SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	FoundationOne CDx™
Device Procode:	PQP
Applicant's Name and Address:	Foundation Medicine, Inc. 150 Second Street Cambridge, MA 02141
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P170019
Date of FDA Notice of Approval:	November 30, 2017

Breakthrough Device: Granted breakthrough device status [previously Expedited Access Pathway (EAP)] on June 15, 2016 because the device (1) is intended to diagnose a life threatening or irreversibly debilitating disease or condition (2) represents a breakthrough technology that provides a clinically meaningful advantage over existing legally marketed technology, and (3) the availability of the device is in the best interest of patients.

II. INDICATIONS FOR USE

FoundationOne CDx™ (F1CDx) is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms. The F1CDx test is a single-site assay performed at Foundation Medicine, Inc.

Table 1. Companion diagnostic indications

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Rubraca® (rucaparib)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS/PRECAUTIONS AND LIMITATIONS

The warnings/precautions and limitations are included in the FoundationOne CDx assay labeling.

V. DEVICE DESCRIPTION

FoundationOne CDx (F1CDx) is a single-site assay performed at Foundation Medicine, Inc. The assay includes reagents, software, instruments and procedures for testing DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.
The assay employs a single DNA extraction method from routine FFPE biopsy or surgical resection specimens, 50-1000 ng of which undergoes whole-genome shotgun

library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, 1 promoter region, 1 non-coding RNA (ncRNA), and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons (refer to Table 2 and Table 3 below for complete list of genes included in F1CDx). In total, the assay therefore detects alterations in a total of 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid-capture–selected libraries will be sequenced to high uniform depth (targeting > 500X median coverage with > 99% of exons at coverage > 100X). Sequence data is processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (amplifications and homozygous deletions), and selected genomic rearrangements (e.g., gene fusions). Additionally, genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) will be reported.

Table 2. Genes with full coding exonic regions included in F1CDx for the detection of substitutions, insertion-deletions (indels), and copy number alterations (CNAs)

ABL1	*BRAF*	*CDKN1A*	*EPHA3*	*FGFR4*	*IKZF1*	*MCL1*	*NKX2-1*	*PMS2*	*RNF43*	*TET2*
ACVR1B	*BRCA1*	*CDKN1B*	*EPHB1*	*FH*	*INPP4B*	*MDM2*	*NOTCH1*	*POLD1*	*ROS1*	*TGFBR2*
AKT1	*BRCA2*	*CDKN2A*	*EPHB4*	*FLCN*	*IRF2*	*MDM4*	*NOTCH2*	*POLE*	*RPTOR*	*TIPARP*
AKT2	*BRD4*	*CDKN2B*	*ERBB2*	*FLT1*	*IRF4*	*MED12*	*NOTCH3*	*PPARG*	*SDHA*	*TNFAIP3*
AKT3	*BRIP1*	*CDKN2C*	*ERBB3*	*FLT3*	*IRS2*	*MEF2B*	*NPM1*	*PPP2R1A*	*SDHB*	*TNFRSF14*
ALK	*BTG1*	*CEBPA*	*ERBB4*	*FOXL2*	*JAK1*	*MEN1*	*NRAS*	*PPP2R2A*	*SDHC*	*TP53*
ALOX12B	*BTG2*	*CHEK1*	*ERCC4*	*FUBP1*	*JAK2*	*MERTK*	*NT5C2*	*PRDM1*	*SDHD*	*TSC1*
AMER1	*BTK*	*CHEK2*	*ERG*	*GABRA6*	*JAK3*	*MET*	*NTRK1*	*PRKAR1A*	*SETD2*	*TSC2*
APC	*C11orf30*	*CIC*	*ERRFI1*	*GATA3*	*JUN*	*MITF*	*NTRK2*	*PRKCI*	*SF3B1*	*TYRO3*
AR	*CALR*	*CREBBP*	*ESR1*	*GATA4*	*KDM5A*	*MKNK1*	*NTRK3*	*PTCH1*	*SGK1*	*U2AF1*
ARAF	*CARD11*	*CRKL*	*EZH2*	*GATA6*	*KDM5C*	*MLH1*	*P2RY8*	*PTEN*	*SMAD2*	*VEGFA*
ARFRP1	*CASP8*	*CSF1R*	*FAM46C*	*GID4 (C17orf39)*	*KDM6A*	*MPL*	*PALB2*	*PTPN11*	*SMAD4*	*VHL*
ARID1A	*CBFB*	*CSF3R*	*FANCA*	*GNA11*	*KDR*	*MRE11A*	*PARK2*	*PTPRO*	*SMARCA4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARCB1*	*WHSC1L1*
ATM	*CCND1*	*CTNNA1*	*FANCG*	*GNAQ*	*KEL*	*MSH3*	*PARP2*	*RAC1*	*SMO*	*WT1*
ATR	*CCND2*	*CTNNB1*	*FANCL*	*GNAS*	*KIT*	*MSH6*	*PARP3*	*RAD21*	*SNCAIP*	*XPO1*
ATRX	*CCND3*	*CUL3*	*FAS*	*GRM3*	*KLHL6*	*MST1R*	*PAX5*	*RAD51*	*SOCS1*	*XRCC2*
AURKA	*CCNE1*	*CUL4A*	*FBXW7*	*GSK3B*	*KMT2A (MLL)*	*MTAP*	*PBRM1*	*RAD51B*	*SOX2*	*ZNF217*
AURKB	*CD22*	*CXCR4*	*FGF10*	*H3F3A*	*KMT2D (MLL2)*	*MTOR*	*PDCD1*	*RAD51C*	*SOX9*	*ZNF703*
AXIN1	*CD274*	*CYP17A1*	*FGF12*	*HDAC1*	*KRAS*	*MUTYH*	*PDCD1LG2*	*RAD51D*	*SPEN*	
AXL	*CD70*	*DAXX*	*FGF14*	*HGF*	*LTK*	*MYC*	*PDGFRA*	*RAD52*	*SPOP*	
BAP1	*CD79A*	*DDR1*	*FGF19*	*HNF1A*	*LYN*	*MYCL*	*PDGFRB*	*RAD54L*	*SRC*	

BARD1	*CD79B*	*DDR2*	*FGF23*	*HRAS*	*MAF*	*MYCN*	*PDK1*	*RAF1*	*STAG2*	
BCL2	*CDC73*	*DIS3*	*FGF3*	*HSD3B1*	*MAP2K1*	*MYD88*	*PIK3C2B*	*RARA*	*STAT3*	
BCL2L1	*CDH1*	*DNMT3A*	*FGF4*	*ID3*	*MAP2K2*	*NBN*	*PIK3C2G*	*RB1*	*STK11*	
BCL2L2	*CDK12*	*DOT1L*	*FGF6*	*IDH1*	*MAP2K4*	*NF1*	*PIK3CA*	*RBM10*	*SUFU*	
BCL6	*CDK4*	*EED*	*FGFR1*	*IDH2*	*MAP3K1*	*NF2*	*PIK3CB*	*REL*	*SYK*	
BCOR	*CDK6*	*EGFR*	*FGFR2*	*IGF1R*	*MAP3K13*	*NFE2L2*	*PIK3R1*	*RET*	*TBX3*	
BCORL1	*CDK8*	*EP300*	*FGFR3*	*IKBKE*	*MAPK1*	*NFKBIA*	*PIM1*	*RICTOR*	*TEK*	

Table 3. Genes with select intronic regions for the detection of gene rearrangements, a promoter region and a ncRNA gene.

ALK introns 18, 19	*BRCA1 introns 2, 7, 8, 12, 16, 19, 20*	*ETV4 introns 5, 6*	*EZR introns 9-11*	*KIT intron 16*	*MYC intron 1*	*NUTM1 intron 1*	*RET introns 7-11*	*SLC34A2 intron 4*
BCL2 3'UTR	*BRCA2 intron 2*	*ETV5 introns 6, 7*	*FGFR1 intron 1, 5, 17*	*KMT2A (MLL) introns 6-11*	*NOTCH2 intron 26*	*PDGFRA introns 7, 9, 11*	*ROS1 introns 31-35*	*TERC ncRNA*
BCR introns 8, 13, 14	*CD74 introns 6-8*	*ETV6 introns 5, 6*	*FGFR2 intron 1, 17*	*MSH2 intron 5*	*NTRK1 introns 8-10*	*RAF1 introns 4-8*	*RSPO2 intron 1*	*TERT Promoter*
BRAF introns 7-10	*EGFR introns 7, 15, 24-27*	*EWSR1 introns 7-13*	*FGFR3 intron 17*	*MYB intron 14*	*NTRK2 Intron 12*	*RARA intron 2*	*SDC4 intron 2*	*TMPRSS2 introns 1-3*

Test Output
The output of the test includes:

Category 1: CDx Claims noted in Table 1 of the Intended Use

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

Genomic findings other than those listed in Table 1 of the intended use statement (i.e., Categories 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

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 Instructions
- Shipping Instructions
- Return Shipping Label

Instruments
The F1CDx assay is intended to be performed with serial number-controlled instruments as indicated in Table 4, below. All instruments are qualified by Foundation Medicine, Inc. (FMI) under FMI's Quality System.

Table 4. Instruments for use with the F1CDx assay

Instrument
Illumina HiSeq 4000
Illumina cBot
Beckman Biomek NXP Span-8 Liquid Handler
Thermo Scientific Kingfisher Flex DW 96

Test Process
All assay reagents included in the F1CDx assay process are qualified by FMI and are compliant with the medical device Quality System Regulation (QSR).

A. Specimen Collection and Preparation

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens are collected and prepared following standard pathology practices. FFPE specimens may be received either as unstained slides or as an FFPE block.

Prior to starting the assay, a Hematoxylin and Eosin (H&E) stained slide is prepared, and then reviewed by a board-certified pathologist to confirm disease ontology and to ensure that adequate tissue (0.6 mm^3), tumor content ($\geq$ 20% tumor) and sufficient nucleated cells are present to proceed with the assay.

B. DNA Extraction

Specimens passing pathology review are queued for DNA extraction which begins with lysis of cells from FFPE tissue by digestion with a proteinase K buffer followed by automated purification using the 96-well KingFisher™ FLEX Magnetic Particle Processor.

After completion of DNA extraction, double-stranded DNA (dsDNA) is quantified by the Quant-iT™ PicoGreen® fluorescence assay using the provided lambda DNA standards (Invitrogen) prior to Library Construction (LC). The sample must yield a minimum of 55 ng of genomic DNA to ensure sufficient DNA for quality control (QC) and to proceed with LC.

C. Library Construction

Library Construction (LC) begins with the normalization of DNA to 50-1000 ng. The normalized DNA samples are randomly sheared (fragmented) to ~200 bp by adaptive focused acoustic sonication using a Covaris LE220 before purification using a 1.8X volume of AMPure® XP Beads (Agencourt®). Solid-phase reversible immobilization (SPRI) purification and subsequent library construction with the NEBNext® reagents (custom-filled kits by NEB), including mixes for end repair, dA addition and ligation,

are performed in 96-well plates (Eppendorf) on a Bravo Benchbot (Agilent) using the "with-bead" protocol[1] to maximize reproducibility and library yield. Indexed (6 bp barcodes) sequencing libraries are PCR amplified with HiFi™ (Kapa) for 10 cycles, and subsequently 1.8X SPRI purified. Purification and dilution for QC are performed.

Following LC, a QC procedure is performed by quantifying single-stranded DNA (ssDNA) from purified libraries using the Quant-iT™ OliGreen® ssDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Libraries yielding insufficient sequencing library are failed.

D. Hybrid Capture

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.8 Mb of the human genome including all coding exons of 309 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track.

After hybridization, the library-bait duplexes are captured on paramagnetic MyOne™ streptavidin beads (Invitrogen) and off-target material is removed by washing one time with 1X SSC at 25°C and four times with 0.25X SSC at 55°C. The PCR master mix is added to directly amplify (12 cycles) the captured library from the washed beads.[3] After 12 cycles of amplification, the samples are 1.8X SPRI purified. Purification and dilution for QC are performed.

Quality Control for Hybrid Capture is performed by measuring dsDNA yield using a Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Captured libraries yielding less than 140 ng of sequencing library are failed.

E. Sequencing

Sequencing is performed using off-board clustering on the Illumina cBot with patterned flow cell technology to generate monoclonal clusters from a single DNA template followed by sequencing using sequencing by synthesis (SBS) chemistry on the Illumina HiSeq 4000. Fluorescently labeled 3′-blocked dNTP's along with a polymerase are incorporated through the flow cell to create a growing nucleotide chain that is excited by a laser. A camera captures the emission color of the incorporated base and then is cleaved off. The terminator is then removed to allow the nucleotide to revert to its natural form and to allow the polymerase to add another base to the growing chain. A new pool of fluorescently labeled 3′-blocked dNTPs are added with each new sequencing cycle. The color changes for each new cycle as a

new base is added to the growing chain. This method allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

F. Sequence Analysis

Sequence data is analyzed using proprietary software developed by FMI. Sequence data is mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9.[4] PCR duplicate read removal and sequence metric collection is performed using Picard 1.47 (http://picard.sourceforge.net) and SAMtools 0.1.12a.[5] Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705.[6] Variant calling is performed only in genomic regions targeted by the test.

Base substitution detection is performed using a Bayesian methodology, which allows for the detection of novel somatic alterations at low mutant allele frequency (MAF) and increased sensitivity for alterations at hotspot sites through the incorporation of tissue-specific prior expectations.[7] Reads with low mapping (mapping quality < 25) or base calling quality (base calls with quality ≤ 2) are discarded. Final calls are made at MAF ≥ 5% (MAF ≥ 1% at hotspots).

To detect indels, de novo local assembly in each targeted exon is performed using the de-Bruijn approach.[8] Key steps are:

- Collecting all read-pairs for which at least one read maps to the target region.
- Decomposing each read into constituent k-mers and constructing an enumerable graph representation (de-Bruijn) of all candidate non-reference haplotypes present.
- Evaluating the support of each alternate haplotype with respect to the raw read data to generate mutational candidates. All reads are compared to each of the candidate haplotypes via ungapped alignment, and a read 'vote' for each read is assigned to the candidate with best match. Ties between candidates are resolved by splitting the read vote, weighted by the number of reads already supporting each haplotype. This process is iterated until a 'winning' haplotype is selected.
- Aligning candidates against the reference genome to report alteration calls.

Filtering of indel candidates is carried out similarly to base substitutions, with an empirically increased allele frequency threshold at repeats and adjacent sequence quality metrics as implemented in GATK: % of neighboring bases mismatches < 25%, average neighboring base quality > 25, average number of supporting read mismatches ≤ 2. Final calls are made at MAF ≥ 5% (MAF ≥ 3% at hotspots).

Copy number alterations (CNAs) are detected using a comparative genomic hybridization (CGH)-like method. First, a log-ratio profile of the sample is acquired by normalizing the sequence coverage obtained at all exons and genome-wide SNPs (~3,500) against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate tumor purity and copy number at each segment. Amplifications are called at segments with ≥ 6 copies (or ≥ 7 for triploid/≥ 8 for tetraploid tumors) and homozygous deletions at 0 copies, in

samples with tumor purity ≥ 20%. Amplifications in ERBB2 are called positive at segments with ≥ 5 copies for diploid tumors.

Genomic rearrangements are identified by analyzing chimeric read pairs. Chimeric read pairs are defined as read pairs for which reads map to separate chromosomes, or at a distance of over 10 megabase (Mb). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five (5) chimeric pairs [three (3) for known fusions] are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (average read mapping quality in the cluster must be 30 or above) and distribution of alignment positions. Rearrangements are annotated for predicted function (e.g., creation of fusion gene).

