Analytical performance evaluation of TruSight Oncology 500 (TSO500) ctDNA kit; a commercial next generation sequencing liquid biopsy platform

Suman Verma, Theodore Naef, Kyle Horvath, Mathew W. Moore, Philip D. Cotter, Shelly Gunn
ResearchDx/PacificDx, Irvine, CA, 92618

Background

An increasing number of clinical laboratories are seeking to add ctDNA sequencing capabilities to their test menu in order to provide tumor biomarker testing support for cancer patients. Yet internally developed liquid biopsy assays require time and resources beyond the capabilities of most commercial and academic laboratories. Liquid biopsy assays require a sophisticated and complex data analysis pipeline to call variants at low allele frequency (AF) with high confidence, posing additional barriers to entry. Commercially available ctDNA kits with integrated data analysis pipelines are a potential solution for laboratories seeking to incorporate liquid biopsy into their test menus. In the current study, we evaluated the analytical performance of the TSO500 ctDNA kit with DRAGEN analysis software (Illumina) for utilization in our clinical laboratory.

Materials & Methods

Intra-laboratory performance evaluation of TSO500 ctDNA kits (Research Use Only) was performed according to College of American Pathologists (CAP) guidelines for the validation of targeted next generation sequencing assays using purchased reference standards and de-identified human normal plasma cell-free (cf) DNA samples. All samples were sequenced at manufacturer recommended multiplexing using the NovaSeq 6000 S2 (8 samples) and S4 (24 samples) reagent kits and NovaSeq Xp 4-lane kit (Illumina).

Samples Used

Sample Name	Sample Type	Expected Mutations	Expected AF	Accuracy	Analytical Sensitivity	Analytical Specificity	Precision	Linearity	Range of Input	LOD
SeraSeq 2.5%	cfDNA reference standard	SNV, INDEL, CNV, FUSION	2.50%	■	■		■	■	■	■
SeraSeq 1%	cfDNA reference standard	SNV, INDEL, CNV, FUSION	1%	■	■		■	■	■	■
SeraSeq 0.5%	cfDNA reference standard	SNV, INDEL, CNV, FUSION	0.50%	■	■		■	■	■	■
SeraSeq 0.1%	cfDNA reference standard	SNV, INDEL, CNV, FUSION	0.10%	■	■			■	■	■
SeraSeq WT	cfDNA reference standard	WT	0%	■		■	■			
D9006P	Healthy Plasma	WT	0%	■		■				
D9077P	Healthy Plasma	WT	0%	■		■				
D9187P	Healthy Plasma	WT	0%	■		■				

Results

At the multiplexing levels tested during this validation, the TSO500 ctDNA kit demonstrated 100% sensitivity in detecting single nucleotide variants (SNVs), Indels, fusions, and copy number variation (CNV) at ≥ 0.5% allele frequency (AF) and 75% sensitivity in detecting SNVs at 0.1% AF using 30 ng sample input amount. Sensitivity for detecting SNVs and INDELS at 0.1% AF improved with increased sample input (92% and 75% respectively at 50 ng). The sensitivity improved further to 100% at 100ng for SNVs, however INDELS, CNVs, and fusion events remained elusive. The assay displayed >95% specificity in detecting all variants.

Analytical Sensitivity

Analytical Sensitivity SNV

AF%	Examined Variants	Detected Variants	Sensitivity
2.5%	12	12	100%
1%	12	12	100%
0.50%	12	12	100%
0.10%	12	9	75%

Analytical Sensitivity Indels

AF%	Examined Varaints	Detected Variants	Sensitivity
2.5%	7	7	100%
1%	7	7	100%
0.50%	7	7	100%
0.10%	7	3	43%

Analytical Sensitivity CNV

AF%	Examined Varaints	Detected Variants	Sensitivity
2.5%	3	3	100%
1%	3	3	100%
0.50%	3	3	100%
0.10%	3	0	0%

Analytical Sensitivity Fusions

AF%	Examined Variants	Detected Variants	Sensitivity
2.5%	3	3	100%
1%	3	3	100%
0.50%	3	3	100%
0.10%	3	0	0%

Analytical Specificity

Analytical specificity SNV/INDEL (>0.1%AF)

TN/(FP+TN) = 4494/(0+4494)= 100%

Analytical specificity CNV

TN/(FP+TN) = 171/ (4+171) = 97.71%

Analytical specificity Fusions

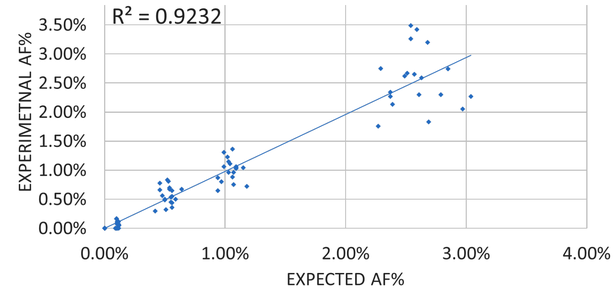
TN/(FP+TN) = 69/ (1+69) = 98.57%

Precision

Assay displayed qualitative precision in detecting all variants types up to 0.5% AF. Less than 20% CV was observed for SNVs ≥0.5% AF and for Indels ≥1% AF.

Range of Input

Linearity for SNV & Indel



Conclusions

Our study demonstrates that TruSight Oncology 500 ctDNA liquid biopsy platform provides a viable alternative for efficient incorporation of liquid biopsy assays into the clinical laboratory for detecting somatic alterations as low as 0.5%. Accurate detection of SNVs as low as 0.1% could potentially be increased with increased sample input amount.

References

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