FOUNDATION**ONE**®**LIQUID CDx**

**FoundationOne® Liquid CDx**
**Technical Information**

## Table of Contents

**FOUNDATIONONE® LIQUID CDx**

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**Technical Information**

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## 1 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, rearrangements in four (4) genes and copy number alterations in three (3) genes. 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.

**Table 1: Companion diagnostic indications**

| Tumor Type | Biomarker(s) Detected | Therapy |
|---|---|---|
| Non-small cell lung cancer (NSCLC) | *ALK* rearrangements | ALECENSA® (alectinib) |
| | *EGFR* Exon 19 deletions and *EGFR* Exon 21 L858R substitution | IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib) |
| Prostate cancer | *BRCA1, BRCA2, ATM* alterations | LYNPARZA® (olaparib) |
| | *BRCA1, BRCA2* alterations | RUBRACA® (rucaparib) |
| Ovarian cancer | *BRCA1, BRCA2* alterations | RUBRACA® (rucaparib) |
| Breast cancer | *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y | PIQRAY® (alpelisib) |

Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients with the tumor types above 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.

## 2 Contraindication
There are no known contraindications.


## 3 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-approved tumor tissue test, if available.


## 4 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 an alteration 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 community.

- 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 nontumor 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, the following: *ASXL1, ATM, CBL, CHEK2, DNMT3A, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2, TP53*, and *U2AF1*. The efficacy of targeting such nontumor somatic alterations (e.g., CH) is unknown.

- 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.

- The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in *ATM*.

- 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.

- Representation of *ALK* rearrangements were limited in the analytical validation studies.

- The representation of *ATM* short variants and rearrangements was limited in the analytical validation studies.

- Performance has not been validated for cfDNA input below the specified minimum input.

## 5 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 fifteen of these genes (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, copy number variants in three genes, and genomic rearrangements in four genes. A subset of targeted regions in 75 genes is baited for enhanced sensitivity.

**Table 2: As part of its FDA-approved intended use, the FoundationOne Liquid CDx assay interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an \*).**

Select regions in 75 genes (indicated in bold) are captured with increased sensitivity. Genes are captured for increased sensitivity with complete exonic (coding) coverage unless otherwise noted.

| **ABL1 [Exons 4-9]** | ACVR1B | **AKT1 [Exon 3]** | AKT2 | AKT3 | **ALK [Exons 20-29, Introns 18,19]** | ALOX12B | AMER1 (FAM123B) | **APC** | **AR** |
|---|---|---|---|---|---|---|---|---|---|
| **ARAF [Exons 4, 5, 7, 11, 13, 15, 16]** | ARFRP1 | ARID1A | ASXL1 | **ATM** | **ATR** | ATRX | AURKA | AURKB | AXIN1 |
| AXL | BAP1 | BARD1 | BCL2 | BCL2L1 | BCL2L2 | BCL6 | BCOR | BCORL1 | BCR* [Introns 8, 13, 14] |
| **BRAF [Exons 11-18, Introns 7-10]** | **BRCA1 [Introns 2, 7, 8, 12, 16, 19, 20]** | **BRCA2 [Intron 2]** | BRD4 | BRIP1 | BTG1 | BTG2 | **BTK [Exons 2, 15]** | C11orf30 (EMSY) | C17orf39 (GID4) |
| CALR | CARD11 | CASP8 | CBFB | CBL | **CCND1** | CCND2 | CCND3 | CCNE1 | CD22 |
| CD70 | CD74* [Introns 6-8] | CD79A | CD79B | **CD274 (PD-L1)** | CDC73 | **CDH1** | **CDK12** | **CDK4** | **CDK6** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CDK8 | CDKN1A | CDKN1B | **CDKN2A** | CDKN2B | CDKN2C | CEBPA | CHEK1 | **CHEK2** | CIC |
| CREBBP | **CRKL** | CSF1R | CSF3R | CTCF | CTNNA1 | **CTNNB1 [Exon 3]** | CUL3 | CUL4A | CXCR4 |
| CYP17A1 | DAXX | DDR1 | **DDR2 [Exons 5, 17, 18]** | DIS3 | DNMT3A | DOT1L | EED | **EGFR [Introns 7, 15, 24-27]** | EP300 |
| EPHA3 | EPHB1 | EPHB4 | **ERBB2** | **ERBB3 [Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25]** | ERBB4 | ERCC4 | ERG | **ERRFI1** | **ESR1 [Exons 4-8]** |
| ETV4* [Intron 8] | ETV5* [Introns 6, 7] | **ETV6* [Introns 5, 6]** | EWSR1* [Introns 7-13] | **EZH2 [Exons 4, 16, 17, 18]** | EZR* [Introns 9-11] | FAM46C | FANCA | FANCC | FANCG |
| FANCL | FAS | FBXW7 | FGF10 | FGF12 | FGF14 | FGF19 | FGF23 | FGF3 | FGF4 |
| FGF6 | **FGFR1 [Introns 1, 5, Intron 17]** | **FGFR2 [Intron 1, Intron 17]** | **FGFR3 [Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17]** | FGFR4 | FH | FLCN | FLT1 | **FLT3 [Exons 14, 15, 20]** | **FOXL2** |
| FUBP1 | GABRA6 | GATA3 | GATA4 | GATA6 | **GNA11 [Exons 4, 5]** | GNA13 | **GNAQ [Exons 4, 5]** | **GNAS [Exons 1, 8]** | GRM3 |
| GSK3B | H3F3A | HDAC1 | HGF | HNF1A | **HRAS [Exons 2, 3]** | HSD3B1 | ID3 | **IDH1 [Exon 4]** | **IDH2 [Exon 4]** |
| IGF1R | IKBKE | IKZF1 | INPP4B | IRF2 | IRF4 | IRS2 | JAK1 | **JAK2 [Exon 14]** | **JAK3 [Exons 5, 11, 12, 13, 15, 16]** |
| JUN | KDM5A | KDM5C | KDM6A | KDR | KEAP1 | KEL | **KIT [Exons 8,9,11,12, 13, 17, Intron 16]** | KLHL6 | KMT2A (MLL) [Introns 6, 8-11, Intron 7] |
| KMT2D (MLL2) | **KRAS** | LTK | LYN | MAF | **MAP2K1 (MEK1) [Exons 2, 3]** | **MAP2K2 (MEK2) [Exons 2-4, 6, 7]** | MAP2K4 | MAP3K1 | MAP3K13 |
| MAPK1 | MCL1 | **MDM2** | MDM4 | MED12 | MEF2B | MEN1 | MERTK | **MET** | MITF |
| MKNK1 | MLH1 | **MPL [Exon 10]** | MRE11A | MSH2 [Intron 5] | MSH3 | MSH6 | MST1R | MTAP | **MTOR [Exons 19, 30, 39 40, 43-45, 47, 48, 53, 56]** |
| MUTYH | MYB* [Intron 14] | **MYC [Intron 1]** | MYCL (MYCL1) | **MYCN** | **MYD88 [Exon 4]** | NBN | **NF1** | NF2 | NFE2L2 |
| NFKBIA | NKX2-1 (TTF-1) | NOTCH1 | NOTCH2 [Intron 26] | NOTCH3 | **NPM1 [Exons 4-6, 8, 10]** | **NRAS [Exons 2, 3]** | NSD3 (WHSC1L1) | NT5C2 | **NTRK1 [Exons 14, 15, Introns 8-11]** |

| NTRK2 [Intron 12] | NTRK3 [Exons 16, 17] | NUTM1* [Intron 1] | P2RY8 | PALB2 | PARK2 | PARP1 | PARP2 | PARP3 | PAX5 |
|---|---|---|---|---|---|---|---|---|---|
| PBRM1 | PDCD1 (PD-1) | PDCD1LG2 (PD-L2) | PDGFRA [Exons 12, 18, Introns 7, 9, 11] | PDGFRB [Exons 12-21, 23] | PDK1 | PIK3C2B | PIK3C2G | PIK3CA [Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)] | PIK3CB |
| PIK3R1 | PIM1 | PMS2 | POLD1 | POLE | PPARG | PPP2R1A | PPP2R2A | PRDM1 | PRKAR1A |
| PRKCI | PTCH1 | PTEN | PTPN11 | PTPRO | QKI | RAC1 | RAD21 | RAD51 | RAD51B |
| RAD51C | RAD51D | RAD52 | RAD54L | RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17, Introns 4-8] | RARA [Intron 2] | RB1 | RBM10 | REL | RET [Introns 7, 8, Exons 11, 13-16, Introns 9-11] |
| RICTOR | RNF43 | ROS1 [Exons 31, 36-38, 40, Introns 31-35] | RPTOR | RSPO2* [Intron 1] | SDC4* [Intron 2] | SDHA | SDHB | SDHC | SDHD |
| SETD2 | SF3B1 | SGK1 | SLC34A2* [Intron 4] | SMAD2 | SMAD4 | SMARCA4 | SMARCB1 | SMO | SNCAIP |
| SOCS1 | SOX2 | SOX9 | SPEN | SPOP | SRC | STAG2 | STAT3 | STK11 (LKB1) | SUFU |
| SYK | TBX3 | TEK | TERC* {ncRNA} | TERT* {Promoter} | TET2 | TGFBR2 | TIPARP | TMPRSS2* [Introns 1-3] | TNFAIP3 |
| TNFRSF14 | TP53 | TSC1 | TSC2 | TYRO3 | U2AF1 | VEGFA | VHL | WHSC1 | WTI |
| XPO1 | XRCC2 | ZNF217 | ZNF703 | | | | | | |

The classification criteria for all CDx variants are outlined at the end of this document.