To determine microsatellite instability (MSI) status, 95 intronic homopolymer repeat loci (10-20 bp long in the human reference genome) with adequate coverage on F1CDx Assay are analyzed for length variability and compiled into an overall MSI score via principal components analysis. Using the 95 loci, for each sample the repeat length is calculated in each read that spans the locus. The means and variances of repeat lengths is recorded. Principal components analysis (PCA) is used to project the 190-dimension data onto a single dimension (the first principal component) that maximizes the data separation, producing an MSI score. Each sample is assigned a qualitative status of MSI-High (MSI-H) or MSI-Stable (MSS); ranges of the MSI score are assigned MSI-H or MSS by manual unsupervised clustering. Samples with low coverage (< 250X median) are assigned a status of MSI-unknown.

Tumor mutational burden (TMB) is measured by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater, and filtering out potential germline variants according to published databases of known germline polymorphisms including Single Nucleotide Polymorphism database (dbSNP) and Exome Aggregation Consortium (ExAC). Additional germline alterations still present after database querying are assessed for potential germline status and filtered out using a somatic-germline/zygosity (SGZ) algorithm. Furthermore, known and likely driver mutations are filtered out to exclude bias of the data set. The resulting mutation number is then divided by the coding region corresponding to the number of total variants counted, or 793 kb. The resulting number is communicated as mutations per Mb unit (mut/Mb).

After completion of the Analysis Pipeline, variant data is displayed in the FMI custom-developed CATi software applications with sequence quality control metrics. As part of data analysis QC for every sample, the F1CDx assay assesses cross-contamination through the use of a SNP profile algorithm reducing the risk of false-positive calls that could occur as a result of an unexpected contamination event. Sequence data is reviewed by trained bioinformatics personnel. Samples failing any QC metrics are automatically held and not released.

G. Report Generation

Approved results are annotated by automated software with CDx relevant information and are merged with patient demographic information and any additional information provided by FMI as a professional service prior to approval and release by the laboratory director or designee.

H. Internal Process Controls Related to the System

Positive Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of ten HapMap cell lines and is used as a positive mutation detection control. One hundred (100) different germline SNPs present across the entire targeted region are required to be detected by the analysis pipeline. If SNPs are not detected as expected, this results in a QC failure as it indicates a potential processing error

Sensitivity Control

The HapMap control pool used as the positive control is prepared to contain variants at 5%-10% MAF which must be detected by the analysis pipeline to ensure expected sensitivity for each run.

Negative Control

Samples are barcoded molecularly at the LC stage. Only reads with a perfect molecular barcode sequence are incorporated into the analysis. The Analysis Pipeline includes an algorithm that analyzes the SNP profile of each specimen to identify potential contamination that may have occurred prior to molecular barcoding, and can detect contamination lower than 1%.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in Table 1 of the F1CDx intended use statement. The approved Cx tests are listed in Table 5 below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm?source=govdelivery.

Table 5. List of FDA approved CDx assays for genes targeted by F1CDx

	Device	Company	Technology	Therapy	Indication
HER2-Amplification	PathVysion HER-2 DNA Probe Kit	Abbott Molecular, Inc.	FISH	Herceptin (trastuzumab)	Breast cancer
	Pathway Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody	Ventana Medical Systems, Inc.	IHC	Herceptin (trastuzumab)	Breast cancer
	Insite HER-2/neu Kit	Biogenex Laboratories, Inc.	IHC	Herceptin (trastuzumab)	Breast cancer
	Spot-Light HER2 CISH Kit	Life Technologies, Inc.	CISH	Herceptin (trastuzumab)	Breast cancer
	Bond Oracle Her2 IHC System	Leica Biosystems	IHC	Herceptin (trastuzumab)	Breast cancer
	HER2 CISH pharmDx Kit	Dako Denmark A/S	CISH	Herceptin (trastuzumab)	Breast cancer
	INFORM HER2 DUAL ISH DNA Probe Cocktail	Ventana Medical Systems, Inc.	Dual ISH	Herceptin (trastuzumab)	Breast cancer
	HercepTest	Dako Denmark A/S	IHC	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
	HER2 FISH pharmDx Kit	Dako Denmark A/S	FISH	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
BRAF-V600	THxID BRAF Kit	bioMerieux	PCR	Mekinist (tramatenib)	Melanoma
	cobas BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	Zelboraf (vemurafenib)	Melanoma
BRAF-600E	THxID BRAF Kit	bioMerieux	PCR	Tafinlar (dabrafemib)	Melanoma
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Tafinlar (dabrafemib) Mekinist (trametinib)	Non-small cell lung cancer
NRAS	Praxis Extended Ras Panel	Illumina	NGS	Vectibix (panitumumab)	Colorectal cancer

	Device	Company	Technology	Therapy	Indication
KRAS	**cobas** KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	Erbitux (cetuximab) Vectibix (panitumumab)	Colorectal cancer
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	Erbitux (cetuximab) Vectibix (panitumumab)	Colorectal cancer
	Praxis Extended Ras Panel	Illumina	NGS	Vectibix (panitumumab)	Colorectal cancer
***ALK* - fusion**	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	Xalkori (crizotinib)	Non-small cell lung cancer
	ALK (D5F3) CDx Assay	Ventana Medical Systems, Inc.	IHC	Xalkori (crizotinib)	Non-small cell lung cancer
***EGFR* – Exon 19 deletions & L858R**	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tarceva (erlotinib)	Non-small cell lung cancer
	therascreen EGFR RGQ PCR Kit	QIAGEN	PCR	Gilotrif (afatinib) Iressa (gefitinib)	Non-small cell lung cancer
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Iressa (gefitinib	Non-small cell lung cancer
***EGFR* T790M**	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tagrisso (osimertinib)	Non-small cell lung cancer
BRCA1/2	FoundationFocus CDx$_{BRCA}$	Foundation Medicine, Inc.	NGS	Rubraca (rucaparib)	Advanced Ovarian

Abbreviations: FISH – fluorescence *in situ* hybridization; IHC – immunohistochemistry; CISH – chromogenic *in situ* hybridization; ISH – in situ hybridization; PCR – polymerase chain reaction; NGS – next generation sequencing.

VII. MARKETING HISTORY

Foundation Medicine, Inc. initially designed and developed the FoundationOne® laboratory developed test (F1 LDT), and the first commercial sample was tested in 2012. The F1 LDT has been used to detect the presence of genomic alterations in FFPE tumor tissue specimens. The F1 LDT is not FDA-cleared or – approved.

The F1CDx assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions. Patients with false positive results may undergo treatment with one of the therapies listed in the above intended use statement without clinical benefit, and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy. For the specific adverse events related to the approved therapeutics, please see approved drug product labels.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

Performance characteristics were established using DNA derived from a wide range of FFPE tissue types; tissue types associated with CDx indications were included in each study. Each study included CDx variants as well as a broad range of representative alteration types (substitution, insertion-deletion, copy number alterations, rearrangements) in various genomic contexts across a number of genes. Analyses of genomic signatures including MSI and TMB were also conducted.

1. Analytical Accuracy/Concordance

a. Comparison to an Orthogonal Method

The detection of alterations by F1CDx was compared to results of an externally validated NGS assay (evNGS). The comparison between short alterations, including base substitutions and short indels, detected by F1CDx and the orthogonal method included 188 samples from 46 different tumor tissue types including ovarian serous carcinoma, breast carcinoma, brain glioblastoma, vulva squamous cell carcinoma, cervix adenocarcinoma, lung, melanoma and colon adenocarcinoma.

A summary of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) is provided in Table 6, below. Overall there were 157 overlapping genes between the F1CDx assay and the orthogonal method. None of the 188 samples were determined to be invalid on either platform.

Table 6. Concordance Summary for short variants (inclusive of both substitutions and indels), base substitutions, and indels with VUS included

Variant Type	F1CDx+/ evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA*(95%CI)	NPA*(95%CI)
All short variants	1282	73	375	284218	94.6% (93.3%-95.8%)	99.9% (99.9%-99.9%)
Substitutions	1111	39	334	242540	96.6% (95.4%-97.6%)	99.9% (99.8%-99.9%)
Indels	171	34	41	41678	83.4% (77.6%-88.2%)	99.9% (99.9%-99.9%)

Since the PPA and NPA were calculated without adjusting for the distribution of samples enrolled using F1 LDT, positive predictive value (PPV) and negative predictive value (NPV) were also estimated conditional on F1CDx. The total number of alterations detected across all 188 samples by either F1CDx and/or evNGS was used to determine NPV. The observed PPV for all short variants was 77.4% [95% confidence interval (CI): 75.3%-79.4%] and for indels PPV was 80.7% [95% CI: 74.7%-85.7%]. The NPV for all short variants was >99.9%. Differences in variants of unknown significance (VUS) alteration calls between the platforms were noted, and is expected based on differences in filtering employed by F1CDx and the NGS comparator. Discordant alterations not related to VUS filtering were primarily caused by deletions with low allelic fraction in homopolymer regions. The F1CDx variant calling pipeline imposes a filter based on MAF of ≥ 0.10% for indels in homopolymer regions to reduce the likelihood of calling false positives resulting from artifacts introduced by the technology. As such, the difference observed was due to varying filter thresholds between the two platforms. For additional concordance for the CDx-associated variants, refer to the Clinical Concordance Studies described in Section X.

b. Comparison to FoundationOne LDT (F1 LDT)

To support the use of retrospective data generated using the F1 LDT as supplemental and supporting data for F1CDx, a concordance study was conducted with the F1CDx assay. This study evaluated a set of 165 specimens with variants in genes interrogated by both assays. The study included DNA derived from 35 different tumor types extracted from tissues with tumor content ranging from 20% to 90%. PPA and NPA between F1CDx and F1 LDT, using the F1 LDT assay as the reference method, was calculated for all alterations, as well as for alterations binned by type: short variants, CNAs and rearrangements. A total of 2325 variants, including 2026 short variants, 266 copy number alterations and 33 rearrangements met the variant inclusion criteria. The study results are in Table 7, below.

Table 7. Summary of concordance between F1CDx and F1 LDT

Variant Type	F1CDx+/ F1 LDT+	F1CDx- /F1 LDT+	F1CDx+/ F1 LDT-	F1CDx- /F1 LDT-	PPA(95%CI)	NPA(95%CI)
All variants	2246	33	46	322890	98.6% (98.0%-99.0%)	99.99% (99.98%-99.99%)
All short variants	1984	19	23	299099	99.1% (98.5%-99.4%)	99.99% (99.9%-99.995%)
Substitutions	1692	10	19	254854	99.4% (98.9%-99.7%)	99.99% (99.99%-99.996%)
Indels	292	9	4	44245	97.0% (94.4%-98.6%)	99.99% (99.98%-99.998%)
All CNAs	230	14	22	19204	94.3% (90.6%-96.8%)	99.9% (99.8%-99.96%)
Amplifications	157	10	12	14671	94.0% (89.3%-97.1%)	99.9% (99.9%-99.96%)
Losses	73	4	10	4533	94.8% (87.2%-98.6%)	99.8% (99.6%-99.9%)

Variant Type	F1CDx+/ F1 LDT+	F1CDx- /F1 LDT+	F1CDx+/ F1 LDT-	F1CDx- /F1 LDT-	PPA(95%CI)	NPA(95%CI)
Rearrangements	32	0	1	4587	100.0% (89.1%-100%])	99.98% (99.99 -99.999%)

The qualitative output for MSI (MSI-H vs MSS) in F1 LDT and F1CDx were evaluated. PPA, NPA and Overall Percent Agreement (OPA) of MSI status between the two assays was calculated for all 165 samples. Of the 165 samples, 5 were MSI-H by F1 LDT and 160 were MSS by F1 LDT, there was one discordant sample observed. The discordant sample was called MSS by F1 LDT and MSI-H by F1CDx. After manual review, the discordant case had an MSI score close to the threshold used to classify MSI status, thus leading to discordant MSI status in the two assays. PPA was 100% with a 95% CI of 47.8-100%, NPA was 99.5% with a 95% CI of 96.6%-99.98% and the OPA was 99.4% with a 95% CI of 96.7%-99.98%.

Furthermore, additional MSI concordance data was reviewed including the following: 1) concordance between the F1 LDT MSI results and a validated mismatch repair immunohistochemistry (MMR IHC) panel and 2) concordance between the F1 LDT and a validated MSI-PCR test. Comparison to MMR IHC was performed on 30 colorectal cancer specimens, and comparison to PCR was performed on a total of 39 FFPE tissue samples (18 colorectal, 20 uterine and one cecum cancer specimen). In this sample set, there were two specimens noted as MSI-ambiguous which were not included in the concordance calculations. Combining IHC and PCR data, the overall concordance was 97% (65/67); see concordance Table 8, below. Additional concordance data will be provided post-market comparing MSI test results generated using F1CDx, to both a validated MMR IHC panel and a validated MSI-PCR test.

Table 8. Concordance Table with FoundationOne LDT (F1 LDT) and a validated MMR IHC and MSI-PCR assay

	IHC			PCR		
F1 LDT	MSI-H	MSS	Total	MSI-H	MSS	Total
MSI-H	1	0	1	17	1	18
MSS	0	29	29	1	18	19
Total	1	29	30	18	19	37

TMB concordance was evaluated by comparing the TMB output in terms of mutations per Mb. Analyses were conducted using all 153 non-zero TMB scores. From linear regression analysis using F1 LDT TMB as the predictor and F1CDx TMB as the outcome, the intercept is -0.27782 (95%CI: -0.662-0.106), and the slope is 0.94064 (95% CI: 0.919-0.963).

2. Analytical Sensitivity

a. Limit of Detection (LoD)

The LoD of alterations assessed by F1CDx was evaluated. The LoDs of five (5) CDx biomarkers representing *EGFR* exon 21 L858R, *EGFR* Exon 20 T790M, *EGFR* exon 19 deletion, *KRAS* codons 12/13 substitution and *BRAF* V600E were determined and is summarized in Table 9, below.

Table 9. Summary of LoD for alterations associated with CDx claims

Alteration	LoD* Allele Fraction (%)
EGFR L858R	2.4%
EGFR Exon 19 deletion	5.1%
EGFR T790M	2.5%
KRAS G12/G13	2.3%
BRAF V600E	2.0%
BRCA1/2** **Alteration in non-repetitive regions or homopolymer repeats < 4 bp**	6%
Alterations in a homopolymer region > 4bp	15.3%

*LoD calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90% for all CDx variants (not including BRCA1/2 variants). LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
**See Summary of Safety and Effectiveness Data for P160018. LoD calculated using probit approach.

Additional categories of alteration types were evaluated for the F1CDx assay platform validation. A single FFPE tumor sample was selected for each of the variant categories. For each sample, six levels of MAF, with 13 replicates per level, were evaluated (total of 78 aliquots per sample). To meet the number of required replicates proposed in CLSI EP17-A2, multiple different pooled DNA extractions from a single FFPE samples and banked DNA samples were used to meet the required variant characteristics. For platform-wide LoD assessment, the indels were grouped together (other than homopolymer repeat context) as they are similar in LoD characteristics. The indels ranged from 1 bp up to 42 bp insertions and deletions up to 276 bp. Indels at homopolymer repeat context had higher LoD, with a dependency on the length of the repeat context. When estimating the LoD of platform substitutions, the analyses excluded certain variants that were fully detected at all dilution levels (N = 13 substitutions, MAF Range 2.2%-15.2%) as they did not reach full dilution and were determined not to be representative of the remaining 166 alterations. Representative LoD for the range of variants detected by the F1CDx platform is summarized in Table 10.

