FOUNDATIONONE® LIQUID CDx

FoundationOne® Liquid CDx
Technical Information

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Intended Use

FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, including rearrangements and copy number losses only in *BRCA1* and *BRCA2*. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling for substitutions and indels to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* Exon 19 deletions and *EGFR* Exon 21 L858R substitution	IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib)
Prostate cancer	*BRCA1*, *BRCA2* alterations	RUBRACA® (rucaparib)

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients who are negative for the mutations listed in Table 1 should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

FoundationOne Liquid CDx is a single-site assay performed at Foundation Medicine, Inc. in Cambridge, MA.

Contraindication

There are no known contraindications.

Warnings and Precautions

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approve tumor tissue test, if possible.

Limitations

- For in vitro diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- Genomic findings other than those listed in Table 1 of the intended use are not prescriptive or conclusive for labeled use of any specific therapeutic product.
- A negative result does not rule out the presence of a mutation in the patient's tumor.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given communities.
- The test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.
- Genomic findings from circulating cell-free DNA (cfDNA) may originate from circulating tumor DNA fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited to: *ASXL1, ATM, CBL, CHEK2, DNMT3A, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2, TP53*, and *U2AF1*.
- The false positive rate of this test was evaluated in healthy donors. The detection rate for unique short variants in apparently healthy patients is 0.82%. Across 30,622 short variants, 58 variants had a detection rate of greater than 5%.
- The analytical accuracy for the FoundationOne Liquid CDx assay has not been demonstrated in all genes.
- The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection.
- The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
- A complete assessment of the impact of cfDNA blood collection tube lot-to-lot variability on the performance of the test has not been evaluated.
- The test is not intended to provide information on cancer predisposition.
- *BRCA1/BRCA2* homozygous deletions and rearrangements were not adequately represented in all analytical studies.
- Performance has not been validated for cfDNA input below the specified minimum input.

Test Principle

The FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. All coding exons of 309 genes are targeted; select intronic or non-coding regions are targeted in *BRCA1* and *BRCA2* (refer to **Table 2** for the complete list of genes reported by FoundationOne Liquid CDx). Hybrid-capture selected libraries are sequenced with deep coverage using the

NovaSeq® 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, and copy number variants and genomic rearrangements in *BRCA1* and *BRCA2*. A subset of targeted regions in 75 genes is baited for increased sensitivity.

Table 2: As part of its FDA-approved intended use, the FoundationOne Liquid CDx assay interrogates 311 genes, including 309 genes with complete exonic (coding) coverage and 2 genes with only select non-coding coverage (indicated with an *).

Select genes and select exons (indicated in bold) are captured with increased sensitivity.

ABL1 [Exons 4-9]	*CALR*	*CYP17A1*	*FGFR4*	*KDM6A*	*MYCL (MYCL1)*	*POLD1*	*SMAD4*
ACVR1B	*CARD11*	*DAXX*	*FH*	*KDR*	***MYCN***	*POLE*	*SMARCA4*
AKT1 [Exon 3]	*CASP8*	*DDR1*	*FLCN*	*KEAP1*	***MYD88 [Exon 4]***	*PPARG*	*SMARCB1*
AKT2	*CBFB*	***DDR2 [Exons 5,17,18]***	*FLT1*	*KEL*	*NBN*	*PPP2R1A*	***SMO***
AKT3	*CBL*	*DIS3*	***FLT3 [Exons 14,15,20]***	***KIT [Exons 8,9,11,12,13 ,17]***	***NF1***	*PPP2R2A*	*SNCAIP*
ALK [Exons 20-29]	***CCND1***	*DNMT3A*	***FOXL2***	*KLHL6*	*NF2*	*PRDM1*	*SOCS1*
ALOX12B	*CCND2*	*DOT1L*	*FUBP1*	*KMT2A (MLL)*	*NFE2L2*	*PRKAR1A*	*SOX2*
AMER1 (FAM123B)	*CCND3*	*EED*	*GABRA6*	*KMT2D (MLL2)*	*NFKBIA*	*PRKCI*	*SOX9*
APC	*CCNE1*	***EGFR***	*GATA3*	***KRAS***	*NKX2-1*	*PTCH1*	*SPEN*
AR	*CD22*	*EP300*	*GATA4*	*LTK*	*NOTCH1*	***PTEN***	*SPOP*
ARAF [Exons 4,5,7, 11,13,15,16]	***CD274 (PD-L1)***	*EPHA3*	*GATA6*	*LYN*	*NOTCH2*	***PTPN11***	*SRC*
ARFRP1	*CD70*	*EPHB1*	***GNA11 [Exons 4,5]***	*MAF*	*NOTCH3*	*PTPRO*	*STAG2*
ARID1A	*CD79A*	*EPHB4*	*GNA13*	***MAP2K1 (MEK1) [Exons 2,3]***	***NPM1 [Exons 4-6,8,10]***	*QKI*	*STAT3*
ASXL1	*CD79B*	***ERBB2***	***GNAQ [Exons 4,5]***	***MAP2K2 (MEK2) [Exons 2-4,6,7]***	***NRAS [Exons 2,3]***	*RAC1*	***STK11***
ATM	*CDC73*	***ERBB3 [Exons 3,6,7, 8,10,12,20,2 1,23,24,25]***	***GNAS [Exons 1,8]***	*MAP2K4*	*NSD3 (WHSC1L1)*	*RAD21*	*SUFU*
ATR	***CDH1***	*ERBB4*	*GRM3*	*MAP3K1*	*NT5C2*	*RAD51*	*SYK*
ATRX	***CDK12***	*ERCC4*	*GSK3B*	*MAP3K13*	***NTRK1 [Exons 14,15]***	*RAD51B*	*TBX3*
AURKA	***CDK4***	*ERG*	*H3F3A*	*MAPK1*	*NTRK2*	*RAD51C*	*TEK*
AURKB	***CDK6***	***ERRFI1***	*HDAC1*	*MCL1*	***NTRK3 [Exons 16,17]***	*RAD51D*	*TERC* {ncRNA}*
AXIN1	*CDK8*	***ESR1 [Exons 4-8]***	*HGF*	***MDM2***	*P2RY8*	*RAD52*	***TERT* {Promoter}***

AXL	*CDKN1A*	***EZH2 [Exons 4,16,17,18]***	*HNF1A*	*MDM4*	***PALB2***	*RAD54L*	*TET2*
BAP1	*CDKN1B*	*FAM46C*	***HRAS [Exons 2,3]***	*MED12*	*PARK2*	***RAF1 [Exons 3,4,6,7,10,14,15,17]***	*TGFBR2*
BARD1	***CDKN2A***	*FANCA*	*HSD3B1*	*MEF2B*	*PARP1*	*RARA*	*TIPARP*
BCL2	*CDKN2B*	*FANCC*	*ID3*	*MEN1*	*PARP2*	***RB1***	*TNFAIP3*
BCL2L1	*CDKN2C*	*FANCG*	***IDH1 [Exon 4]***	*MERTK*	*PARP3*	*RBM10*	*TNFRSF14*
BCL2L2	*CEBPA*	*FANCL*	***IDH2 [Exon 4]***	***MET***	*PAX5*	*REL*	***TP53***
BCL6	*CHEK1*	*FAS*	*IGF1R*	*MITF*	*PBRM1*	***RET [Exons 11,13-16]***	*TSC1*
BCOR	***CHEK2***	*FBXW7*	*IKBKE*	*MKNK1*	*PDCD1 (PD-1)*	*RICTOR*	*TSC2*
BCORL1	*CIC*	*FGF10*	*IKZF1*	*MLH1*	***PDCD1LG2 (PD-L2)***	*RNF43*	*TYRO3*
BRAF [Exons 11-18]	*CREBBP*	*FGF12*	*INPP4B*	***MPL [Exon 10]***	***PDGFRA [Exons 12,18]***	***ROS1 [Exons 31,36-38,40]***	*U2AF1*
BRCA1 *{Introns 2, 7, 8, 12, 16, 19, 20}*	***CRKL***	*FGF14*	*IRF2*	*MRE11A*	***PDGFRB [Exons 12-21,23]***	*RPTOR*	***VEGFA***
BRCA2 *{Intron 2}*	*CSF1R*	*FGF19*	*IRF4*	*MSH2*	*PDK1*	*SDHA*	*VHL*
BRD4	*CSF3R*	*FGF23*	*IRS2*	*MSH3*	*PIK3C2B*	*SDHB*	*WHSC1*
BRIP1	*CTCF*	*FGF3*	*JAK1*	*MSH6*	*PIK3C2G*	*SDHC*	*WT1*
BTG1	*CTNNA1*	*FGF4*	***JAK2 [Exons 14]***	*MST1R*	***PIK3CA [Exons 2,3,5-8,10,14,19,21] (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)***	*SDHD*	*XPO1*
BTG2	***CTNNB1 [Exon 3]***	*FGF6*	***JAK3 [Exons 5,11,12,13,15,16]***	*MTAP*	*PIK3CB*	*SETD2*	*XRCC2*
BTK [Exons 2,15]	*CUL3*	***FGFR1***	*JUN*	***MTOR [Exons 19,30,39,40, 43-45,47,48,53, 56]***	*PIK3R1*	*SF3B1*	*ZNF217*
C11orf30 (EMSY)	*CUL4A*	***FGFR2***	*KDM5A*	*MUTYH*	*PIM1*	*SGK1*	*ZNF703*
C17orf39 (GID4)	*CXCR4*	***FGFR3 [Exons 7, 9 (alternative designation exon 10),14, 18]***	*KDM5C*	***MYC***	*PMS2*	*SMAD2*	

Table 3 and **Table 4** describe the criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function rendering the sample *BRCA+*.

Table 3: Classification Criteria for Deleterious Tumor *BRCA* Variants

Qualification Criteria	Sequence Classification	Methodology
A *BRCA1/2* alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons anywhere in the gene coding region, except: 3' of and including *BRCA2* K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions -/+ 2bp of exon starts/ends
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements
	Deleterious missense mutations	Curated list (**Table 4**)

Table 4: Deleterious *BRCA* Missense Alterations

***BRCA1* Alterations (Protein Change)**				***BRCA2* Alterations (Protein Change)**	
M1V	C61G	D1692H	G1788V	M1V	R2659T
M1T	C61Y	D1692Y	P1812A	M1T	R2659K
M1R	C64R	R1699W	A1823T	M1R	E2663V
M1I	C64G	R1699Q	V1833M	M1I	S2670L
M18T	C64Y	G1706R	W1837R	D23N	I2675V
L22S	C64W	G1706E	V1838E	D23Y	T2722K
I26N	R71G	A1708E		S142N	T2722R
T37K	R71K	S1715R		S142I	D2723H
C39R	R71T	S1722F		V159M	D2723G
C39G	R71M	V1736A		V211I	G2724W
C39Y	S770L	G1738R		V211L	G2748D
C39W	R1495T	G1738E		Y600C	A2911E
H41R	R1495M	K1759N		K1530N	E3002K
C44S	R1495K	L1764P		R2336P	R3052W
C44Y	E1559K	I1766N		R2336L	D3095G
C44F	E1559Q	I1766S		R2336H	D3095E
C47S	T1685A	G1770V		T2412I	N3124I
C47Y	T1685I	M1775K		R2602T	N3187K
C47F	D1692N	M1775R		W2626C	
C61S	M1689R	C1787S		I2627F	

The output of the test includes:

Category 1: Companion Diagnostic (CDx) claims noted in Table 1 of the Intended Use

Category 2: cfDNA Biomarkers with Strong Evidence of Clinical Significance in cfDNA

Category 3: Biomarkers with Evidence of Clinical Significance in tissue supported by:
3A: strong analytical validation using cfDNA
3B: analytical validation using cfDNA

Category 4: Other Biomarkers with Potential Clinical Significance

FoundationOne Liquid CDx cfDNA Blood Specimen Collection Kit Contents

Test Kit Contents
The test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen preparation and shipping instructions
- Two FoundationOne Liquid CDx cfDNA blood collection tubes (8.5 mL nominal fill volume per tube)
- Return shipping label

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Foundation Medicine laboratory. The FoundationOne Liquid CDx assay is intended to be performed with serial number-controlled instruments.

FoundationOne Liquid CDx Sample Collection and Test Ordering
To order FoundationOne Liquid CDx, the test order form in the test kit must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to Specimen Preparation Instructions and Shipping Instructions included in the test kit.

