNA (RNA) sequences.
- The ASPYRE-Lung panel comprises 114 actionable DNA and RNA variants in *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1* covering all genomic biomarker classes across the 11 genes recommended for analysis in NSCLC patients.
- ASPYRE-Lung is easily implemented on common laboratory equipment comprising thermocyclers and real-time PCR systems and requires minimal training.
- Results obtained from ASPYRE-Lung are highly concordant with orthogonal testing by NGS-based methods with overall PPA and NPA 97.2% and 99.963%, respectively.
- While ASPYRE-Lung is validated for NSCLC FFPE lung tissue, these data demonstrate that this novel technology may be applied to a variety of challenging patient sample input types, including cytology preps such as FNA, FNA rinse, peritoneal fluid, and pleural effusions.

DISCUSSION

Together, these results demonstrate high concordance of ASPYRE-Lung and NGS across different types of clinical sample preparation methods. In addition, we found high reproducibility between sites, with 75/77 samples returning the same result across all three sites, or 108/110 (98%) identical positive variant calls.

ASPYRE-Lung assay was easy to adopt and run at each site. It is a cost-effective testing method requiring no specialist expertise or complicated bioinformatics. It can rapidly inform genomic data on small and challenging tissue samples, and therefore, it can enable more patients with NSCLC to benefit from highly active and well-tolerated targeted therapeutics.

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