SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Next Generation Sequencing Oncology Panel, Somatic Variant Detection System

Device Trade Name: xT CDx

Device Procode: PQP

Applicant's Name and Address: Tempus Labs, Inc. (Tempus)
600 W. Chicago Ave
Chicago, IL 60654

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P210011

Date of FDA Notice of Approval: April 28, 2023

II. INDICATIONS FOR USE

xT CDx is a qualitative Next Generation Sequencing (NGS)-based *in vitro* diagnostic device intended for use in the detection of substitutions (single nucleotide variants (SNVs) and multi-nucleotide variants (MNVs)) and insertion and deletion alterations (INDELs) in 648 genes, as well as microsatellite instability (MSI) status, using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens, and DNA isolated from matched normal blood or saliva specimens, from previously diagnosed cancer patients with solid malignant neoplasms.

The test is intended as a companion diagnostic (CDx) to identify patients who may benefit from treatment with the targeted therapies listed in the Companion Diagnostic Indications table in accordance with the approved therapeutic product labeling.

Additionally, xT CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with previously diagnosed solid malignant neoplasms. Genomic findings other than those listed in the Companion Diagnostic Indications table are not prescriptive or conclusive for labeled use of any specific therapeutic product.

xT CDx is a single-site assay performed at Tempus Labs, Inc., Chicago, IL.

Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Colorectal cancer (CRC)	KRAS wild type (absence of mutations in codons 12 or 13)	Erbitux (cetuximab)
Colorectal cancer (CRC)	KRAS wild type (absence of mutations in exons 2, 3, or 4) and NRAS wild type (absence of mutations in exons 2, 3, or 4)	Vectibix (panitumumab)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the xT CDx labeling.

V. DEVICE DESCRIPTION

xT CDx is a single-site assay performed at Tempus Labs, Inc. located at 600 West Chicago Avenue Suite 510, Chicago, IL 60654. The assay includes reagents, software, instruments (qualified by Tempus), and procedures for testing DNA extracted from FFPE tumor specimens and matched normal saliva or blood specimens.

Extracted DNA undergoes whole-genome shotgun library construction and hybridization-based capture of specified regions from 648 cancer-related genes (including intronic overhang and selected promoter regions) (Appendix 1), and 239 loci for MSI. Using the Illumina NovaSeq 6000 platform, hybrid-capture-selected libraries are sequenced to highly uniform depth (targeting ≥ 500x median coverage of tumor samples, with ≥ 95% of exons at ≥ 150x coverage and ≥ 98% of exons at ≥ 100x coverage). Sequence data is processed using a customized analysis pipeline designed to detect substitutions (SNVs and MNVs) and INDELs in coding and noncoding genomic regions targeted by the assay. Additionally, MSI status is reported by an MSI classification algorithm to classify tumors into three categories: Microsatellite Instability High (MSI-H), Microsatellite Stable (MSS) and Equivocal (MSI cannot be determined).

Test Output

The output of the test is generated via curation by automated software using information from several databases including information derived from the FDA-recognized content

of Memorial Sloan Kettering's Precision Oncology Knowledge Base (MSK OncoKB, https://www.oncokb.org), and consists of results representing three categories:

Level 1: CDx claims for KRAS and NRAS as noted in the Intended Use

Level 2: Cancer Mutations with Evidence of Clinical Significance

Level 3: Cancer Mutations with Potential Clinical Significance

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

Test Kit Contents

xT CDx includes a specimen collection and shipping kit (the Specimen Kit). The Specimen Kit contains the following components (Table 1).

Table 1. Specimen Kit contents

Blood Kit	Saliva Kit	Tissue Kit*
• Informational Brochures (2) • Document Checklist • Specimen ID Label • Seal Sticker • Cold Pack • Prelabeled FedEx Clinical Shipping Pak • 3x5 Biohazard Bag w/ Absorbent Sheet • Blood Collection tubes	• Informational Brochures (2) • Document Checklist • Specimen ID Label • Seal Sticker • Prelabeled FedEx Clinical Shipping Pak • 3x5 Biohazard Bag w/ Absorbent Sheet • Saliva Collection tube	• Informational Brochure w/ Specimen Requirements • Seal Sticker • 12x15 Biohazard bag • Prelabeled FedEx Clinical Shipping Pak • Microscope Slide cases (2)

* For tissue collection, Tempus Labs, Inc. (Tempus) receives either previously prepared slides or blocks.

