A concordance study of the ArcherDx Reveal ctDNA 28 NGS panel and Biocept's Target-Selector™ mutation assay using ctDNA collected in Biocept's CEE-sure blood collection tube

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Background

- The ability to build complex, targeted libraries, free of chemically induced mutations is of major importance for the detection of circulating tumor DNA (ctDNA) from solid tumors found in liquid biopsies. DNA damage occurring as a result of sample handling, polymerase errors or chemical preservatives can create low abundance, falsely positive signals indistinguishable from cancer-derived mutations. A critical component of this, often overlooked, is the proper collection and preservation of the sample paired with the right isolation and detection methodology.

- We undertook to evaluate and compare the performance of samples collected in Biocept CEE-Sure blood collection tubes by generating NGS libraries using the ArcherDX Reveal ctDNA 28 panel. We utilized remnant patient DNA isolated from plasma collected and processed at our CAP/CLIA certified laboratory in San Diego, CA.

Study Design

Sample	Biocept Result	Mutation Level
1	KRAS Ex2	high
2	KRAS Ex2	low
3	BRAF V600	high
4	BRAF V600	low
5	EGFR L858	high
6	EGFR L858	low
7	EGFR Del19	high
8	EGFR Del19	low
9	EGFR T790M	high
10	EGFR T790M	low
11	Negative	N/A
12	Negative	N/A

Table 1: circulating DNA from patient blood collected in the patented Biocept CEE-sure was extracted from plasma and used in Target-Selector™ assays specific for EGFR (Del19, L858R, T790M), KRAS or BRAF mutations. In certain cases CTC enumeration and biomarker testing was also performed. Sequencing of the amplified Target-Selector™ product was used to confirm the presence of the mutation. The remnant DNA samples were de-identified and sent blinded to ArcherDx and processed through their Reveal ctDNA workflow. **Each sample contained either a high or low mutational burden for one of the 5 clinically actionable markers listed along with two samples which tested negative for all five**.

CEE-Sure BCT

Figure 1: CEE-Sure™ BCT is designed to both prevent blood coagulation, and to preserve the cells from lysing due to age or transport conditions. CEE-Sure™ allows for room temperature shipment of whole blood for nucleated cell isolation, and for cfDNA and cfRNA detection.

Manufacturing	ISO 9001
Tube Volume	8.0 ml liquid capacity
Tube Type	Plastic or Glass
Shelf Life	Plastic: 12 months Glass: 24 months
Storage Temperature	18°C – 25°C
Shipping Temperature	15°C – 30°C
Stability	Blood: Up to 4 days DNA: Up to 8 days

Advantages

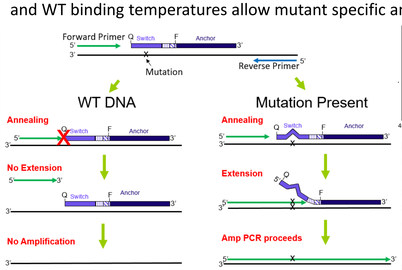
- Ships at room temperature
- Patented preservative stabilizes cellular and genetic content
- ACD prevents blood coagulation
- Choice of Plastic or Glass
- Used commercially in Biocept's own CLIA lab for 2+ years for CTC capture and ctDNA applications
- Proprietary solution
- Manufactured in accordance with SO 9001

Biocept
Completing the Answer™

Testing and Tools

Target Selector Test

Figure 3: Target Selector is a targeted hotspot mutation test that enriches for rare mutations in a large excess of WT DNA. The test is specific to small regions of interest, highly sensitive, and validated at 7 mutant copies in a background of 14,000 WT at >98% sensitivity. Target-Selector™ probes block WT amplification but allow mutant DNA amplification. A mismatch (mutation) under the switch region creates a large annealing temperature discrimination between the mutan and WT binding temperatures allow mutant specific amplification.