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

As part of its FDA-approved intended use, copy number alterations and rearrangements are reported in the genes listed in

**Table** 3**.**

**Table 3: Genes for which copy number alterations and rearrangements are reported for tumor profiling by FoundationOne Liquid CDx**

| Alteration Type | Genes |
|---|---|
| Copy Number Alterations | *BRCA1, BRCA2, ERBB2* |
| Rearrangements | *ALK, BRCA1, BRCA2* |

## 6 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.

## 7 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: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032B.pdf

## 8 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

## 9 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 4** 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 4 Representation of tumor types and variants across validation studies**

| Study Title | Cancer Types Represented | # Unique Samples | # of Sample Replicates | # of Unique Genes | # of Unique | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Subs | Indels | Rearrang. | Copy Number Amplif. | Copy Number Losses |
| Contrived Sample Functional Characterization (CSFC) Study | Breast cancer Colorectal cancer Lung cancer Contrived samples | 13 | 1843 | 228 | 563 | 81 | 11 | 1 | 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 | 0 | 2 |
| FoundationOne Liquid CDx to Validated NGS cfDNA Assay Concordance: *PIK3CA* mutations | Breast cancer | 412 | N/A | 1 | 32 | 5 | 0 | 0 | 0 |
| Orthogonal Concordance | 23 cancer types Contrived samples | 278 | N/A | 64 | 541 | 12 | 11 | 3 | 0 |
| LoD Estimation | Prostate Contrived samples | 10 | 877 | 286 | 1490 | 247 | 32 | 13 | 3 |
| LoB | Healthy Donors | 28 | 79 | 322 | 26134 | 4482 | 911 | 222 | 42 |
| Potentially Interfering Substances | Contrived samples | 9 | 336 | 18 | 16 | 11 | 11 | 1 | 2 |
| Hybrid Capture Bait Specificity | 25 cancer types Contrived samples | 3546 | N/A | 324 | N/A | N/A | N/A | N/A | N/A |
| Reagent Stability | Contrived samples | 8 | 142 | 279 | 1090 | 215 | 32 | 17 | 2 |
| Reagent Interchangeability | Contrived samples | 8 | 192 | 20 | 15 | 11 | 11 | 1 | 1 |
| Precision study 1 | Breast cancer Colon cancer Lung cancer Ovarian cancer Prostate cancer Skin cancer Contrived samples | 47 | 1121 | 280 | 900 | 229 | 63 | 49 | 5 |
| Precision study 2 | Lung cancer Prostate cancer Stomach cancer Colorectal cancer Bile duct cancer Breast cancer | 10 | 230 | 6 | 6 | 4 | 0 | 0 | 0 |
| DNA Extraction | Colorectal cancer Prostate cancer Breast cancer Lung cancer Skin cancer | 6 | 72 | 161 | 265 | 53 | 2 | 0 | 0 |
| Whole Blood Sample Stability | Lung cancer Colorectal cancer Prostate cancer Breast cancer | 11 | 22 | 66 | 75 | 15 | 1 | 0 | 0 |

| Study Title | Cancer Types Represented | # Unique Samples | # of Sample Replicates | # of Unique Genes | # of Unique | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Subs | Indels | Rearrang. | Copy Number Amplif. | Copy Number Losses |
| Inverted Tube Whole Blood Sample Stability | Lung cancer Colorectal cancer Breast cancer Ovarian cancer Prostate cancer | 130 | 260 | 237 | 594 | 91 | 5 | 5 | 0 |
| Cross Contamination | Contrived samples | 5 | 376 | 39 | 9 | 5 | 4 | 21 | 1 |
| Guard Banding | Contrived samples | 10 | 375 | 20 | 17 | 12 | 12 | 1 | 1 |
| 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 | 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 | 0 | 1 |
| Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in ovarian cancer | Ovarian cancer | 217 | N/A | 2 | 48 | 49 | 3 | 0 | 0 |
| Clinical validation study for detection of PIK3CA mutations in breast cancer | Breast | 359 | N/A | 1 | 28 | 4 | 0 | 0 | 0 |
| Clinical validation study for ALK rearrangements in NSCLC | Lung cancer | 249 | N/A | 1 | 13 | 1 | 11 | 1 | 0 |
| Clinical validation study for BRCA1, BRCA2, and ATM alterations in prostate cancer | Prostate cancer | 333 | N/A | 3 | 48 | 75 | 10 | 0 | 1 |

| Study Title | Cancer Types Represented | # Unique Samples | # of Sample Replicates | # of Unique Genes | # of Unique | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Subs | Indels | Rearrang. | Copy Number Amplif. | Copy Number Losses |
| Blood Collection Tube Equivalence | Ovarian cancer<br>Breast cancer<br>Colorectal cancer<br>Prostate cancer<br>Lung cancer<br>Skin cancer<br>Stomach cancer | 60 | 192 | 116 | 135 | 39 | 13 | 5 | 0 |
| Automation Line Equivalence | Contrived samples | 8 | 187 | 303 | 1926 | 337 | 63 | 61 | 4 |
| Variant Report Curation | Breast cancer<br>Colorectal cancer<br>Lung cancer<br>Prostate cancer<br>Skin cancer | 19 | 57 | 183 | 300 | 104 | 15 | 11 | 2 |
| Pan-tumor performance (includes historical analysis) | 20 cancer types | 19868 | N/A | 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 | N/A |
| FoundationOne Liquid LDT to FoundationOne Liquid CDx Concordance | 25 cancer types | 927 | N/A | 73 | 1815 | 376 | 109 | 46 | N/A |

\* Variants detected include variants classified as VUS and benign.

## 9.1    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). 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 5).**

**Table 5. 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 | 96.1% [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 6**).

**Table 6. 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 7**).

**Table 7. Concordance of CDx alterations called between FoundationOne Liquid CDx and the comparator assay (n = 74)**

| 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%] |
| *PIK3CA* base substitutions | 49 | 100% [92.7%-100.0%] | 100% [99.9%-100.0%] |

| Targeted Alteration | *n* | PPA [95% CI] | NPA [95% CI] |
|---|---|---|---|
| *ALK* rearrangements | 1 | 100% [2.5%-100.0%] | 99.9% [99.7%-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.

### 9.2 Concordance – FoundationOne 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 8**). As summarized in **Table 9**, 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 the validated NGS tumor tissue assay for the detection of deleterious alterations in *BRCA1* or *BRCA2*.

**Table 8. 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 9. 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 (95% CI) | NPA (95% CI) |
|---|---|---|---|---|---|---|
| **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%) |

### 9.3 Concordance – Comparison to an Orthogonal cfDNA NGS Method #2

The accuracy of using FoundationOne Liquid CDx as a companion diagnostic to identify breast cancer patients harboring *PIK3CA* alterations was assessed with residual plasma samples from the SOLAR-1 clinical trial. Of the remaining plasma samples, 542 were evaluable by the externally-validated NGS method and produced valid results. 418 were evaluable by FoundationOne Liquid CDx, of which 192 positive variants were detected across 188 patients, with four patients possessing two positive variants each. The distribution of counts per positive variant is listed in **Table 10**.

**Table 10. Distribution of variants detected with FoundationOne Liquid CDx evaluable samples.**

| Protein Effect in PIK3CA | # Variant Calls (188 Positive Samples) |
|---|---|
| C420R | 3 |
| E542K | 25 |
| E545A | 1 |
| E545G | 2 |
| E545K | 50 |
| H1047L | 9 |
| H1047R | 100 |
| H1047Y | 1 |
| Q546R | 1 |
| **Total** | **192** |

A total of 412 valid samples generated valid results with both assays. The primary analysis using NGS Method #2 as the reference assay achieved a PPA [95% CI] of 97.06% [93.27%, 99.04%], and an NPA [95% CI] of 91.74% [87.52%, 94.88%]. The contingency table for this comparison is provided in **Table 11**, with counts representing number of samples (versus number of variant calls).

The sample counts in the core 2x2 white boxes total to 412 samples. There were seven samples evaluable with FoundationOne Liquid CDx but failed (italicized in **Table 11**), as well as three samples missing from reference assay data. There were five samples unevaluable by the reference assay; three of these aligned with the 418 evaluable FoundationOne Liquid CDx samples, while two were among the 130 samples not evaluable due to insufficient plasma.

**Table 11. Contingency table comparing FoundationOne Liquid CDx with the reference assay, primary analysis with 412 cases.**

| | | Reference Assay | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Positive** | **Negative** | **Not Evaluable** | **Missing** | **Total** | |
| **FoundationOne Liquid CDx** | **Positive** | 165 | 20 | 2 | 1 | **188** | PPA$_{F1L}$: 89.19% [83.80%, 93.27%] |
| | **Negative** | 5 | 222 | 1 | 2 | **230** | NPA$_{F1L}$: 97.80% [94.93%- 99.28% |
| | **Evaluable but Failed** | 0 | 7 | 0 | 0 | 7 | |
| | **Not Evaluable** | 35 | 93 | 2 | 0 | 130 | |
| | **Total** | 205 | 342 | 5 | 3 | 555 | |
| | | PPA$_{ONC}$: 97.06% [93.27%, 99.04%] | NPA$_{ONC}$: 91.74% [87.52%, 94.88%] | | | | OPA: 93.93% [91.17%, 96.04%] |

## 9.4    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. 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 12.** The median LoD for targeted short variant, rearrangement, and copy number alterations were consistent with the platform LoD (**Table 13**).