Instruments

xT CDx uses the Illumina NovaSeq 6000 Sequencer, a high throughput sequencing system employing sequencing-by-synthesis chemistry. xT CDx is intended to be performed with serial number-controlled instruments. All instruments are qualified by Tempus Labs, Inc. (Tempus) under Tempus' Quality System.

Principles of Operation

All assay reagents included in the xT CDx assay process are qualified by Tempus Labs, Inc. and are compliant with the medical device Quality System (QS) regulation.

1. **Specimen Collection and Preparation**

   FFPE tumor specimens received either as unstained slides or as an FFPE block, and matched normal specimens, are collected using materials supplied in the Specimen Kit and prepared following standard pathology practices. Preparation and review of a Hematoxylin and Eosin (H&E) slide is performed as a laboratory service in accordance with standard laboratory practices, prior to initiation of the xT CDx assay. H&E stained slides are reviewed by a board-certified pathologist to ensure that adequate tissue, tumor content and sufficient nucleated cells are present to satisfy minimum tumor content (tumor purity). Specifically, the minimum recommended tumor purity for detection of variants by xT CDx is 20%, with macrodissection required for specimens with tumor purity lower than 20%.

2. **DNA Extraction**

   Genomic DNA is extracted from tumor tissue specimens and patient-matched normal specimens. Specimen specific extraction methods are used for each sample type. The recovered DNA is quantified and qualified. The DNA is then mechanically sheared prior to library preparation. The minimum amount of DNA required to perform the test is 50 ng.

3. **Library Preparation**

   Library preparation is performed using the Illumina KAPA DNA Hyper Prep Kit with unique dual indices. First, end repair and A-tailing occurs to create end-repaired, 5'-phosphorylated, 3'-dA-tailed dsDNA fragments on the sheared DNA. Then DNA pair adapters with 3'-dTMP overhangs are ligated to 3'-dA-tailed molecules. The libraries are amplified with high fidelity, low-bias PCR. After bead-based purification of libraries, library yield and quality are assessed.

4. **Hybrid Capture**

   Resultant libraries are enriched by hybridization to a set of custom probes that are subsequently immobilized onto magnetic beads and unbound fragments are then washed away. To reduce non-specific binding of untargeted regions, blockers are included in the hybridization step. The enriched DNA targets are amplified. The amplified libraries are assessed and pooled for sequencing.

5. **Sequencing**

   The amplified target-captured tumor and normal libraries are sequenced to a median depth of 500x and 150x, respectively, on an Illumina NovaSeq 6000 System using patterned flow cell technology. Each tumor sample is required to have unique reads above 8,000,000. Samples with insufficient coverage are failed. A matched normal

specimen is sequenced in order to subtract germline variants from the tumor sequencing results to more accurately report somatic variants.

6. Data Analysis

A. Read Alignment and BAM Generation

FASTQ files are aligned to the Genome Reference Consortium Human Build 37 (GRCh37). During this process, remaining adapter sequences are trimmed. A Sequence Alignment Map (SAM) output file is generated which is then converted to a Binary Alignment Map (BAM) file. This file is then sorted by chromosome, indexed, and PCR duplicate FASTQ entries are marked.

B. Variant Calling – SNVs, MNVs and INDELs

The analysis pipeline identifies SNVs, MNVs and INDELs. SNVs and MNVs reflect changes in the identity of a nucleotide (or nucleotides) at a specific position (or positions) without any change to the total number of nucleotides detected. SNVs are single nucleotide variants, in which the identity of only a single nucleotide is changed. MNVs are considered to be two or more adjacent or nearby SNVs detected on the same haplotype in an individual. INDELs refer to both insertions and deletions, in which the total number of nucleotides is altered relative to wild type (i.e., additional nucleotides are present, or nucleotides are absent). Paired sample variant calling is performed on tumor samples and their respective matched normal samples. Filtering is performed to remove low quality sequence data and sources of sequencing artifacts.