For more detailed information, including Performance Characteristics, please find the FDA Summary of Safety and Effectiveness Data at: [Placeholder]

1. Instruments

The FoundationOne Liquid CDx device is intended to be performed with the following instruments, as identified by specific serial numbers:

Illumina NovaSeq 6000
Beckman Biomek NXP Span-8 Liquid Handler
Thermo Scientific Kingfisher Flex DW 96
Bravo Benchbot
Hamilton STARTlet-STAR Liquid Handling Workstation

2. Performance Characteristics

Performance characteristics were established using contrived and clinical circulating cfDNA derived from blood specimens extracted from a wide range of tumor types. **Table 5** below provides a summary of the number of tumor types and variants included in each study. As summarized in this table, each study included a broad range of representative alteration types (substitutions, insertion-deletions, copy number alterations, rearrangements) in various genomic contexts across a number of genes. The validation studies included >7,000 sample replicates, >31,000 unique variants [includes variants classified as variants of unknown significance (VUS) and/or benign], >30 tumor types, representing all 324 genes targeted by the assay.

Table 5. Representation of tumor types and variants across validation studies

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique				
				Targeted Genes	Subs	Indels	Rearrang.	Copy Number Losses
Contrived Sample Functional Characterization (CSFC) Study	Breast cancer Colorectal cancer Lung cancer Contrived samples	13	1843	228	563	81	11	1
FoundationOne Liquid CDx to Validated NGS Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	Prostate cancer Ovarian cancer	279	N/A	2	100	87	9	2
Orthogonal Concordance	23 cancer types Contrived samples	278	N/A	64	541	12	11	0
LoD Estimation	Prostate Contrived samples	10	877	286	1490	247	32	3
LoB	Healthy Donors	28	79	322	26134	4482	911	42
Potentially Interfering Substances	Contrived samples	9	336	18	16	11	11	2
Hybrid Capture Bait Specificity	25 cancer types Contrived samples	3546	N/A	324	N/A	N/A	N/A	N/A
Reagent Stability	Contrived samples	8	142	279	1090	215	32	2
Reagent Interchangeability	Contrived samples	8	192	20	15	11	11	1
Precision study 1	Breast cancer Colon cancer Lung cancer Ovarian cancer Prostate cancer Skin cancer Contrived samples	47	1121	280	900	229	63	5
Precision study 2	Lung cancer Prostate cancer Stomach cancer Colorectal cancer Bile duct cancer Breast cancer	10	230	6	6	4	0	0
DNA Extraction	Colorectal cancer Prostate cancer Breast cancer Lung cancer Skin cancer	6	72	161	265	53	2	0
Whole Blood Sample Stability	Lung cancer Colorectal cancer Prostate cancer Breast cancer	11	22	66	75	15	1	0
Inverted Tube Whole Blood Sample Stability	Lung cancer Colorectal cancer Breast cancer Ovarian cancer Prostate cancer	130	260	237	594	91	5	0
Cross Contamination	Contrived samples	5	376	39	9	5	4	1
Guard Banding	Contrived samples	10	375	20	17	12	12	1

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique				
				Targeted Genes	Subs	Indels	Rearrang.	Copy Number Losses
Clinical validation for detection of *EGFR* exon 19 deletions and L858R alterations: non-inferiority study	Lung cancer	177	N/A	1	5	7	N/A	N/A
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in prostate cancer	Prostate cancer	199	N/A	2	44	55	8	1
Blood Collection Tube Equivalence	Ovarian cancer Breast cancer Colorectal cancer Prostate cancer Lung cancer Skin cancer Stomach cancer	60	192	116	135	39	13	0
Automation Line Equivalence	Contrived samples	8	187	303	1926	337	63	4
Variant Report Curation	Breast cancer Colorectal cancer Lung cancer Prostate cancer Skin cancer	19	57	183	300	104	15	2
Pan-tumor performance (includes historical analysis)	20 cancer types	19868	N/A	N/A	N/A	N/A	N/A	N/A
Molecular Index Barcode Performance	25 cancer types Contrived samples	7637	N/A	324	N/A	N/A	N/A	N/A
FoundationOne Liquid LDT to FoundationOne Liquid CDx Concordance	25 cancer types	927	N/A	73	1815	376	109	N/A

* Variants detected include variants classified as VUS and benign.

2.1 Concordance – Comparison to an Orthogonal cfDNA NGS Method #1

The detection of short variants and rearrangements by the FoundationOne Liquid CDx assay was compared to that of an externally validated NGS assay in 74 genes common to both assays across 278 samples that represented an array of tumor types (>50 unique disease ontologies across 23 cancer types, **Table 9**). The cancer types (# samples) included lung [NSCLC (75) and other (3)]; breast (54); prostate (32); colorectal [colon (27) and rectal (6)]; liver (11); ovarian (6); pancreas (9); gastrointestinal (7); bile duct (2); esophageal (5); skin (6); cervical (1); anal (1); bladder (1); gallbladder (1); salivary gland (2); thymus (1); thyroid (3); uterine (2); fallopian tube (1); head and neck (1); soft tissue (1); and unknown primary (19). The study included samples selected from clinical FoundationOne Liquid testing (n=268) and contrived samples consisting of fragmented gDNA diluted in clinical cfDNA to represent rare alterations (n=10).

Using the externally validated NGS assay as the comparator, the analysis demonstrated a short variant PPA of 96.2% with a 95% two-sided CI of [94.8%-97.4%]. The short variant NPA was >99.9% with a 95% two-sided CI of [99.9%-100.0%]. The respective PPA of base substitutions and indels with a 95% two-sided CI was 96.1% [94.6%-97.3%] and 100.0% [85.2%-100.0%]. The respective NPA and 95% two-sided CI of base substitutions and indels was >99.9% [99.9%-100.0%] and 100.0% [99.89%-100.0%] (**Table 6**).

Table 6. Concordance of short variants called in FoundationOne Liquid CDx and the comparator assay (n= 902 positive variants, n= 152,832 negative variants* by the comparator assay)

Variant Type	FoundationOne Liquid CDx(+) Comparator(+)	FoundationOne Liquid CDx(-) Comparator(+)	FoundationOne Liquid CDx(+) Comparator(-)	FoundationOne Liquid CDx(-) Comparator(-)	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	868	34	8	152824	96.2% [94.8%-97.4%]	>99.9% [99.9%-100.0%]	>99.9% [99.9%-100.0%]
Base Substitutions	845	34	8	149511	96.2% [94.6%-97.3%]	>99.9% [99.9%-100.0%]	>99.9% [99.9%-100.0%]
Indels	23	0	0	3361	100.0% [85.2%- 100.0%]	100.0% [99.9%- 100.0%]	100.0% [99.9%- 100.0%]

* Variants detected include variants classified as VUS and benign.

For the concordance of rearrangement detection between FoundationOne Liquid CDx and the comparator assay, the observed rearrangement PPA was 100.0%, with a 95% two-sided CI of [59.0%-100.0%]. The NPA was 99.8%, with a 95% two-sided CI [99.5%-100.0%] (**Table 7**).

Table 7. Concordance of rearrangements called in FoundationOne Liquid CDx and the comparator assay (n= 7 positive, n=1685 negative* as determined by the comparator assay)

	Comparator (+)	Comparator (-)	Total
FoundationOne Liquid CDx (+)	7	3	10
FoundationOne Liquid CDx (-)	0	1682	1682
Total	7	1685	1692
	PPA: 100.0% [59.0% - 100.0%]	**NPA: 99.8% [99.5% - 100.0%]**	**OPA: 99.8% [99.5% - 100.0%]**

* Variants detected include variants classified as VUS and benign.

Assessment of a subset of highly-actionable alterations were compared between the two assays. The analysis resulted in a PPA of 100% across all eligible highly-actionable alterations called in the comparator assay (**Table** 8).

Table 8. Concordance of highly actionable alterations called between FoundationOne Liquid CDx and the comparator assay (n = 73)

Targeted Alteration	*n*	PPA [95% CI]	NPA [95% CI]
BRCA1 short variants	1	100% [2.5%-100.0%]	100% [98.7%-100.0%]
BRCA2 short variants	2	100% [15.8%-100.0%]	100% [99.3%-100.0%]
EGFR exon 19 deletions	11	100% [71.5%-100.0%]	100% [99.7%-100.0%]
EGFR L858R	10	100% [69.2%-100.0%]	100% [98.7%-100.0%]

These data demonstrate that the FoundationOne Liquid CDx assay and an externally-validated NGS assay are highly concordant across the 74 genes common between the two panels.

2.2 Concordance – FoundationeOne Liquid CDx to validated NGS tumor tissue assay (*BRCA1* and *BRCA2* alterations)

Samples from a total of 279 prostate and ovarian cancer patients were tested and the concordance evaluated between FoundationOne Liquid CDx and the validated NGS tumor tissue assay for the detection of deleterious alterations in *BRCA1* or *BRCA2*. As summarized below, a PPA of 88.03% and an NPA of 95.68% were observed on a sample level (**Table 9**). As summarized in **Table 10**, an overall PPA of 87.28% and an NPA of 99.83% were observed at the variant level. Some discordance is expected based on biological differences and sampling times between tumor tissue and plasma samples. Considering the impact of biological differences between analytes, these data demonstrate a high concordance between FoundationOne Liquid CDx and FoundationOne for the detection of deleterious alterations in *BRCA1* or *BRCA2*.

Table 9. Concordance (by sample) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in *BRCA1* or *BRCA2*

		NGS Tumor Tissue Assay	
		Positive	**Negative**
FoundationOne Liquid CDx	**Positive**	103	7
	Negative	14	155
		PPA: 88.03% [80.91%-92.74%]	**NPA:** 95.68% [91.35%-97.89%]

Table 10. Concordance (by variant) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in *BRCA1* or *BRCA2*

	F1LCDx+ /Tissue+	F1L CDx- /Tissue+	F1L CDx+ /Tissue-	F1L CDx-/ Tissue-	PPA (%) CI_1	NPA (%) CI_1
Substitutions	77	6	29	20255	92.77 (85.11, 96.64)	99.86 (99.79, 99.90)
Indels	65	3	31	16362	95.59 (87.81, 98.49)	99.81 (99.73, 99.87)
Rearrangements	4	3	7	1939	57.14 (25.05, 84.18)	99.64 (99.26, 99.83)
Copy number loss	5	10	1	263	33.33 (15.18, 58.29)	99.62 (97.89, 99.93)
Total	151	22	68	38819	87.28 (81.50, 91.45)	99.83 (99.78, 99.86)

2.3 Limit of Detection (Analytical Sensitivity)

The LoD for each variant type was established by processing a total of 1,069 sample replicates across ten contrived (enzymatically fragmented cell-line gDNA) samples representing short variants, rearrangements, and copy number alterations (losses). The LoD was determined using the conservative hit rate approach for the majority of variants. A probit model was used when appropriate (when ≥3 dilution levels with hit rates between 10% and 90% were observed). LoD by hit rate was defined as the mean VAF value (for short variants and rearrangements) or mean tumor fraction value (for copy number alterations) at the lowest dilution level tested with at least 95% detection across replicates. The hit rate was computed as the number of replicates with positive variant calls per the total number of replicates tested at each level of the targeted VAF (short variants and rearrangements) or tumor fraction (copy number alterations). Short variants with hit rates of at least 95% at all dilution levels or hit rates below 95% for all dilution levels were excluded from analysis as LoD could not be reliably estimated.

The median estimated LoD for CDx alterations are presented in **Table 11** The median LoD for targeted short variant, rearrangement, and copy number alterations were consistent with the platform LoD (**Table 12**).

Table 11: LoD estimation for CDx alterations

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD
BRCA1	Indels	1	0.38% VAF*
	Substitutions	8	0.34% VAF
	Rearrangement[1]	1	0.87% VAF
BRCA2	Substitutions	17	0.37% VAF
	Indels	2	0.36% VAF
	BRCA2- EDA Truncation[1]	1	0.48% VAF
	Copy Number Loss[1]	1	48.1% TF
EGFR	Indels (exon 19 deletions)	2	0.27% VAF
	Substitutions (L858R substitutions)	2	0.34% VAF

The estimated LoDs for *BRCA1* and *BRCA2* subs and indels were confirmed at values higher than the LoDs established in Table 15 (see Precision: Reproducibility and Reproducibility section below, Tables 19 and 20 for confirmed LoD values).
*The accuracy of %VAF/%TF have not been analytically validated.
[1]The LoD for these alterations was determined using clinical specimens.

