FOUNDATIONACT™

Analytic validation of the FoundationACT™ ctDNA assay

Liquid biopsy tests have arrived with the potential to change diagnostic, prognostic, and therapeutic approaches in patient care. When a tissue-based biopsy is not feasible, blood-based assays test circulating cell-free DNA, which has been shed by a tumor. In the absence of uniform clinical standards for liquid biopsies, however, the responsibility for evaluating an assay's quality lies with the ordering clinician.

An assay's rigorous validation study offers the best insight into its design and performance. The entire workflow is challenged to quantify the likelihood of a false negative or false positive result, because either can negatively affect patient care.

Approach to the analytical validation of FoundationACT

Foundation Medicine created a rigorous validation framework for its new circulating tumor DNA (ctDNA) assay, FoundationACT, building on guidelines from New York State, Nex-StoCT and College of American Pathologists (CAP). The approach tested four classes of genomic alterations in challenging genomic contexts: base substitutions, insertions/deletions (indels), copy number variations, and rearrangements/fusions at levels intended to push the limit of detection.

To challenge sensitivity and specificity, the study used a demanding and diverse sample pool, including:

- 267 cancer patient samples
- 117 mixtures from cell lines
- 42 synthetic DNA samples engineered for complexity

Specificity, sensitivity, accuracy, and precision were tested in a target region including: 62 total genes with 61 genes across exons and 6 genes across introns commonly involved in rearrangements.

Performance in each class of genomic alterations (e.g., base substitutions, indels, copy number variations, and rearrangements/fusions) was tested separately.

Results[1]

FoundationACT passed rigorous analytic validation, exceeding all target performance specifications.

- **Specificity:** 97.6-99.9% Positive Predictive Value (PPV). PPV is the most clinically meaningful measure of specificity for a next generation assay as per base specificity will always be >99.99% due to the large number of bases interrogated.
- **Sensitivity:** ≥95% for all four classes of alterations.
- **Orthogonal tests:** Base substitutions, indels, and copy number variations were orthogonally validated using ddPCR for MAF<5% and FoundationOne for MAF>5%. Breakpoint PCR was used to validate gene rearrangements/fusions.
- **Reproducibility:** Thirty-six samples tested in triplicate under the same 100% (intra-batch precision) and different 96.8% (inter-batch precision) conditions.

Specification	MAF/Tumor Fraction	Sensitivity	Positive Predictive Value (PPV)
Base Substitutions	≥0.5%	>98.9%	>99.9%
Insertions/Deletions (1-40 bp)	≥1%	>99%	98.8%
Rearrangements/Fusions	≥1%	>99%	98.0%
Copy Number Variations (CNV)*	≥20%	95.3%	97.6%
	<20%	Will vary depending on CNV level and tumor fraction.	
Per-Base Specificity		>99.999%	

* Copy-number ≥8 in genes with at least 4 targets

Summary

FoundationACT achieved very high specificity and sensitivity. It proved reliable and robust for the analysis of whole blood, plasma, and ctDNA. If offers clinicians a high degree of assurance for the detection of four classes of alterations in 62 genes.

1. Data on file at Foundation Medicine. Accessed April 6, 2016.

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