Establishing the Sensitivity, Specificity, Interlaboratory Reproducibility, and Analytical Limit Of Detection of The UltraSEEK™ Liquid Biopsy Application Using Well-Defined Seraseq Reference Material

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INTRODUCTION

A goal of liquid biopsy is to obtain ctDNA data by performing a non-invasive test on a patient with a solid tumor cancer when tissue failed or could not be biopsied due to patient safety concerns. Although the clinical relevance of the variant allele fraction (VAF) of liquid biopsies is being studied, it is critical to establish test performance so the binary present/absent result can be confidently reported. Since residual clinical samples are limiting to such studies that require replicate testing, patient-like commercial controls containing known variants at pre-defined VAFs has become an accepted approach. This study was designed to determine the analytical performance of the UltraSEEK™ Lung Panel (Agena Bioscience, San Diego, CA) using the Seraseq® ctDNA v2 reference materials (Seracare, Milford, MA).

The UltraSEEK Lung Panel is a liquid biopsy test for the MALDI-TOF-based MassARRAY® system that detects 67 variants across 5 genes (EGFR, KRAS, BRAF, ERBB2, PIK3CA) and 3 variant types (SNV, insertion, deletion) that are relevant to NSCLC. The UltraSEEK chemistry is optimized for low and poor quality samples, such as fragmented cfDNA, providing a targeted, multiplexed method for detecting rare variant events, with an analytical sensitivity to less than 1% and as low as 0.1% on 10-20ng of input cfDNA.

The Seraseq ctDNA v2 reference material is a patient-like circulating tumor DNA (ctDNA) reference standard with a size distribution, amplification yield and low complexity consistent with native DNA.

This reference material contains 9 cancer-relevant somatic variants that have been precisely quantitated and are detectable by the UltraSEEK Lung Panel.

MATERIALS AND METHODS

The Seraseq ctDNA Mutation Mix v2 reference materials* at variant allele frequency of 2%, 1%, 0.5%, 0.25%, 0.125%, and Wild-Type (WT) were provided to each of the 5 testing laboratories in a blinded format. In addition, each site included a patient derived cfDNA sample known to be wild-type or from a normal healthy individual along with a no-template control in each run (Figure 1). Two whole process technical replicates were performed per instrument per site. As several sites contained multiple MassARRAY instruments, the workflow was completed in 20 independent runs across 8 MassARRAY instruments using multiple operators.

Thirty-three nanograms of input material was used for each replicate such that the variants could be reliably represented at 0.125% in the input material.

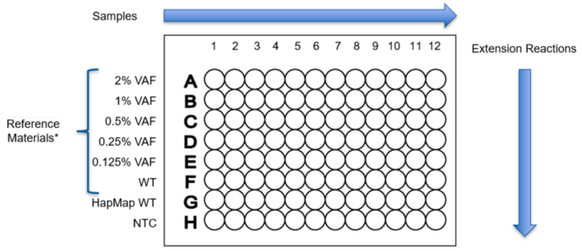

Figure 1: Sample plate layout

The UltraSEEK chemistry amplifies and detects a set of pre-defined loci harboring the variants of interest (Figure 2).

Step 1: Mutant and wild-type nucleotide sequences are amplified in the initial PCR reaction.
Step 2: A single base extension (SBE) enriches for the desired sequence(s) using a mutant-specific terminator.
Step 3: The UltraSEEK extension reaction utilizes a single variant-specific chain terminator labeled with biotin for solid phase capture. This enrichment step involves capture, wash, and elution of the mutant alleles.
Step 4: The mutant-specific extension products are measured on the MassARRAY Analyzer using time-of-flight for detection. The MassARRAY Typer software provides a simple readout of variants detected within the sample run.
Data was exported from Typer software (v4.1) and analyzed using the UltraSEEK Report (v2) with common analysis parameters across all sites.