The platform LoD for short variants, rearrangements, and copy number losses are presented in **Table 12**. A total of 864 short variants were included in the platform LoD analysis. The enhanced sensitivity region of the bait set contains 269 of the short variants analyzed and the standard sensitivity region of the bait set contains 595 of the short variants analyzed. The estimated LoD for short variants is 0.40% for the enhanced sensitivity region and 0.82% of the standard sensitivity region. The median LoD is 30.4% tumor fraction for copy number losses.

Because a major component driving the detectability of a variant is genomic context (repetitiveness of the reference genomic region), the LoD analysis for short variants was also evaluated within categories based on genomic context as summarized in **Table** 13.

Table 12: LoD estimation by variant type

Alteration Type	Number of Variants in Analysis	Bait Set Region	Median LoD	Quartile 1 to Quartile 3 LoD Range
Short Variants	269	Enhanced Sensitivity	0.40% VAF	0.33% - 0.50% VAF
	595	Standard Sensitivity	0.82% VAF	0.70% - 0.98% VAF
Rearrangements	7	Enhanced Sensitivity	0.37% VAF	0.26% - 0.47% VAF
	1	Standard Sensitivity	0.90% VAF	NA
Copy Number Losses	2	NA	30.4% TF	NA

VAF = variant allele frequency
TF = tumor fraction
*The accuracy of %VAF/%TF have not been analytically validated

Table 13: LoD by variant subtype based on genomic context

Region	Alteration Subtype	N	Minimum LoD (VAF/TF)[1]	1st Quantile LoD (VAF/TF)[1]	Median LoD (VAF/TF)[1]	3rd Quantile LoD (VAF/TF)[1]
Enhanced Sensitivity Region	Short Variants: Enhanced Sensitivity Region Total	269	0.20%	0.33%	0.40%	0.50%
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	10	0.23%	0.29%	0.31%	0.36%
	Insertion/Deletion in a repetitive region of 4 to 6 base pairs	23	0.28%	0.37%	0.48%	0.56%
	Insertion/Deletion in a repetitive region of >=7 base pairs	6	0.33%	0.48%	0.58%	0.82%
	Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	229	0.20%	0.33%	0.39%	0.49%
	Substitution in a repetitive region of >7 base pairs	1	0.32%	0.32%	0.32%	0.32%
Standard Sensitivity Region	Short Variants: High Sensitivity Region Total	595	0.40%	0.70%	0.82%	0.98%
	Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	18	0.46%	0.68%	0.87%	1.00%
	Insertion/Deletion in a repetitive region of 4 to 6 base pairs	32	0.61%	0.75%	0.87%	0.95%
	Insertion/Deletion in a repetitive region of >=7 base pairs	11	0.59%	1.07%	1.15%	1.20%
	Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	524	0.40%	0.70%	0.81%	0.96%
	Substitution in a repetitive region of >7 base pairs	8	0.69%	0.83%	0.96%	1.28%
Enhanced Sensitivity Region	Rearrangements	7	0.20%	0.26%	0.37%	0.47%
Enhanced/ Standard Sensitivity Region	Rearrangements	1	0.28%	0.28%	0.28%	0.28%
Standard Sensitivity Region	Rearrangements	1	0.90%	0.90%	0.90%	0.90%
NA	Copy Number Losses	1	12.70%	12.70%	12.70%	12.70%

[1]VAF reported for short variant and rearrangement LoD, tumor fraction reported for copy number loss LoD. The accuracy of %VAF/%TF have not been analytically validated

The median LoD for highly-actionable, non-CDx alterations evaluated for LoD are presented in **Table 14.** The median LoD for these targeted short variants are consistent with the platform LoD presented in **Table** 12.

Table 14: LoD for non-CDx alterations

Gene	Alteration Subtype	Number of Samples Evaluated	Median LoD*
ATM	Indels	1	0.51% VAF
BRAF	Substitutions	1	0.33% VAF
KRAS	Substitutions	2	0.33% VAF
MET	Indels	1	0.41% VAF
NRAS	Substitutions	2	0.42% VAF
PALB2	Indels	1	0.37% VAF
	Substitutions	1	0.51% VAF
PIK3CA	Substitutions	6	0.34% VAF

VAF = variant allele frequency
*The accuracy of %VAF have not been analytically validated.

2.4 Limit of Blank (LoB)

Per CLSI EP17-A2, the limit of blank (LoB) was established by profiling plasma samples from 30 asymptomatic donors with no diagnosis of cancer with 4 replicates per sample. All donors were over the age of 60 with a median age of 68 and included 15 smokers and 15 non-smokers.

As would be expected in a sampling of human plasma, especially plasma from an aged population, a small number of alterations were detected. Across 30,622 short variants, which include variants classified as VUS/benign, five variants of unknown significance had a detection rate significantly exceeding 5% on an individual variant basis: *TSC1* 965T>C, *IRF4* 1ins87, *MSH3* 186_187insGCCGCAGCGCCCGCAGCG, *IGF1R* 568C>T, WHSC1 1582C>A.

All other variants were determined to have an LoB of 0, based on the detection rate not significantly exceeding 5%. Each cancer-related alteration detected in this study was detected in replicates from a single donor, indicating that these are likely true variants present in the sample. On a per variant basis (number of unique variants detected at least once across all replicates divided by the total number of unique variants included in the analysis), the overall detection rate for short variants in this study was 0.82%.

Across 264 copy number alterations and 894 rearrangements, zero variants were detected. These results demonstrate the high specificity of FoundationOne Liquid CDx.

2.5 Potentially Interfering Substances

To evaluate the robustness of the FoundationOne Liquid CDx results in the presence of potentially interfering exogenous and endogenous substances, a total of 11 potential interferents were evaluated. These potential interferents included six endogenous substances (albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin and triglycerides) and five exogenous substances (DNA from another source [the microorganism *Staphylococcus epidermidis*], excess anticoagulant, proteinase K, ethanol and molecular index barcodes).

A total of 340 samples were tested to evaluate the potential interference of albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin, triglycerides, DNA from another source (the microorganism *Staphylococcus epidermidis*), excess anticoagulant, proteinase K, ethanol, and molecular index barcodes. An

assessment of the cfDNA yield obtained during the DNA isolation, purification, and quantification steps, as well as at library construction QC (LCQC) and hybrid capture QC (HCQC) was performed. The process success rates for each step are listed in
Table 15.

Table 15: Process success rates with interfering substances

Process	# Failed	# Pass	Total	Success Rate (%)	95% CI LB (%)	95% CI UB (%)
DNA Extraction	0	180	180	100.00	97.97	100.00
LC	1	339	340	99.71	98.37	99.99
HC	3	336	339	99.12	97.44	99.82
Sequencing	0	336	336	100.00	98.91	100.00

For each potential interferent, concordance of alteration calls was calculated relative to a control sample without interferent. The pre-defined variants included 27 short variants, 17 rearrangements, and 3 copy number variants. Of the 11 potential interferents tested across 16 conditions, concordance for all variant calls was 100% for 8 conditions and ≥97% for all conditions (**Table 16)**.

Table 16: Concordance per substance for variants ≥1x LoD

Substance	Concordance	95% CI LB	95% CI UB	N
Triglycerides, 37 mmol/L (or 33 g/L)	100.00%	91.00%	100.00%	40
Hemoglobin, 2.0 g/L	100.00%	91.00%	100.00%	39
Albumin, 60 g/L	97.56%	87.00%	100.00%	41
Bilirubin (conjugated), 0.2 g/L	100.00%	92.00%	100.00%	42
Bilirubin (unconjugated), 0.2 g/L	97.44%	87.00%	100.00%	39
Cholesterol Level 2, 3.88 mmol (150 mg/dL)	97.56%	87.00%	100.00%	41
Cholesterol Level 1, 6.47mmol (250 mg/dL)	97.37%	86.00%	100.00%	38
Staphylococcus epidermidis, 1 x 106 CFU/mL	100.00%	91.00%	100.00%	39
Anticoagulant, 5X nominal volume	100.00%	91.00%	100.00%	41
Proteinase K, +0.6 mg/mL	98.00%	89.00%	100.00%	50
Proteinase K, +0.3 mg/mL	100.00%	92.00%	100.00%	46
Ethanol, +2.5%	97.96%	89.00%	100.00%	49
Ethanol, +5.0%	97.92%	89.00%	100.00%	48
Molecular Index barcodes, +5%	97.22%	85.00%	100.00%	36
Molecular Index barcodes, +15%	100.00%	93.00%	100.00%	48
Molecular Index barcodes, +30%	100.00%	93.00%	100.00%	49

Taken together, these data indicate that the FoundationOne Liquid CDx assay is robust to potential specimen-related endogenous substances and exogenous contaminants or interferents.

2.6 Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage of targeted regions in FoundationOne Liquid CDx using 3,546 validation study samples. Results show that targeted genomic regions have consistently high, uniform coverage. For each genomic region associated with a predefined subset of highly-actionable alterations, between 94% to 100% of samples possessed the expected level of coverage. An in-depth, platform-wide examination of the FoundationOne Liquid CDx baitset through the analysis of HapMap process control samples

revealed that, on average, 98.8% and 94.1% of platform-wide baited coding and non-coding regions, respectively, met their expected coverage levels. Samples assessed in this study consistently demonstrated high quality uniform and deep coverage across the entire genomic region targeted by the assay.

2.7 Carryover/Cross-Contamination

The study demonstrated that the risk of cross contamination (intra-plate), and carry-over contamination (inter-plate) of samples during the processing of the FoundationOne Liquid CDx assay is low. A total of 376 wells were examined for intra- and inter-plate contamination by processing and sequencing of contrived samples derived from cell lines at high input concentrations with known genomic backgrounds. Unique variants of each cell line were characterized by independent control sequencing runs. The samples were arrayed in a checkerboard fashion across four 96-well PCR plates to detect cross-contamination events. A cross-contamination rate of 0.53% (2/376) was observed in this study. These data demonstrate a low probability of cross contamination during the FoundationOne Liquid CDx process.

2.8 Precision: Repeatability and Reproducibility

Precision was evaluated for alterations associated with CDx claims, as well as tumor mutation profiling variants. Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three reagent lots, two sequencers, and two processing runs.

2.8.1 Results for a subset of highly-actionable alterations

A set of 39 unique samples were used to evaluate the precision of FoundationOne Liquid CDx for detecting a set of highly-actionable variants, including 8 contrived samples representing various targeted alterations and 31 clinical samples. The samples representing CDx alterations are summarized in **Table 17**. Additional non-CDx variants were evaluated as summarized in **Table 18.**

Table 17: CDx sample set assessed for precision

Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
BRCA1, BRCA2 alterations	6 contrived samples
EGFR exon 19 deletions and *EGFR* exon 21 L858R alterations	5 contrived samples
*BRCA1 E23fs*17*	Ovary cancer
BRCA1 Q780*	Ovary high grade serous carcinoma
BRCA1 Rearrangement	Unknown primary malignant neoplasm
BRCA2 G267*	Ovary serous carcinoma
BRCA2 Loss (15 of 26)	Prostate acinar adenocarcinoma
BRCA2 Loss *(26 of 26)*	Prostate acinar adenocarcinoma
BRCA2 S2988fs*12	Ovary cancer
BRCA2- EDA Truncation	Prostate cancer
EGFR E746_A750del	Non-small cell lung carcinoma
EGFR L858R	Non-small cell lung carcinoma
EGFR L858R	Non-small cell lung carcinoma

Table 18: Non-CDx sample set assessed for precision

Targeted Alteration	Number of Contrived Samples
ATM alterations	1 contrived samples
BRAF substitution	2 contrived samples
EGFR exon 20 T790M substitution	2 contrived samples
KRAS codon 12, 13 substitutions	3 contrived samples
METexon14 skipping	2 contrived samples

Targeted Alteration	Number of Contrived Samples
NRAS exon 2,3,4 substitutions	2 contrived samples
PALB2 alterations	2 contrived samples
PIK3CA alterations	4 contrived samples
BRAF V600E	Skin melanoma
BRAF V600K	Skin melanoma
KRAS G12L	Colon adenocarcinoma
KRAS Q61R	Colon adenocarcinoma
MET exon 14 splice site 2888-17_2888-3del15	Non-small cell lung carcinoma
MET exon 14 splice site 3005_3028+3>C	Non-small cell lung carcinoma
PIK3CA E542K	Breast carcinoma
PIK3CA E545K	Breast carcinoma
PIK3CA H1047R	Breast cancer

Target alterations were assessed at two target levels each (near LoD and 2-3x LoD) for the contrived samples, and at one target level (1-1.5x LoD) for clinical cfDNA samples. Each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Across 47 samples (31 clinical specimens at one dilution level and 8 contrived samples across two dilution levels), a total of 57 unique alterations were evaluated. The repeatability of CDx alterations is summarized in **Table** 19 and reproducibility of CDx alterations is summarized in
Table 20.