Table 10. Summary representative LoD for platform alterations

Variant Category	Subcategory*	N	Range LoD** Allele Fraction (%)
Base Substitutions	known	21***	1.8-7.9
	other	166***	5.9-11.8
Indels at non-homopolymer context, including insertions up to 42bp and deletions up to 276bp	known	3	4.5-6.5
	other	17	6.0-10.2

Variant Category	Subcategory*	N	Range LoD** Allele Fraction (%)
Indels at homopolymer context	5bp repeat	8	10.0-12.2
	6bp repeat	2	13.6-13.7
	7bp repeat	4	16.3-20.4
	8bp repeat	3	17.0-20.0

*Alterations classified as" known" are defined as those that are listed in COSMIC. Alterations classified as "other" include truncating events in tumor suppressor genes (splice, frameshift and nonsense) as well as variants that appear in hotspot locations but do not have a specific COSMIC association, or are considered variants of unknown significance (VUS) due to lack of reported evidence and conclusive change in function.
**LoD calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
***Data includes an alteration in the *TERT* promoter 124C>T (LoD of 7.9%) *TERT* is the only promoter region interrogated by F1CDx and is highly enriched for repetitive context of poly-Gs, not present in coding regions.

b. Limit of Blank (LoB)

The limit of blank (LoB) of zero was confirmed using the mutation calls from mutation-negative FFPE samples (19 distinct samples with four replicates per sample), with a percentage of false-positive results less than 5% (type I error risk α=0.05). Seventy-five (75) samples (19 × 4 – 1, because one failed to meet the Library Construction QC process specification) were used for the assessment of LoB. It was confirmed that each replicate of LoB sample was negative for variants included in the LoD analysis.

c. Analytical Sensitivity – Tumor Purity

The minimum tumor fraction required to support the robustness of the F1CDx for select CDx alterations and platform validation was evaluated. For each sample, six levels of tumor content, with 13 replicates per level, were evaluated, so a total of 78 aliquots per sample. The analytical sensitivity of two (2) CDx biomarkers representing *ALK* fusion and *ERBB2* amplification were determined and is summarized in Table 11, below. The data show that for *ALK* fusion the assay is robust down to 2.6% tumor purity, therefore at the minimal required tumor purity required for the assay, 20%, the assay is robust at detecting this alteration. For *ERBB2* amplification, the data show that the assay is robust at detecting this alteration down 25.3% tumor purity. Similarly, additional categories representing four alteration types were also evaluated for the F1CDx assay platform validation and results summarized in Table 12. In addition, analytical sensitivity based on tumor purity was also evaluated for MSI-H. Performance of TMB at the recommended tumor purity of ≥ 20%, was found to be robust based on the LoD of short variants.

Table 11. Summary of analytical sensitivity for tumor purity for alterations associated with CDx claims

Alteration	Tumor Purity (%)*
ALK fusion	2.6%**
ERBB2 amplification	25.3%***

* Sensitivity calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
**The number of chimeric reads for the sample evaluated is 16 at the indicated tumor fraction.
***The number of copy number amplifications for the sample evaluated is 6.

Table 12. Summary analytical sensitivity for tumor purity for platform validation

Variant Category	N	Range Tumor Purity (%)*
Copy Number Amplifications (CN > 10)	8	9.6%-18.5%
Copy Number Amplifications ($6 \leq CN \leq 10$)	7	19.5%-58.3%**
Copy Number: Homozygous Deletions	3	33.4%-33.4%
Genomic Rearrangements	3	9.2%-14.9%
MSI-High	3	8.3%-15.8%

* Sensitivity calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
**Max represents VUS alteration at calling threshold.

3. Analytical Specificity

a. Interfering Substances

To evaluate the potential impact of endogenous and exogenous interfering substances on the performance of the F1CDx assay, this study evaluated five FFPE specimens representing five tumor types (ovary, lung, colorectal, breast cancer and melanoma). The specimens included representative variant types (substitution, indel, amplification, homozygous deletion and rearrangement), and were tested in duplicate (Table 13). An additional 54 short alterations (base substitutions and indels) were assessed. The addition of interfering substances including melanin (endogenous), ethanol (exogenous), proteinase K (exogenous), and molecular index barcodes (MIB) (exogenous) was evaluated to determine if they were impactful to F1CDx, and the results were compared to the control (no interferents) condition.

Table 13. Summary of tumor types and variant types included in study

Tumor Type	Gene (and variant as relevant)	Variant type
Colorectal	*FGFR1*	Rearrangement
	BCL2L1	Amplification
	AXIN1 c.1058G>A (R353H)	Substitution
	SOX9 c.768_769insGG (R257fs*23)	Indel
Breast	*ERBB2*	Amplification
	AKT1	Amplification
	CCND1	Amplification
Lung	*CDKN2A*	Homozygous Deletion
	CDKN2B	Homozygous Deletion
	EGFR	Amplification
Ovary	*BRCA1* c.5263_5264insC (Q1756fs*74)	Indel
	ERCC4 c.2395C>T	Substitution
	TP53 c.779_779delC (S261fs*84)	Indel
Melanoma	*BRAF* c.1799T>A (V600E)	Substitution
	TP53 c. 856G>A (E286K)	Substitution
	IGF1R	Amplification

Interfering substances included melanin, ethanol, proteinase K, and molecular index barcodes, as noted in Table 14, below. Each of the five FFPE specimens were tested in either two or four replicates each, resulting in a total of 170 data points across the five specimens (10 without interferent, 80 for evaluation of melanin, ethanol and proteinase K and 80 for molecular index barcodes) were assessed in this study.

Table 14. Interfering Substance Evaluated

Substances	Level	# Samples	# Replicates/Sample
No interferent	–	5	2
Melanin	0.025 µg/mL	5	2
Melanin	0.05 µg/mL	5	2
Melanin	0.1 µg/mL	5	2
Melanin	0.2 µg/mL	5	2
Proteinase K	0.04 mg/mL	5	2
Proteinase K	0.08 mg/mL	5	2
Ethanol	5%	5	2
Ethanol	2.5%	5	2
MIB	0	5	4
MIB	5%	5	4
MIB	15%	5	4
MIB	30%	5	4

Substances were considered as non-interfering if, when compared to no interferent, the DNA yield was sufficient to meet the standard processing requirements of DNA isolation (≥ 55 ng), the quality was sufficient to create products per the specification of library construction (≥ 545 ng) and hybrid capture (≥ 140 ng), and the sample success rate (fraction of samples that met all process requirements and specification), across all replicates in aggregate, was ≥ 90%. Sequence analysis was assessed as percent agreement for each specimen and was calculated as the number of replicates with the correct alteration call reported per the total number of replicates processed. Percent agreement (fraction of correct calls) was computed across the replicates. The results were aggregated across all variants in all five FFPE specimens, and concordance was assessed within each treatment category across variants. The acceptance for concordance required a minimum of 90% of correct calls within each treatment category. All samples tested at all interfering substance levels met all process requirements and specifications; indicating that the sample quality was not impacted by the interfering substances. The concordance for each variant for the melanin, proteinase K and MIB evaluations was 100%, and 95.3% for the ethanol evaluation. Each met the acceptance criterion of ≥ 90% indicating that the test performance was not affected by the tested interferents. In addition to the variants selected to represent specific alteration types summarized in Table 13 above, samples included in the study harbored 54 additional short alterations (substitutions and indels) which were found to be 100% concordant across all replicates for each variant.

See Summary of Safety and Effectiveness Data for P160018 for additional interference studies wherein the interference of necrotic tissue, triglycerides, hemoglobin, and xylene, in addition to ethanol, proteinase K, and MIBs, was evaluated in ovarian tissue and assessed *BRCA1/2* alterations.

b. *In silico* Analysis – Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage at the base level for targeted regions included in F1CDx. Lack of bait specificity and/or insufficient bait inclusion would result in regions of diminished high quality mapped reads due to the capture of off-target content. This analysis showed that all regions that may harbor alterations associated with companion diagnostic claims consistently have high quality (MQS ≥ 30), deep coverage ≥ 250X. When assessing the entire gene set, 91.45% of individual bases within targeted introns platform-wide had ≥ 100X coverage.

4. Carryover/Cross-Contamination

No carryover or cross-contamination was observed when alternating positive and negative samples for *BRCA1* and *BRCA2* variants, assessed in a checker-board pattern (see Summary of Safety and Effectiveness Data for P160018). In addition, data from plates evaluated in multiple analytical validation studies, containing high-level confirmed *ERBB2* amplifications, *EGFR* T790M alterations or *ALK* fusions were examined for cross-contamination in adjacent wells containing confirmed negative samples. No contamination was detected.

5. Precision and Reproducibility

Precision was evaluated for alterations associated with CDx claims, as well as representative alterations to support platform-level performance including, MSI, TMB and MAF of short 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 different sequencers and three different reagent lots, across multiple days (typical assay workflow spans 10 days) of performance by multiple operators. A full factorial design for this study was carried out with four (4) replicates per reagent lot/sequencer combination for samples with 36 replicates.

A set of 47 unique FFPE samples containing a variety of variants were evaluated. In total, 717 alterations were assessed, including variants associated with CDx claims (Table 15), and additional variants intended to demonstrate platform level validation (Table 16). The platform-level study included a total of 443 substitutions, 188 indels, 55 copy number amplifications, 13 copy number loss and 18 rearrangements in the variant set across the samples.

Table 15. Sample set with CDx variants

Gene	# of Unique Samples	Alteration	Tumor Type
EGFR	3	Exon 19 Deletion	NSCLC
	2	Exon 21 L858R	
	2	Exon 20 T790M	
KRAS	3	Codons 12/13 substitution	CRC
ALK	3	Fusion	NSCLC
BRAF	3	V600E/V600K	Melanoma
ERBB2 (HER2)	3	Amplification	Breast

Table 16. Sample set for platform validation

Alterations Type	# of Unique Samples	Alteration Size	Genomic Context
Substitution	3	-	-
Short Insertion	2	1-2bp	Homopolymer Repeats
Short Insertion	2	1-2bp	Dinucleotide Repeats
Short Insertion	2	3-5bp	-
Short Insertion	2	> 5bp	-
Short Deletion	2	1-2bp	Homopolymer Repeats
Short Deletion	2	1-2bp	Dinucleotide

Alterations Type	# of Unique Samples	Alteration Size	Genomic Context
			Repeats
Short Deletion	2	3-5bp	-
Short Deletion	2	> 5bp	-
Amplification	3	-	-
Homozygous Deletion	3	-	-
Rearrangement	3	-	-

A contamination level of 1.9% was observed, wherein < 1% is required. As such, one sample and its associated aliquots were excluded from repeatability and reproducibility concordance and MSI analyses. In total, 18 samples to support CDx validation and 28 samples for platform validation were included in the final analysis. The data for process failures and no call rate are summarized in Table 17, below. There were no replicates that generated a no call result for base substitution, indel, CNA or rearrangement calls.

Table 17. Summary data including process failures and no call rates for samples included in the precision study

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Replicates with Pre-sequencing Process Failure	% Replicates with MSI No Call	% Replicates with TMB No Call	Mean TMB
Sample 1	SUB	*EGFR* T790M	0	0.0% (0 of 36)	0.0% (0 of 36)	2.1
Sample 2	SUB	*EGFR* T790M	0	0.0% (0 of 36)	0.0% (0 of 36)	8.37
Sample 3	RE	*ALK* Fusion	0	0.0% (0 of 36)	0.0% (0 of 36)	1.51
Sample 4	RE	*ALK* Fusion	0	0.0% (0 of 36)	19.4% (7 of 36)	3.36
Sample 5	RE	*ALK* Fusion	0	0.0% (0 of 36)	11.1% (4 of 36)	3.12
Sample 6	Indel	*EGFR* Exon 19 Deletion	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	5.04
Sample 7	Indel	*EGFR* Exon 19 Deletion	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	4.21
Sample 8	Indel	*EGFR* Exon 19 Deletion	0	0.0% (0 of 36)	25.0% (9 of 36)	2.8
Sample 9	SUB	*KRAS* Codons 12/13 SUB	0	0.0% (0 of 36)	100.0% (36 of 36)	3.33
Sample 10	SUB	*KRAS* Codons 12/13 SUB	0	0.0% (0 of 36)	0.0% (0 of 36)	4.17
Sample 11	SUB	*BRAF* V600E/V600K	1 (HC)	2.9% (1 of 35)	0.0% (0 of 35)	14.91
Sample 12	SUB	*BRAF* V600E/V600K	0	0.0% (0 of 36)	0.0% (0 of 36)	23.54
Sample 13	SUB	*EGFR* Exon 21 L858R	2 (HC)	0.0% (0 of 34)	8.8% (3 of 34)	3

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Replicates with Pre-sequencing Process Failure	% Replicates with MSI No Call	% Replicates with TMB No Call	Mean TMB
Sample 14	Amplification	*ERBB2* (HER2) Amplification	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	4
Sample 15	Amplification	*ERBB2* (HER2) Amplification	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	4.82
Sample 16	Amplification	*ERBB2* (HER2) Amplification	2 (HC)	0.0% (0 of 34)	0.0% (0 of 34)	7.9
Sample 17	SUB	*KRAS* Codons 12/13 SUB	0	0.0% (0 of 36)	0.0% (0 of 36)	6.37
Sample 18	SUB	*EGFR* Exon 21 L858R	1 (HC)	0.0% (0 of 35)	2.9% (1 of 35)	11.21
Sample 19	CNA/RE/ SUB	Amplification	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	6.59
Sample 20	CNA/SUB/ Indel	Homozygous Deletion	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	10.95
Sample 21	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	1 (LC)	0.0% (0 of 35)	0.0% (0 of 35)	42.91
Sample 22	CNA/SUB/ Indel	Short Deletion (3-5bp)	0	0.0% (0 of 36)	0.0% (0 of 36)	2.7
Sample 23	SUB/Indel	Short Insertion (1-2bp with homopolymer repeats)	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	21.47
Sample 24	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	0	0.0% (0 of 36)	0.0% (0 of 36)	67.31
Sample 25	CNA/SUB/ Indel	Short Deletion (1-2bp with homopolymer repeats)	0	0.0% (0 of 36)	0.0% (0 of 36)	63.71
Sample 26	SUB/Indel	Short Deletion (1-2bp with homopolymer repeats)	0	0.0% (0 of 36)	0.0% (0 of 36)	51.83
Sample 27	CNA/SUB/ Indel	SUB	0	0.0% (0 of 36)	0.0% (0 of 36)	12.44