### Table 12: LoD estimation for CDx alterations

| Gene | Alteration Subtype | Number of Samples Evaluated | Median LoD |
|---|---|---|---|
| *ATM* | Indels | 1 | 0.51% VAF |
| | Rearrangement (*ATM-EXPH5* Truncation[1]) | 1 | 1.13% VAF |
| *BRCA1* | Substitutions | 8 | 0.34% VAF |
| | Indels | 1 | 0.38% 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* | Substitutions (L858R substitutions) | 2 | 0.34% VAF |
| | Indels (exon 19 deletions) | 2 | 0.27% VAF |
| *PIK3CA* | Substitutions | 6 | 0.34% VAF |
| *ALK* | Rearrangement (*ALK-EML4*) | 1 | 0.24% VAF |
| | Rearrangement (*NPM1-ALK* Rearrangement) | 1 | 0.94% VAF |

The estimated LoDs for *BRCA1* and *BRCA2* subs and indels were confirmed at values higher than the LoDs established in Table 17(see Precision: Reproducibility and Reproducibility section below,

**Table** 21 and **Table 22** for confirmed LoD values).

[1]The LoD for these alterations was determined using clinical specimens.

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

The platform LoD for short variants, rearrangements, and copy number losses are presented in **Table 13**. 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 14**.

**Table 13: 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 | N/A |
| Copy Number Amplifications | 8 | N/A | 21.7% TF | 19.8% - 25.2% TF |

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

**Table 14: 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% |
| | 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% |
|---|---|---|---|---|---|---|
| Standard Sensitivity Region | 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 Amplifications | 8 | 19.8% | 19.8% | 21.7% | 25.2% |

1VAF reported for short variant and rearrangement LoD, tumor fraction reported for copy number alterations 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 15.** The median LoD for these targeted short variants are consistent with the platform LoD presented in **Table 13.**

**Table 15: LoD for non-CDx alterations**

| Gene | Alteration Subtype | Number of Samples Evaluated | Median LoD* |
|---|---|---|---|
| *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 |
| *ERBB2* | Copy Number Amplification | 1 | 19.8% TF |

VAF = variant allele frequency
TF = tumor fraction
[1] LoD for these alterations was determined using clinical specimens.
*The accuracy of %VAF/%TF have not been analytically validated


## 9.5   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%. On a per variant basis (number of variants detected across all replicates divided by the total number of variants included in the analysis across all replicates), the overall detection rate for short variants in this study was 0.027% (**Table 16**).

**Table 16: Detection rate for each reporting category in LoB study**

| Category | Unique Variant Detection Rate (Unique variants detected) / (total unique variants analyzed) | Total Variant Detection Rate (Total variants detected) / (total variants analyzed[1]) |
|---|---|---|
| Level 1 | 0% (0 of 292) | 0% (0 of 23,068) |
| Level 2 | 0% (0 of 10) | 0% (0 of 790) |
| Level 3 | 0% (0 of 18) | 0% (0 of 1,422) |
| Level 4 | 0.82% (47 of 5,760) | 0.024% (107 of 455,040) |
| VUS | 0.83% (203 of 24,542) | 0.029% (555 of 1,938,818) |
| All categories | 0.82% (250 of 30,622) | 0.027% (662 of 2,419,138[1]) |

[1] total variants analyzed = unique variants * 79 replicates

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

## 9.6 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 17**.

**Table 17 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** 18**).**
**Table 18: Concordance per substance for variants ≥1x LoD**

| Substance | Concordance | 95% CI LB (Exact) | 95% CI UB (Exact) | N |
|---|---|---|---|---|
| Triglycerides, 37 mmol/L (or 33 g/L) | 100.00% | 91.19% | 100.00% | 40 |
| Hemoglobin, 2.0 g/L | 100.00% | 90.97% | 100.00% | 39 |
| Albumin, 60 g/L | 97.56% | 87.14% | 99.94% | 41 |
| Bilirubin (conjugated), 0.2 g/L | 100.00% | 91.59% | 100.00% | 42 |
| Bilirubin (unconjugated), 0.2 g/L | 97.44% | 86.52 % | 99.94% | 39 |
| Cholesterol Level 2, 3.88 mmol (150 mg/dL) | 97.56% | 87.14% | 99.94% | 41 |
| Cholesterol Level 1, 6.47mmol (250 mg/dL) | 97.37% | 86.19% | 99.93% | 38 |
| Staphylococcus epidermidis, 1 x 106 CFU/mL | 100.00% | 90.97% | 100.00% | 39 |
| Anticoagulant, 5X nominal volume | 100.00% | 91.40% | 100.00% | 41 |
| Proteinase K, +0.6 mg/mL | 98.00% | 89.35% | 99.95% | 50 |
| Proteinase K, +0.3 mg/mL | 100.00% | 92.29% | 100.00% | 46 |
| Ethanol, +2.5% | 97.96% | 89.15% | 99.95% | 49 |
| Ethanol, +5.0% | 97.92% | 88.93% | 99.95% | 48 |
| Molecular Index barcodes, +5% | 97.22% | 85.47% | 99.93% | 36 |
| Molecular Index barcodes, +15% | 100.00% | 92.60% | 100.00% | 48 |
| Molecular Index barcodes, +30% | 100.00% | 92.75% | 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.

## 9.7   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.

## 9.8   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.

## 9.9 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.

### 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 19**. Additional non-CDx variants were evaluated as summarized in **Table 20.**

**Table 19: CDx sample set assessed for precision**

| CDx Biomarker | Targeted Alteration | Disease Ontology of Patient from which Sample was Derived |
|---|---|---|
| *ALK* rearrangements | *ALK-EML4* Rearrangement | Contrived sample |
| | *ALK-EML4* Rearrangement | Lung adenocarcinoma |
| | *ALK-NPM1* Rearrangement | Contrived sample |
| *ATM* alterations | *ATM* 5318delA | Contrived sample |
| | *ATM* I2012fs*4 | Prostate cancer |
| | *ATM* splice site 8850+1G>A | Prostate cancer |
| | *ATM-EXPH5* Truncation | Prostate cancer |
| *BRCA1* and *BRCA2* alterations | *BRCA1 E23fs*17* | Ovary cancer |
| | *BRCA1* Q780* | Ovary high grade serous carcinoma |
| | *BRCA1* Rearrangement | Unknown primary malignant neoplasm |
| | *BRCA1_2475delC* | Contrived sample |
| | *BRCA1_2612C>TT* | Contrived sample |
| | *BRCA2_3599_3600delGT* | Contrived sample |
| | *BRCA2_4284_4285insT* | Contrived sample |
| | *BRCA2_5351delA* | Contrived sample |
| | *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* exon 19 deletions and *EGFR exon 21 L858R* alterations | *EGFR E746_A750del* | Non-small cell lung carcinoma |
| | *EGFR_E746_A750del* | Contrived sample |
| | *EGFR* L858R | Contrived sample |
| | *EGFR* L858R | Non-small cell lung carcinoma (2) |
| *PIK3CA* alterations | *PIK3CA E542K* | Contrived sample |
| | *PIK3CA E542K, D549N* | Contrived sample |
| | *PIK3CA H1047R* | Contrived sample |
| | *PIK3CA E542K* | Breast carcinoma |
| | *PIK3CA E545K* | Breast carcinoma |
| | *PIK3CA H1047R* | Breast cancer |

**Table 20: Non-CDx sample set assessed for precision**

| Non-CDx Targeted Alteration | Targeted Alteration | Disease Ontology of Patient from which Sample was Derived |
|---|---|---|
| BRAF alterations | BRAF L597R | Contrived sample |
| | BRAF V600E | Contrived sample |
| | BRAF V600E | Skin melanoma |
| | BRAF V600K | Skin melanoma |
| EGFR exon 20 T790M substitution | EGFR exon 20 T790M substitution | Contrived sample |
| KRAS alterations | KRAS G12D | Contrived sample |
| | KRAS G13D | Contrived sample |
| | KRAS G12L | Colon adenocarcinoma |
| | KRAS Q61R | Colon adenocarcinoma |
| MET exon 14 alterations | MET 3029-1G>T | Contrived sample |
| | MET 3933delC | Contrived sample |
| | 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 |
| NRAS alterations | NRAS exon 2,3,4 substitutions | Contrived sample |
| PALB2 alterations | PALB2 2422G>T | Contrived sample |
| | PALB2 2724delA | Contrived sample |
| ERBB2 CNA | ERBB2 CNA | Contrived sample |
| | ERBB2 CNA | Breast carcinoma |

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 and reproducibility of CDx alterations tested at >1x LoD is summarized in