Table 19: Repeatability of CDx alterations targeted in precision study at >1x LoD*

Variant Type	Alteration	Concordant Pairs	Repeatability (%)	95% CIs (%)	Level Tested**
Short variant	*BRCA1*_2338C>T	12/12	100	(73.54, 100)	1.11% VAF
Short variant	*BRCA1*_2475delC	12/12	100	(73.54, 100)	0.61% VAF
Short variant	*BRCA1*_2475delC	12/12	100	(73.54, 100)	0.93% VAF
Short variant	*BRCA1*_2612C>TT	11/11	100	(71.51, 100)	0.51% VAF
Short variant	*BRCA1*_68_69delAG	12/12	100	(73.54, 100)	0.66% VAF
Short variant	*BRCA1*_P871fs*32	12/12	100	(73.54, 100)	1.08% VAF
Rearrangement	*BRCA1-BRCA1*	12/12	100	(73.54, 100)	0.87% VAF
Short variant	*BRCA2*_3599_3600delGT	12/12	100	(73.54, 100)	0.58% VAF
Short variant	*BRCA2*_3599_3600delGT	12/12	100	(73.54, 100)	0.92% VAF
Short variant	*BRCA2*_4284_4285insT	12/12	100	(73.54, 100)	0.94% VAF
Short variant	*BRCA2*_4284_4285insT	11/11	100	(71.51, 100)	1.26% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	1.22% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	1.85% VAF
Short variant	*BRCA2*_5351delA	11/11	100	(71.51, 100)	1.07% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	2.24% VAF
Short variant	*BRCA2*_5465_5466insA	12/12	100	(73.54, 100)	0.92% VAF
Short variant	*BRCA2*_5465_5466insA	11/11	100	(71.51, 100)	1.19% VAF
Short variant	*BRCA2*_8961_8964delGAGT	12/12	100	(73.54, 100)	1.07% VAF
Short variant	*BRCA2*_c.799G>T	10/12	83.33	(51.59, 97.91)	0.5% VAF
Short variant	*BRCA2*_c.9097_9098insA	6/11	54.55	(23.38, 83.25)	0.71% VAF
Short variant	*BRCA2*_c.9097_9098insA	10/12	83.33	(51.59, 97.91)	1.03% VAF
Copy Number Loss	*BRCA2*_loss	11/12	91.67	(61.52, 99.79)	39.43% TF
Rearrangement	*BRCA2*-EDA	11/11	100	(71.51, 100)	0.48% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.44% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.66% VAF
Short variant	*EGFR*_2369C>T	11/11	100	(71.51, 100)	0.36% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.65% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	1.26% VAF

Variant Type	Alteration	Concordant Pairs	Repeatability (%)	95% CIs (%)	Level Tested**
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.46% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.68% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.68% VAF
Short variant	*EGFR*_2573T>G	11/11	100	(71.51, 100)	0.95% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.64% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	1.64% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.51% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.74% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.93% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	1.2% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	0.51% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	1.01% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	0.34% VAF

*Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD
**The accuracy of %VAF/%TF have not been analytically validated.

Table 20: Reproducibility of CDx alterations targeted in precision study at >1x LoD*

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	VAF/TF Level Tested
Short variant	*BRCA1*_2338C>T	24/24	100	(85.75, 100)	1.11%
Short variant	*BRCA1*_2475delC	24/24	100	(85.75, 100)	0.61%
Short variant	*BRCA1*_2475delC	24/24	100	(85.75, 100)	0.93%
Short variant	*BRCA1*_2612C>TT	23/23	100	(85.18, 100)	0.51%
Short variant	*BRCA1*_68_69delAG	24/24	100	(85.75, 100)	0.66%
Short variant	*BRCA1*_P871fs*32	24/24	100	(85.75, 100)	1.08%
Rearrangement	*BRCA1-BRCA1*	24/24	100	(85.75, 100)	0.87%
Short variant	*BRCA2*_3599_3600delGT	24/24	100	(85.75, 100)	0.58%
Short variant	*BRCA2*_3599_3600delGT	24/24	100	(85.75, 100)	0.92%
Short variant	*BRCA2*_4284_4285insT	24/24	100	(85.75, 100)	0.94%
Short variant	*BRCA2*_4284_4285insT	23/23	100	(85.18, 100)	1.26%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	1.22%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	1.85%
Short variant	*BRCA2*_5351delA	23/23	100	(85.18, 100)	1.07%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	2.24%
Short variant	*BRCA2*_5465_5466insA	24/24	100	(85.75, 100)	0.92%
Short variant	*BRCA2*_5465_5466insA	23/23	100	(85.18, 100)	1.19%
Short variant	*BRCA2*_799G>T	22/24	91.67	(73.0, 98.97)	0.5%
Short variant	*BRCA2*_8961_8964delGAGT	24/24	100	(85.75, 100)	1.07%
Short variant	*BRCA2*_9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%
Short variant	*BRCA2*_c.799G>T	22/24	91.67	(73.0, 98.97)	0.5%
Short variant	*BRCA2*_c.9097_9098insA	5/23	21.74	(7.46, 43.7)	0.71%
Short variant	*BRCA2*_c.9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%
Copy Number Loss	*BRCA2* loss	21/24	87.5	(67.64, 97.34)	39.43% TF
Rearrangement	*BRCA2*-EDA	23/23	100	(85.18, 100)	0.48%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.44%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.66%
Short variant	*EGFR*_2369C>T	23/23	100	(85.18, 100)	0.36%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.65%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	1.26%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.46%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.68%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.68%
Short variant	*EGFR*_2573T>G	23/23	100	(85.18, 100)	0.95%

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	VAF/TF Level Tested
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.64%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	1.64%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.51%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.74%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.93%
Short variant	*EGFR*_E746_A750del	23/23	100	(85.18, 100)	1.2%
Short variant	*EGFR*_E746_A750del	23/23	100	(85.18, 100)	0.51%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	1.01%
Short variant	*EGFR*_E746_A750del	22/22	100	(84.56, 100)	0.34%

*Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD
**The accuracy of %VAF/%TF have not been analytically validated.

As observed in the **Table 19** and **Table 20** above, three BRCA2 positive samples (c.799G>T, c.9097_9098insA, and a *BRCA2* loss) demonstrated poor performance for both repeatability and reproducibility. For the *BRCA2* specimen harboring the c.799G>T, the average %VAF was determined to be 0.5%, near the LoD of 0.4% for this variant type. The *BRCA2* c.9097_9098insA variant is an insertion of an A in a highly repetitive homopolymer region of eight As, which impacts sensitivity. In the LoD study, a 93% hit rate was observed at the highest level tested, 1.16% VAF, indicating that the levels evaluated in this precision analysis were below the LoD for this variant. The replicates for the clinical sample harboring the BRCA2 loss were processed at below the minimum cfDNA input.

Of 53 targeted alterations, repeatability of 100% was observed for 43 alterations and ≥90% repeatability was observed for 53 alterations. For the targeted variants assessed, the overall repeatability was 96.39% (95% two-sided exact CIs [95.28%, 97.30%]).

Of 55 targeted alterations, reproducibility of 100% was observed for 42 alterations and ≥90% reproducibility was observed for 55 alterations. For the targeted variants assessed, the overall reproducibility was 97.33% (95% 2-sided exact CIs [96.67 %, 97.89%]). The reproducibility of non-CDx alterations tested at ≥1x LoD are summarized in **Table 21**.

Table 21: Reproducibility of non-CDx alterations targeted in precision study at ≥1x LoD

Variant Type	Alteration	Concordant Replicates	Repeatability (%)	95% two-sided exact CIs (%)	Level Tested*
Short variant	ATM_5318delA	24/24	100	(85.75, 100)	0.77% VAF
Short variant	ATM_5318delA	23/23	100	(85.18, 100)	1.04% VAF
Short variant	ATM_6034_6035insCAGAAGTA	23/23	100	(85.18, 100)	0.86% VAF
Short variant	ATM_8850+1G>A	24/24	100	(85.75, 100)	0.56% VAF
Short variant	BRAF_1790T>G	22/23	95.65	(78.05, 9.89)	0.42% VAF
Short variant	BRAF_1790T>G	24/24	100	(85.75, 100)	0.85% VAF
Short variant	BRAF_1798_1799GT>AA	23/24	95.83	(78.88, 9.89)	0.36% VAF
Short variant	BRAF_1799T>A	23/23	100	(85.18, 100)	0.72% VAF
Short variant	BRAF_1799T>A	24/24	100	(85.75, 100)	1.38% VAF
Short variant	BRAF_1799T>A	24/24	100	(85.75, 100)	0.44% VAF
Short variant	KRAS_182A>G	24/24	100	(85.75, 100)	0.53% VAF
Short variant	KRAS_34_35GG>CT	24/24	100	(85.75, 100)	0.49% VAF
Short variant	KRAS_35G>A	24/24	100	(85.75, 100)	0.89% VAF
Short variant	KRAS_35G>A	23/23	100	(85.18, 100)	1.12% VAF
Short variant	KRAS_38G>A	24/24	100	(85.75, 100)	0.55% VAF

Variant Type	Alteration	Concordant Replicates	Repeatability (%)	95% two-sided exact CIs (%)	Level Tested*
Short variant	KRAS_38G>A	24/24	100	(85.75, 100)	0.82% VAF
Short variant	KRAS_38G>A	23	100	(85.18, 100)	0.57% VAF
Short variant	KRAS_38G>A	24/24	100	(85.75, 100)	0.92% VAF
Short variant	MET_2888-17_2888-3del15	24/24	100	(85.75, 100)	1.17% VAF
Short variant	MET_3005_3028+3>C	24/24	100	(85.75, 100)	1.67% VAF
Short variant	MET_3029-1G>T	15/24	62.50	(40.59, 81.2)	0.21% VAF
Short variant	MET_3029-1G>T	21/23	91.30	(71.96, 8.93)	0.30% VAF
Short variant	MET_3933delC	24/24	100	(85.75, 100)	0.69% VAF
Short variant	MET_3933delC	24/24	100	(85.75, 100)	0.96% VAF
Short variant	NRAS_34G>T	24/24	100	(85.75, 100)	0.69% VAF
Short variant	NRAS_34G>T	24/24	100	(85.75, 100)	0.96% VAF
Short variant	NRAS_35G>A	24/24	100	(85.75, 100)	0.84% VAF
Short variant	*NRAS*_c.35G>A	19/23	82.61	(61.22, 95.05)	0.48% VAF
Short variant	PALB2_2422G>T	23/23	100	(85.18, 100)	0.47% VAF
Short variant	PALB2_2422G>T	24/24	100	(85.75, 100)	0.92% VAF
Short variant	PALB2_2724delA	24/24	100	(85.75, 100)	0.52% VAF
Short variant	PALB2_2724delA	24/24	100	(85.75, 100)	0.74% VAF
Short variant	*PIK3CA*_1624G>A	24/24	100	(85.75, 100)	0.89% VAF
Short variant	*PIK3CA*_1633G>A	24/24	100	(85.75, 100)	0.45% VAF
Short variant	*PIK3CA*_1633G>A	24/24	100	(85.75, 100)	0.66% VAF
Short variant	*PIK3CA*_1633G>A	24/24	100	(85.75, 100)	0.5% VAF
Short variant	*PIK3CA*_1634A>C	24/24	100	(85.75, 100)	0.52% VAF
Short variant	*PIK3CA*_1634A>C	23/23	100	(85.18, 100)	0.70% VAF
Short variant	*PIK3CA* _1637A>G	22/23	95.65	(78.05, 9.89)	0.49% VAF
Short variant	*PIK3CA*_1637A>G	24/24	100	(85.75, 100)	0.92% VAF
Short variant	*PIK3CA*_1645G>A	24/24	100	(85.75, 100)	0.48% VAF
Short variant	*PIK3CA*_1645G>A	24/24	100	(85.75, 100)	0.73% VAF
Short variant	*PIK3CA*_3140A>G	23/23	100	(85.18, 100)	0.41% VAF
Short variant	*PIK3CA*_3140A>G	24/24	100	(85.75, 100)	0.76% VAF
Short variant	*PIK3CA*_3140A>G	24/24	100	(85.75, 100)	1.04% VAF

*The accuracy of %VAF/%TF have not been analytically validated.