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Replicates with Pre-sequencing Process Failure	% Replicates with MSI No Call	% Replicates with TMB No Call	Mean TMB
Sample 28	RE/SUB/ Indel	Short Insertion (1-2bp with homopolymer repeats)	1 (HC)	0.0% (0 of 35)	8.6% (3 of 35)	5.87
Sample 29	CNA/ SUB	SUB	2 (1 LC, 1 HC)	0.0% (0 of 34)	0.0% (0 of 34)	1.15
Sample 30	CNA/RE/ SUB/Indel	RE	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	3.67
Sample 31	RE/SUB/Indel	Short Insertion (1-2bp with dinucleotide repeats)	0	0.0% (0 of 36)	0.0% (0 of 36)	1.26
Sample 32	CNA/SUB/ Indel	Short Deletion (3-5bp)	0	0.0% (0 of 36)	0.0% (0 of 36)	6.83
Sample 33	SUB/Indel	Short Insertion (3-5bp)	0	0.0% (0 of 36)	0.0% (0 of 36)	48.23
Sample 34	CNA/RE/ SUB/Indel	Short Insertion (3-5bp)	0	0.0% (0 of 36)	0.0% (0 of 36)	5.18
Sample 35	RE/SUB/Indel	SUB	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	4.21
Sample 36	RE/SUB/Indel	Short Insertion (>5bp)	0	5.6% (2 of 36)	94.4% (34 of 36)	1.82
Sample 37	CNA/RE/ SUB/Indel	Short Deletion (>5bp)	0	0.0% (0 of 36)	0.0% (0 of 36)	8.09
Sample 38	CNA/RE/ SUB/Indel	Short Insertion (>5bp)	1 (LC)	0.0% (0 of 35)	0.0% (0 of 35)	2.2
Sample 39	CNA /SUB	Amplification	1 (HC)	0.0% (0 of 35)	0.00% (0 of 35)	3.67
Sample 40	CNA/RE/ SUB/Indel	RE	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	12.65
Sample 41	CNA/ SUB/Indel	Homozygous Deletion	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	8.22
Sample 42	CNA/RE/ SUB/Indel	Short Insertion (1-2bp with dinucleotide repeats)	0	0.0% (0 of 36)	0.0% (0 of 36)	10.16
Sample 43	RE/SUB	Short Deletion (>5bp)	0	0.0% (0 of 36)	13.9% (5 of 36)	2.24
Sample 44	CNA /SUB	Homozygous Deletion	1 (HC)	0.0% (0 of 35)	0.0% (0 of 35)	7.28
Sample 45	CNA/RE/ SUB/Indel	Amplification	0	0.0% (0 of 36)	0.0% (0 of 36)	6.23
Sample 46	CNA/RE/	RE	1 (HC)	0.0% (0 of 35)	5.7% (2 of 35)	4.18

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Replicates with Pre-sequencing Process Failure	% Replicates with MSI No Call	% Replicates with TMB No Call	Mean TMB
	SUB/Indel					

*Samples 1-18 were selected to represent alterations associated with CDx claims and Samples 19-46 were selected for platform-wide analysis.
** While all possible alterations harbored in the sample were included in the analysis, each sample was selected based on a specific alteration in accordance with the study protocol.
Abbreviations: HC – Hybrid Capture, LC – Library Construction SUB – substitution, Indel – Insertion or Deletion, CNA – Copy Number Alteration, RE – Rearrangement.

Within the assessment of repeatability and reproducibility for CDx variants, all variants from all 18 samples had 100.0% positive call (PC) rates with two-sided 95% CIs ranging from (89.7%, 100.0%) to (90.3%, 100.0%). Percent of negative calls at each CDx variant location for wild-type samples was 100.0% with two-sided 95% CIs ranging from (99.0%, 100.0%) to (99.1%, 100.0%).

The platform-level repeatability and reproducibility, across the 717 variants, showed high overall negative call rates ranging from 99.7% to 100.0% for all 28 samples, and the overall positive call rates were also high for 27 samples ranging from 95.3% to 100.0% (except for sample TRF147811, with an overall positive call rate 85.4% (82.3%, 88.2%). The overall positive call rates and negative call rates are summarized in Table 18, below. The platform-level study included a total of 443 substitutions, 188 indels, 55 copy number amplifications, 13 copy number loss, and 18 rearrangements that were detected at least once by F1CDx in any replicate tested in the precision study.

Table 18. Positive and negative call rates for platform variants (N=717)

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	PC Rate (exact 95% CI)	PC Rate (exact 95% CI)
Sample 19	CNA/RE/SUB	Amplification	100.00% (99.40%-100.00%)	99.98% (99.95%-99.99%)
Sample 20	CNA/ SUB/Indel	Homozygous Deletion	99.37% (98.38%-99.83%)	99.96% (99.92%-99.98%)
Sample 21	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	100.00% (99.10%-100.00%)	99.97% (99.95%-99.99%)
Sample 22	CNA/ SUB/Indel	Short Deletion (3-5bp)	97.84% (96.89%-98.56%)	99.84% (99.78%-99.89%)
Sample 23	SUB/Indel	Short Insertion (1-2bp with homopolymer repeats)	99.81% (98.94%-100.00%)	99.98% (99.95%-99.99%)
Sample 24	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	99.60% (97.81%-99.99%)	99.94% (99.90%-99.97%)
Sample 25	CNA/ SUB/Indel	Short Deletion (1-2bp with homopolymer repeats)	98.33% (97.11%-99.14%)	99.98% (99.96%-100.00%)

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	PC Rate (exact 95% CI)	PC Rate (exact 95% CI)
Sample 26	SUB/Indel	Short Deletion (1-2bp with homopolymer repeats)	100.00% (99.83%-100.00%)	99.97% (99.94%-99.99%)
Sample 27	CNA/ SUB/Indel	SUB	100.00% (99.32%-100.00%)	99.98% (99.96%-100.00%)
Sample 28	RE/ SUB/Indel	Short Insertion (1-2bp with homopolymer repeats)	96.46% (94.14%-98.05%)	99.96% (99.92%-99.98%)
Sample 29	CNA/ SUB	SUB	98.67% (97.27%-99.46%)	99.98% (99.96%-100.00%)
Sample 30	CNA/RE/SUB/ Indel	RE	96.27% (95.39%-97.02%)	99.87% (99.82%-99.91%)
Sample 31	RE/SUB/Indel	Short Insertion (1-2bp with dinucleotide repeats)	98.23% (97.48%-98.80%)	99.66% (99.58%-99.73%)
Sample 32	CNA/ SUB/Indel	Short Deletion (3-5bp)	98.32% (97.57%-98.89%)	99.92% (99.88%-99.95%)
Sample 33	SUB/Indel	Short Insertion (3-5bp)	99.30% (98.90%-99.58%)	99.90% (99.86%-99.94%)
Sample 34	CNA/RE/SUB/ Indel	Short Insertion (3-5bp)	85.42% (82.27%-88.20%)	99.89% (99.84%-99.93%)
Sample 35	RE/SUB/Indel	SUB	97.75% (96.42%-98.68%)	99.98% (99.95%-99.99%)
Sample 36	RE/SUB/Indel	Short Insertion (>5bp)	95.30% (92.97%-97.03%)	99.96% (99.93%-99.98%)
Sample 37	CNA/RE/SUB/ Indel	Short Deletion (>5bp)	100.00% (98.31%-100.00%)	99.89% (99.84%-99.93%)
Sample 38	CNA/RE/SUB/ Indel	Short Insertion (>5bp)	100.00% (99.25%-100.00%)	99.96% (99.93%-99.98%)
Sample 39	CNA /SUB	Amplification	96.83% (94.90%-98.17%)	99.94% (99.90%-99.97%)
Sample 40	CNA/RE/SUB/ Indel	RE	95.97% (94.06%-97.40%)	99.98% (99.96%-100.00%)
Sample 41	CNA/ SUB/Indel	Homozygous Deletion	100.00% (99.42%-100.00%)	99.93% (99.89%-99.96%)
Sample 42	CNA/RE/SUB/ Indel	Short Insertion (1-2bp with dinucleotide repeats)	100.00% (99.30%-100.00%)	99.95% (99.91%-99.97%)
Sample 43	RE/SUB	Short Deletion (>5bp)	100.00% (99.05%-100.00%)	100.00% (99.98%-100.00%)
Sample 44	CNA /SUB	Homozygous Deletion	96.99% (95.39%-98.15%)	99.84% (99.79%-99.89%)
Sample 45	CNA/RE/SUB/ Indel	Amplification	100.00% (98.95%-100.00%)	99.93% (99.89%-99.96%)
Sample 46	CNA/RE/SUB/ Indel	RE	99.80% (99.29%-99.98%)	99.98% (99.96%-100.00%)

*Samples 1-18 were selected to represent alterations associated with CDx claims and Samples 19-46 were selected for platform-wide analysis.

** While all possible alterations harbored in the sample were included in the analysis, each sample was selected based on a specific alteration in accordance with the study protocol.

Abbreviations: PC – Positive Call, NC – Negative Call, SUB – substitution, Indel – Insertion or Deletion, CNA – Copy Number Alteration, RE – Rearrangement.

For the assessment of MSI, 100.0% positive call rates and negative call rates for all samples were observed, with the two sided 95% CIs ranging from (88.2%, 100.0%) to (88.5%, 100.0%) for positive call rates and (87.9%, 100.0%) to (88.55%, 100.0%) for negative call rates. For TMB determination, 46 samples included non-zero TMB scores and were included in the assessment of repeatability and reproducibility. Summary of the performance, including the standard deviation (SD) and coefficient of variation (CV) is shown Table 19, below. Twenty-three samples met the ≤ 20% CV requirements for this study.

Table 19. TMB precision analysis based on all samples with non-zero TMB scores

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Mean TMB Value	# Valid Results	Repeatability		Reproducibility		Average Median Exon Coverage
					SD	%CV	SD	%CV	
Sample 1	SUB	*EGFR* T790M	2.1	36	0.9391	44.70%	1.0447	49.80%	648.7
Sample 2	SUB	*EGFR* T790M	8.37	36	2.0437	24.40%	2.1012	25.10%	697.9
Sample 3	RE	*ALK* RE	1.51	36	0.5815	38.60%	0.6001	39.90%	592.3
Sample 4	RE	*ALK* RE	3.36	36	0.594	17.70%	0.6212	18.50%	712.8
Sample 5	RE	*ALK* RE	3.12	36	0.7572	24.30%	0.7608	24.40%	722.3
Sample 6	Indel	*EGFR* Exon 19 Deletion	5.04	35	1.2615	25.00%	1.2979	25.80%	388.6
Sample 7	Indel	*EGFR* Exon 19 Deletion	4.21	35	0.9544	22.70%	1.0088	24.00%	622
Sample 8	Indel	*EGFR* Exon 19 Deletion	2.8	36	0.6641	23.70%	0.6885	24.60%	776.9
Sample 9	SUB	*KRAS* Codons 12/13 SUB	3.33	36	0.6062	18.20%	0.7644	23.00%	825.3
Sample 10	SUB	*KRAS* Codons 12/13 SUB	4.17	36	1.243	29.80%	1.4987	36.00%	707
Sample 11	SUB	*BRAF* V600E/V600K	14.91	35	3.1604	21.20%	3.3605	22.50%	319.2
Sample 12	SUB	*BRAF* V600E/V600K	23.54	36	1.8078	7.70%	1.8277	7.80%	706.3
Sample 13	SUB	*EGFR* Exon 21 L858R	3	34	1.0863	36.20%	1.1141	37.10%	743.2
Sample 14	Amplification	*ERBB2* (HER2) Amplification	4	35	1.6585	41.50%	1.8719	46.80%	804.5
Sample 15	Amplification	*ERBB2* (HER2) Amplification	4.82	35	0.5479	11.40%	0.6082	12.60%	535.1
Sample 16	Amplification	*ERBB2* (HER2) Amplification	7.9	34	0.554	7.00%	0.593	7.50%	783.3
Sample 17	SUB	*KRAS* Codons	6.37	36	1.4682	23.00%	1.5366	24.10%	732.1

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Mean TMB Value	# Valid Results	Repeatability		Reproducibility		Average Median Exon
		12/13 SUB							
Sample 18	SUB	*EGFR* Exon 21 L858R	11.2	35	2.1352	19.10%	2.1561	19.20%	743.5
Sample 19	CNA/RE/SUB	Amplification	6.59	35	1.0807	16.40%	1.3041	19.80%	708.2
Sample 20	CNA/ SUB/Indel	Homozygous Deletion	10.95	35	0.7975	7.30%	0.936	8.50%	429.2
Sample 21	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	42.91	35	0.6259	1.50%	0.7054	1.60%	765
Sample 22	CNA/ SUB/Indel	Short Deletion (3-5bp)	2.7	36	0.4696	17.40%	0.4696	17.40%	741
Sample 23	SUB/Indel	Short Insertion (1-2bp with homopolymer repeats)	21.47	35	0.6681	3.10%	0.7558	3.50%	565.7
Sample 24	SUB/Indel	Short Deletion (1-2bp with dinucleotide repeats)	67.31	36	1.2633	1.90%	1.7042	2.50%	672.1
Sample 25	CNA/ SUB/Indel	Short Deletion (1-2bp with homopolymer repeats)	63.71	36	1.7275	2.70%	1.9587	3.10%	730.6
Sample 26	SUB/Indel	Short Deletion (1-2bp with homopolymer repeats)	51.83	36	1.0879	2.10%	1.2238	2.40%	614.7
Sample 27	CNA/ SUB/Indel	SUB	12.44	36	0.5556	4.50%	0.6515	5.20%	672.5
Sample 28	RE/ SUB/Indel	Short Insertion (1-2bp with homopolymer repeats)	5.87	35	0.6259	10.70%	0.6347	10.80%	700.2
Sample 29	CNA/ SUB	SUB	1.15	34	1.7122	149.00 %	1.8914	164.60 %	740.3
Sample 30	CNA/RE/SUB /Indel	RE	3.67	35	1.2768	34.80%	1.5155	41.30%	732.4
Sample 31	RE/SUB/Indel	Short Insertion (1-2bp with dinucleotide repeats)	1.26	36	0	0.00%	0	0.00%	732.3
Sample 32	CNA/ SUB/Indel	Short Deletion (3-5bp)	6.83	36	2.4023	35.20%	2.4413	35.80%	689.7
Sample 33	SUB/Indel	Short Insertion (3-5bp)	48.23	36	1.1046	2.30%	1.2945	2.70%	824.1
Sample 34	CNA/RE/SUB	Short Insertion	5.18	36	0.5158	10.00%	0.5668	10.90%	574

Sample*	Alteration Type(s) Assessed	Alteration for Selection**	Mean TMB Value	# Valid Results	Repeatability		Reproducibility		Average Median Exon
	/Indel	(3-5bp)							
Sample 35	RE/SUB/Indel	SUB	4.21	35	0.6259	14.90%	0.641	15.20%	765.9
Sample 36	RE/SUB/Indel	Short Insertion (>5bp)	1.82	36	0.7857	43.20%	0.8201	45.10%	815.7
Sample 37	CNA/RE/SUB /Indel	Short Deletion (>5bp)	8.09	36	1.0665	13.20%	1.2517	15.50%	791.4
Sample 38	CNA/RE/SUB /Indel	Short Insertion (>5bp)	2.2	35	0.9301	42.40%	0.9586	43.70%	746.5
Sample 39	CNA /SUB	Amplification	3.67	35	0.9463	25.80%	1.0407	28.30%	704.5
Sample 40	CNA/RE/SUB /Indel	RE	12.65	35	0.4732	3.70%	0.5019	4.00%	645.9
Sample 41	CNA/ SUB/Indel	Homozygous Deletion	8.22	35	0.8349	10.20%	0.8505	10.40%	761.3
Sample 42	CNA/RE/SUB /Indel	Short Insertion (1-2bp with dinucleotide repeats)	10.16	36	0.2425	2.40%	0.297	2.90%	714.8
Sample 43	RE/SUB	Short Deletion (>5bp)	2.24	36	1.3058	58.30%	1.4511	64.80%	685.3
Sample 44	CNA /SUB	Homozygous Deletion	7.28	35	1.5508	21.30%	1.6205	22.30%	769.3
Sample 45	CNA/RE/SUB /Indel	Amplification	6.23	36	1.3755	22.10%	1.3755	22.10%	687.2
Sample 46	CNA/RE/SUB /Indel	RE	4.18	35	1.6427	39.30%	1.7542	42.00%	478.5

*Samples 1-18 were selected to represent alterations associated with CDx claims and Samples 19-46 were selected for platform-wide analysis.
** While all possible alterations harbored in the sample were included in the analysis, each sample was selected based on a specific alteration in accordance with the study protocol.
Abbreviations: SD – Standard Deviation, CV – Coefficient of Variation, SUB – substitution, Indel – Insertion or Deletion, CNA – Copy Number Alteration, RE – Rearrangement.

a. Reagent Lot-to-Lot Reproducibility

Three lots of critical reagents were assessed for four replicates in a full factorial design. Reagents were evaluated as internally prepared kits for each process step (LC, HC, sequencing). The variant level pairwise APA/ANAs among three reagent lots and the corresponding 95% confidence interval were calculated. The use of three different lots of reagents did not impact performance as 27 of 28 samples (96.4%) had pairwise agreement estimates (APA and ANA) above 95%. One sample had APA estimates below 90% (85.9% to 88.7%). The putative source of variability was determined to be non-focal copy number amplifications with low copy numbers close to the calling threshold; no specific reagent lot performed differently among three lots for this sample.

b. Instrument-to-Instrument Reproducibility

Four replicates for each sample were sequenced on each of three Illumina HiSeq4000 sequencers, in a full factorial design. The variant level pairwise APA/ANAs among three sequencers and the corresponding 95% CI per sample were calculated. The use of three different sequencers did not impact performance as 27 of 28 samples (96.4%) had pairwise agreement estimates (APA and ANA) of at least 97%. One sample had APA estimates below 90% (86.6% to 89.2%). The putative source of variability was determined to be non-focal copy number amplifications with low copy numbers close to the calling threshold; no specific sequencer performed differently among three lots for this sample.