**Table** 21.

**Table 21 Repeatability and Reproducibility of CDx alterations targeted in precision study at >1x LoD\***

| Variant Type | Alteration | Repeatability [%] {95% CI [%]} | Reproducibility [%] {95% CI [%]} | Level Tested\*\* |
|---|---|---|---|---|
| ATM Short variant | ATM_5318delA | 100 (73.54, 100) | 100 (85.75, 100) | 0.77% VAF |
| | ATM_5318delA | 100 (71.51, 100) | 100 (85.18, 100) | 1.04% VAF |
| | ATM_6034_6035insCAGAAGTA | 100 (71.51, 100) | 100 (85.18, 100) | 0.86% VAF |
| | ATM_8850+1G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.56% VAF |
| ATM Rearrangement | ATM-EXPH5 Truncation | 100 (73.54, 100) | 100 (85.75, 100) | 1.13% VAF |
| BRCA1 Short variant | BRCA1_2338C>T | 100 (73.54, 100) | 100 (85.75, 100) | 1.11% VAF |
| | BRCA1_2475delC | 100 (73.54, 100) | 100 (85.75, 100) | 0.61% VAF |
| | BRCA1_2475delC | 100 (73.54, 100) | 100 (85.75, 100) | 0.93% VAF |
| | BRCA1_2612C>TT | 100 (71.51, 100) | 100 (85.18, 100) | 0.51% VAF |
| | BRCA1_68_69delAG | 100 (73.54, 100) | 100 (85.75, 100) | 0.66% VAF |
| | BRCA1_P871fs\*32 | 100 (73.54, 100) | 100 (85.75, 100) | 1.08% VAF |
| BRCA1 Rearrangement | BRCA1-BRCA1 | 100 (73.54, 100) | 100 (85.75, 100) | 0.87% VAF |

| Variant Type | Alteration | Repeatability [%] {95% CI [%]} | Reproducibility [%] {95% CI [%]} | Level Tested** |
|---|---|---|---|---|
| *BRCA2* Short Variant | *BRCA2*_3599_3600delGT | 100 (73.54, 100) | 100 (85.75, 100) | 0.58% VAF |
| | *BRCA2*_3599_3600delGT | 100 (73.54, 100) | 100 (85.75, 100) | 0.92% VAF |
| | *BRCA2*_4284_4285insT | 100 (73.54, 100) | 100 (85.75, 100) | 0.94% VAF |
| | *BRCA2*_4284_4285insT | 100 (71.51, 100) | 100 (85.18, 100) | 1.26% VAF |
| | *BRCA2*_5351delA | 100 (73.54, 100) | 100 (85.75, 100) | 1.22% VAF |
| | *BRCA2*_5351delA | 100 (73.54, 100) | 100 (85.75, 100) | 1.85% VAF |
| | *BRCA2*_5351delA | 100 (71.51, 100) | 100 (85.18, 100) | 1.07% VAF |
| | *BRCA2*_5351delA | 100 (73.54, 100) | 100 (85.75, 100) | 2.24% VAF |
| | *BRCA2*_5465_5466insA | 100 (73.54, 100) | 100 (85.75, 100) | 0.92% VAF |
| | *BRCA2*_5465_5466insA | 100 (71.51, 100) | 100 (85.18, 100) | 1.19% VAF |
| | *BRCA2*_8961_8964delGA GT | 100 (73.54, 100) | 100 (85.75, 100) | 1.07% VAF |
| | *BRCA2*_c.799G>T | 83.33 (51.59, 97.91) | 91.67 (73.0, 98.97) | 0.5% VAF |
| | *BRCA2*_c.9097_9098insA | 54.55 (23.38, 83.25) | 21.74 (7.46, 43.7) | 0.71% VAF |
| | *BRCA2*_c.9097_9098insA | 83.33 (51.59, 97.91) | 91.67 (73.0, 98.97) | 1.03% VAF |
| *BRCA2* Copy Number Loss | *BRCA2*_loss | 91.67 (61.52, 99.79) | 87.5 (67.64, 97.34) | 39.43% TF |
| *BRCA2* Rearrangement | *BRCA2*-EDA | 100 (71.51, 100) | 100 (85.18, 100) | 0.48% VAF |
| *EGFR* Short variant | *EGFR*_2369C>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.44% VAF |
| | *EGFR*_2369C>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.66% VAF |
| | *EGFR*_2369C>T | 100 (71.51, 100) | 100 (85.18, 100) | 0.36% VAF |
| | *EGFR*_2369C>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.65% VAF |
| | *EGFR*_2369C>T | 100 (73.54, 100) | 100 (85.75, 100) | 1.26% VAF |
| | *EGFR*_2573T>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.46% VAF |
| | *EGFR*_2573T>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.68% VAF |
| | *EGFR*_2573T>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.68% VAF |
| | *EGFR*_2573T>G | 100 (71.51, 100) | 100 (85.18, 100) | 0.95% VAF |
| | *EGFR*_2573T>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.64% VAF |
| | *EGFR*_2573T>G | 100 (73.54, 100) | 100 (85.75, 100) | 1.64% VAF |
| | *EGFR*_E746_A750del | 100 (73.54, 100) | 100 (85.75, 100) | 0.51% VAF |
| | *EGFR*_E746_A750del | 100 (73.54, 100) | 100 (85.75, 100) | 0.74% VAF |
| | *EGFR*_E746_A750del | 100 (73.54, 100) | 100 (85.75, 100) | 0.93% VAF |
| | *EGFR*_E746_A750del | 100 (71.51, 100) | 100 (85.18, 100) | 1.2% VAF |
| | *EGFR*_E746_A750del | 100 (71.51, 100) | 100 (85.18, 100) | 0.51% VAF |
| | *EGFR*_E746_A750del | 100 (73.54, 100) | 100 (85.75, 100) | 1.01% VAF |
| | *EGFR*_E746_A750del | 100 (71.51, 100) | 100 (84.56, 100) | 0.34% VAF |
| *PIK3CA* Short variant | *PIK3CA*_1624G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.89% VAF |
| | *PIK3CA*_1633G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.45% VAF |
| | *PIK3CA*_1633G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.66% VAF |
| | *PIK3CA*_1633G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.5% VAF |
| | *PIK3CA*_1634A>C | 100 (73.54, 100) | 100 (85.75, 100) | 0.52% VAF |
| | *PIK3CA*_1634A>C | 100 (71.51, 100) | 100 (85.18, 100) | 0.70% VAF |
| | *PIK3CA*_1637A>G | 90.91 (58.72, 99.77) | 95.65 (78.05, 9.89) | 0.49% VAF |
| | *PIK3CA*_1637A>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.92% VAF |
| | *PIK3CA*_1645G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.48% VAF |
| | *PIK3CA*_1645G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.73% VAF |
| | *PIK3CA*_3140A>G | 100 (71.51, 100) | 100 (85.18, 100) | 0.41% VAF |
| | *PIK3CA*_3140A>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.76% VAF |
| | *PIK3CA*_3140A>G | 100 (73.54, 100) | 100 (85.75, 100) | 1.04% VAF |
| *ALK* Rearrangement | *ALK*_EML4 | 100 (73.54, 100) | 100 (85.75, 100) | 0.64% VAF |
| | *ALK*_EML4 | 100 (71.51, 100) | 100 (85.18, 100) | 0.89% VAF |
| | *ALK*_EML4 | 100 (73.54, 100) | 100 (85.75, 100) | 1.39% VAF |
| | *ALK*-NPM1 | 100 (73.54, 100) | 100 (85.75, 100) | 0.64% 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

As observed in the

**Table** 21 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 repeatability and reproducibility of non-CDx alterations tested at ≥1x LoD are summarized in **Table 22**.

**Table 22: Repeatability and Reproducibility of non-CDx alterations targeted in precision study at ≥1x LoD**

| Variant Type | Alteration | Repeatability [%] {95% CI [%]) | Reproducibility [%] {95% CI [%]) | Level Tested |
|---|---|---|---|---|
| *BRAF* Short variant | BRAF_1790T>G | 90.91 (58.72, 99.77) | 95.65 (78.88, 99.89) | 0.42% VAF |
| | BRAF_1790T>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.85% VAF |
| | BRAF_1798_1799GT>AA | 91.67 (61.52, 99.79) | 95.83 (78.88, 99.89) | 0.36% VAF |
| | BRAF_1799T>A | 100 (71.51, 100) | 100 (85.18, 100) | 0.72% VAF |
| | BRAF_1799T>A | 100 (73.54, 100) | 100 (85.75, 100) | 1.38% VAF |
| | BRAF_1799T>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.44% VAF |
| *KRAS* Short variant | KRAS_182A>G | 100 (73.54, 100) | 100 (85.75, 100) | 0.53% VAF |
| | KRAS_34_35GG>CT | 100 (73.54, 100) | 100 (85.75, 100) | 0.49% VAF |
| | KRAS_35G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.89% VAF |
| | KRAS_35G>A | 100 (71.51, 100) | 100 (85.18, 100) | 1.12% VAF |
| | KRAS_38G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.55% VAF |
| | KRAS_38G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.82% VAF |
| | KRAS_38G>A | 100 (71.51, 100) | 100 (85.18, 100) | 0.57% VAF |
| | KRAS_38G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.92% VAF |
| *MET* Short variant | MET_2888-17_2888-3del15 | 100 (73.54, 100) | 100 (85.75, 100) | 1.17% VAF |
| | MET_3005_3028+3>C | 100 (73.54, 100) | 100 (85.75, 100) | 1.67% VAF |
| | MET_3029-1G>T | 81.82 (48.22, 97.72) | 91.30 (71.96, 98.93) | 0.30% VAF |
| | MET_3933delC | 100 (73.54, 100) | 100 (85.75, 100) | 0.69% VAF |
| | MET_3933delC | 100 (73.54, 100) | 100 (85.75, 100) | 0.96% VAF |
| *NRAS* Short variant | NRAS_34G>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.69% VAF |
| | NRAS_34G>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.96% VAF |
| | NRAS_35G>A | 100 (73.54, 100) | 100 (85.75, 100) | 0.84% VAF |
| | NRAS_c.35G>A | 63.64 (30.79, 89.07) | 82.61 (61.22, 95.05) | 0.48% VAF |

| Variant Type | Alteration | Repeatability [%] {95% CI [%]) | Reproducibility [%] {95% CI [%]) | Level Tested |
|---|---|---|---|---|
| *PALB2* Short variant | PALB2_2422G>T | 100 (71.51, 100) | 100 (85.18, 100) | 0.47% VAF |
| | PALB2_2422G>T | 100 (73.54, 100) | 100 (85.75, 100) | 0.92% VAF |
| | PALB2_2724delA | 100 (73.54, 100) | 100 (85.75, 100) | 0.52% VAF |
| | PALB2_2724delA | 100 (73.54, 100) | 100 (85.75, 100) | 0.74% VAF |
| *ERBB2* CN Amplification | *ERBB2* amplification | 100 (73.54, 100) | 100 (85.75, 100) | 35.78% VAF |
| | *ERBB2* amplification | 100 (73.54, 100) | 100 (85.75, 100) | 39.79% VAF |
| | *ERBB2* amplification | 100 (73.54, 100) | 100 (85.75, 100) | 61.73% VAF |