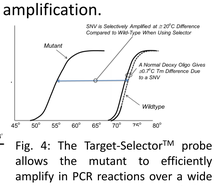


Fig. 4: The Target-Selector™ probe allows the mutant to efficiently amplify in PCR reactions over a wide extension temperature window compared to the wild-type template which is efficiently blocked. This can produce a 20°C window of preferential amplification of mutant template over WT during PCR.

Target Selector Workflow

Archer ctDNA Reveal 28

The ArcherDX Reveal ctDNA test is a 28 gene NGS panel that targets key oncogene activating mutations, drug resistance mutations, and yields full coverage TP53. The Reveal ctDNA assay utilizes Anchored Multiplex PCR (AMP) to enrich, tag, and efficiently capture short ctDNA fragments.

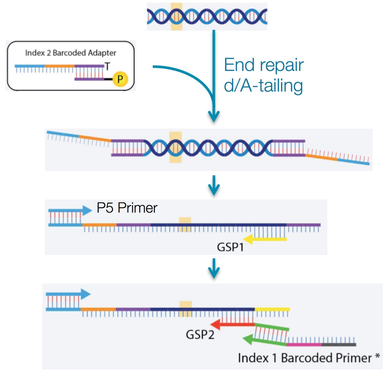


Fig 5: ctDNA inputs, naturally fragmented prior to extraction from plasma, are end-repaired, A-tailed, and tagged by ligation to a molecular barcode-containing adapter. Target enrichment with AMP is achieved by two rounds of nested PCR between region-specific primers (GSP1 and GSP2) and a universal primer (P5 Primer) . Fully indexed, target enriched libraries are produced in under 7 hours (left). Archer ctDNA Reveal 28 target list (right).

Reveal Gene List

ALK	KRAS
AKT1	MAP2K1
AR	MAP2K2
BRAF	MET
CTNNB1	NRAS
DDR2	NTRK1
EGFR	NTRK3
ERBB2	PIK3CA
ESR1	PDGFRA
FGFR1	RET
HRAS	ROS1
IDH1	SMAD4
IDH2	MTOR
KIT	TP53

Biocept Results

CTC Platform

Sample #	Accession #	Cancer Type	CK+ CTCs	CK- CTCs	ALK	ROS1	FGFR1
1	16-01431	lung	0	31	Not Det.	Not Det.	Not Det.
2	16-01195	colon	-	-	-	-	-
3	16-01350	melanoma	-	-	-	-	-
4	16-01064	lung	0	21	Not Det.	Not Det.	-
5	16-01513	lung	-	-	-	-	-
6	16-01493	lung	-	-	-	-	-
7	16-01494	lung	-	-	-	-	-
8	16-01512	lung	-	-	-	-	-
9	16-01400	lung	-	-	-	-	-
10	16-01414	lung	-	-	-	-	-
11	16-01498	lung	0	11	-	-	-
12	16-01516	unknown	2	24	Not Det.	Not Det.	-

Table 2: Additional information on patients tested in the study from the Biocept Clinical Laboratory using the target selector tumor cell capture platform. Cytokeratin negative CTCs were found in 4 of the patients including the two where no other genetic alterations were found. Highlighted cells indicated signal was detected. Dash lines mean the test was not ordered.

ctDNA Platform

Sample #	T790M MT/WT	T790M %	L858R MT/WT	L858R %	Del19 MT/WT	Del19 %	KRAS MT/WT	KRAS %	BRAF MT/WT	BRAF %
1	0/1912	0	0/1912	0	0/1912	0	493/1912	26%	0/2589	0
2	-	-	-	-	-	-	120/14219	<1%		
3	-	-	-	-	-	-	-	-	690/17890	3.86%
4	5/1532	<1%	29/1256	2.2	34/2006	1.6	0/1194	1.2	12/1512	0.79%
5	0/2625	0	33/2592	1.2	0/2325	0	-	-	-	
6	0/1231	0	1/1229	<1%	0/1231	0	-	-	-	
7	5/707	<1%	0/712	0	25/686	3.5	-	-	-	
8	2/1752	<1%	0/1751	0	3/1751	<1.0	-	-	-	
9	350/13126	2.67%	0/13475	0	1/13474*	0	-	-	-	
10	1/1342	<1%	0/1343	0	4/1339	0.30%	-	-	-	
11	0/1336	0	0/1398	0	0/1398	0	0/1481	0	0/1094	0
12	0/5693	0	0/5693	0	0/5693	0	0/7547	0	0/5530	0

