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Abstract # A20

Validation of the QuantStudio5 Instrument for Use in Biocept's TargetSelector™ ctDNA Lung Cancer Assays

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Background

Liquid biopsies represent a non-invasive alternative to traditional tissue biopsies, enabling the detection and tracking of cancer driver mutations from a simple blood draw. Biocept's Target Selector™ test platform offers the unique ability to analyze biomarkers from both circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). Here we performed a clinical concordance study, comparing the ABI 7900 to the newer ABI QuantStudio 5 (QS5) for integration of the QS5 into Biocept's CAP/CLIA certified laboratory. TargetSelector™ ctDNA mutation tests were validated for five targets including *EGFR* (Del19, L858R, or T790M), *BRAF*, and *KRAS*, all markers integral to devising personalized therapies for non-small cell lung cancer (NSCLC) patients.

TargetSelector™ ctDNA Assay Workflow & Diagram

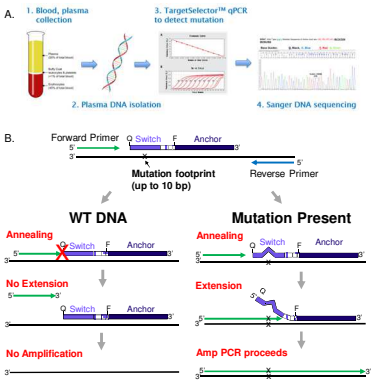

Figure 1: A. TargetSelector™ ctDNA assay incorporates qPCR and Sanger DNA sequencing to identify mutations. B. TargetSelector™ assay is a targeted mutation test which applies the blocker (switch + anchor) to block WT DNA amplification but allows mutant DNA amplification. One specific blocker covers variants on a short stretch of target DNA (up to 10 bp for nucleotide variants).

Methods

Prior to assessing clinical samples, extensive analytical validation was performed with DNA extracted from cancer cell lines containing the relevant *EGFR*, *BRAF* or *KRAS* mutations. TargetSelector™ ctDNA mutant assays procedurally incorporate real-time PCR and DNA sequencing. The analytical validation was conducted to compare the performance of the ABI 7900 vs QS5 instruments within Biocept's ctDNA testing platform. Evaluation of 3000 samples across the five TargetSelector™ assays demonstrated single mutant copy detection sensitivity on the QS5 platform, with >99% sensitivity and >99% specificity for each of the ctDNA mutant assays. Following analytical evaluation, synchronized aliquots of 13 patient ctDNA samples, extracted from whole blood collected in Biocept CEE-Sure™ Blood Collection tubes, were used to test the performance of both the ABI 7900 and QS5 instruments in the TargetSelector™ ctDNA assays.

Results

EGFR, *BRAF* and *KRAS* TargetSelector™ assays that incorporate the ABI QS5 vs the ABI 7900 enable more sensitive ctDNA testing, as demonstrated by analytical validation and subsequent analyses of clinical samples. In patient samples, TargetSelector™ tests using the QS5 identified all of the mutations detected by the same assays using the ABI 7900 platform. At the same time, the QS5 instrument enabled identification of additional mutations not detected in the assays where the ABI 7900 was used.

EGFR Del19 TargetSelector™ Assay

Total Population 232	Condition Positive 120	Condition Negative 112
Test Outcome Positive	True Positive 120	False Positive 1
Test Outcome Negative	False Negative 0	True Negative 111
Accuracy > 99%	Sensitivity > 99%	False Pos. Rate < 1%
	False Neg. Rate < 1%	Specificity > 99%

Table 1: Analytical validation of *EGFR* Del19 TargetSelector™ assay showed >99% sensitivity and >99% specificity.

EGFR L858R TargetSelector™ Assay

Total Population 250	Condition Positive 138	Condition Negative 112
Test Outcome Positive	True Positive 138	False Positive 1
Test Outcome Negative	False Negative 0	True Negative 111
Accuracy > 99%	Sensitivity > 99%	False Pos. Rate < 1%
	False Neg. Rate < 1%	Specificity > 99%

Table 2: Analytical validation of *EGFR* L858R TargetSelector™ assay showed >99% sensitivity and >99% specificity.