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

**Precision of Platform Variants**

Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,240 variants were evaluated with variant types including substitutions, indels, rearrangements, and copy number alterations. The number of variants in each variant bin are summarized in **Table 23.**

**Table 23: 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** |
| 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 |
| **Rearrangements** | **60** |
| **Copy Number Alterations** | **54** |
| Copy Number Amplification | 49 |
| Copy Number Loss | 5 |
| **Total** | **1240** |

The overall repeatability for all variants were 99.47% with 95% 2-sided exact CIs (99.45%, 99.48%). The repeatability result for each variant type are summarized in **Table 24.**

**Table 24: 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) |
| Rearrangements | 33105 | 33480 | 98.88 | (98.76, 98.99) |
| Copy Number Alterations | 29880 | 30132 | 99.16 | (99.05, 99.26) |

The overall reproducibility results were 99.59% with the 95% 2-sided exact CIs (99.58%, 99.60%). The reproducibility result for each variant type are summarized in **Table 25.**

**Table 25: 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) |
| Rearrangements | 66723 | 67260 | 99.20 | (99.13, 99.27) |
| Copy Number Alterations | 60115 | 60534 | 99.31 | (99.24, 99.7) |

**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 26** and

**Table** 27. 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 26.** A summary of the Confirmation of LoD and Precision results for CDx variants are provided in

**Table** 27.

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

| Target Alteration | LoD | Mean Level Tested2 | 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) |
| *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% VAF1 | 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% VAF1 | 0.48% VAF | 100 | (85.18, 100) |
| *EGFR E746_A750del* | 0.27% VAF | 0.34% VAF | 100 | (84.56, 100) |
| *EGFR* L858R | 0.34% VAF | 1.64% VAF | 100 | (85.75, 100) |
| *EGFR* L858R | 0.34% VAF | 0.64% 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) |
| *ALK-EML4* Rearrangement | 0.24% MAF | 1.39 %MAF | 100 | (85.75, 100) |

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

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

| Target Alteration | LoD | Mean Level Tested1 | Reproducibility (%) | 95% Two-sided exact CIs (%) |
|---|---|---|---|---|
| *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) |
| *ERBB2* CNA | 19.8% TF | 61.73% TF | 100 | (85.75, 100) |

*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 28**.

**Table 28: CDx variant confirmation of LoD and precision in clinical specimens**

| Target Alteration | LoD | Mean Level Tested1 | 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%] |
| *PIK3CA* 1637A>G | 0.34% | 0.44% | 91.7% | [74.2%, 97.7%] |

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

As summarized in **Table 28** 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** 29.

**Table 29: Non-CDx variant confirmation of LoD and precision in a clinical specimen**

| Target Alteration | LoD | Mean Level Tested1 | Reproducibility (95% CI) | 95% CIs (%) |
|---|---|---|---|---|
| *BRCA2* 8524C>T | 0.37% | 0.57% | 100% | [85.7%, 100%] |

| | | | | | |
|---|---|---|---|---|---|
| *NRAS* 34G>T | 0.42% | 0.55% | 91.3% | [73.2%, 97.6%] | |

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

## 9.10  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.

## 9.11  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%).

## 9.12  Stability

### 9.12.1    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 30**. 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 30: Concordance analysis between 3 months and baseline**

| | Reagent Lot | Timepoint | | Total # Replicates | Concordance Percentage | 95% C.I. | |
|---|---|---|---|---|---|---|---|
| | Lot #1 | 1 | 1921 | 1966 | 97.71% | 96.95% | 98.28% |
| | Lot #2 | 1 | 2083 | 2148 | 96.97% | 96.16% | 97.62% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variant Calls** | Lot #3 | 1 | 2086 | 2139 | 97.52% | 96.77% | 98.10% |

### 9.12.2 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 31.**

**Table 31: 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** 32). 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 32: 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%, 97.2%] |
| | Inverted | 147 | 150 | 98% | [94.3%, 99.3%] |
| LC | Upright | 135 | 136 | 99.3% | [96%, 99.9%] |
| | Inverted | 146 | 146 | 100% | [97.4%, 100%] |

| | | | | | |
|---|---|---|---|---|---|
| HC | Upright | 134 | 135 | 99.3% | [95.9%, 99.9%] |
| | Inverted | 143 | 146 | 97.9% | [94.1%, 99.3%] |
| 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 33**.

**Table 33: Concordance of detected alterations between baseline sample and experimental conditions for inverted tube stability study**

| Variant Type | Temp. | Tube Position | Exp. Time Point | N Variants Detected at Baseline Time Point | N Variants Detected at Exp. Time Point | N Variants Agree | 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 | 49 | 98% | [89.5%, 99.6%] | 612 | 612 | 100% | [100%, 100%] |
| **Short variants** | 04°C | Upright | Day 10 | 50 | 51 | 50 | 100% | [92.9%, 100%] | 613 | 612 | 100% | [100%, 100%] |
| **Short variants** | 04°C | Inverted | Day 14 | 59 | 58 | 58 | 98.3% | [90.9%, 99.7%] | 610 | 611 | 100% | [100%, 100%] |
| **Short variants** | 04°C | Upright | Day 14 | 44 | 44 | 44 | 100% | [92.0%, 100%] | 611 | 611 | 100% | [100%, 100%] |
| **Short variants** | 04°C | Inverted | Day 15 | 37 | 37 | 37 | 100% | [90.6%, 100%] | 611 | 611 | 100% | [100%, 100%] |
| **Short variants** | 04°C | Upright | Day 15 | 52 | 52 | 52 | 100% | [93%, 100%] | 611 | 611 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Inverted | Day 10 | 78 | 77 | 76 | 97.1% | [91.1%, 99.2%] | 627 | 628 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Upright | Day 10 | 44 | 44 | 44 | 100% | [92.0%, 100%] | 613 | 613 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Inverted | Day 14 | 46 | 48 | 46 | 100% | [92.3%, 100%] | 611 | 609 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Upright | Day 14 | 42 | 41 | 41 | 97.6% | [87.7%, 99.6%] | 610 | 611 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Inverted | Day 15 | 44 | 44 | 44 | 100% | [92.0%, 100%] | 613 | 613 | 100% | [100%, 100%] |
| **Short variants** | 25°C | Upright | Day 15 | 49 | 48 | 48 | 97.8% | [89.3%, 99.6%] | 616 | 617 | 100% | [100%, 100%] |

| Variant Type | Temp. | Tube Position | Exp. Time Point | N Variants Detected at Baseline Time Point | N Variants Detected at Exp. Time Point | N Variants Agree | 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** | 35°C | Inverted | Day 10 | 15 | 15 | 15 | 100% | [79.6%, 100%] | 609 | 609 | 100% | [100%, 100%] |
| **Short variants** | 35°C | Upright | Day 10 | 35 | 35 | 35 | 100% | [90.1%, 100%] | 609 | 609 | 100% | [100%, 100%] |
| **Short variants** | 35°C | Inverted | Day 14 | 55 | 55 | 55 | 100% | [93.4%, 100%] | 611 | 611 | 100% | [100%, 100%] |
| **Short variants** | 35°C | Upright | Day 14 | 48 | 47 | 46 | 95.7% | [86.0%, 98.8%] | 609 | 610 | 100% | [100%, 100%] |
| **Short variants** | 35°C | Inverted | Day 15 | 39 | 39 | 38 | 97.4% | [86.8%, 99.5%] | 610 | 610 | 100% | [100%, 100%] |
| **Short variants** | 35°C | Upright | Day 15 | 28 | 29 | 28 | 100% | [87.9%, 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.

## 9.13  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** 34) 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 35**).

**Table 34: 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 35: 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.

### 9.14  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 36**).

**Table 36: Summary of process pass and failure rate at each guard banding DNA input level**

| Process | Input Level | | # of Pass | Pass Rate (%) |
|---|---|---|---|---|
| | -33% | 20ng | 20/20 | 100 |
| | -20% | 24ng | 20/20 | 100 |
| | Recommended lower limit | 30ng | 20/20 | 100 |
| | Low input | 45ng | 20/20 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| LC | 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.