6. Reagent Lot Interchangeability

Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ assay and F1CDx. For reagent lot interchangeability performance data, see the Summary of Safety and Effectiveness for P160018.

7. Stability

a. Reagent Stability

Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ Assay and F1CDx. For reagent stability performance data, see the Summary of Safety and Effectiveness Data for P160018. The claimed reagent stability is 4 months for the LC and HC kits, and 3 months for the sequencing kits.

b. DNA Stability

Stability of DNA was evaluated through a retrospective review of data generated using the F1 LDT assay. Samples from 47 unique clinical specimens from 21 different tissues of origin were evaluated. The sample set covered 200 alterations inclusive of nucleotide changes, indels, CNAs (amplifications and losses) and rearrangements. Duration of DNA storage at time of testing ranged from 48 to 464 days, with a median of 184 days and a mean of 199 days. For this study, 199 of 200 alteration calls were concordant. A 242-day old sample with a single alteration call (*FGF10* amplification) that met inclusion criteria was discordant; however, this sample was classified as not meeting all QC criteria due to other data quality issues. Sixteen other samples had concordant calls with DNA age > 242 days. Based on this data, DNA stored in accordance with internal procedures can be considered stable for up to six months. An additional prospective DNA stability study is underway.

c. FFPE Slide Stability

The FFPE Slide Stability Study is an ongoing study with data summarized for T_0, T_1 (30 days) and T_2 (6 months) time points. This study evaluated the stability of FFPE tumor tissue prepared as slides prior to DNA extraction for use with F1CDx. The study evaluated five tumor samples including ovarian, lung, colorectal cancer, melanoma and breast cancer that contained a variety of DNA alterations, as described in Table 20, below. The five samples were selected to include specific alteration types

that were reflective of the CDx-alterations (shown in Table 21); but were found to contain additional alterations as well (13 CNAs, one rearrangement, 53 base substitutions and five indels). To assess stability of pre-cut FFPE tissue for genomic alterations, the agreement between results from the defined time points for each sample were calculated by comparing the alteration call reported at each follow-up time point to the alteration call at baseline (T_0). Alterations at the 30-day time point and the 6-month time point were in 100% agreement with the Day 0 baseline results (T_0). Therefore, FFPE slides stored in accordance with internal procedures can be considered stable for at least 6 months. Further assessment at Months 12 and 15 will demonstrate stability of FFPE slides beyond 6 months.

Table 20. Stability Results at baseline, 30 days and 6 months

	Baseline Call (T_0)		**Percent Agreement to T_0**	**Percent Agreement to T_0**
Tissue	**Gene**	**Variant Effect**	**30 days (T_1)**	**6 months (T_2)**
Ovarian	*BRCA1*	c.1340_1341insG, p.H448fs*8	100.0% (2/2)	100.0% (2/2)
Lung	*KRAS*	c.34G>T, p.G12C	100.0% (2/2)	100.0% (2/2)
CRC	*PIK3CA*	c.3139C>T, p.H1047Y	100.0% (2/2)	100.0% (2/2)
Melanoma	*CDKN2A*	Homozygous Deletion	100.0% (2/2)	100.0% (2/2)
Melanoma	*CDKN2B*	Homozygous Deletion	100.0% (2/2)	100.0% (2/2)
Breast	*ERBB2*	Amplification	100.0% (1/1)	100.0% (2/2)

Table 21. Percent agreement for each variant type

Variant type	**# of variants**	**30 days (T_1) Percent Agreement (# agreement/total)**	**95% 2-sided CI LB, UB**	**6 months (T_2) Percent Agreement (# agreement/total)**	**95% 2-sided CI LB, UB**
Copy Number	13	100.0% (23/23)	85.2%, 100.0%	100.0% (26/26)	86.8%, 100.0%
Rearrangement	1	100.0% (2/2)	15.8%, 100.0%	100.0% (2/2)	15.8%, 100.0%
Substitution	53	100.0% (98/98)	96.3%, 100.0%	100.0% (106/106)	96.6%, 100.0%
Ins/Dels	5	100.0% (7/7)	59.0%, 100.0%	100.0% (10/10)	69.2%, 100.0%

Abbreviations: LB – Lower Bound, CV – Upper Bound.

8. General Lab Equipment and Reagent Evaluation

a. DNA Amplification

Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDx$_{BRCA}$ assay and F1CDx. For DNA amplification performance data, see the Summary of Safety and Effectiveness Data for P160018.

b. DNA Extraction

The performance of DNA extraction from FFPE tumor specimens was evaluated. The DNA extraction procedure for the F1CDx assay was assessed by testing FFPE specimens including two samples per tissue type for ten different tissue types including lung, breast, ovarian, melanoma, colorectal, brain, hepatic, pancreatic, thyroid, and bladder with different representative types of alterations. Samples were

run in duplicate for a total of 240 extractions, employing two different KingFisher Flex Magnetic Particle Processors (120 extractions per processor) and comparing across three extraction reagent lots (80 extractions per reagent lot). Average DNA yield was calculated across 12 replicates for each sample. All average DNA yields were significantly above the minimum requirement of 55 ng, with the minimum being 758.3 ng. Only one sample aliquot out of 240 replicates failed the DNA yield specification, and the success rates based on the reagent lot and the equipment were 98.8% (79/80) and 99.2% (119/120), respectively, passing the acceptance criteria (≥ 90%). Concordance of all genomic alterations detected was also analyzed for all variant types across 12 replicates for each sample. Table 22 provides a summary of concordance across replicates. A study with and additional ten samples will be completed post-market.

Table 22. Summary of Concordance Across Replicates of DNA Extraction Study

Group	$N_{concordance}$	N_{total}	Concordance (95% CI)
Substitutions (All MAF)	2700	2969	90.9% (89.9% 91.9%)
Substitutions (MAF > 10%)	1631	1637	99.6% (99.2% 99.9%)
Substitutions (All MAF, excluding hypermutated sample)*	1663	1685	98.7% (98% 99.1%)
Indel (All)	465	476	97.7% (95.9% 98.8%)
Copy Number: Amplification	307	314	97.8% (95.4% 99%)
Copy Number: Loss	132	144	91.7% (85.9% 95.3%)
Rearrangement	84	90	93.3% (85.9% 97.2%)

*One sample included in the study was hypermutated, harboring many alterations near LoD and exhibited evidence of external contamination. Concordance of substitutions was 80.8% for this sample.

9. Guard banding/Robustness

Guard banding studies were completed to evaluate the performance of the F1CDx assay and the impact of process variation with regard to uncertainty in the measurement of DNA concentration at various stages of the process. Guard bands were evaluated relative to observed and measured process variability for Library Construction (LC), Hybrid Capture (HC), and Sequencing. Each of the three guard banding experiments demonstrated reliable and robust performance at all DNA input levels evaluated. A total of 255 samples were processed; 90 to assess DNA input into LC, 90 to assess DNA input into HC, and 75 to assess DNA input into sequencing. For LC input, five samples were run in triplicate over six different DNA input levels representing -20% and -50% from the lower limit (50 ng) to +20% and +50% from the upper limit (1000 ng) needed for LC (n = 90). Five samples were run in triplicate over six DNA input levels representing -25% and -50% from the lower limit (0.5 µg) to +25% and +50% from the upper limit (2.0 µg) for HC input. The third component of the guard banding study evaluated the captured DNA input into the sequencing reaction. Five samples were run in triplicate over five different DNA input levels

representing ±10% and ±20% from the required amount needed for sequencing (1.75 nM; n=75). Concordance of detected alterations was calculated for each condition across successful replicates. Study design and results are shown below in Tables 23-1 through 23-4, stratified by variant type.

Table 23-1. Summary of the success rate per process and per input level, and concordance of substitutions (SUB) among successful replicates.

Process	Input Level	# of sample failures	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	184	184	100.0% (98.0%, 100.0%)
LC	40 ng	0/15	192	192	100.0% (98.1%, 100.0%)
LC	50 ng	0/15	191	192	99.5% (97.1%, 100.0%)
LC	1000ng	0/15	192	192	100.0% (98.1%, 100.0%)
LC	1200 ng	0/15	191	192	99.5% (97.1%, 100.0%)
LC	1500 ng	0/15	190	192	99.0% (96.3%, 99.9%)
HC	0.25 µg	15/15	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	30	30	100.0% (88.4%, 100.0%)
HC	0.5 µg	1/15	166	166	100.0% (97.8%, 100.0%)
HC	2.0 µg	0/15	192	192	100.0% (98.1%, 100.0%)
HC	2.5 µg	0/15	192	192	100.0% (98.1%, 100.0%)
HC	3.0 µg	0/15	192	192	100.0% (98.1%, 100.0%)
Seq	1.4 nM	0/15	192	192	100.0% (98.1%, 100.0%)
Seq	1.575 nM	1/15	180	180	100.0% (98.0%, 100.0%)
Seq	1.75 nM	1/15	184	184	100.0% (98.0%, 100.0%)
Seq	1.925 nM	0/15	192	192	100.0% (98.1%, 100.0%)
Seq	2.1 nM	0/15	192	192	100.0% (98.1%, 100.0%)

*All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-2. Summary of the success rate per process and per input level, and concordance of insertions and deletions (INDEL) among successful replicates.

Process	Input Level	# of sample failures	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	17	17	100.% (80.5%, 100.0%)
LC	40 ng	0/15	18	18	100.0% (81.5%, 100.0%)
LC	50 ng	0/15	18	18	100.0% (81.5%, 100.0%)
LC	1000ng	0/15	18	18	100.0% (81.5%, 100.0%)
LC	1200 ng	0/15	18	18	100.0% (81.5%, 100.0%)
LC	1500 ng	0/15	18	18	100.0% (81.5%, 100.0%)

Process	Input Level	# of sample failures	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
HC	0.25 µg	15/15	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	4	4	100.0% (39.8%, 100.0%)
HC	0.5 µg	1/15	18	18	100.0% (81.5%, 100.0%)
HC	2.0 µg	0/15	18	18	100.0% (81.5%, 100.0%)
HC	2.5 µg	0/15	18	18	100.0% (81.5%, 100.0%)
HC	3.0 µg	0/15	18	18	100.0% (81.5%, 100.0%)
Seq	1.4 nM	0/15	18	18	100.0% (81.5%, 100.0%)
Seq	1.575 nM	1/15	16	16	100.0% (79.4%, 100.0%)
Seq	1.75 nM	1/15	17	17	100.0% (80.5%, 100.0%)
Seq	1.925 nM	0/15	18	18	100.0% (81.5%, 100.0%)
Seq	2.1 nM	0/15	18	18	100.0% (81.5%, 100.0%)

* All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-3. Summary of the success rate per process and per input level, and concordance of rearrangements (RE) among successful replicates.

Process	Input Level	# of sample failures	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	6	6	100.0% (54.1%, 100.0%)
LC	40 ng	0/15	6	6	100.0% (54.1%, 100.0%)
LC	50 ng	0/15	6	6	100.0% (54.1%, 100.0%)
LC	1000ng	0/15	6	6	100.0% (54.1%, 100.0%)
LC	1200 ng	0/15	6	6	100.0% (54.1%, 100.0%)
LC	1500 ng	0/15	6	6	100.0% (54.1%, 100.0%)
HC	0.25 µg	15/15	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	2	2	100.0% (15.8%, 100.0%)
HC	0.5 µg	1/15	6	6	100.0% (54.1%, 100.0%)
HC	2.0 µg	0/15	6	6	100.0% (54.1%, 100.0%)
HC	2.5 µg	0/15	6	6	100.0% (54.1%, 100.0%)
HC	3.0 µg	0/15	6	6	100.0% (54.1%, 100.0%)
Seq	1.4 nM	0/15	8	9	88.9% (51.8%, 99.7%)
Seq	1.575 nM	1/15	9	9	100.0% (66.4%, 100.0%)
Seq	1.75 nM	1/15	8	8	100.0% (63.1%, 100.0%)
Seq	1.925 nM	0/15	8	9	88.9% (51.8%, 99.77%)
Seq	2.1 nM	0/15	7	9	77.8% (40.0%, 97.2%)

*All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

Table 23-4. Summary of the success rate per process and per input level, and concordance of copy number alterations (CN) among successful replicates.

Process	Input Level	# of sample failures	# of concordant successes	# of variant comparisons	Success Rate (95% CI) (Number of Concordant comparisons)
LC	25 ng	1/15	128	128	100.0% (97.2%, 100.0%)
LC	40 ng	0/15	132	132	100.0% (97.2%, 100.0%)
LC	50 ng	0/15	132	132	100.0% (97.2%, 100.0%)
LC	1000ng	0/15	132	132	100.0% (97.2%, 100.0%)
LC	1200 ng	0/15	132	132	100.0% (97.2%, 100.0%)
LC	1500 ng	0/15	132	132	100.0% (97.2%, 100.0%)
HC	0.25 µg	15/15	0	0	NA* (no samples sequenced)
HC	0.375 µg	12/15	13	14	92.9% (66.1%, 99.8%)
HC	0.5 µg	1/15	107	108	99.1% (95.0%, 100.0%)
HC	2.0 µg	0/15	129	132	97.7% (93.5%, 99.5%)
HC	2.5 µg	0/15	129	132	97.7% (93.5%, 99.5%)
HC	3.0 µg	0/15	130	132	98.5% (94.6%, 99.8%)
Seq	1.4 nM	0/15	131	132	99.2% (95.9%, 100.0%)
Seq	1.575 nM	1/15	122	128	95.3% (90.1%, 98.3%)
Seq	1.75 nM	1/15	128	128	100.0% (97.2%, 100.0%)
Seq	1.925 nM	0/15	130	132	98.5% (94.6%, 99.8%)
Seq	2.1 nM	0/15	131	132	99.2% (95.9%, 100.0%)

*All samples failed at the input level of 0.25 µg and as a result, there is no data available to present for that level.