EGFR T790M TargetSelector™ Assay

Total Population 250	Condition Positive 138	Condition Negative 112
Test Outcome Positive	True Positive 138	False Positive 0
Test Outcome Negative	False Negative 0	True Negative 112
Accuracy > 99%	Sensitivity > 99%	False Pos. Rate < 1%
	False Neg. Rate < 1%	Specificity > 99%

Table 3: Analytical validation of *EGFR* T790M TargetSelector™ assay showed >99% sensitivity and >99% specificity.

BRAF TargetSelector™ Assay

Total Population 247	Condition Positive 135	Condition Negative 112
Test Outcome Positive	True Positive 134	False Positive 0
Test Outcome Negative	False Negative 1	True Negative 112
Accuracy > 99%	Sensitivity > 99%	False Pos. Rate < 1%
	False Neg. Rate < 1%	Specificity > 99%

Table 4: Analytical validation of *BRAF* TargetSelector™ assay showed >99% sensitivity and >99% specificity.

KRAS TargetSelector™ Assay

Total Population 248	Condition Positive 136	Condition Negative 112
Test Outcome Positive	True Positive 136	False Positive 0
Test Outcome Negative	False Negative 0	True Negative 112
Accuracy > 99%	Sensitivity > 99%	False Pos. Rate < 1%
	False Neg. Rate < 1%	Specificity > 99%

Table 5: Analytical validation of *KRAS* TargetSelector™ assay showed >99% sensitivity and >99% specificity.

Clinical Concordance Study between ABI 7900 and QuantStudio5

	ABI 7900			QuantStudio 5		
Sample #	T790M	L858R	Del19	T790M	L858R	Del19
4	×	×	×	×	×	√
7	×	×	√	×	×	√
8	×	×	×	×	×	√
9	×	×	×	×	×	×
10	×	×	√	×	√	×
11	√	×	√	√	×	√
12	×	×	×	×	×	×
13	√	×	×	√	√	×

	ABI 7900		QuantStudio 5	
Sample #	*BRAF*	*KRAS*	*BRAF*	*KRAS*
1	×	×	×	√
2	×	-	√	-
3	×	-	×	-
5	×	-	×	-
6	×	×	×	√

Table 6: Clinical samples were tested in parallel on 2 platforms, followed by sequencing confirmation. TargetSelector™ assays using QS5 identified additional mutations compared to ABI 7900, as highlighted. ×, not detected; √, detected; -, not applicable.

Clinical Performance of TargetSelector™ ctDNA Assays on QuantStudio5

ctDNA Assay	Tested	Detected	% of Detection
EGFR T790M	518	48	9%
EGFR L858R	518	38	7%
EGFR Del19	518	78	15%
BRAF	353	8	2%
KRAS	138	29	21%

Table 7: Whole blood samples from cancer patients were collected in Biocept's CEE-Sure™ Blood Collection tubes from September 2017 to December 2017. Plasma and ctDNA were isolated, and TargetSelector™ ctDNA assays were then performed in Biocept's CAP/CLIA certified laboratory. Note: these samples comprise various cancer types including lung, breast, colorectal, and melanoma cancers.

Conclusions

- Implementation of the QuantStudio 5 real-time PCR instrument into Biocept's TargetSelector™ ctDNA assays has improved performance over the older TargetSelector™ platform that utilized the ABI 7900.
- The more sensitive QS5-based TargetSelector™ assays increase the likelihood of identifying molecular drivers linked to a patient's cancer.
- Liquid biopsy detection of *EGFR*, *BRAF* and *KRAS* mutant ctDNA provides a minimally invasive means to gain valuable information towards developing personalized treatment strategies, monitoring therapeutic response, and identifying potential resistance mechanisms, all of which are vital for disease management and NSCLC patient care.

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