## 9.15  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 37** 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 37. F1L/FACT samples per tumor type and pass rates**

| Tumor Type | Sample Size | DNA Extraction Pass Rate (≥25 ng$^2$) | DNA Extraction Pass Rate (≥36 ng$^1$) | LC Yield Pass Rate | HC Yield Pass Rate | Median Coverage Pass Rate | Overall Pass Rate (≥36 ng$^1$) | Overall Pass Rate (≥25 ng$^2$) |
|---|---|---|---|---|---|---|---|---|
| Rare Tumors | 1164 | 97.0% | 86.4% | 99.9% | 100.0% | 93.8% | 94.0% | 97.0% |
| Biliary Cancer | 171 | 99.4% | 95.3% | 100.0% | 100.0% | 98.8% | 97.1% | 99.4% |
| Bladder Cancer | 166 | 97.6% | 85.5% | 100.0% | 100.0% | 93.2% | 98.8% | 97.6% |

| Tumor Type | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Breast Cancer | 2775 | 97.6% | 87.7% | 99.9% | 100.0% | 96.4% | 95.3% | 97.6% |
| Cholangiocarcinoma | 377 | 98.9% | 96.0% | 99.7% | 100.0% | 98.7% | 96.8% | 98.9% |
| Colorectal Cancer (CRC) | 1640 | 98.5% | 92.4% | 99.9% | 100.0% | 97.5% | 96.9% | 98.5% |
| Endocrine-Neuro Cancer | 75 | 100.0% | 85.3% | 100.0% | 100.0% | 100.0% | 93.3% | 100.0% |
| Endometrial Cancer | 231 | 98.3% | 88.3% | 100.0% | 100.0% | 96.5% | 95.6% | 98.3% |
| Esophagus Cancer | 291 | 99.7% | 92.4% | 100.0% | 100.0% | 97.6% | 96.6% | 99.7% |
| Glioma Cancer | 59 | 94.9% | 72.9% | 100.0% | 100.0% | 100.0% | 76.8% | 94.9% |
| Head and Neck Cancer | 154 | 96.1% | 81.8% | 100.0% | 100.0% | 89.2% | 95.3% | 96.1% |
| Kidney Cancer | 203 | 99.0% | 87.7% | 100.0% | 100.0% | 95.0% | 95.0% | 99.0% |
| Liver Cancer | 109 | 98.2% | 95.4% | 100.0% | 100.0% | 100.0% | 95.3% | 98.2% |
| Lung Non-Small Cell Lung Carcinoma (NSCLC) | 5919 | 98.2% | 88.8% | 99.8% | 100.0% | 95.5% | 95.4% | 98.2% |
| Melanoma | 257 | 96.5% | 79.8% | 100.0% | 100.0% | 92.7% | 93.1% | 96.5% |
| Ovary Cancer | 496 | 97.8% | 88.5% | 100.0% | 100.0% | 95.9% | 94.2% | 97.8% |
| Pancreas Cancer | 1359 | 98.8% | 94.0% | 99.9% | 100.0% | 97.8% | 95.5% | 98.8% |
| Peripheral Nervous System (PNS) | 44 | 100.0% | 90.9% | 100.0% | 100.0% | 100.0% | 93.2% | 100.0% |
| Prostate Cancer | 1778 | 97.3% | 87.7% | 99.9% | 100.0% | 96.9% | 95.1% | 97.3% |
| Small Cell Cancer | 135 | 98.5% | 93.3% | 100.0% | 100.0% | 99.2% | 99.2% | 98.5% |
| Soft Tissue Sarcoma | 130 | 97.7% | 83.1% | 100.0% | 100.0% | 95.3% | 92.1% | 97.7% |
| Stomach Cancer | 267 | 98.9% | 89.1% | 100.0% | 100.0% | 98.1% | 93.2% | 98.9% |
| Thyroid Cancer | 50 | 98.0% | 86.0% | 100.0% | 100.0% | 100.0% | 81.6% | 98.0% |
| Unspecified | 856 | 98.5% | 89.1% | 100.0% | 100.0% | 95.5% | 96.3% | 98.5% |
| Unknown Primary Carcinoma (CUP) | 1162 | 98.1% | 89.7% | 100.0% | 100.0% | 95.2% | 95.7% | 98.1% |

[1] 36 ng of extracted cfDNA allows for sufficient cfDNA to process 30 ng of cfDNA
[2] 25 ng of extracted cfDNA allows for sufficient cfDNA to process 20 ng of cfDNA

**Table 38** 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 38: 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%) |

## 9.16  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 39**.

**Table 39. 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%] |
| Copy Number Amplifications | 173 | 32 | 0 | 59463 | 84.4% [78.7%-89.1%] | 99.8% [>99.9%-100.0%] | 99.8% [>99.9%-100.0%] |
| 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, rearrangement, and copy number amplification PPA values, with 95% two-sided CI, were: 95.9% [95.1%-96.6%], 88.0% [82.1%-92.5%], and 84.4% [78.7%-89.1%]. 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.

### 9.17  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.

### 9.18  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.

# 10  Clinical Validation Studies

## 10.1  Clinical Bridging Study: Detection of *ALK* Rearrangements to Determine Eligibility for Treatment with Alectinib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) harboring *ALK* rearrangements for treatment with alectinib was assessed through a clinical bridging study using screening (i.e., pre-alectinib treatment) plasma samples from Cohort A of the Blood First Assay Screening Trial (BFAST, BO29554).

The BFAST trial is a Phase II/III multicenter study, in which Cohort A evaluated the safety and efficacy of alectinib as a treatment for patients with advanced or metastatic NSCLC who tested positive for an *ALK* rearrangement as determined by a blood-based NGS assay (CTA).

The concordance between FoundationOne Liquid CDx and the CTA was evaluated as summarized in **Table** .

**Table 40**: **Concordance between FoundationOne Liquid CDx and the CTA for the detection of** *ALK* **rearrangements**

|  | CTA Pos | CTA Neg | Total |
|---|---|---|---|
| **FoundationOne Liquid CDx Positive** | 63 | 0 | 63 |
| **FoundationOne Liquid CDx Negative** | 12 | 174 | 186 |
| **Missing** | 4 | 9 | 13 |
| **Total** | 79 | 183 | 262 |

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 84.0% [73.7%, 91.4%]
- NPA [95% CI] : 100% [ 97.9%, 100.0%]

After adjusting for a 5% prevalence of *ALK* rearrangements in the intended use population, the Positive Predictive Value (PPV), and Negative Predictive Value (NPV) calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [95% CI]: 100.0% [94.3%, 200.0%]
- NPV [95% CI]: 93.5% [89.0%, 96.6%]

The estimated Overall Response Rate (ORR) and the corresponding 95% confidence intervals was 88.9% [78.4%, 95.4%] for the FoundationOne Liquid CDx *ALK*-positive population which is comparable with the observed ORR and the corresponding 95% confidence intervals of 87.4% [78.5%, 93.5%] for the CTA *ALK*- positive population (BFAST Cohort A).

A sensitivity analysis was performed to estimate the clinical efficacy of treating patients with alectinib when considering missing FoundationOne Liquid CDx results. The estimated ORR and the corresponding 95% confidence intervals were 90.4% [90.1%, 90.6%] for the patient population that are both CTA *ALK+* and FoundationOne Liquid CDx *ALK+*, demonstrating the robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

## 10.2  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** .

**Table 41: Agreement analysis results for EGFR exon 19 deletion and L858R separately.**

| Exon 19 deletion | PPAC1F | 95.5% | NPAC1F | 95.6% |
|---|---|---|---|---|
| | PPAC1C2 | 97.7% | NPAC1C2 | 98.9% |
| | PPAC2F | 95.5% | NPAC2F | 96.0% |
| | PPAC2C1 | 96.2% | NPAC2C1 | 99.4% |

| L858R | PPAC1F | 100.0% | NPAC1F | 95.6% |
|---|---|---|---|---|
| | PPAC1C2 | 92.9% | NPAC1C2 | 98.9% |
| | PPAC2F | 100.0% | NPAC2F | 94.7% |
| | PPAC2C1 | 96.0% | NPAC2C1 | 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** .

**Table 42: 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 .**

**Table 43: 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 .**

**Table 44: Point estimate and one-Sided 95% upper confidence limit of ζPPA1, ζNPA1, ζPPA2, and ζNPA**

|  | Point Estimate | Mean one-sided 95% upper confidence limit |
|---|---|---|
| **ζPPA1** | -2.3% | 2.3% |
| **ζNPA1** | 3.3% | 6.6% |
| **ζPPA2** | -1.6% | 4.7% |
| **ζNPA2** | 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.

## 10.3  Clinical Bridging Study: Detection of *BRCA1/BRCA2/ATM* Alterations to Determine Eligibility for Treatment with Olaparib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castrate-resistant prostate cancer (mCRPC) harboring *BRCA1, BRCA2 or ATM* alterations for treatment with olaparib was assessed through a clinical bridging study using screening (i.e., pre-olaparib treatment) plasma samples from Cohort A of the PROfound trial.

The PROfound trial is a Phase III, open label, randomized study to assess the efficacy and safety of olaparib (Lynparza™) versus enzalutamide or abiraterone acetate in men with metastatic castration-resistant prostate cancer who have failed prior treatment with a new hormonal agent and have homologous recombination repair gene mutations. Only Cohort A patients with either *BRCA1*, *BRCA2* or *ATM* mutations were tested with the FoundationOne Liquid CDx assay.

In total, 4425 patients were screened and 387 (9.6%) were randomized into the PROfound study by the CTA. Of these 387 patients, 245 patients were randomized in cohort A. 181 out of the 245 randomized patients in cohort A both consented to the use of their sample for ctDNA CDx development and had a plasma sample available for testing. In total, 181/245 (73.9%) of the Cohort A patients were tested using the FoundationOne Liquid CDx assay. Of these, 139 (76.8%) Cohort A patients had a successful FoundationOne Liquid CDx test result and 42 Cohort A patients had a failed FoundationOne Liquid CDx test result. This represents 56.7% (139/245) of total Cohort A patients with a FoundationOne Liquid CDx result. In addition, 250 non-HRRm patient samples were randomly selected for ctDNA testing from the screen-failed population to determine the NPA/NPV of the

FoundationOne Liquid CDx assay. 194/250 (77.6%) screen failed non-HRRm patients were successfully tested using the FoundationOne Liquid CDx assay.