2.8.2 Precision of Platform Variants

Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,126 variants were evaluated with variant types including substitutions and indels. The number of variants in each variant bin are summarized in **Table 22.**

Table 22: Number of each variant type

Variant Category	N
Substitutions	**898**
Substitution in a non-repetitive region or a repetitive region of <=7 base pairs	882
Substitution in a repetitive region of >7 base pairs	16
Indels	**228**

Variant Category	N
Insertion/Deletion in non-repetitive region or a repetitive region of <=3 base pairs	52
Insertion/Deletion in a repetitive region of 4 to 6 base pairs	118
Insertion/Deletion in a repetitive region of >=7 base pairs	58
Total	**1126**

The overall repeatability for all short variants was 99.51% with 95% 2-sided exact CIs (99.49%, 99.53%). The repeatability results for each variant type are summarized in **Table 23.**

Table 23: Assessment of repeatability of tumor mutation profiling variants per type

Variant Type	# of Concordant Pairs	# of Total Pairs	Repeatability (%)	95% two-sided exact CIs (%)
Substitution	498765	501084	99.54	(99.52, 99.56)
Indels	126475	127224	99.41	(99.37, 99.45)

The overall reproducibility results were 99.62% with the 95% 2-sided exact CIs (99.61%, 99.63%). The reproducibility result for each variant type are summarized in **Table 24.**

Table 24: Assessment of reproducibility of tumor mutation profiling variants per type

Variant Type	# of Concordant Replicates	# of Total Replicates	Reproducibility (%)	95% two-sided exact CIs (%)
Substitution	1002981	1006658	99.63	(99.62, 99.65)
Indels	254509	255588	99.58	(99.55, 99.60)

2.8.3 Confirmation of LoD and Precision in Clinical Specimens

Twenty-nine clinical cfDNA samples targeting variants at near the estimated LoD were evaluated to confirm LoD and precision in clinical specimens. The mean level tested in most cases were higher than the estimated LoD as shown in **Table 25** and **Table 26**. Twenty-six had 100% reproducibility, one had 95.8% reproducibility, and two samples had reproducibility below 90%. Of these two samples, one contained a *BRCA2* loss that had 87.5% reproducibility. This sample was processed with a cfDNA input mass below the recommended minimum and was also below LoD. The other sample harbored a *BRCA2* substitution (c.799G>T) with 91.67% reproducibility. The average VAF of this variant was 0.5% across replicates, which is near the LoD for this variant type (median LoD of 0.4% VAF). A summary of the Confirmation of LoD and Precision results for CDx variants are provided in **Table 25.** A summary of the Confirmation of LoD and Precision results for CDx variants are provided in **Table 26**.

Table 25: CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[2]	Reproducibility (%)	95% Two-sided exact CIs (%)
*BRCA1 E23fs*17*	0.38% VAF	0.66% VAF	100	(85.75, 100)
BRCA1 Q780*	0.34% VAF	1.11%VAF	100	(85.75, 100)
BRCA1 Rearrangement	0.87% VAF[1]	0.87% VAF	100	(85.75, 100)
BRCA2 799G>T	0.40% VAF	0.50% VAF	91.67	(73.0, 98.97)
BRCA2 Loss	48.1% TF	39.43%TF	87.50	(67.64, 97.34)
BRCA2 S2988fs*12	0.36% VAF	1.07% VAF	100	(85.75, 100)
BRCA2- EDA Truncation	0.48% VAF[1]	0.48% VAF	100	(85.18, 100)
EGFR E746_A750del	0.27% VAF	0.34% VAF	100	(84.56, 100)

Target Alteration	LoD	Mean Level Tested[2]	Reproducibility (%)	95% Two-sided exact CIs (%)
EGFR L858R	0.34% VAF	1.64% VAF	100	(85.75, 100)
EGFR L858R	0.34% VAF	0.64% VAF	100	(85.75, 100)

1 LoD determined in this confirmation of LoD and precision study
2 The accuracy of %VAF/%TF have not been analytically validated.

Table 26: Non-CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (%)	95% Two-sided exact CIs (%)
*ATM I2012fs*4*	0.51% VAF	0.86% VAF	100	(85.18, 100)
ATM splice site 8850+1G>A	0.51% VAF	0.56% VAF	100	(85.75, 100)
BRAF V600E	0.33% VAF	0.44% VAF	100	(85.75, 100)
BRAF V600K	0.33% VAF	0.36% VAF	95.8	(78.88, 99.89)
EGFR T790M	0.34% VAF	1.26% VAF	100	(85.75, 100)
KRAS G12L	0.33% VAF	0.49% VAF	100	(85.75, 100)
KRAS Q61R	0.33% VAF	0.53% VAF	100	(85.75, 100)
MET exon 14 splice site 2888-17_2888-3del15	0.41% VAF	1.17%	100	(85.75, 100)
MET exon 14splice site 3005_3028+3>C	0.41% VAF	1.67% VAF	100	(85.75, 100)
PIK3CA E542K	0.34% VAF	0.89% VAF	100	(85.75, 100)
PIK3CA E545K	0.34% VAF	0.5% VAF	100	(85.75, 100)
PIK3CA H1047R	0.34% VAF	1.04% VAF	100	(85.75, 100)

1 The accuracy of %VAF/%TF have not been analytically validated.

A second study with 10 samples targeting variants at 1-1.5x LoD was performed to confirm LoD and precision in clinical specimens. Similar to above, each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Each sample was tested across 24 replicates. Six samples were included in the primary analysis for samples with ≥30 ng DNA input. Three had 100% reproducibility, one had 95.7% reproducibility, one had 91.7% reproducibility, and one had 91.3% reproducibility. The other four samples had a majority of sample replicates with DNA input <30 ng. A summary of the Confirmation of LoD and Precision results for CDx alterations are provided in **Table 27**.

Table 27: CDx variant confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (95% CI)	95% CIs (%)
BRCA1 1395T>A	0.34%	0.51%	100%	[86.2%, 100%]
BRCA2 5351_5352insA	0.36%	0.34%	87.5%	[69.0%, 95.7%]
EGFR 2235_2249del	0.27%	0.45%	95.7%	[79.0%, 99.2%]

1 The accuracy of %VAF/%TF have not been analytically validated.

As summarized in Table 27 above, all CDx variants with ≥30 ng DNA input had reproducibility ≥95% with the exception of one variant (BRCA2 5351_5352insA) which was tested at a variant allele fraction below the LoD.

Additionally, one of the 10 samples evaluated in this study targeted a non-CDx *BRCA2* substitution. Reproducibility of 100% was observed as summarized in **Table 28**.

Table 28: Non-CDx *BCRCA2* variant confirmation of LoD and precision in a clinical specimen

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (95% CI)	95% CIs (%)
BRCA2 8524C>T	0.37%	0.57%	100%	[85.7%, 100%]

[1] The accuracy of %VAF/%TF have not been analytically validated.

2.9 Reagent Lot Interchangeability

The interchangeability of critical reagent lots for library construction (LC), hybrid capture (HC) and sequencing within the FoundationOne Liquid CDx assay was evaluated by testing eight (8) contrived samples from either enzymatically fragmented cell line genomic DNA containing alterations of interest or enzymatically fragmented plasmid DNA. Each of the contrived samples was tested in triplicate using two different lots each of LC, HC, and sequencing reagents. Eight reagent pairings were assessed. A total of eight analyses for each specimen were completed. 192 tests in total were included in this study. Four Master Pool Libraries (MPLs) were evaluated on each of two flowcells on a NovaSeq 6000 sequencer, using two different Sequencing reagent lots. Of the 49 alterations assessed in the sample set, 43 had a percent agreement greater than 90% (39 alterations had percentage agreement equal to 100%, one had percent agreement equal to 95.83%, one had percent agreement equal to 95.65%, and two had percent agreement equal to 91.67%), exceeding the pre-specified acceptance criteria. For the remaining six alterations the observed detection rates for these variants were similar to the predicted detection rate based on the LoD analysis. These results demonstrate the interchangeability of critical reagent lots in the FoundationOne Liquid CDx assay.

2.10 Variant Curator Precision

This study was performed to evaluate the precision of genomic variant call curation, following analysis by the FoundationOne Liquid CDx analysis pipeline. This was established by analyzing targeted alterations, including CDx alterations, and platform-wide alterations within samples used in the FoundationOne Liquid CDx Precision and LoD and Precision Confirmation Study. The study design reflected the intermediate precision design and evaluated curator precision in reporting of targeted and platform alterations. A total of 19 samples were selected for this study. Three curators were chosen randomly amongst all qualified curators to curate variant calls in a set of randomly chosen replicates from each of the 19 samples. The variant calls were generated from each sample per curator. The overall average percent agreement for targeted alterations was 93.3% (95% CI; 83.80%, 98.15%), and for platform genomic alterations was 99.14% (95% CI; 98.47%, 99.57%).

2.11 Stability

2.11.1 Reagent Stability

The reagent stability of FoundationOne Liquid CDx is assessed by analyzing data from each of eight samples in triplicate, per each of three different lots of LC, HC, and sequencing reagents. A total of nine analyses for each specimen are completed for each of six time points assessed. A total of 72 tests will be assessed per time period; a total of 432 samples and six time points will be included in this study overall. Each of the three sample Master Library Pools (MPLs), representing three LC and HC reagent lots will be evaluated per time point on a NovaSeq 6000 sequencer, using three different sequencing reagent lots. The analysis of baseline timepoint zero (T0) identified the baseline variant calls for each sample. Concordance of 12,511 variant alterations will be assessed across future time points for sample aliquots derived from eight DNA samples.

To date, timepoint the 3-month timepoint has been analyzed for reagent Lot #1, Lot #2, and Lot #3. Variants at the experimental time points are ≥90% concordant with the baseline variant call values as presented in **Table 29**. Current data demonstrate LC, HC, and sequencing reagent stability for up to 3 months. This study is ongoing and further evaluation will be performed to validate reagent stability over 12 months.

Table 29: Concordance analysis between 3 months and baseline

	Reagent Lot	Timepoint		Total # Replicates	Concordance Percentage	95% C.I.

			# of Replicates Concordant			Lower	Upper
Variant Calls	Lot #1	1	1921	1966	97.71%	96.95%	98.28%
	Lot #2	1	2083	2148	96.97%	96.16%	97.62%
	Lot #3	1	2086	2139	97.52%	96.77%	98.10%

2.11.2 Whole Blood Specimen Stability

Whole blood stability and the impact of tube inversion was evaluated in freshly collected whole blood samples from the following five cancer types: non-small cell lung cancer (NSCLC), colorectal cancer (CRC), prostate, breast, and ovarian cancer. The recommended storage temperature is 18°C - 25°C. In this study, stress conditions were simulated through extended storage at elevated (35°C ± 2°C) and reduced (4° ± 2°C) temperatures.

In this interim analysis, 22 samples (11 sample pairs) were tested, including baseline (within 24 hours of collection) and experimental time points (after 10, 14, or 15 days of storage).

Overall, 100% of samples yielded a cfDNA input ≥30ng. The success rate for DNAx yield, and LC yield were 100% and the success rate of the HC yield was 96.3%. The variant analysis was conducted for variants at ≥2x LoD. For the aggregate 11 pairs of samples processed and reported, 100% agreement was observed between the baseline and experimental timepoint for short variants and rearrangements for each experimental time point. The percent agreement per sample also resulted in 100% agreement between the baseline and experimental timepoint for short variants and rearrangements. The data is summarized in **Table 30.**

Table 30: Aggregate percent agreement per temperature and experimental timepoint

Temperature	Experimental Timepoint	N	Short Variants [95% two-sided CI]	Rearrangements
4°C	7 Days	4	100.00 [89.72, 100.00]	100.00 [39.76, 100.00]
	14 Days	3	100.00 [91.40, 100.00]	N/A
	15 Days	3	100.00 [83.89, 100.00]	N/A
35°C	14 Days	1	N/A	N/A

The impact of potential interferents originating from the FoundationOne Liquid cfDNA blood collection tube (BCT) stopper on the performance of the FoundationOne Liquid CDx assay was assessed by evaluating stability of whole blood in tubes stored in an upright or inverted position at 4°C± 2°C , 25°C± 2°C, and 35°C± 2°C for various durations (10, 14, and 15 days).