10. Tissue Comparability

A large-scale retrospective analysis was conducted, using 80,715 specimens from 43 tissue types, to establish the comparability of assay performance across tumor tissue types. The goal of the study was to demonstrate that genomic profiling can be performed on DNA derived from FFPE specimens from any tissue type. The retrospective analysis of data included specimens assayed using the F1 LDT assay under the same or substantially similar testing protocols, reagents, and conditions used for F1CDx. DNA extraction and post-DNA extraction data were assessed for comparability of performance across tissue types. The data set for analysis consisted of routine clinical samples analyzed using the F1 LDT from March 25, 2015 to March 13, 2017.

Table 24 below includes a summary of the tissue types included in the study. Thirty-nine of the 43 tissue types had ≥ 90% of specimens passing DNA extraction QC. Specimen DNA extraction pass rates for the remaining four tumor tissue types: lung, pancreas, pelvis and prostate, were 89.6%, 89%, 89%, and 79.7%, respectively. Each of these four tissue types has characteristically small biopsies.

Table 24. Summary of tissue types included in the tissue comparability study

Tissue Group	Number of Samples in Tissue Group	Biopsy Sites Included	% Passing DNA Extraction Yield QC	% Passing LC Yield QC	% Passing HC Yield QC	% Passing Median Exon Coverage QC
Abdomen	1161	Abdomen, Abdominal wall	91.2	99.8	99.1	98.3
Adrenal Gland	689	Adrenal Gland	90.9	99.8	99.8	98.7
Anus	115	Anus	97.3	99.1	99.1	99.1
Appendix	177	Appendix	97.3	99.3	100.0	100.0
Bladder	974	Bladder	98.1	99.8	99.6	99.3
Brain	5840	Brain, Pituitary gland, Pituitary, Central nervous system (CNS), Pineal, Pineal Gland	96.1	99.8	99.6	99.2
Breast	3304	Breast	96.0	99.7	99.4	98.7
Cervix	346	Cervix	96.3	99.3	100.0	99.3
Chest Wall	820	Chest Wall	92.0	99.6	99.4	99.3
Colon	5560	Colon, Rectum	97.4	99.9	99.6	99.2
Diaphragm	112	Diaphragm	94.8	100.0	99.0	97.8
Esophagus	902	Esophagus	96.1	100.0	99.7	98.6
Fallopian Tube	211	Fallopian Tube	99.0	100.0	100.0	100.0
Gallbladder	373	Gallbladder	96.6	100.0	100.0	99.7
Gastro-esophageal junction	301	Gastro-esophageal junction	93.8	100.0	99.2	98.8
Head and Neck	1777	Head and neck, Tongue, Trachea, Mouth, Nasal Cavity, Nasopharynx and Paranasal Sinuses, Tonsil, Eye, Larynx, Head or Neck, Ear, Lacrimal Gland	93.4	99.7	99.4	99.1
Kidney	945	Kidney	96.5	99.8	98.8	99.2
Liver	12112	Liver	90.4	99.6	99.6	98.7
Lung	15700	Lung, Pleura	89.6	99.6	99.3	98.8
Lymph Node	7785	Lymph Node	93.0	99.7	99.5	98.9
Malignant effusions	1587	Pleural Fluid, Peritoneal Fluid, Pericardial Fluid	94.4	99.8	99.4	98.8
Mediastinum	493	Mediastinum	91.0	99.8	99.5	99.5
Omentum	1761	Omentum	94.6	99.9	99.7	98.9
Ovary	1442	Ovary	98.6	99.8	99.2	99.8
Pancreas	1764	Pancreas	89.0	99.3	98.7	97.9
Pancreatobiliary	166	Bile Duct	91.0	100.0	98.4	96.7
Pelvis	863	Pelvis	89.4	99.7	99.6	99.3
Pericardium	74	Pericardium	100.0	100.0	100.0	100.0
Peritoneum	1027	Peritoneum	92.4	99.9	99.6	98.0
Prostate	1450	Prostate	79.5	99.0	97.3	96.1

Tissue Group	Number of Samples in Tissue Group	Biopsy Sites Included	% Passing DNA Extraction Yield QC	% Passing LC Yield QC	% Passing HC Yield QC	% Passing Median Exon Coverage QC
Rare Tissues *	176	Penis (43), Testes (30), Testis (21), Urethra (32), Heart (32), Thymus (14), Parathyroid (1), Peripheral Nervous System (PNS; 1), Placenta (2)	94.2	100.0	97.4	100.0
Salivary Gland	302	Parotid Gland, Salivary Gland	95.2	99.6	97.3	99.2
Skin	1617	Skin	95.9	99.3	99.5	99.0
Small Intestine	1025	Small Intestine, Duodenum, Ampulla of Vater	97.2	99.7	99.5	99.1
Soft Tissue	3356	Soft Tissue, Retroperitoneum, Muscle	91.6	99.8	99.5	99.0
Spleen	86	Spleen	94.9	100.0	98.7	97.3
Stomach	1199	Stomach	97.1	99.8	99.7	99.1
Thyroid	355	Thyroid, Thyroid Gland	95.0	99.4	99.7	99.7
Unknown	509	Other, Not Provided	90.4	99.2	98.7	99.2
Ureter	116	Ureter	96.0	100.0	100.0	100.0
Uterus	1308	Uterus, Endometrium	99.0	99.9	99.8	99.7
Vagina	327	Vagina	97.0	99.3	99.7	99.7
Vulva	71	Vulva	100.0	100.0	98.6	100.0
Whipple Resection	437	Whipple Resection	98.7	100.0	99.7	99.7

*Biopsy sites with < 50 samples were combined into a single 'Rare Tissues' group for analysis and the number of each is indicated.

Of specimens entering the assay at LC, 39 of 43 tissue types had ≥ 90% of specimens resulting in a patient report being issued. The four tissue types below 90% include pancreatobiliary, appendix, pericardium, and prostate, and had pass rates of 83%, 88%, 79%, and 84%, respectively. For these four tissue types, the most frequent cause of failure was low tumor purity with no alterations detected.

The mean LC yields across tissue types were 7,050–8,643 ng. The percent of specimens passing the LC yield criteria of > 545ng for each tissue types ranged from 98%-100%. After HC, the mean yields across tissue types ranged from 434-576 ng, well above the minimum requirement of 140 ng. The percent of specimens passing HC across tissue types ranged from ranged from 97%-100%.

The average median exon coverage assessed across tissue types ranged from 702X-793X. The percent of specimens passing QC for median coverage across tissue types ranged from 96%-100%. Uniformity of coverage was assessed by calculating the

average percent of targets with > 100X coverage across tissue types, and ranged from 99.0%-99.8%. The percentage of specimens passing this QC metric ranged 98%-100%. The average sequencing error rate, assessed across tissue types, is 0.0028-0.0031, well below the required error rate of 0.01 for assay acceptance.

B. Animal Studies

No animal studies were conducted using the F1CDx assay.

C. Additional Studies

No additional studies were conducted using the F1CDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Multiple clinical concordance studies were conducted to support the companion diagnostic (CDx) claims indicated in Table 1 of the intended use statement. For these studies, the F1CDx test was compared to an approved CDx test (Table 25) using samples representative of the intended use population for that specific device.

Table 25. FDA Approved CDx devices used as comparator method for concordance studies

Biomarker	Comparator Method
EGFR exon 19 deletions and L858R	**cobas**® *EGFR* Mutation Test v2
EGFR T790M	**cobas**® EGFR Mutation Test v1 **cobas**® EGFR Mutation Test v2
ALK rearrangements	Ventana *ALK* (D5F3) CDx Assay Vysis *ALK* Break-Apart FISH Probe Kit
KRAS	*therascreen*® KRAS RGQ PCR Kit
ERBB2 (HER2) Amplifications	Dako HER2 FISH pharmDx® Kit
BRAF V600	**cobas**® *BRAF* V600 Mutation Test THxID™ *BRAF* kit
BRCA1 and *BRCA2*	FoundationFocus CDx_{BRCA}

CDx claims were based on a non-inferiority (NI) statistical testing approach using the enrichment design presented in the paper by Li (2016)[9], when the concordance study sample is not a random sample from the companion diagnostic (F1CDx) intended use population and a reference standard is not available. F1CDx was compared to FDA-approved CDxs. The agreements were calculated based on the methods described in the paper by Li (2016)[9]. All studies based on NI passed the acceptance criteria specified in each study protocol. The clinical concordance studies, with the exception of *ALK* and *EGFR* T790M, were subject to pre-screening bias, therefore the concordance results may be overestimated and the failure rate may be underestimated. Details regarding the pre-screening method(s) are included in the study summaries below.

A. FoundationOne CDx Concordance Study for *EGFR* Exon 19del/L858R

Clinical validity of F1CDx assay was established as a companion diagnostic used for identifying patients with advanced NSCLC who may be eligible for treatment with

Gilotrif® (afatinib), Iressa® (gefitinib), or Tarceva® (erlotinib). A study was performed using 282 retrospective samples from patients with NSCLC. Samples used in this study underwent pre-screening using the F1 LDT or prescreening by an external vendor to enrich for positive samples. The prescreen failure rate using the F1 LDT was 3.7% and is unknown for the external vendor. The *EGFR* diagnostic results from the F1CDx assay were compared against those obtained from the approved **cobas**® v2 *EGFR* Mutation Test (Roche Molecular Systems). Samples were tested using **cobas**® *EGFR* mutation test (CCD1) with an approximately equal number of mutation positive and negative samples, followed by testing with F1CDx and a second, replicate testing of **cobas**® *EGFR* mutation test (CCD2). NSCLC tumor samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age and gender data were available and were found to be similar to the pivotal study EURTAC for Tarceva® (erlotinib).

A total of 406 samples were tested for the full analysis. The following steps were followed to exclude ineligible or failed samples sequentially:

1. Eighty-four (84) samples identified as duplicate samples with other pairing samples based on germline single nucleotide polymorphism analysis were removed.
2. Fourteen (14) samples whose *EGFR* testing was not done for CCD1 were removed.
3. Thirteen (13) samples with missing or invalid results for CCD1 were removed.
4. Ten (10) samples with F1CDx testing as "TIFA (tumor insufficient for analysis)" were removed.
5. Three (3) samples whose *EGFR* results were not evaluable due to no tumor or no tissue for CCD2 were removed.

After all the above five steps, 282 samples remained for analysis. Among those, there were 267 samples with complete records and 15 samples with missing results in either F1CDx and/or CCD2. Missing data was caused by process failures or samples not meeting assay specifications.

Two separate concordance analyses were performed: one with samples with complete records only (N = 267), and the other with all 282 samples, where missing data was handled by multiple imputation. Data from concordance testing is summarized in Table 26, below.

Table 26. Concordance Table with CCD1, CCD2 and F1CDX results with eligible samples

	CCD1+				**CCD1-**			
	CCD2+	**CCD2-**	**CCD2 missing**	**Total**	**CCD2+**	**CCD2-**	**CCD2 missing**	**Total**
F1CDx+	106	0	0	106	1	1*	0	2
F1CDx-	2**	1	0	3	3	153	0	156
F1CDx Missing	3	0	0	3	1	9	2	12
Total	111	1	0	112	5	163	2	170

*****The sample noted here was the only sample where both replicates of the **cobas**® v2 assay reported negative results but F1CDx reported positive for L858R with AF 33%. Upon further review, F1CDx identified a second somatic mutation in-cis (on same allele) as that of L858R with identical AF only 17bp downstream: *EGFR* A864P. Therefore, it is suspected that this second mutation interfered with the allele-specific PCR primers of **cobas**® v2, and thus L858R went undetected.
******The samples noted here were reported as positive for both replicates of **cobas**® v2 for Exon 19del, but negative by F1CDx. For one sample, F1CDx detected the exon19 deletion, but incorrectly annotated the variant as 2 frameshift mutations. This would have been corrected by manual curation review, which was not part of this concordance study. For the second sample, F1CDx identified an 18bp exon 19 insertion event, with protein effect K745_E746insIPVAIK. As **cobas**® v2 is not designed to detect insertion events at exon 19, this result may indicate an error by **cobas**® v2.

Based on samples with complete data only (15 samples with missing F1CDx results excluded), the agreement analysis results are shown in Tables 27A and 27B. The agreements comparing F1CDx and CCD2 were calculated both with and without adjusting for the prevalence of *EGFR* Exon19delL858R in the original trial for **cobas**® *EGFR* mutation test.

Table 27A. PPA and NPA for *EGFR* Exon 19del /L858R Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	99.1%
PPA_{C1F}	97.2%
NPA_{C1C2}	97.5%
NPA_{C1F}	98.7%
PPA_{C2C1}	96.4%
PPA_{C2F}	95.5%
NPA_{C2C1}	99.4%
NPA_{C2F}	99.4%

Table 27B. PPA and NPA for *EGFR* Exon 19 del/L858R Adjusted for Prevalence (22.1%)

	% Agreement
PPA_{C1C2}	99.1%
PPA_{C1F}	97.2%
NPA_{C1C2}	97.5%
NPA_{C1F}	98.7%
PPA_{C2C1}	91.7%
PPA_{C2F}	92.1%
NPA_{C2C1}	99.7%
NPA_{C2F}	99.4%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bounds of one-sided 95% Cl for adjusted agreements are computed using bootstrap and the results are shown in Table 28, below.

Table 28. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, ζ_{NPA2} (complete data only)

Parameter	Mean	95% one-sided upper bound
ζ_{PPA1}	1.9%	4.6%
ζ_{NPA1}	-1.3%	0.6%
ζ_{PPA2}	-0.3%	3.4%
ζ_{NPA2}	0.4%	1.7%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

The mutations detected by **cobas**® *EGFR* mutation test include all the mutations detected by *therascreen*® *EGFR* RGQ PCR Kit, as well as a few additional exon19 deletions/L858R variants. Several concordance studies comparing the **cobas**® *EGFR* mutation test and therascreen® *EGFR* RGQ PCR Kit have been reported in literature[10, 11, 12], supporting that these two assays are equivalent. Additionally, a post-market concordance study will also be completed comparing F1CDx to the therascreen® *EGFR* RGQ PCR Kit.

B. <u>FoundationOne CDx Concordance Study for *EGFR* T790M</u>

Clinical validity of F1CDx was established as a companion diagnostic device used for identifying NSCLC patients harboring *EGFR* T790M that may be eligible for treatment with Tagrisso® (osimertinib). The patient samples and corresponding demographic information were obtained from AstraZeneca in connection with the clinical studies entitled AURA (NCT01802632), AURA2 (NCT02094261) and AURA3 (NCT02151981). Samples in these clinical studies were screened for *EGFR* T790M mutation status in these studies using the **cobas®** v1 or v2 assay. The clinical trial results were used as the comparator method results (CCD1). An approximately equal number of *EGFR* T790M mutation positive and negative samples were then tested with **cobas®** v2 assay (CCD2) and F1CDx. The T790M status was evaluated based on the agreement between both the F1CDx and the **cobas®** assay results and the agreement between the two replicates of the **cobas®** assay results. The summary statistics of age and sex were similar to the estimates from the pivotal trial AURA2 study.

A total of 354 samples were tested for the full analysis. The following steps were followed to exclude ineligible or failed samples sequentially:

1. One (1) sample identified as a duplicate sample with the other pairing sample based on germline single nucleotide polymorphism analysis was excluded.
2. One (1) sample with invalid results from CCD1 was excluded.