C. Variant Annotation

Predicted functional effect and clinical interpretation for each mutation is curated by automated software using information from several databases and clinical evidence, including information derived from the FDA-recognized content (https://www.fda.gov/media/152847/download) of Memorial Sloan Kettering's Precision Oncology Knowledge Base (MSK OncoKB, https://www.oncokb.org) and Tempus' custom proprietary database using criteria that include known evolutionary models, functional data, clinical data, known gene-disease relationships, hotspot regions within genes, internal and external somatic databases, primary literature, and the unique combination of data from having paired tumor/normal DNA.

To determine that variant calling can be performed with high levels of confidence, there are four quality control steps performed as part of xT CDx sequence data analyses:

- Alignment and Coverage Quality Control: This first process computes a series of statistics on the output of the reference genome alignment process such as read mapping rates, on target rates, and PCR duplication rates. The second process computes the depth and uniformity of coverage for all bases analyzed by the assay. The goal is to confirm that all bases within the target regions are sequenced to sufficient depth so that if a variant were present, it would be called successfully down to the lower Limit of Detection (LoD) of the assay.

- Sequence Data Quality Control: This includes per base quality scores for both forward and reverse reads which are output from the sequencer.

- Variant Quality Control: This includes sequencing of control samples and analysis of results against specifications.

- Sample Provenance Quality Control: In order to confirm that the specimen is free from exogenous DNA which may result in false positive results, the tumor sample is analyzed for common germline polymorphisms at low variant allele frequency (VAF). If the ratio of known germline polymorphisms to all mutations exceeds a pre-specified cutoff, the sample will be flagged as potentially contaminated by another human specimen. Concomitantly, the tumor and normal sample are compared against one another along a pre-specified set of common germline polymorphisms in order to confirm that both the normal and tumor specimens were derived from the same individual. If the discordance in common germline polymorphism minor allele frequency between the tumor specimen and the normal specimen exceeds a pre-specified threshold, the analysis will be flagged for further review.

D. MSI Status Calling

xT CDx analyzes 239 microsatellite loci that are frequently unstable in tumors with mismatch repair deficiencies to determine the frequency of DNA slippage events. The information detected is used by the MSI classification algorithm to classify tumors into three categories: Microsatellite Instability High (MSI-H), Microsatellite Stable (MSS) and Equivocal (MSI cannot be determined).

To be an MSI locus mapping read, the read must be mapped to the MSI locus during the alignment step in the xT CDx bioinformatics pipeline. The mapping read must also contain the 5 base pairs in both the front and rear flank of the microsatellite, with any number of the expected repeating units in between. The minimum required number of reads must map to a microsatellite in both the normal sample and the tumor sample for it to be included in the test. A prespecified threshold of microsatellite loci on the panel must be included for the algorithm to be run. If these thresholds are not met, no MSI status is reported.

Each microsatellite is tested for instability, as measured by changes in the number of repeat units in the tumor sample compared to the normal sample using the Kolmogorov-Smirnov test. If p :S 0.05, the locus is considered unstable and used to predict the binary MSI-H status based on the proportion of unstable microsatellites. The proportion of unstable microsatellites is entered into a univariate logistic regression classifier.

The classifier groups samples into three categories, MSI-H, MSS and equivocal, based on where this probability falls relative to two probability thresholds, a high confidence MSS threshold and a high confidence MSI-H threshold. If the probability that a sample is MSI-H exceeds the high confidence MSI-H threshold, the sample is classified as MSI-H. If the probability that a sample is MSI-H falls below the high confidence MSS threshold, the sample is considered MSS. For samples where the probability falls between the high confidence MSS and high confidence MSI-H threshold, the MSI status cannot be called with confidence and the xT CDx report will not include a definitive status, mark the MSI result as "equivocal" and will recommend further testing. In addition, if the tumor proportion falls below the established LoD for the MSI test (30% tumor purity), the xT CDx report will not include an MSI status (result not determined) and will recommend further testing.