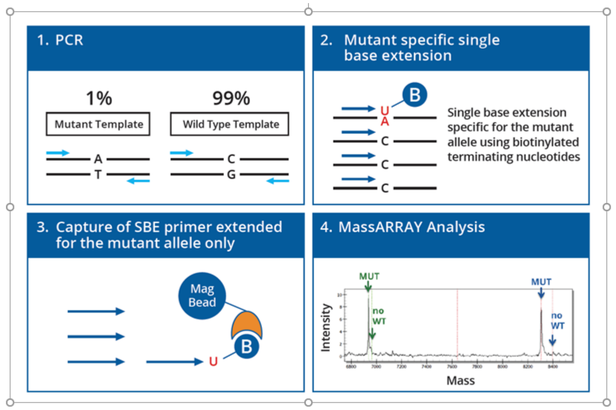

Figure 2: UltraSEEK workflow

RESULTS

	BRAF V600E	EGFR Ins.[1]	EGFR Del.[2]	EGFR L858R	EGFR T790M	ERBB2 Ins.[3]	KRAS G12D	PIK3CA E545K	PIK3CA H1047R	Inter-Laboratory Range	Overall Sensitivity
2% VAF	20	20	20	20	20	20	20	20	20	100-100%	100%
1% VAF	20	19	20	20	20	20	20	20	20	97-100%	99.4%
0.5% VAF	20	6	20	20	20	20	20	19	19	89-96%	91%
0.25% VAF	20	4	20	17	20	8	14	8	13	57-94%	69.9%
0.125% VAF	15	0	19	9	17	8	4	1	7	37-66%	44.4%
LoD[4]	0.25%	1.0%	0.125%	0.50%	0.25%	0.50%	0.50%	0.50%	0.50%		

[1]EGFR p.E746_A750delELREA c.2236_2250del15, [2]EGFR p.D770_N771insG c.2310_2311insGGT, [3]ERBB2 p.A775_G776insYVMA c.2324_2325ins12, [4]Limit of Detection

Table 1: Limit of detection and analytical sensitivity for all 9 expected variants from 20 independent measurements

Study Summary

This study evaluated the interlaboratory performance and reproducibility of the UltraSEEK Lung Panel, a liquid biopsy test for the MALDI-TOF-based MassARRAY system that detects 67 variants across 5 genes (EGFR, KRAS, BRAF, ERBB2, PIK3CA) and 3 variant types (SNV, insertion, deletion) that are relevant to NSCLC.

Samples were patient-like reference standards containing 9 targeted variants at 6 predefined variant frequencies along with a patient derived cfDNA sample and NTC per run. Each variant/frequency was analysed in 20 independent measurements across 5 laboratories and 8 instruments. In addition, sensitivity was determined across 8180 known wild-type measurements.

The UltraSEEK Lung Panel achieved a Limit of detection down to 0.125% (0.125-1%) and specificity of 99.8%. No significant differences were observed across the 5 participating laboratories.

This study demonstrates that the UltraSEEK Lung panel is a reliable and reproducible method for ctDNA analysis. This technology provides an easy-to-use, high-performance, and adaptable solution for disease detection and therapeutic intervention monitoring.

CONCLUSIONS

The reference materials utilized in this study include a variety of different commonly found variants across 3 different variant classes, including single nucleotide variants (SNV, n=6), insertion (2) and deletion (1) variants, and fragmented to mimic the size distribution of patient derived ctDNA.

The Limit of Detection (LoD) was determined for each of the 9 variants across the 3 variant classes and are detected by the UltraSEEK Lung Panel as the lowest Mutant Allele Frequency (MAF) yielding a variant detected rate of at least 95% for the targeted variant (as defined in CLSI Guideline EP17-A2). The lowest limit of detection achieved was 0.125% for the EGFR Deletion and the highest of 1% with the EGFR Insertion. This observation confirmed expectations that deletion and insertion variant types would fall toward the outer limits of detection due to amplification bias of differentially sized mutant DNA compared to the wild-type template in a PCR reaction. The overall LoD for the SNV class was 0.5% MAF.

This study generated an overall specificity of 99.8% across 8180 known negative variant positions in both the reference materials and patient derived cfDNA samples. No significant differences in LoD or Sensitivity were observed across the 5 participating laboratories.