First, the success rate of the FoundationOne Liquid CDx assay for processing samples was assessed at the DNA extraction (DNAx), Library Construction (LC), Hybrid Capture (HC) and Sequencing step, based on product in-process quality control (QC) criteria. Samples stratified by the upright and the inverted condition exhibited comparable success rates above 94% at DNAx, LC, HC and Seq (**Table 31**). Thus, the stopper of the FoundationOne Liquid cfDNA BCT does not impact FoundationOne Liquid CDx test performance when stored between 4 and 35°C for up to 15 days.

Table 31: Process success rate by tube position

Process	Tube Position	# Passing Samples	# Total Samples	Success Rate (%)	95% 2-sided CIs (%)
DNA Extraction	Upright	139	147	94.6%	[89.6%, 100%]
	Inverted	147	150	98%	[94.3%, 100%]
LC	Upright	135	136	99.3%	[96%, 100%]
	Inverted	146	146	100%	[97.4%, 100%]
HC	Upright	134	135	99.3%	[95.9%, 100%]
	Inverted	143	146	97.9%	[94.1%, 100%]
Sequencing	Upright	134	134	100%	[97.2%, 100%]
	Inverted	143	143	100%	[97.4%, 100%]

Stability was also evaluated by comparing concordance between baseline and experimental samples. Positive percent agreement (PPA) and negative percent agreement (NPA) for alteration calls at ≥ 2x LoD were computed along with the corresponding two-sided 95% score confidence interval (CI) across all replicates by variant category using the baseline detection as reference. Note that NPA is under-estimated as variants not detected at any of the treatment conditions were not used in the analysis set and hence counted against the NPA calculation.

Concordance between baseline and experimental results from all samples in the upright and inverted position combined demonstrated > 99% PPA and NPA for the detection of short variants and rearrangements. Copy number alterations were only detected in samples treated in the inverted tube position and therefore, not included in this analysis. Furthermore, stratification by the treatment condition (2 tube positions × 3 temperatures × 3 durations) revealed >99.0% PPA and NPA for short variants and rearrangements across the combinations of tube positions, temperatures and durations tested. The data also demonstrate that the detection of copy number alterations is not impacted by the storage of blood in the inverted position at 35°C for up to 14 days. The concordance results by variant type for each of the experimental conditions are provided in **Table 32**.

Table 32: Concordance of detected alterations between baseline sample and experimental conditions for inverted tube stability study

Variant Type	Temperature	Tube Position	Exp. Time Point	N Variants Detected at Baseline Time Point	N Variants Detected at Exp. Time Point	PPA	PPA [95% CI]	N Variants Not Detected at Baseline Time Point	N Variants Not Detected at Exp. Time Point	NPA	NPA [95% CI]
Short variants	04°C	Inverted	Day 10	50	50	98%	[89.3%, 99.6%]	612	612	100%	[100%, 100%]
Short variants	04°C	Upright	Day 10	50	51	100%	[92.6%, 100%]	613	612	100%	[100%, 100%]
Short variants	04°C	Inverted	Day 14	59	58	98.3%	[90.9%, 99.7%]	610	611	100%	[100%, 100%]
Short variants	04°C	Upright	Day 14	44	44	100%	[91.8%, 100%]	611	611	100%	[100%, 100%]
Short variants	04°C	Inverted	Day 15	37	37	100%	[90.4%, 100%]	611	611	100%	[100%, 100%]
Short variants	04°C	Upright	Day 15	52	52	100%	[93%, 100%]	611	611	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 10	78	77	97.1%	[90.2%, 99.2%]	627	628	100%	[100%, 100%]
Short variants	25°C	Upright	Day 10	44	44	100%	[91.6%, 100%]	613	613	100%	[100%, 100%]

Variant Type	Temperature	Tube Position	Exp. Time Point	N Variants Detected at Baseline Time Point	N Variants Detected at Exp. Time Point	PPA	PPA [95% CI]	N Variants Not Detected at Baseline Time Point	N Variants Not Detected at Exp. Time Point	NPA	NPA [95% CI]
Short variants	25°C	Inverted	Day 14	46	48	100%	[92.1%, 100%]	611	609	100%	[100%, 100%]
Short variants	25°C	Upright	Day 14	42	41	97.6%	[87.4%, 99.6%]	610	611	100%	[100%, 100%]
Short variants	25°C	Inverted	Day 15	44	44	100%	[91.6%, 100%]	613	613	100%	[100%, 100%]
Short variants	25°C	Upright	Day 15	49	48	97.8%	[88.4%, 99.6%]	616	617	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 10	15	15	100%	[79.6%, 100%]	609	609	100%	[100%, 100%]
Short variants	35°C	Upright	Day 10	35	35	100%	[90.1%, 100%]	609	609	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 14	55	55	100%	[93.4%, 100%]	611	611	100%	[100%, 100%]
Short variants	35°C	Upright	Day 14	48	47	95.7%	[85.8%, 98.8%]	609	610	100%	[100%, 100%]
Short variants	35°C	Inverted	Day 15	39	39	97.4%	[86.8%, 99.5%]	610	610	100%	[100%, 100%]
Short variants	35°C	Upright	Day 15	28	29	100%	[87.5%, 100%]	613	612	100%	[100%, 100%]

These results demonstrate that blood is stable in the FoundationOne Liquid CDx cfDNA BCT when stored between 4°C and 35°C for up to 15 days, in an upright or inverted position. Additional data will be generated to further evaluate whole blood stability and potential interference of the blood collection tube cap.

2.12 DNA Extraction

DNA extraction evaluated 72 samples across five cancer types: lung cancer (including NSCLC), colorectal cancer (CRC), prostate cancer, breast cancer, and skin cancer (melanoma, sarcoma), using three reagent lots and two KingFisher Magnetic Particle processors.

Reproducibility of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments and extraction reagent lots were analyzed utilizing a factorial design (3 reagent lots × 2 KingFisher instruments × 2 replicates). The success rate of the DNAx yield for three reagent lots range from 95.8% to 100.0% and two King Fisher instruments range from 97.2% to 100.0%.
Variant calls included in the concordance analysis were identified based on the majority call across all 12 replicates for a given disease ontology. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were computed across the replicates for each somatic alteration for each sample, and aggregated by variant type (deletion, insertion, rearrangement, and substitution) for variants at ≥1x LoD. The percent agreement results by disease ontologies are: 90.3% - 99.8 % for PPA, and 99.1% - 100.0% for NPA (**Table 33**) The percent agreement results across all variant types (deletion, insertion, rearrangement and substitution) evaluated at ≥1x LoD are: 90.6% - 96.8% for PPA and 98.9% - 100.0% for NPA (**Table 34**).

Table 33: Concordance summary by disease ontology at 1x LoD for DNA extraction study

Disease Ontology	Positive Detected/ Positive Total	PPA [95% two-sided CI]	Negative Detected/ Negative Total*	NPA [95% two-sided CI]	Overall Detected/ Total*	OPA [95% two-sided CI]
Breast Cancer	347/348	99.7% [98.4%,100.0%]	3144/3144	100.0% [99.9%,100.0%]	3491/3492	100.0% [99.8%,100.0%]
Colorectal Cancer (CRC)	1122/1188	94.4% [93.0%,95.7%]	2284/2304	99.1% [98.7%,99.5%]	3406/3492	97.5% [97.0%,98.0%]
Lung Cancer	431/432	99.8% [98.7%,100.0%]	3053/3060	99.8% [99.5%,99.9%]	3484/3492	99.8% [99.5%,99.9%]
Non-Small Cell Lung Cancer (NSCLC)	600/612	98.0% [96.6%,99.0%]	2878/2880	99.9% [99.7%,100.0%]	3478/3492	99.6% [99.3%,99.8%]
Prostate Cancer	486/492	98.8% [97.4%,99.6%]	2987/3000	99.6% [99.3%,99.8%]	3473/3492	99.5% [99.2%,99.7%]
Skin Cancer	455/504	90.3% [87.4%,92.7%]	2987/2988	100.0% [99.8%,100.0%]	3442/3492	98.6% [98.1%,98.9%]

* Variants detected include variants classified as VUS and benign

Table 34: Concordance summary by variant type at 1x LoD for DNA extraction study

Variant Type	Positive Detected/ Positive Total	PPA [95% two-sided CI]	Negative Detected/ Negative Total*	NPA [95% two-sided CI]	Overall Detected/ Total*	OPA [95% two-sided CI]
Deletions	386/ 408	94.6% [91.9%, 96.6%]	2036/ 2040	99.8% [99.5% 99.9%]	2422/ 2448	98.9% [98.4% 99.3%]
Insertions	163/ 180	90.6% [85.3%, 94.4%]	819/ 828	98.9% [97.9% 99.5%]	982/ 1008	97.4% [96.2% 98.3%]
Rearrangements	23/ 24	95.8% [78.9%, 99.9%]	120/ 120	100.0% [97.0% 100.0%]	143/ 144	99.3% [96.2% 100.0%]
Substitutions	2869/ 2964	96.8% [96.1%, 97.4%]	14358/ 14388	99.8% [99.7% 99.9%]	17227/ 17352	99.3% [99.1% 99.4%]

* Variants detected include variants classified as VUS and benign

These results demonstrate robustness of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments, extraction reagent lots, and cancer types.

2.13 Guard Banding/Robustness

The purpose of this validation study was to evaluate the impact on FoundationOne Liquid CDx test performance due to potential process variation with regard to uncertainty in the measurement of DNA concentration. This guard banding evaluation assessed the DNA input into each of the main process steps of the FoundationOne Liquid CDx assay (LC, HC, and sequencing).
Guard bands were evaluated relative to calculated process variability for LC, HC, and sequencing. The assessment of multiple DNA input levels into LC demonstrated robust performance and tolerance of various DNA input levels. The observed results of HC guard banding showed that the HC process is robust within the predefined specifications 1000ng to 2000ng of DNA input into HC. For sequencing, the observed distribution of coverage indicated robust performance within the predefined specifications of 1.0nM of DNA input concentration into sequencing (as summarized in **Table 35**).

Table 35: Summary of process pass and failure rate at each guard banding DNA input level

Process	Input Level		# of Pass	Pass Rate (%)
LC	-33%	20ng	20/20	100
	-20%	24ng	20/20	100
	Recommended lower limit	30ng	20/20	100
	Low input	45ng	20/20	100
	Mid-point	55ng	20/20	100
	Upper limit	80ng	20/20	100
	+20%	96ng	19/20*	95
	+33%	106ng	20/20	100
HC	-50%	500ng	18/20	90
	-20%	800ng	20/20	100
	Lower limit	1000ng	20/20	100
	Upper limit	2000ng	20/20	100
	+20%	2400ng	20/20	100
	+50%	3000ng	18/20	90
Sequencing	-50%	0.5nM	20/20	100
	-20%	0.8nM	20/20	100
	Normal input	1.0nM	20/20	100
	+20%	1.2nM	20/20	100
	+50%	1.5nM	20/20	100

* This one failure was due to failure of HC PICO DNA yield rather than LC PICO DNA yield.

2.14 Pan-Tumor Performance

A large-scale retrospective analysis was performed to demonstrate consistent test performance of FoundationOne Liquid CDx across samples derived from patients with different tumor types. This was evaluated by comparing in-process QC metrics across tumor types using historical data from samples processed in Foundation Medicine's clinical laboratory using two prior versions of the FoundationOne Liquid CDx assay. The FoundationOne Liquid CDx assay was developed based on two versions of the FoundationOne Liquid LDT assay, each of which targeted a subset of the genomic regions targeted by FoundationOne Liquid CDx. FoundationACT (FACT) targeted 62 genes and FoundationOne Liquid targeted 70 genes. The workflow is substantially similar between the assays. In

order to support the use of historical data in this study, the regions commonly baited by the two previous assay versions and by FoundationOne Liquid CDx were evaluated for comparability of test performance (Section 2.15).