*not confirmed by sequencing

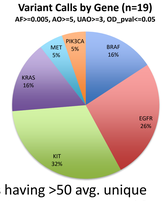
Table 3: Complete testing results for the study patients using Biocept's target selector circulating tumor DNA platform. Tabular numbers indication copy #/mL of plasma tested for either mutant or WT species of DNA. Blue highlight indicates mutation detected. Dashed lines indicate test was not ordered for this patient.

Archer Results

Library Quality

Sample	ng/uL	uL Input	ng Input	nM Yield	AVERAGE_UNIQUE_DNA_AND_AMBIG_START_SITES_PER_GSP2	Avg. Unique Reads per GSP2	UNIQUE_FRAGMENT_TOTAL
16-01431	0.107	50	5.35	12.75	121.43	445.69	218015
16-01195	1.04	30	31.2	31.93	165.97	1626.88	495736
16-01350	1.42	30	42.6	150.28	251.3	3582.94	892171
16-01064	0.195	30	5.85	17.10	132.31	474.55	227398
16-01513	0.342	30	10.26	47.93	169.28	1325.01	414318
16-01493	0.154	30	4.62	6.44	107.35	300.56	115502
16-01494	0.176	30	5.28	7.30	89.28	219.43	104696
16-01512	<0.05	35	NA	1.74	42.68	73.23	62279
16-01400	1.21	30	36.3	128.68	246.84	2466.75	681210
16-01414	0.116	30	3.48	6.08	95.96	233.99	120997
16-01498	0.194	50	9.7	14.04	97.82	319.48	146512
16-01516	0.509	50	25.45	55.01	156.49	1333.93	426587

Variant Frequency



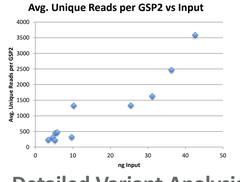


Fig 6: High complexity libraries having >50 avg. unique start sites per region-specific primer were generated from ctDNA inputs ranging from 3.48-42.60 ng. The average number of unique reads per primer increases with increased input mass (left). Archer Analysis leverages features of AMP to filter variant calls using metrics including unique molecular observations (UAO) to increase specificity of results. Archer Analysis also implements outlier detection (OD_pval) to distinguish variants from instrument noise to increase sensitivity.

Detailed Variant Analysis

Name	Gene	Mutation	AF	Chromosome	Position	Reference	Alternate	Score	Depth	AO	UDP	UAO	OD_Pval
16-01431	KRAS	p.G12D	0.1007	chr12	25398284	C	T	3134.36	486	52	160	29	1.44E-15
16-01195	KRAS	p.G12V	0.3243	chr12	25398284	C	A	41671.2	2168	703	458	189	1.44E-15
16-01350	BRAF	p.V600K	0.6854	chr7	140453136	AC	TT	0	16227	11122	.	.	1.55E-15
16-01350	BRAF	p.V600E	0.6868	chr7	140453136	A	T	626628	16257	11165	1203	673	1.55E-15
16-01350	BRAF	p.V600M	0.6865	chr7	140453137	C	T	627053	16304	11193	1195	671	1.55E-15
16-01064	PIK3CA	p.E545K	0.0673	chr3	178936091	G	A	2674.36	654	44	189	26	1.44E-15
16-01064	MET		0.0397	chr7	116412043	G	T	1757.73	781	31	185	19	1.44E-15
16-01493	KRAS	p.G12R	0.0147	chr12	25398285	C	G	543.943	546	8	186	8	1.44E-15
16-01493	KIT	p.D572G	0.0067	chr4	55593649	A	G	230.555	1052	7	237	5	0.00386763
16-01493	KIT	p.T574A	0.0073	chr4	55593654	A	G	273	1098	8	234	7	0.000478108
16-01494	KIT	p.T574A	0.0056	chr4	55593654	A	G	176	891	5	196	3	0.025918618
16-01494	EGFR	p.E746_A750delELREA	0.0914	chr7	55242464	AGGAATTAAGAGAAGC	A	22552.1	372	34	.	.	1.44E-15
16-01494	EGFR	p.T790M	0.0258	chr7	55249071	C	T	500.743	310	8	103	5	9.55E-12
16-01512	KIT	p.D572G	0.0232	chr4	55593649	A	G	219	259	6	81	5	2.91E-06
16-01512	KIT	p.S583G	0.0227	chr4	55593682	A	G	176	220	5	63	4	0.000210155
16-01400	EGFR	p.L747_E749delLRE	*0.2112	chr7	55242465	GGAATTAAGA	G	.	3878	819	.	.	.
16-01400	EGFR	p.A750P	*0.2112	chr7	55242478	G	C	1517.91	4629	28	493	7	1.44E-15
16-01400	EGFR	p.T790M	0.0925	chr7	55249071	C	T	25762.7	4920	455	569	156	1.55E-15
16-01498	KIT	p.K558E	0.0059	chr4	55593606	A	G	208	1012	6	218	6	0.014716424