Of the 139 successfully tested Cohort A patients, 111 patients were reported as *BRCA1/BRCA2/ATM* mutation positive and 28 randomized patients were reported as biomarker negative by FoundationOne Liquid CDx. Therefore, the FoundationOne Liquid CDx ctDNA biomarker positive subgroup comprises 111 patients with *BRCA1, BRCA2, and/or ATM* mutations.

Sample accountability for this clinical bridging study is summarized in **Table 45**.

**Table 45: Sample accountability for olaparib clinical bridging study**

| Description | Number of patients |
|---|---|
| Patients randomized into PROfound | 387 |
| Patients with qualifying *BRCA1, BRCA2,* or *ATM* alterations (Cohort A) | 245 |
| Cohort A patients with samples tested by FoundationOne Liquid CDx | 181 |
| FoundationOne Liquid CDx results available | 139 |
| Cohort A patients, biomarker positive by FoundationOne Liquid CDx | 111 |

**Table 46** shows the agreement analysis between CLIA CTA (tissue test) and the FoundationOne® Liquid CDx results for PROfound patients, including Invalid and Not Tested results

**Table 46: Summary of agreement analyses for FoundationOne® Liquid CDx compared against CTA tissue test, including Invalid and Not Tested results**

| | | CTA Results (n=495) | |
|---|---|---|---|
| | | Biomarker[a] positive | Biomarker[a] negative |
| **FMI F1 Liquid CDx assay** | **Biomarker[a] positive** | 111 | 16 |
| | **Biomarker[a] negative** | 28 | 178 |
| | **Biomarker[a] Invalid** | 42 | 56 |
| | **Not Tested** | 64 | 0 |
| | | | |
| **Agreement analyses (only Valid results included)** | **PPA (95% CI[b])** | 79.9 (72.2, 86.2) [111/139] | |
| | **NPA (95% CI[b])** | 91.8 (87.0, 95.2) [178/194] | |
| | **OPA (95% CI[b])** | 86.8 (82.7, 90.2) [289/333] | |
| | **PPV (95% CI[b])** | 66.6 (56.0, 77.2) | |
| | **NPV (95% CI[b])** | 95.7 (94.3, 97.1) | |

[a] Biomarker refers to patients with eligible *BRCA/ATM* mutations
[b] Confidence intervals calculated using Clopper-Pearson method

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:
- PPA [95% CI]: 79.9% [72.2%, 86.2%]
- NPA [95% CI] : 91.8% [87.0%, 95.2%]

After adjusting for a 17.1% prevalence of BRCA1/2 and ATM alterations in the intended use population, the Positive Predictive Value (PPV), and Negative Predictive Value (NPV) calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [95% CI]: 66.6% [56.0%, 77.2%]
- NPV [95% CI]: 95.7% [94.3%, 97.1%]

The estimated radiological progression-free survival (rPFS) hazard ratio (HR) and the corresponding 95% confidence intervals were 0.331 [0.21, 0.53] for the FoundationOne Liquid CDx biomarker positive population, which were comparable with the observed rPFS HR and the corresponding 95% confidence intervals of 0.34 [0.25, 0.47] for the CTA biomarker positive population (PROfound Cohort A).

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 in All Patients. After imputing the missing FoundationOne Liquid CDx results, the median rPFS HR and corresponding [95% CI] across the imputed datasets was 0.44 [0.32, 0.59], demonstrating robustness of the analysis to missing FoundationOne Liquid CDx results.

## 10.4 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**
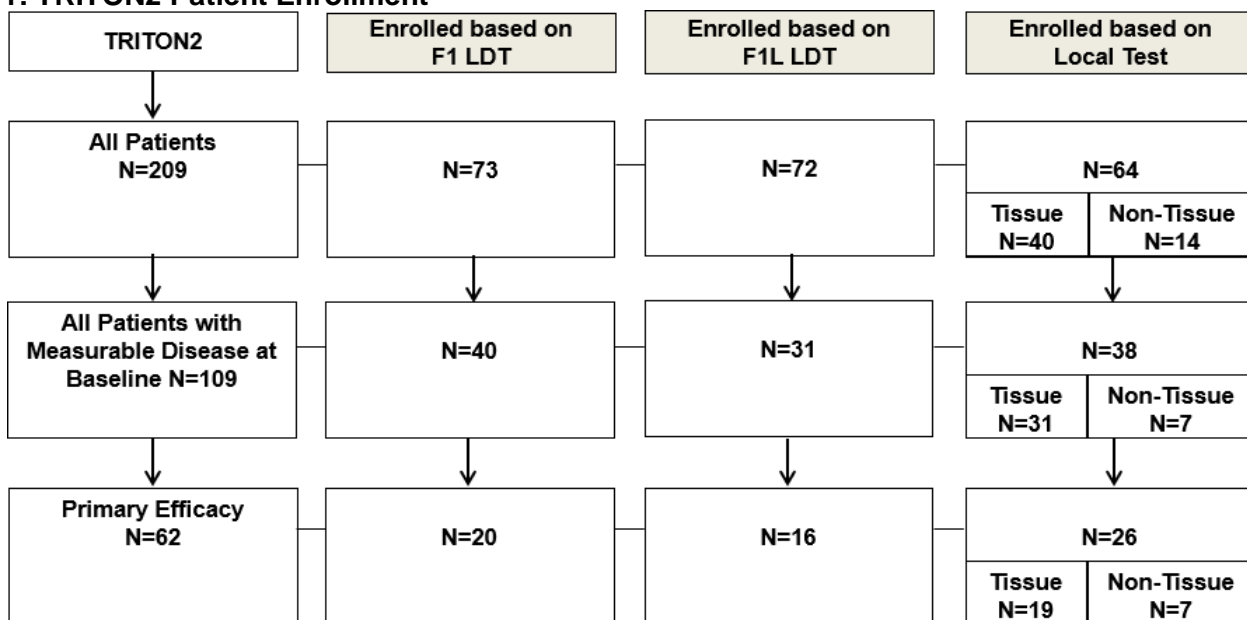
Pre-rucaparib treatment plasma samples were available for 92% (192/209) of the patients. FoundationOne Liquid CDx data were available for 93% (178/192) of the patients with samples tested; inadequate input material resulted in FoundationOne Liquid CDx test data being unavailable for 14 patients. In total, 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** .

**Table 45: 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 |

**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**  and

**Table** .

**Table 46: 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 47: 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%]

**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** ).

**Table 48: ORR 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.

## 10.5 Clinical Validation Study: Detection of *BRCA1* and *BRCA2* Alterations to Determine Eligibility of Ovarian Cancer Patients for Treatment with Rucaparib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with rucaparib was demonstrated using pre- rucaparib treatment blood samples from the ARIEL2 study.

The bridging study was conducted to evaluate: 1) the concordance between *BRCA1* and *BRCA2* alteration status by the 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.

The ARIEL2 study is complete and enrolled 491 patients (All Patients). Pre-rucaparib treatment plasma samples were available for 55% (271/491) of patients dosed in ARIEL2. FoundationOne Liquid CDx data were available for

80% (217/271) of the patients with samples tested; 49 failures were due to insufficient remaining plasma volume or insufficient DNA extraction yield. In total, FoundationOne Liquid CDx results were available for 44% (217/491) of All Patients.

Of the 64 patients in the primary efficacy population, FoundationOne Liquid CDx results were available for 42% (27/64) and used for concordance and efficacy analyses. The sample accountability for this clinical validation study is summarized in **Table 49**.

**Table 49: Sample accountability for rucaparib ovarian clinical bridging study**

| Description | Number |
|---|---|
| All Patients | 491 |
| Total samples available | 271 |
| Patients with FoundationOne Liquid CDx data (All Patients) | 217 |
| Patients with FoundationOne Liquid CDx data (Primary Efficacy Population) | 27 |

The concordance between FoundationOne Liquid CDx and CTA test results was evaluated in All Patients and is summarized in

**Table** 50. The Primary Efficacy Population is summarized in **Table 51.**

**Table 50: Concordance between FoundationOne Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in All Patients**

| | CTA Positive | CTA Negative | Total |
|---|---|---|---|
| **FoundationOne Liquid CDx Positive** | 60 | 4 | 64 |
| **FoundationOne Liquid CDx Negative** | 4 | 149 | 153 |
| **Missing** | 60 | 214 | 274 |
| **Total** | 124 | 367 | 491 |

The PPA and NPA between FoundationOne Liquid CDx and the CTA were determined using the CTA as the reference for All Patients:
- PPA [95% CI]: 93.8% [84.8%, 98.3%]
- NPA [95% CI]: 97.4% [93.4%, 99.3%]

**Table 51: Concordance between FoundationOne Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in the primary efficacy population**

| | CTA Positive | CTA Negative | Total |
|---|---|---|---|
| **FoundationOne Liquid CDx Positive** | 26 | 0 | 26 |
| **FoundationOne Liquid CDx Negative** | 0 | 1 | 1 |
| **Missing** | 35 | 2 | 37 |
| **Total** | 61 | 3 | 64 |

The PPA and NPA between FoundationOne Liquid CDx and the CTA were determined using the CTA as the reference for the Primary Efficacy Population:

- PPA [95% CI]: 100% [86.8%, 100.0%]
- NPA [95% CI]: 100% [ 2.5%, 100.0%]

*BRCA1* and *BRCA2* alteration status was verified retrospectively by FoundationOne Liquid CDx in 41% (26/64) of the patients in the Primary Efficacy Population.