The sample set for this analysis included 19,868 distinct samples from 25 tumor type categories that had previously been tested using the Foundation Medicine FoundationOne Liquid and FoundationACT assays, previous versions of FoundationOne Liquid CDx. **Table 36** below includes a summary of the tissue types included in the study. Overall, 98.1% of samples yielded ≥25ng DNA, which corresponds to a DNA input mass of 20ng for library construction (LC). A total of 89.1% of samples yielded ≥36ng of DNA which corresponds to a DNA input mass of 30ng for LC. The proportion of samples with an LC yield greater than the minimum mass of 500ng was 99.9%, with one sided 95% confidence interval of [99.8%, 99.9%]. The proportion of samples with an HC yield greater than the minimum mass of 1000ng was 100%, with one sided 95% confidence interval of [99.99%, 100%]. The proportion of samples which met coverage requirements was 96.2%, with one sided 95% confidence interval of [95.9%, 96.3%]. The proportion of samples that generated a passing or qualified result after sequencing was 95.4%, with one sided 95% confidence interval of [95.1%, 95.6%].

Table 36. F1L/FACT samples per tumor type and pass rates

Tumor Type	Sample Size	DNA Extraction Pass Rate (≥25 ng)	DNA Extraction Pass Rate (≥36 ng)	LC Yield Pass Rate	HC Yield Pass Rate	Median Coverage Pass Rate	Overall Pass Rate (≥36 ng)
Rare Tumors	1164	97.0%	86.4%	99.9%	100.0%	93.8%	94.0%
Biliary Cancer	171	99.4%	95.3%	100.0%	100.0%	98.8%	97.1%
Bladder Cancer	166	97.6%	85.5%	100.0%	100.0%	93.2%	98.8%
Breast Cancer	2775	97.6%	87.7%	99.9%	100.0%	96.4%	95.3%
Cholangiocarcinoma	377	98.9%	96.0%	99.7%	100.0%	98.7%	96.8%
Colorectal Cancer (CRC)	1640	98.5%	92.4%	99.9%	100.0%	97.5%	96.9%
Endocrine-Neuro Cancer	75	100.0%	85.3%	100.0%	100.0%	100.0%	93.3%
Endometrial Cancer	231	98.3%	88.3%	100.0%	100.0%	96.5%	95.6%
Esophagus Cancer	291	99.7%	92.4%	100.0%	100.0%	97.6%	96.6%
Glioma Cancer	59	94.9%	72.9%	100.0%	100.0%	100.0%	76.8%
Head and Neck Cancer	154	96.1%	81.8%	100.0%	100.0%	89.2%	95.3%
Kidney Cancer	203	99.0%	87.7%	100.0%	100.0%	95.0%	95.0%
Liver Cancer	109	98.2%	95.4%	100.0%	100.0%	100.0%	95.3%
Lung Non-Small Cell Lung Carcinoma (NSCLC)	5919	98.2%	88.8%	99.8%	100.0%	95.5%	95.4%
Melanoma	257	96.5%	79.8%	100.0%	100.0%	92.7%	93.1%
Ovary Cancer	496	97.8%	88.5%	100.0%	100.0%	95.9%	94.2%
Pancreas Cancer	1359	98.8%	94.0%	99.9%	100.0%	97.8%	95.5%
Peripheral Nervous System (PNS)	44	100.0%	90.9%	100.0%	100.0%	100.0%	93.2%
Prostate Cancer	1778	97.3%	87.7%	99.9%	100.0%	96.9%	95.1%
Small Cell Cancer	135	98.5%	93.3%	100.0%	100.0%	99.2%	99.2%

Tumor Type	Sample Size	DNA Extraction Pass Rate (≥25 ng)	DNA Extraction Pass Rate (≥36 ng)	LC Yield Pass Rate	HC Yield Pass Rate	Median Coverage Pass Rate	Overall Pass Rate (≥36 ng)
Soft Tissue Sarcoma	130	97.7%	83.1%	100.0%	100.0%	95.3%	92.1%
Stomach Cancer	267	98.9%	89.1%	100.0%	100.0%	98.1%	93.2%
Thyroid Cancer	50	98.0%	86.0%	100.0%	100.0%	100.0%	81.6%
Unspecified	856	98.5%	89.1%	100.0%	100.0%	95.5%	96.3%
Unknown Primary Carcinoma (CUP)	1162	98.1%	89.7%	100.0%	100.0%	95.2%	95.7%

Table 37 summarizes the overall sample pass rate across tumor types as well as performance metrics from key QC points in the process. These results demonstrate comparable test performance across tumor types.

Table 37: Summary of F1L/FACT sample data

QC Metric	QC Pass Rate Across Tumor Types	Tumor Types with ≥ 90% QC Pass Rate
Overall report Pass/Qualified rate	76.8%~99.2%	23/25 (92%)
Library Construction	99.7%~100%	25/25 (100%)
Hybridization Capture	100%	25/25 (100%)
Median exon coverage	89.2%~100%	24/25 (96%)

2.15 Concordance – FoundationOne Liquid Laboratory Developed Test to FoundationOne Liquid CDx

In order to support the use of historical data from the FoundationOne Liquid LDT to evaluate performance across cancer types, a study was performed to evaluate concordance between FoundationOne Liquid CDx and the FoundationOne Liquid LDT across the genomic regions targeted by both assays. This study evaluated the concordance of 927 unique samples processed on both the FoundationOne Liquid laboratory developed test (LDT) and FoundationOne Liquid CDx assays. A total of 3,366 alterations, consisting of only those in common between the assays were evaluated. The concordance analysis using FoundationOne Liquid LDT or FoundationOne Liquid CDx as the reference assay is summarized by variant category in **Table 38**.

Table 38. Concordance between FoundationOne Liquid LDT (F1L LDT) and FoundationOne Liquid CDx (F1L CDx)

Variant/ Mutation Type	F1L CDx+ F1L LDT+	F1L CDx- F1L LDT+	F1L CDx+ F1L LDT-	F1L CDx- F1L LDT -	PPA [95% CI]	NPA [95% CI]	OPA [95% CI]
All Short Variants	2871	123	32	1171180	95.9% [95.1%-96.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Base Substitutions	2415	104	31	999032	95.9% [95.0%-96.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Indels	456	19	1	172148	96.0% [93.8%-97.6%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]
Rearrangements	147	20	24	59587	88.0% [82.1%-92.5%]	>99.9% [>99.9%-100.0%]	99.9% [99.9%-99.9%]
Total	3191	175	166	1290230	94.8% [94.0%-95.5%]	>99.9% [>99.9%-100.0%]	>99.9% [>99.9%-100.0%]

The overall PPA between FoundationOne Liquid LDT and FoundationOne Liquid CDx assays, with FoundationOne Liquid LDT as the reference assay, was 94.8% with a 95% two-sided CI of [94.0%-95.5%]. The respective short variant and rearrangement PPA values, with 95% two-sided CI, were: 95.9% [95.1%-96.6%] and 88.0% [82.1%-92.5%]. These results support the agreement between FoundationOne Liquid LDT and FoundationOne Liquid CDx and the applicability of the tumor comparability analysis performed using historical FoundationOne Liquid data.

2.16 Molecular Index Barcode Performance

To evaluate the molecular index barcode performance, a total of 7,641 sequenced samples from FoundationOne Liquid CDx validation studies were analyzed with the FoundationOne Liquid CDx assay.

The overall coefficient of variation (% CV) of sequencing coverage across all barcodes was 8.95% for the enhanced sensitivity regions and 7.64% for the standard sensitivity regions. This observed small % CV includes both sample variability and barcode variability as these two components were confounded and inseparable. Results demonstrated that all 480 barcodes analyzed are detectable with low differences in sample coverage variance between barcodes, indicating comparable performance of the barcodes.

2.17 Automation Line Equivalence

An intermediate precision study was performed to establish equivalence between the Hamilton instrumentation and the Biomek/Bravo instrumentation. The study consisted of eight contrived samples run in triplicate across four runs and both instrumentation platforms resulting in a total of 192 sample replicates included in the study overall. The analysis evaluated the negative call rate (NCR) and positive call rate (PCR) for 1,309 variants from eight contrived samples. The PCR and NCR were also evaluated by the seven variant categories.

The Mann-Whitney test was used for the comparison of PCR and NCR across liquid handling platforms for each sample, all samples in aggregate, and for each variant type. The NCR across platforms for each analysis set (per sample, all samples in aggregate, per variant type) were not statistically significant ($p > 0.05$). by sample and by variant type. The PCR across platforms were not statistically significant ($p > 0.05$) with the exception of contrived sample #3, the aggregate of all samples, and substitutions in a non-repetitive region or a repetitive region of ≤7 base pairs. The PCRs for the Hamilton liquid handling platform were slightly higher than the PCRs for the Biomek/Bravo platform (92.08% versus 90.15% for sample #3, 90.75% versus 89.67% for all samples, and 91.14 versus 90.10% for substitutions in a non-repetitive region or repetitive region of ≤7 base pairs). The statistical significance observed was due to large sample sizes allowing for the detection of slight differences that are likely not meaningful in practice; therefore, the Hamilton and Biomek/Bravo liquid handling platforms are considered to be interchangeable in the FoundationOne Liquid CDx assay.

Clinical Validation Studies

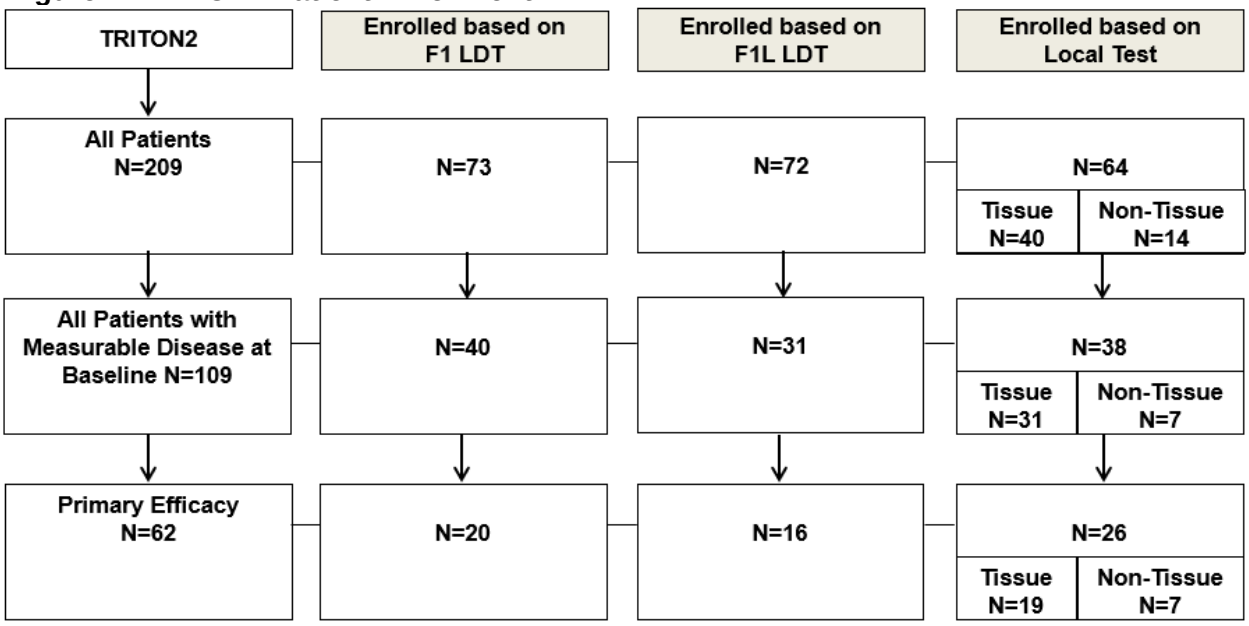
1. Clinical Bridging Study: Detection of *BRCA1* and *BRCA2* Alterations to Determine Eligibility of mCRPC Patients for Treatment with Rucaparib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castration-resistant prostate cancer (mCRPC) harboring breast cancer gene 1 or 2 (*BRCA1* or *BRCA2*) alterations for treatment with rucaparib was demonstrated using pre-rucaparib treatment blood samples from clinical trial NCT0952534 (TRITON2). The clinical data supporting the use of rucaparib in the proposed indication was submitted as New Drug Application (NDA) 209115/S-004.