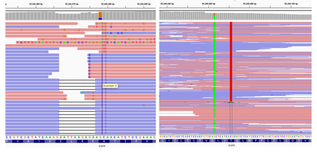


Fig 7: ctDNA enrichment in the Reveal ctDNA assay enhances the ability to detect low abundance variants. AMP chemistry and Archer Analysis detect complex mutations such as polynucleotide BRAF:p.V600K and EGFR deletions. EGFR deletions such as those identified here are oncogenic drivers; a secondary resistance mutation (T790M) was identified in both samples exhibiting the deletion (one example shown to the left). * Allele fractions for the complex EGFR:p.L747_E749del;A750P event are approximate.

Study Results

Patient	Gene	Mutation Level	Biocept	ArcherDx	Biocept Other	Archer Other
1	KRAS	High	X	X	CK- CTCs,	None
2	KRAS	Low	X	X	None	None
3	BRAF	High	X	X	None	None
4	BRAF	Low	X		CK- CTCs, EGFR T790M, L858R, Del19	PIK3CA E545K,MET
5	EGFR L858R	High	X		None	None
6	EGFR L858R	Low	X		None	KRAS G12R, KIT (D572G, T574A)
7	EGFR Del19	High	X	X	EGFR T790M	EGFR T790M
8	EGFR Del19	Low	X		EGFR T790M	KIT (D572G, E583G)
9	EGFR T790M	High	X	X	EGFR Del19*	EGFR (Del19,A750P)
10	EGFR T790M	Low	X		None	None
11	Negative	N/A	X	X	CK - CTCs	KIT K558E
12	Negative	N/A	X	X	CK+ CTCs, CK- CTCs	None

*not confirmed by sequencing

Table 3: Concordance of the tests in 7 of 12 patients. The Biocept test outperformed on sensitivity for the most common markers, but the ArcherDX panel added important additional markers that could guide patient treatment in certain cases. Overall the tests complement one another. For instance one could test the most common markers from Biocept and if negative follow up with a larger panel to look for less common markers that may guide treatment toward therapeutics in clinical trials.

Conclusions

- In this pilot evaluation, the tumor status of 12 patients were tested using both the Biocept Target Selector Assay and the Archer ctDNA reveal 28 NGS panel.
- Archer was able to produce high quality libraries from the stored sample, indicating that little DNA damage occurred during preservation in the CEE-Sure tube.
- The two methods were shown to have good concordance and while the target selector tests excelled at finding low abundance markers, the Archer panel uncovered mutations untested in the Biocept profiling.
- This study demonstrates that both breadth of coverage and depth of analysis are important factors for today's oncologist when considering which diagnostics to perform on their patient. The rise of the liquid biopsy provides an powerful, informative, non-invasive option when looking for actionable biomarkers.