3. Thirty-eight (38) samples were excluded due to low tumor content (N = 35), or not processed due to no tissue remaining (N = 3) in F1CDx.
4. Two (2) samples with no tissue left were excluded.

After all the above four steps, 312 samples remained for analysis. Among those, there were 227 samples with complete records and 82 samples with missing results in either F1CDx and/or CCD2. Of the 82 samples that were assigned as missing data for F1CDx, 78 samples had no sequencing results from F1CDx and 4 samples were had a QC status as "Fail" after curation. CCD2 had 22 samples with missing data in total, in which 19 samples also had missing values in F1CDx.

Two separate concordance analyses were performed: one included samples with complete records only (N = 227), and the other with all 312 samples, where missing data was handled by multiple imputation. Data from concordance testing is summarized in Table 29 below.

Table 29. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples

	CCD1+				**CCD1-**			
	CCD2+	**CCD2-**	**CCD2 missing**	**Total**	**CCD2+**	**CCD2-**	**CCD2 missing**	**Total**
F1CDx+	87	19	1	107	8	15	0	23
F1CDx -	1	4	0	5	0	93	2	95
F1CDx Missing	21	4	8	33	1	37	11	49
Total	109	27	9	145	9	145	13	167

Based on samples with complete data only (85 samples with missing F1CDx and/or CCD2 results removed), the agreement analysis results are shown in Tables 30A and 30B. The agreements comparing F1CDx and CCD2 were calculated both with and without adjusting for the prevalence of *EGFR* T790M in the original trial for **cobas**® *EGFR* Mutation Test v2.

Table 30A. PPA and NPA for *EGFR* T790M, Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	79.3%
PPA_{C1F}	95.5%
NPA_{C1C2}	93.1%
NPA_{C1F}	80.2%
PPA_{C2C1}	91.7%
PPA_{C2F}	99.0%
NPA_{C2C1}	82.4%

Table 30B. PPA and NPA for *EGFR* T790M, Adjusted for Prevalence (62.5%)

	% Agreement
PPA_{C1C2}	79.3%
PPA_{C1F}	95.5%
NPA_{C1C2}	93.1%
NPA_{C1F}	80.2%
PPA_{C2C1}	95.0%
PPA_{C2F}	98.9%
NPA_{C2C1}	72.9%

	% Agreement
NPA_{C2F}	74.0%

	% Agreement
NPA_{C2F}	67.5%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bound of one-sided 95% Cl for adjusted agreements were computed using bootstrap and the results are shown in Table 31, below.

Table 31. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, ζ_{NPA2} (complete data only)

	Mean	95% one-sided upper bound
ζ_{PPA1}	-16.2%	-9.9%
ζ_{NPA1}	12.9%	18.1%
ζ_{PPA2}	-3.8%	-0.6%
ζ_{NPA2}	5.5%	11.2%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

Due to the limited availability of samples, a large percentage of samples with volume $< 0.6mm^3$ were included in this study (N = 94), while routine F1CDx testing requires samples with volume $\geq 0.6mm^3$. Subgroup analysis was performed based on samples with volume $\geq 0.6mm^3$ (N = 133). The concordance data for this subgroup is shown in Table 32 and agreement calculations are shown in Table 33, below.

Table 32. Concordance Table with CCD1, CCD2 and F1CDx results with samples with volume ≥ 0.6mm^3

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
F1CDx +	54	9	63	3	5	8
F1CDx -	0	4	4	0	58	58
Total	54	13	67	63	183	66

Table 33. Agreement analysis based on samples with volume ≥ 0.6mm^3

	% Agreement Volume ≥ 0.6mm^3 (N = 133)
PPA_{C1C2}	80.6%
PPA_{C1F}	94.0%
NPA_{C1C2}	95.5%
NPA_{C1F}	87.9%
PPA_{C2C1}	96.7%
PPA_{C2F}	100.0%
NPA_{C2C1}	74.7%
NPA_{C2F}	76.6%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bound of one-sided 95% Cl for adjusted agreements were computed for samples with volume ≥ 0.6mm^3, using bootstrap and the results are shown in Table 34, below.

Table 34. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, ζ_{NPA2} based on samples with volume ≥ 0.6mm^3

		Volume ≥ 0.6mm^3 (N = 133)
Mean	ζ_{PPA1}	-13.4%
	ζ_{NPA1}	7.6%
	ζ_{PPA2}	-3.3%
	ζ_{NPA2}	-1.7%
Upper bound of one-sided 95% CI	ζ_{PPA1}	-7.5%
	ζ_{NPA1}	13.6%
	ζ_{PPA2}	-1.0%
	ζ_{NPA2}	5.6%
95% one-sided CI	ζ_{PPA1}	(-20.9%, -7.5%)
	ζ_{NPA1}	(3%, 13.6%)
	ζ_{PPA2}	(-6.5%, -1.0%)
	ζ_{NPA2}	(-9.2%, 5.6%)

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

A difference in detection sensitivity between CCD1 and CCD2 was also observed, with CCD1 appearing to be more sensitive than CCD2. This could be attributed to the fact that CCD1 was run 2-3 years ago using freshly biopsied tissue, while CCD2 testing was recently performed using DNA extracted from archival FFPE sections. The clinical outcome data in the subset of the patient population for which an *EGFR* T790M mutation was detected by F1CDx with a MAF < 5% but considered negative by the **cobas**® *EGFR* Mutation Test v2 has not been established but is ongoing and will be provided post-market. Additionally, a limitation statement for the device has been included to inform users of the lack of clinical outcome data for Tagrisso® (osimertinib) in this subset of the patient population (MAF < 5%).

C. FoundationOne CDx Concordance Study for *ERBB2* (HER2)

Clinical validity of F1CDx as a companion diagnostic device used to identify patients eligible for treatment with approved HER2-directed therapies including Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), and Perjeta® (pertuzumab) was established. A study was performed using 317 pre-screened retrospective samples obtained from patients with advanced breast cancer. The failure rate for pre-screening is not known; however, the sample set is enriched for samples with low-level (ratio 2-3) HER2+ samples representing 27% of samples compared to the expected range of 8-10% reported in literature.[13,14] The *ERBB2* amplification positive results from the F1CDx assay were compared against those obtained from the approved HER2 FISH pharmDx® Kit (Dako Denmark A/S). The samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age and ethnicity data were available. Age data was compared to the Danish Study for the Danish Breast Cancer Group clinical trial 89-D in 1990 and was found to have a similar distribution, though the mean age was higher for the concordance samples. Data from concordance testing is summarized in Table 35, below.

Table 35. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples

	CCD1+			CCD1-		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1CDx +	101	2	103	3	3	6
F1CDx -	12	10	22	6	180	186
Total	113	12	125	9	183	192

The key statistics of PPA and NPA between F1CDx and the two replicates of the Dako assay (CCD1 and CCD2) were estimated based on the data in Table 35, and the results are shown in Tables 36A and 36B below, with and without adjusting for the prevalence of the *ERBB2*/HER2 amplification mutation in the IU population, as estimated by the ASCO guideline.

Table 36A. PPA and NPA for *ERBB2*, Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	90.4%
PPA_{C1F}	82.4%
NPA_{C1C2}	95.3%
NPA_{C1F}	96.9%
PPA_{C2C1}	92.6%
PPA_{C2F}	85.2%
NPA_{C2C1}	93.8%
NPA_{C2F}	97.4%

Table 36B. PPA and NPA for *ERBB2*, Adjusted for Prevalence (17.5%)

	% Agreement
PPA_{C1C2}	90.4%
PPA_{C1F}	82.4%
NPA_{C1C2}	95.3%
NPA_{C1F}	96.9%
PPA_{C2C1}	80.4%
PPA_{C2F}	78.4%
NPA_{C2C1}	97.9%
NPA_{C2F}	98.0%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bounds of one-sided 95% Cl for adjusted agreements were computed using bootstrap and the results are shown in Table 37, below.

Table 37. Point estimate and 95% one-sided upper bounds of ξ_{PPAI}, ξ_{NPAI}, ξ_{PPA2}, and ξ_{NPA2}

	Point Estimate	95% one-sided upper bound CI
ξ_{PPAI}	8.0%	12.8%
ξ_{NPAI}	-1.56%	1.0%
ξ_{PPA2}	1.99%	9.3%
ξ_{NPA2}	-0.14%	1.7%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$;
$\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$; $\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

To assess the impact of prevalence for the main results of this study, a sensitivity analysis was performed using the lower and upper bound of the prevalence guideline of 15% and 20%. The sensitivity analysis showed that there was no impact on the study conclusion. The distribution of age is similar to the IU population for all samples tested. However, there was a large number of missing demographic data from the sample population. For missing data analysis using multiple imputation, the results show that based on the missing at random (MAR) assumption, the invalid test results did not affect the conclusion of this study. Given the observed PPA (unadjusted PPA_{C1F} of 82.4% and PPA_{C2F} of 85.2%) a limitation statement was

included in the F1CDx device labeling and patient report informing physicians of the need for reflex testing to an alternative FDA approved CDx test for patients who have an *ERBB2* amplification detected with copy number equal to 4 (baseline ploidy of tumor +2) as the discordances observed between the F1CDx test and the HER2 FISH pharmDx® Kit predominantly occurred within this group of patients.

D. FoundationOne CDx Concordance Study for *ALK*

Clinical validity of F1CDx as a companion diagnostic device used to identify non-small cell lung cancer (NSCLC) patients eligible for treatment with approved *ALK*-directed therapies including Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib) was established. The study was performed using 175 tumor samples from patients with histologically-confirmed NSCLC including enrolled patients as well as screen failures from the clinical trial NCT02075840, Roche study number BO28984 (also known as the ALEX study), which is a randomized, active controlled, multicenter phase III open-label study designed to evaluate the efficacy and safety of alectinib compared with crizotinib treatment in participants with treatment-naïve *ALK* rearrangement positive advanced NSCLC. In this concordance study, the majority of the samples were from the IU population of the clinical trial NCT02075840**.** Samples were screened using CCD1, the FDA approved Ventana *ALK* (D5F3) CDx Assay ("Ventana IHC", Ventana Medical Systems, Inc). An approximately equal number of *ALK* rearrangement positive and negative samples were then tested with F1CDx and a second replicate with CCD2, the FDA approved Vysis *ALK* Break-Apart FISH Probe Kit ("Vysis FISH", Abbott Molecular).

The available tissue samples limited prospective testing to only CCD1 and F1CDx, and it was not possible to obtain another replicate for the Ventana IHC. Therefore, the retrospective Vysis FISH results, available from the ALEX study, were used as a surrogate for the second replicate of FDA approved comparator method (CCD2). The *ALK* diagnostic results from the F1CDx panel were compared against those obtained from the Ventana IHC assay and Vysis FISH assay. The screened samples were compared against the IU population with respect to age, gender, race, brain metastasis, ECOG, and smoking status and were found to be similar to the IU population of the PROFILE 1014 (NCT01154140) trial used for the approval of the Ventana *ALK* IHC assay.

A total of 487 samples were received, including 162 samples that had an insufficient number of slides available. In addition, 8 blocks of samples which were not intended to be used for this study were accidentally included in the shipment and 6 duplicates samples were removed. In addition, there were 33 samples with failed tumor nuclei assessment during F1CDx pathology review or no tissue remaining. After eliminating ineligible samples, there were 278 (= 487-162-6-8-33) samples left. Five additional samples were removed from analysis due to CCD1 screening failure. Thus, 273 (= 278-5) samples were left for processing. Among those samples, there were 175 samples with complete records and 98 samples with missing results in either F1CDx and/or CCD2.

Two separate concordance analyses were performed: one included samples with complete records only (N = 175), and the other with all 273 samples, where missing data was handled by multiple imputation. Data from concordance testing is summarized in Table 38.

Table 38. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples.

	CCD1 +				CCD1 -			
	CCD2 +	CCD2 -	CCD2 missing	Total	CCD2 +	CCD2 -	CCD2 missing	Total
F1CDx+	78	1	9	88	3	0	0	3
F1CDx-	6*	7	3	16	5	75	1	81
F1CDx missing	19	3	8	30	0	30	25	55
Total	103	11	20	134	8	105	26	139

*Two samples harbored *ALK* rearrangements that were detected by F1CDx but were classified as negative based on the study protocol.

Based on samples with complete data only (98 samples with missing F1CDx and/or CCD2 results removed), the agreement analysis results are shown in Tables 39A and 39B. The agreements comparing F1CDx and CCD2 were calculated both with and without adjusting for the prevalence of *ALK* in the original trial [PROFILE 1014 (NCT01154140) trial] for Ventana *ALK* IHC assay.

Table 39A. PPA and NPA for ALK Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	91.3%
PPA_{C1F}	85.9%
NPA_{C1C2}	90.4%
NPA_{C1F}	96.4%
PPA_{C2C1}	91.3%
PPA_{C2F}	88.0%
NPA_{C2C1}	90.4%
NPA_{C2F}	98.8%

Table 39B. PPA and NPA for ALK Adjusted for Prevalence (16.5%)

	% Agreement
PPA_{C1C2}	91.3%
PPA_{C1F}	85.9%
NPA_{C1C2}	90.4%
NPA_{C1F}	96.4%
PPA_{C2C1}	65.2%
PPA_{C2F}	73.6%
NPA_{C2C1}	98.1%
NPA_{C2F}	99.8%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bounds of one-sided 95% Cl for adjusted agreements were computed using bootstrap and the results are shown in Table 40.

Table 40. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, and ζ_{NPA2}.

	Point estimate	95% one-sided upper bound
ζ_{PPA1}	5.43%	9.78%
ζ_{NPA1}	-6.02%	-2.40%
ζ_{PPA2}	-8.40%	3.37%
ζ_{NPA2}	-1.63%	-0.71%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

Due to the limited availability of samples, some samples with volume < 0.6mm^3 were included in this study (N=34), while for routine F1CDx testing requires a volume of ≥ 0.6mm^3. Subgroup analysis was performed based on samples with volume ≥ 0.6mm^3 (N=141). The concordance data for this subgroup is shown in Table 41 and agreement calculations are shown in Table 42, below.

Table 41. Concordance Table with CCD1, CCD2 and F1CDx results with samples with volume ≥ 0.6mm^3

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
F1CDx+	63	1	64	2	0	2
F1CDx-	4	6	10	3	62	65
Total	67	7	74	5	67	66

Table 42. Agreement analysis results based samples with volume ≥ 0.6mm^3

	% Agreement Volume ≥ 0.6mm^3 (N = 141)
PPA_{C1C2}	90.5%
PPA_{C1F}	86.5%
NPA_{C1C2}	92.5%
NPA_{C1F}	97.0%
PPA_{C2C1}	70.6%
PPA_{C2F}	78.1%
NPA_{C2C1}	98.0%
NPA_{C2F}	99.7%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.

NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bound of one-sided 95% Cl for adjusted agreements were computed for samples with volume ≥ $0.6mm^3$, using bootstrap and the results are shown in Table 43, below.