A bridging study was conducted to evaluate: 1) the concordance between BRCA1 and BRCA2 alteration status by the clinical trial assay (CTA) and FoundationOne Liquid CDx, and 2) the clinical efficacy of rucaparib treatment in patients that would be eligible for therapy based on BRCA1 and BRCA2 alteration status as determined by FoundationOne Liquid CDx.

A total of 209 patients (All Patients) from TRITON2 were included in NDA 209115/S-004. Genomic status was determined using the FoundationOne laboratory developed test [LDT] (F1 LDT), the FoundationOne Liquid LDT (F1L LDT), or a local test, as summarized in **Figure 1**.

Figure 1: TRITON2 Patient Enrollment



Pre-rucaparib treatment plasma samples were available for 92% (192/209) of the patients. FoundationOne Liquid CDx data were available for 93% (178/192) of the patients with samples tested; inadequate input material resulted in FoundationOne Liquid CDx test data being unavailable for 14 patients. In total, FoundationOne Liquid CDx data were available for 85% (178/209) of All Patients.

Of the 62 patients in the Primary Efficacy Population (those patients with measurable visceral and/or nodal disease at baseline), FoundationOne Liquid CDx test data were obtained for 84% (52/62) and used for concordance and efficacy analyses. The sample accountability for this clinical bridging study is summarized in **Table 39**.

Table 39: Sample accountability for rucaparib prostate clinical bridging study

Description	Number
All Patients in TRITON2	209
Total samples available for retesting by FoundationOne Liquid CDx	192
Patients with evaluable FoundationOne Liquid CDx data and cfDNA input ≥ 30ng (All Patients)	161
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (All Patients)	178
Primary efficacy population in TRITON2	62
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 30ng (Primary Efficacy Population)	48
Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (Primary Efficacy Population)	52

1.1 Concordance between FoundationOne Liquid CDx and the CTAs

The concordance of BRCA status between FoundationOne Liquid CDx and CTA test results were evaluated in all patients as summarized in **Table 40** and **Table 41**.

Table 40: Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥30ng

All Patients		CTA		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
FoundationOne Liquid CDx	***BRCA* Positive**	75	1	76
	***BRCA* Negative**	16	69	85
	***BRCA* Unknown**	2	1	3
	Total	93	71	164

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥30ng, were determined using the CTA as the reference for all patients.

PPA (95% CI): 82.4% (73.0%, 89.6%)
NPA (95% CI): 98.6% (92.3%, 100.0%)

Table 41: Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥20ng

All Patients		CTA		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
FoundationOne Liquid CDx	***BRCA* Positive**	82	1	83
	***BRCA* Negative**	18	77	95
	***BRCA* Unknown**	3	2	5
	Total	103	80	183

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥20ng, were determined using the CTA as the reference for all patients.

- PPA [95% CI]: 82.0% [73.1%, 89.0%]
- NPA [95% CI]: 98.7% [93.1%, 100%]

1.2 Efficacy Based on FoundationOne Liquid CDx Results

BRCA1 and *BRCA2* alteration status were verified retrospectively by FoundationOne Liquid CDx in 66% (41/62) of the patients in the Primary Efficacy Population. The ORR [95% CI] in the Primary Efficacy Population was 46.3% [30.7%-62.6%] in *BRCA* positive patients determined by FoundationOne Liquid CDx, which is comparable to the ORR of 43.5% [31.0%-56.7%] in patients identified by CTA (**Table 42**).

Table 42: ORR and duration of response in the primary efficacy population by CTA and FoundationOne Liquid CDx test results

Primary Efficacy Population	**FoundationOne Liquid CDx**		**CTA**
	***BRCA* Positive N=38 (≥ 30 ng cfDNA input)**	***BRCA* Positive N = 41 (≥ 20 ng cfDNA input)**	***BRCA* Positive N = 62**
Confirmed ORR (CR + PR), n (%)	18 (47.4)	19 (46.3)	27 (43.5)
95% CI(%)	31.0 – 64.2	30.7 - 62.6	31.0 – 56.7

Abbreviations: *BRCA* = breast cancer gene, includes *BRCA1* and *BRCA2*; CI = confidence interval; CTA = clinical trial assay; ORR = objective response rate; CR = complete response; PR = partial response.

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the unknown FoundationOne Liquid CDx results was performed using the multiple imputation method and demonstrated that the drug efficacy in the FoundationOne Liquid CDx positive population was robust to missing FoundationOne Liquid CDx results.

2. FoundationOne Liquid CDx Concordance Study for *EGFR* Exon 19 deletion and *EGFR* Exon 21 L858R Alteration

Clinical validity of FoundationOne Liquid CDx assay was established as a companion diagnostic to identify patients with advanced NSCLC who may be eligible for treatment with TARCEVA® (erlotinib), IRESSA® (gefitinib), or TAGRISSO® (osimertinib). Two hundred and eighty retrospective samples from NSCLC patients were included in this study, which were tested for *EGFR* exon 19 deletion and exon 21 L858R alterations (*EGFR* alterations) by the FoundationOne Liquid CDx assay and the previously approved **cobas®** *EGFR* Mutation Test v2 (Roche Molecular Systems, referred to cobas assay). Both *EGFR* alteration-positive and *EGFR* alteration-negative samples (based on CTA results) were selected from the screen failed population of an unrelated clinical trial in NSCLC. To avoid selection bias, the samples were selected starting with a specific testing date until the predefined number of 150 *EGFR* alteration-positive and 100 *EGFR* alteration-negative samples were fulfilled. Samples were tested across two replicates by the cobas assay (denoted as CCD1 and CCD2) and one replicate by FoundationOne Liquid CDx. The tested samples, from NSCLC patients, were compared against the intended use (IU) population with respect to gender to ensure the screening population is representative of the IU population. The variant calls were evaluated based on the agreement between both the FoundationOne Liquid CDx and the cobas assay results and between the two cobas assay replicates. For any samples in which there was insufficient plasma to process both CCD1 and CCD2, processing was not performed. In total there were 177 samples with complete test results available for analysis. The agreement analysis results between FoundationOne Liquid CDx and the cobas assay for the detection of *EGFR* exon 19 deletions and L858R alterations are presented in **Table 43**.

Table 43: Agreement analysis results for EGFR exon 19 deletion and L858R separately.

Exon 19 deletion	PPA_{C1F}	95.5%	NPA_{C1F}	95.6%
	PPA_{C1C2}	97.7%	NPA_{C1C2}	98.9%
	PPA_{C2F}	95.5%	NPA_{C2F}	96.0%
	PPA_{C2C1}	96.2%	NPA_{C2C1}	99.4%
L858R	PPA_{C1F}	100.0%	NPA_{C1F}	95.6%
	PPA_{C1C2}	92.9%	NPA_{C1C2}	98.9%
	PPA_{C2F}	100.0%	NPA_{C2F}	94.7%
	PPA_{C2C1}	96.0%	NPA_{C2C1}	98.0%

The concordance of *EGFR* mutations as detected by FoundationOne Liquid CDx and the cobas assay were assessed and the data are summarized in **Table 44**.

Table 44: Concordance among CCD1, CCD2 and FoundationOne Liquid CDx results with eligible samples (n=177)

	CCD1+			**CCD1-**		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FoundationOne Liquid CDx+	80	4	84	1	3	4
FoundationOne Liquid CDx-	2	0	2	0	87	87
Total	82	4	86	1	90	91

The agreement analysis results between FoundationOne Liquid CDx and the cobas assay are presented in **Table** 45.

Table 45: Agreement analysis results

	PPA	**NPA**
CCD2|CCD1*	95.3%	98.9%
CCD1|CCD2**	96.1%	98.7%
FoundationOne Liquid CDx|CCD1*	97.7%	95.6%
FoundationOne Liquid CDx |CCD2**	97.7%	95.4%

* CCD1: the 1st replicate of cobas assay as the reference
** CCD2: the 2nd replicate of cobas assay as the reference

The estimates of ζPPA1, ζPPA2, ζNPA1 and ζNPA2 and the corresponding one-sided 95% upper bounds confidence limit computed using the bootstrap method are presented in **Table 46.**

Table 46: Point estimate and one-Sided 95% upper confidence limit of ζPPA1, ζNPA1, ζPPA2, and ζNPA

	Point Estimate	**Mean one-sided 95% upper confidence limit**
ζ_{PPA1}	-2.3%	2.3%
ζ_{NPA1}	3.3%	6.6%
ζ_{PPA2}	-1.6%	4.7%
ζ_{NPA2}	3.3%	6.6%

Based on these results, FoundationOne Liquid CDx has been demonstrated to be non-inferior to the cobas assay for the detection of *EGFR* exon 19 deletions and *EGFR* exon 21 L858R mutations. This study establishes the clinical validity of the FoundationOne Liquid CDx assay for identifying patients eligible for treatment with erlotinib, gefitinib, and osimertinib.

FOUNDATIONONE®LIQUID CDx

Specimen Instructions

Peripheral Whole Blood

Use only tubes provided inside the FoundationOne®Liquid CDx Specimen Collection and Shipping Kit. Other tubes will not be accepted.

Instructions For Use

Accurate analysis of cell-free DNA requires proper collection technique and handling of the sample. Failure to adhere to these instructions can compromise results by diluting cell-free DNA with DNA from white blood cell lysis.

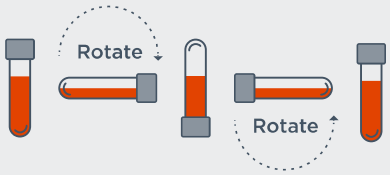
☐ 1 Check special tubes provided in FoundationOne Liquid CDx kits to confirm liquid is clear and without cloudiness or crystals.

☐ 2 Label tubes with date of collection and two patient identifiers.

☐ 3 Collect two tubes of whole blood (8.5mL per tube).

- Prevent backflow: tubes contain chemical additives and it is important to avoid backflow into patient.
- Collect specimen by venipuncture according to CLSI H3-A6.[1]
- Fill tubes completely (8.5mL per tube).

☐ 4 Remove the tube from adapter and immediately **mix by gentle inversion 8 to 10 times.** Inadequate or delayed mixing may result in inaccurate test results. One inversion is a complete turn of the wrist, 180°, and back per the figure below.



☐ 5 Place specimen, completed test requisition form (TRF) (remember to include patient's diagnosis), insurance information, available reports, and accompanying documents into the FoundationOne Liquid CDx Specimen Collection and Shipping Kit (copies of pathology reports and/or other clinical documentation).

- Confirm each tube is labeled with the supplied labels indicating the date of collection and two unique patient identifiers (label included in kit).

☐ 6 Preferably on the same day of collection, ship via FedEx priority overnight delivery at ambient temperature. Do not freeze or refrigerate blood samples.

Temperature is important. Keep at room temperature (43-99° F, 6-37° C).

DO NOT FREEZE.

Shipping Instructions

1. Place the samples, test requisition form, insurance information, and any other attachments into the FoundationOne Liquid CDx Specimen Collection and Shipping Kit.
2. Place the specimen kit (including samples and paperwork) into the provided FedEx shipping pack, first ensuring that primary specimen containers (e.g. tubes) are labeled with two patient-specific identifiers. Seal the shipping pack.
3. If using shipping pack provided in this kit (recommended), recording the Kit ID # will allow you to properly track specimen. If you use a different shipping pack, consider recording that pack's tracking number.
4. Call 800.463.3339 to request a pick-up or drop the package at your site's designated FedEx pick-up location and ship sealed shipping pack to:

Foundation Medicine, Inc.
150 Second Street
Cambridge, MA 02141
Phone: 888.988.3639

Abbreviated Statement

Us ex eos et earunt, sitat ma coruptaquam eum fuga. Ga. Pudi quias mi, alibusa debis acc lo rem abo. Nam qui aceptatem dolorerum quam hari dolupta cumquatqui acias pra doluptatem. Luptaecat eos parum dus maiorio mincto deligen ditibus doluptat xim hou nonse quunt ius endit od ut asi nus non cuptas aut rerupici dicium dolorum derae pore laccatae et excerumeniet elestio mintis alique erro incipis es endunturi cu omnis odit, a om nus magnam, imporrovit voloremporem utatur sequibus.

FPO

Reference

1. Clinical and Laboratory Standards Institute. H3-A6, Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard-sixth edition.

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