The ORR [95% CI] in the primary efficacy population was 53.8% [33.4%-73.4%] in *BRCA* Positive patients as determined by FoundationOne Liquid CDx, which is comparable to the ORR of 54.1% [40.8%-66.9%] in patients identified by the CTA **(Table 52)**.

The median DOR [95% CI] was 225 days [115, 403] in FoundationOne Liquid CDx *BRCA* Positive patients from the Primary Efficacy Population. This is similar to the median DOR of 288 days [170, 403] for the Primary Efficacy Population in *BRCA* Positive patients by the CTA.

**Table 52: ORR and duration of response in the primary efficacy population by CTA and FoundationOne Liquid CDx test results**

|  | FoundationOne Liquid CDx *BRCA* Positive n = 26 | CTA *BRCA* Positive n = 61 |
|---|---|---|
| **Confirmed ORR (CR + PR), % (n)** | 53.8% (14) | 54.1% (33) |
| 95% CI | 33.4%, 73.4% | 40.8%, 66.9% |
| **Duration of Response (days)** |  |  |
| Median | 225 | 288 |
| 95% CI | 115 – 403 | 170 – 403 |

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.

The ORR [95% CI] in All Patients was evaluated for *BRCA* Positive and *BRCA* Negative patients. The ORR in *BRCA* Positive patients identified from FoundationOne Liquid CDx was 40.6% [28.5%-53.6%] compared to the ORR of 46.8% [37.8%-55.9%] in *BRCA* Positive patients based on the CTA. The ORR in *BRCA* Negative patients by FoundationOne Liquid CDx and the CTA was 5.9% [2.7%-10.9%] and 13.1% [9.8%-17.0%], respectively.

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 in All Patients. After imputing the missing FoundationOne Liquid CDx results, the weighted ORR [95% CI] across the imputed datasets was 45.2% [36.3%-54.1%].

## 10.6  Clinical Bridging Study: Detection of *PIK3CA* Alterations to Determine Eligibility for Treatment with Alpelisib

Clinical validity of using FoundationOne Liquid CDx to identify breast cancer patients harboring *PIK3CA* alterations eligible for treatment with alpelisib was assessed through retrospective testing of plasma samples collected prior to study treatment from advanced or metastatic breast cancer patients enrolled in clinical trial CBYL719C2301 (SOLAR-1). A total of 395 patients were enrolled based on CTA1 results and 177 patients were enrolled based on CTA2 results. All 395 patients enrolled based on CTA1 results were retrospectively tested by CTA2. This clinical bridging study was performed based on CTA2 results.

Samples with ≥30 ng from 375 patients were tested by FoundationOne Liquid CDx. Excluding those with invalid results for either CTA2 or CDx (4 and 12, respectively), the primary efficacy analyses were conducted using data

from the 359 subjects who were CTA2-evaluable and CDx-evaluable. A concordance analysis was conducted with the CTA2-evaluable and FoundationOne Liquid CDx-evaluable samples as summarized in Error! Reference source not found.**.**

**Table 53: Concordance between FoundationOne Liquid CDx and CTA2**

| CDx | CTA2 | | | |
|---|---|---|---|---|
| | **+** | **-** | **Invalid** | **Total** |
| **+** | 165 | 0 | 1 | 166 |
| **-** | 65 | 129 | 3 | 197 |
| **Invalid** | 7 | 5 | 0 | 12 |
| **Total** | 237 | 134 | 4 | 375 |

Samples not tested are excluded from the analysis.
Samples tested with cfDNA input < 30 ng are excluded from the analysis.

The point estimates of PPA and NPA between FoundationOne Liquid CDx and the CTA2 assay and the corresponding 95% confidence intervals were:
- PPA [95% CI]: 71.7% [65.4%, 77.5%]
- NPA [95% CI]: 100% [97.2%, 100%]

The primary efficacy analysis in the *PIK3CA* alteration positive population identified by FoundationOne Liquid CDx was based on PFS by local investigator assessment per RECIST 1.1 criteria. Clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne Liquid CDx-positive population with cfDNA input ≥30 ng (N=165) was demonstrated with an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm (HR = 0.46, 95% CI: 0.30, 0.70).

As summarized in Error! Reference source not found., the PFS hazard ratio for the 165 tissue CTA2-positive, FoundationOne Liquid CDx- positive patients was 0.46 (95% CI: 0.30, 0.70). Median PFS was 11.0 months for the alpelisib plus fulvestrant arm versus 3.6 months for the placebo plus fulvestrant arm.

**Table 57: Progression-free survival in the CTA2-positive, FoundationOne Liquid CDx-positive patients (primary analysis set)**

| Progression free survival (months) | Alpelisib 300mg qd + Fulvestrant N=84 | Placebo qd + Fulvestrant N=81 | HR (95% CI) Alpelisib 300mg qd + Fulv /Placebo qd + Fulv[1] |
|---|---|---|---|
| No of events (%) | 54 (64.3) | 67 (82.7) | 0.46 (0.30, 0.70) |
| PD (%) | 52 (61.9) | 61 (75.3) | |
| Death (%) | 2 (2.4) | 6 (7.4) | |
| No of censored (%) | 30 (35.7) | 14 (17.3) | |
| Median (95% CI)[2] | 11.0 (7.3, 15.9) | 3.6 (2.4, 5.8) | |

[1] Hazard ratio (HR) estimated using Cox regression model stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor, and adjusted for clinically relevant covariates, as well as the imbalanced covariates.

[2] The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982).
   CDx results from samples tested with cfDNA input < 30 ng are treated as missing.
   PD = progressive disease

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multivariate imputation by chained equations (MICE) method. After imputing the missing FoundationOne Liquid CDx results, the hazard ratio was estimated to be 0.63 (95% CI: 0.45, 0.87), demonstrating robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

# 11 CDx Classification Criteria

## 11.1 CDx classification criteria for *ALK* rearrangements, qualifying NSCLC patients for therapy with ALECENSA® (alectinib):

- The *ALK* rearrangement must have pathogenic driver status (FMI driver status of "known" or "likely")

- AND the disease type must be NSCLC

- AND one of the following two conditions must hold:

  1. The partner gene is *EML4*, or

  2. The *ALK* breakpoint occurs within *ALK* intron 19

## 11.2 CDx classification criteria for *EGFR* alterations, qualifying NSCLC patients for therapy with IRESSA® (gefitinib), TAGRISSO® (osimertinib), TARCEVA® (erlotinib):

- Base substitutions resulting in *EGFR* L858R

- In-frame deletions occurring within *EGFR* exon 19

## 11.3 CDx classification criteria for *BRCA1*, *BRCA2*, and *ATM* alterations, qualifying prostate cancer patients for therapy with LYNPARZA® (olaparib):

**Table 57**, **Table 58,** and **Table 59** describe the criteria for classifying *BRCA1*, *BRCA2, or ATM* alterations known to be deleterious to protein function

### Table 57: Classification Criteria for *BRCA1, BRCA2,* and *ATM*

| Deleterious Variant Criteria | Sequence Classification | CDx Classifier Methodology |
|---|---|---|
| A gene alteration that includes any of the sequence classifications | Protein truncating mutations | Sequence analysis identifies premature stop codons or frameshift indels 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: within ± 2bp of exon starts/ends, or callable splice variants in Table 59 |
| | Homozygous deletions | Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size.<br><br>Only reported for BRCA1 and *BRCA2*. Not reported for ATM. |
| | Large protein truncating rearrangements | Sequence analysis identifies protein truncating rearrangements |
| | Deleterious missense mutations | Curated list (Table 58) |

### Table 58: Deleterious Missense Alterations

| *BRCA1* Protein Effect (PE) | *BRCA2* Protein Effect (PE) | *ATM* Protein Effect (PE) |
|---|---|---|
| M1V | M1R | M1T |
| M1I | M1I | R2032K |
| C61G | V159M | R2227C |

| BRCA1<br>Protein Effect (PE) | BRCA2<br>Protein Effect (PE) | ATM<br>Protein Effect (PE) |
|---|---|---|
| C64Y | V211L | R2547_S2549del |
| R71G | V211I | G2765S |
| R71K | R2336P | R2832C |
| R1495M | R2336H | S2855_V2856delinsRI |
| E1559K | | R3008C |
| D1692N | | R3008H |
| D1692H | | |
| R1699W | | |
| A1708E | | |
| G1788V | | |

**Table 59: Intronic Variants**

| Gene | Chromosome | Position | Ref | Alt | dbSNP |
|---|---|---|---|---|---|
| *ATM* | chr11 | 108128198 | T | G | rs730881346 |
| *ATM* | chr11 | 108214102 | AGTGA | A | rs730881295 |

**11.4 CDx classification criteria for *BRCA1* and *BRCA2* alterations, qualifying prostate cancer or ovarian cancer patients for therapy with RUBRACA® (rucaparib):**

**Table** and **Table 61** describe the criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function rendering the sample *BRCA+*.

**Table 60: 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 61**) |

**Table 61**: 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 |

| BRCA1 Alterations (Protein Change) | | | | BRCA2 Alterations (Protein Change) | |
|---|---|---|---|---|---|
| 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 | |

## 11.5 CDx classification criteria for PIK3CA alterations, qualifying breast cancer patients for therapy with PIQRAY® (alpelisib):

Presence of PIK3CA mutation(s): H1047R; E545K; E542K; C420R; E545A; E545D [1635G>T only]; E545G; Q546E; Q546R; H1047L; or H1047Y