Table 43. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, and ζ_{NPA2} ζ_{NPA2} based on samples with volume ≥ $0.6mm^3$

		Volume ≥ $0.6mm^3$ (N = 141)
Point estimate	ζ_{PPA1}	4.05%
	ζ_{NPA1}	-7.56%
	ζ_{PPA2}	-4.48%
	ζ_{NPA2}	-1.69%
Upper bound of one-sided 95% CI	ζ_{PPA1}	9.46%
	ζ_{NPA1}	-1.49%
	ζ_{PPA2}	5.39%
	ζ_{NPA2}	-0.59%
95% one-sided CI	ζ_{PPA1}	(0.00%, 9.46%)
	ζ_{NPA1}	(-9.00%, -1.49%)
	ζ_{PPA2}	(-20.38%, 5.39%)
	ζ_{NPA2}	(-2.81%, -.0.59%)

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

E. FoundationOne CDx Concordance Study for *KRAS*

Clinical validity of F1CDx as a companion diagnostic device used to identify colorectal cancer patients that may not benefit from certain *EGFR* inhibitor treatments, including Erbitux® (cetuximab) or Vectibix® (panitumumab), due to alterations in *KRAS*. The study was performed using 342 retrospective samples obtained from patients with advanced front-line or later-line colorectal cancer (CRC). Samples used in this study underwent pre-screening using the F1 LDT or prescreening by an external vendor to enrich for positive samples. The prescreen failure rate using the F1 LDT was 3.7% and is unknown for the external vendor. The *KRAS* diagnostic results from the F1CDx assay were compared against those obtained from the approved *therascreen® KRAS* RGQ PCR Kit (QIAGEN). The samples used for this study were not obtained from a clinical trial and had limited demographic data available. For this study age, gender and ethnicity data were available. Age and gender characteristics were found to be similar between the F1CDx concordance study and the pivotal studies, with the percentage of male samples in the concordance study being slightly lower compared to the pivotal studies (CRYSTAL and PRIME). Data from concordance testing is summarized in Table 44, below.

Table 44. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples

	CCD1+				CCD1-			
	CCD2+	**CCD2-**	**CCD2 missing**	**Total**	**CCD2+**	**CCD2-**	**CCD2 missing**	**Total**
F1CDx+	173	0	2	175	0	0	0	0
F1CDx-	0	2	0	2	1	154	7	162
F1CDx Missing	0	0	0	0	0	3	0	3
Total	173	2	2	177	1	157	7	165

The key statistics of PPA and NPA between F1CDx and the two replicates of the *therascreen*® assay (CCD1 and CCD2) were estimated based on the result in Table 44. Twelve (12) samples were assigned as missing data, including 3 samples with missing data in F1CDx and 9 samples with missing data in CCD2.

Based on complete data only (12 samples with missing F1CDx or CCD2 results removed), the agreement analysis results are shown in Tables 45A and 45B below, both with and without adjustment for the prevalence of the *KRAS* mutation in the IU population based on the CRYSTAL study for cetuximab (35.6%) and PRIME study for panitumumab (40%).

Table 45A. PPA and NPA for *KRAS*, Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	98.9%
PPA_{C1F}	98.9%
NPA_{C1C2}	99.4%
NPA_{C1F}	100.0%
PPA_{C2C1}	99.4%
PPA_{C2F}	99.4%
NPA_{C2C1}	98.7%
NPA_{C2F}	100.0%

Table 45B. PPA and NPA for *KRAS* Adjusted for Prevalence (35.6% for cetuximab and 40% for panitumumab)

	% Agreement Cetuximab	**% Agreement Panitumumab**
PPA_{C1C2}	98.9%	98.9%
PPA_{C1F}	98.9%	98.9%
NPA_{C1C2}	99.4%	99.4%
NPA_{C1F}	100.0%	100.0%
PPA_{C2C1}	98.8%	99.0%
PPA_{C2F}	98.8%	99.0%
NPA_{C2C1}	99.4%	99.2%
NPA_{C2F}	100.0%	100.0%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bounds of one-sided 95% CI for adjusted agreements were computed using bootstrap and the results are shown in Table 46, below.

Table 46. Point estimate and 95% one-sided upper bounds of ξ_{PPAl} , ξ_{NPAl}, ξ_{PPA2}, and ξ_{NPA2}

	Point estimate (Cetuximab)	Upper Bound (one-sided 95% CI) (Cetuximab)	Point estimate (Panitumumab)	Upper Bound (one-sided 95% CI) (Panitumumab)
ξ_{PPAl}	0.0%	0.0%	0.0%	0.0%
ξ_{NPAl}	-0.7%	0.0%	-0.7%	0.0%
ξ_{PPA2}	0.0%	0.0%	0.0%	0.0%
ξ_{NPA2}	-0.6%	0.0%	-0.8%	0.0%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

Multiple imputation was used to impute the missing data and showed that missing data did not impact study conclusions. Given the level of agreement observed for F1CDx and the *therascreen*® assay using colorectal cancer samples, the accuracy of F1CDx for detecting *NRAS* mutations and additional benefit-risk considerations, reporting of *NRAS* mutations will be included in F1CDx and post-market clinical concordance data with the FDA approved Praxis Extended Ras Panel will be provided post-approval.

F. FoundationOne CDx Concordance Study for *BRAF*

Clinical validity of the F1CDx as a companion diagnostic device used to identify melanoma patients that may be eligible for treatment with approved *BRAF*-directed therapies was established. The study was performed using 305 retrospective samples obtained from patients with advanced melanoma. In total, 157 samples used in this study underwent pre-screening using the F1 LDT and 27 were prescreened by an external vendor to enrich for positive samples. The prescreen failure rate using the F1 LDT was 3.7% and is unknown for the external vendor. The *BRAF* diagnostic results from the F1CDx assay were compared against those obtained from the approved **cobas**® *BRAF* V600 mutation test (Roche Molecular Systems, Inc). Samples were screened using **cobas**® *BRAF* V600 mutation test (CCD1) and an approximately equal number of V600E positive and negative samples were then tested by F1CDx, and a second replicate was tested by **cobas**® *BRAF* V600 mutation test (CCD2). In total, 305 samples met protocol requirements and completed testing for inclusion in the final concordance analysis. These samples were not obtained from a clinical trial and had demographic data limited to age and gender. The distributions of age and gender to the intended use population (BRIM-3 trial) was found to be comparable.

There were total 384 samples enrolled in the study. Ineligible samples include 24 samples that were found to be identical to other samples already included in the study and 17 samples that do not meet the inclusion/exclusion criteria for one or more assays due to low tumor content or absence of tumor DNA based on pathology review before testing began. In addition, there were 2 samples with CCD1 screening failures.

Those samples were removed from the analysis. Among those samples remained in the study, there were 305 samples with complete records and 36 samples with missing results in either F1CDx and/or CCD2.

Two separate concordance analyses were performed: one with samples with complete records only (N = 305), and the other with all 341 samples were missing data was handled by multiple imputation. Data from concordance testing is summarized in Table 47, below.

Table 47. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples

	CCD1+			CCD1-		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1CDx+	166	0	166	3	14	17
F1CDx-	1	0	1	0	121	121
Total	167	0	167	3	135	138

Based on samples with complete data only (36 samples with missing F1CDx and/or CCD2 results removed), the agreement analysis results are shown in Tables 48A and 48B both with and without adjusting for the prevalence of *BRAF* V600 mutation in the original trial (BRIM3 trial) for **cobas**® *BRAF* V600 mutation test.

Table 48A. PPA and NPA for *BRAF* Unadjusted for Prevalence

	% Agreement
PPA_{C1C2}	100.0%
PPA_{C1F}	99.4%
NPA_{C1C2}	97.8%
NPA_{C1F}	87.7%
PPA_{C2C1}	98.2%
PPA_{C2F}	99.4%
NPA_{C2C1}	100.0%
NPA_{C2F}	89.6%

Table 48B. PPA and NPA for BRAF Adjusted for Prevalence (33.8%)

	% Agreement
PPA_{C1C2}	100.0%
PPA_{C1F}	99.4%
NPA_{C1C2}	97.8%
NPA_{C1F}	87.7%
PPA_{C2C1}	95.9%
PPA_{C2F}	99.4%
NPA_{C2C1}	100.0%
NPA_{C2F}	89.6%

PPA_{C1C2} is the PPA between CCD1 and CCD2 conditional on CCD1.
PPA_{C1F} is the PPA between CCD1 and F1CDx conditional on CCD1.
PPA_{C2C1} is the PPA between CCD1 and CCD2 conditional on CCD2.
PPA_{C2F} is the PPA between CCD2 and F1CDx conditional on CCD2.
NPA_{C1C2} is the NPA between CCD1 and CCD2 conditional on CCD1.
NPA_{C1F} is the NPA between CCD1 and F1CDx conditional on CCD1.
NPA_{C2C1} is the NPA between CCD1 and CCD2 conditional on CCD2.
NPA_{C2F} is the NPA between CCD2 and F1CDx conditional on CCD2.

The differences of the PPAs and NPAs and the corresponding upper bounds of one-sided 95% Cl for adjusted agreements were computed using bootstrap and the results are shown in Table 49, below.

Table 49. Point estimate and 95% one-sided upper bounds of ζ_{PPA1}, ζ_{NPA1}, ζ_{PPA2}, and ζ_{NPA2}

	Point estimate	95% one-sided upper bound
ζ_{PPA1}	0.60%	1.80%
ζ_{NPA1}	10.14%	14.49%
ζ_{PPA2}	-3.50%	0.00%
ζ_{NPA2}	10.37%	14.90%

$\zeta_{PPA1}=(PPA_{C1C2}- PPA_{C1F})$; $\zeta_{PPA2}=(PPA_{C2C1}- PPA_{C2F})$; $\zeta_{NPA1}=(NPA_{C1C2}- NPA_{C1F})$
$\zeta_{NPA2}=(NPA_{C2C1}- NPA_{C2F})$

Because **cobas**® *BRAF* V600 mutation test has lower sensitivity for detection of dinucleotide mutations, a separate analysis was conducted that included only eligible samples without dinucleotide mutations. As there were 32 dinucleotide samples detected by F1CDx, a total of 273 (= 305-32) samples were available for this analysis. The concordance results are summarized in Table 50.

Table 50. Concordance Table with CCD1, CCD2 and F1CDx results with eligible samples excluding samples with dinucleotide mutations detected by F1CDx

	CCD1+			CCD1-		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1CDx+	149	0	149	1	1*	2
F1CDx-	1**	0	1	0	121	121
Total	150	0	150	1	122	123

*The sample noted here was the only sample where both replicates of the **cobas**® assay reported negative results but F1CDx reported positive. The allele frequency of this sample was 3.45% with the computational tumor purity of 10%. Per Table 4 of the **cobas**® assay insert, the **cobas**® assay can correctly detect all *BRAF* V600E mutant specimens that have a minimum % mutant DNA above 5% and when the minimum tumor content is at least 15%. Thus, the discordance may be explained by F1CDx's sensitivity in the lower % mutant DNA and low tumor purity condition.
The sample noted here was the only sample where both replicates of the **cobas® assay reported positive results but F1CDx reported negative. A mutation was recorded having protein effect V600_K601>E, which is a non-frameshift deletion of 3 nucleotides with CDS effect 1799_1801delTGA. This more complex mutation does result in V600E, but because of annotation differences to the canonical V600E, it was called negative by F1CDx.

A single run of the THxID™ *BRAF* Kit was performed on the dinucleotide mutation samples detected by F1CDx. Of the 32 dinucleotide samples detected by F1CDx, only 29 samples had enough DNA material for further testing. An additional 29 negative samples with matching sex and age characteristics were included for the analysis. 7

samples did not produce results with the THxID assay. Among those, one sample did not have enough DNA material to proceed and six did not generate valid results after repeat testing. Data from concordance testing of dinucleotide samples with available data are summarized in Table 51.

Table 51. Concordance of *BRAF* dinucleotide samples

Dinucleotide Samples	THxID+	THxID-	THxID Missing	Total
F1CDx+	26	0	3	29
F1CDx-	1	24	4	29
Total	27	24	7	58

The concordance results excluding missing data indicated 100.0% PPV with two-sided 95% exact CI of (86.8%, 100.0%) and 96.0% NPV with two-sided 95% exact CI of (79.6%, 100.0%). A study using the THxID™ *BRAF* kit (bioMérieux) will be conducted post-market to provide a better evaluation of V600E and V600K concordance. Further, additional clinical concordance data will be provided post-market to support the *BRAF* clinical claim in NSCLC patients.

G. FoundationOne CDx Concordance with FoundationFocus CDx$_{BRCA}$ for *BRCA1* and *BRCA2*

F1CDx and FoundationFocus CDx$_{BRCA}$ use the same reagents, equipment and procedures with exception of the allowance for a lower DNA input into library construction and incremental enhancements to the analysis pipeline. The differences were shown to have no impact on assay performance through the guard band study which included ovarian tissue and a comprehensive validation of the analysis pipeline which included robust regression testing. As such, the F1CDx and FoundationFocus CDx$_{BRCA}$ are considered concordant. Details for the clinical study in which the assay was shown to be effective in identify patients with ovarian cancer that may benefit from rucaparib treatment can be found in PMA P160018.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Analytical performance studies were conducted with the F1CDx test using FFPE tissue or DNA extracted from FFPE tissue from a variety of cancer indications. When the test is used in accordance with the directions provided, the sensitivity for detecting the tested variants is shown in Tables 9-12, above. The clinical benefit of the F1CDx test in the detection of alterations listed in Table 1 of the intended use statement was demonstrated in seven clinical concordance studies using previously approved CDx tests as the comparator methods. All studies based on non-inferiority (NI) statistical testing approach passed the acceptance criteria specified in each study protocol. Additional post-market data will be provided for certain clinical claims (i.e., *EGFR* T790M and *BRAF*) to further support the clinical validity of the device. The concordance observed between the F1CDx test and the approved companion diagnostics tests supports the effectiveness of the F1CDx test to identify patients whose tumors are positive for the alterations listed in Table 1 of the intended use and may respond to the associated therapeutics listed in the same table.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. The F1CDx assay is an in vitro diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from an existing (archival) tissue samples routinely collected as part of the diagnosis and patient care.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit, and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

C. Benefit-Risk Determination

Risk associated with the F1CDx assay include the possibility of inaccurate results that may lead to mismanagement of patients. The F1CDx assay has demonstrated non-inferiority to the companion diagnostics indicated in Section X above, and therefore does not introduce additional risks above other approved devices. Since the device was accepted into the Breakthrough Devices Program, the device review included an appropriate balance of pre- and post-market data collection. As such, for biomarkers and associated therapeutics for which direct clinical concordance has not been provided, but the accuracy of the test in detecting the biomarkers has been demonstrated, the respective clinical concordance data will be provided post-market.

Additional factors to be considered in determining probable risks and benefits for the F1CDx included: analytical performance of the device, representation of variants, additional and ongoing analytical testing. Additional analytical testing will be performed in the post approval setting.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the F1CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indication for use. Data from the clinical concordance studies, support the performance of F1CDx assay as an aid for the identification of cancer patients for whom the therapies listed Table 1 of the Intended Use statement may be indicated.

XIV. CDRH DECISION

CDRH issued an approval order on November 30, 3017. The final conditions of approval cited in the approval order are described below.

1. Provide additional clinical concordance data to support the performance of your device within the appropriate clinical contexts. Please perform concordance testing against additional approved CDx devices for their respective approved clinical indications.

2. Provide clinical response data for NSCLC patients with an EGFR T790M mutation detected with mutant allele frequency (MAF) < 5% who were subsequently treated with Tagrisso® (osimertinib). This will support the clinical performance of your device for patients detected as positive by F1CDx (with MAF < 5%) who were considered negative by another approved CDx.

3. Provide results from additional testing of clinical samples to establish the analytical performance characteristics of your device for all variant types and genomic signatures that may be detected. Please ensure that the samples adequately represent the ranges of CNAs, rearrangements, MSI and TMB that are detected by your device, with consideration given to the fusion partners (for rearrangements) and the reportable ranges (for MSI and TMB).

4. Provide software documentation for validating and implementing software changes required to generate the test report.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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