7. Controls

A. Matched-Normal Control

DNA is extracted from a patient-matched normal specimen, for use as a matched normal control from either blood or saliva. One matched normal control is required for each patient.

B. Positive Control

A positive control sample containing known variants at defined allele frequencies is included with each sequencing run. Positive controls are rotated in order to evaluate variants from different genes and at different frequencies. The positive control is a contrived material with synthetically derived variants. Data generated from the positive control sample is analyzed using the same data analysis pipeline as patient samples, and frequencies of the detected mutations are reviewed to determine if (1) the known mutations are among those called, and (2) the observed frequencies for the known mutations match their pre-defined acceptable range, which is determined as three standard deviations from the average of the observed values. If known mutations in the positive control sample are not called, and the observed frequency of any mutation differs from expected value by more than the established boundary this results in a quality control (QC) failure.

C. No Template Control (NTC)

A NTC is included at nucleic acid extraction, library preparation, and hybridization/capture The NTCs have predefined acceptability criteria in each step.

8. Quality Metrics

Reporting takes in account the quality metrics outlined in Table 2. Quality metrics are assessed across the following categories:

- Batch-level: Metrics that are quantified per sequencing run; if the external control fails these criteria, no results are reported for the entire batch of samples.
- Sample-level: Metrics that are quantified per sample; no device results are generated for samples failing these metrics.
- Paired-sample level: Assessed to confirm that given tumor and matched normal samples are derived from the same individual.
- Analyte-level: Metrics that are quantified for individual alteration types and positions. Variants passing analyte-level metrics are reported.

Table 2. Summary of xT CDx post-sequencing key quality control metrics

Metric	Batch/Sample/Analyte	Required Value
Positive Control	Batch level	Known sequence mutations are detected and pass acceptance criteria
On Target Rate (Normal)	Sample level	≥ 50%
Tumor On Target Rate (Tumor)	Sample level	≥ 50%
Total Unique Reads (Normal)	Sample level	≥ 5,000,000
Total Unique Reads (Tumor)	Sample level	≥ 8,000,000
Total Reads (Normal)	Sample level	≥ 10,000,000
Total Reads (Tumor)	Sample level	≥ 10,000,000
PCR Duplication Rate (Normal)	Sample level	< 85%
PCR Duplication Rate (Tumor)	Sample level	< 85%

Metric	Batch/Sample/Analyte	Required Value
Fingerprint Score	Paired-sample level	> 0.65 to demonstrate that there are similar common SNVs between the tumor and normal samples
Hotspot substitutions	Analyte level	VAF ≥ 3%
Non-hotspot substitutions	Analyte level	VAF ≥ 5%
Hotspot INDELs	Analyte level	VAF ≥ 5%
Non-hotspot INDELs	Analyte level	VAF ≥ 10%

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved CDx alternatives for the detection of NRAS and KRAS biomarkers to direct the use of specific therapies using FFPE CRC specimens. The approved CDx tests are listed in Table 3, below.

Table 3. Alternative FDA-approved CDx assays for CDx biomarkers dentified by xT CDx

Biomarker	Device	Company	Technology	Therapy
NRAS Variants	*Praxis Extended Ras Panel*	*Illumina*	NGS[1]	Vectibix (panitumumab)
	FoundationOne CDx	*Foundation Medicine, Inc.*	NGS[1]	Vectibix (panitumumab)
KRAS Variants	*Cobas KRAS Mutation Test*	*Roche Molecular Systems, Inc.*	PCR[2]	Erbitux (cetuximab) Vectibix (panitumumab)
	therascreen KRAS RGQ PCR Kit	*QIAGEN*	PCR[2]	Erbitux (cetuximab) Vectibix (panitumumab)
	Praxis Extended Ras Panel	*Illumina*	NGS[1]	Erbitux (cetuximab) Vectibix (panitumumab)

Biomarker	Device	Company	Technology	Therapy
	FoundationOne CDx	*Foundation Medicine, Inc.*	NGS[1]	Erbitux (cetuximab) Vectibix (panitumumab)
	ONCO/Reveal Dx Lung and Colon Cancer Assay (O/RDx-LCCA)	*Pillar Biosciences, Inc.*	NGS[1]	Erbitux (cetuximab) Vectibix (panitumumab)

[1] NGS = Next Generation Sequencing
[2] PCR = Polymerase Chain Reaction

For additional details see the FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools

VII. MARKETING HISTORY

xT CDx has not been marketed in the United States or any foreign country.

Tempus initially designed and developed the Tempus xT Laboratory-Developed Test (LDT), and the first commercial sample was tested in 2017. The Tempus xT LDT has been used to detect the presence of genomic alterations in FFPE tumor tissue specimens by sequencing of patient-matched tumor and normal tissue. Tempus xT LDT is not FDA-cleared or approved.

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 results may be inappropriately treated with one of the therapies listed in the above intended use statement and may experience adverse reactions associated with inappropriate 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 FDA therapeutic product labeling.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Sample Coverage

The sequencing read depth of the device was evaluated by sequencing duplicate libraries from 10 normal diploid samples using worst-case run conditions for detection of somatic alterations. The mean coverage (read depth) for all targeted regions across all samples ranged from 508x to 1218x (with an overall mean of 905x). All sequenced libraries had more than 98% of exons sequenced with a read depth ≥ 150x. The interlibrary median mean coverage for all targeted hotspots ranged from 564.1x to 1557.4x (mean of 1041.6x). The coverage of target regions enables calling of variants at the xT CDx reporting thresholds, which are 3% VAF for substitutions and 5% for INDELs at hotspots, and 5% for substitutions and 10% for INDELs at non-hotspots.

2. Accuracy – Comparison to an Orthogonal Method

i. CDx Accuracy – KRAS and NRAS mutation detection in CRC samples

To support the use of xT CDx as a companion diagnostic for cetuximab and panitumumab, a total of 349 banked CRC samples were tested by xT CDx and compared to results obtained with the original CDx assays; the FDA approved *QIAGEN therascreen KRAS RGQ PCR Kit* (*therascreen*) and the *Illumina Praxis Extended RAS Panel* (*Praxis*), respectively. Concordance between xT CDx results and these approved CDx devices is presented in Section X. Summary of Primary Clinical Studies.

In short, the agreement study was conducted using the comparison of xT CDx to two (2) orthogonal methods (FDA approved *Praxis* and *therascreen* assays). A total set of 190 patient-matched tumor and normal CRC samples was sequenced with xT CDx and *Praxis*, and a total set of 250 patient-matched tumor and normal CRC samples was sequenced with xT CDx and *therascreen*. The reported mutations from xT CDx were compared with results of *Praxis* and *therascreen* assays and used to calculate the PPA and NPA. Of the 84 CDx variants positively identified by *Praxis*, 83 were identified by xT CDx yielding a PPA of 98.8% (95% CI: 93.54-99.97%); of the 88 CDx variants positively identified by *therascreen*, 87 were identified by xT CDx, yielding a PPA of 98.86% (95% CI: 93.83-99.97%). Of the 8400 CDx variants identified as negative by *Praxis*, 8400 were identified as negative by xT CDx, yielding a NPA of 100.0% (95% CI: 99.96-100.0%); of the 1134 CDx variants identified as negative by *therascreen*, 1134 were identified as negative by xT CDx, yielding a NPA of 100.0% (95% CI: 99.68-100%). These results are shown in Section X. Summary of Primary Clinical Studies (Tables 58-70).

In addition to concordance with FDA approved *Praxis* and *therascreen* assays using clinical study samples, analytical accuracy of detecting KRAS and NRAS mutations by xT CDx was also evaluated by comparison with results obtained using a validated NGS-based orthogonal method (OM1), using 69 patient-matched tumor and normal CRC

samples. Summary of these CDx analytical accuracy results, as well as a breakdown by KRAS and NRAS, are provided below in Table 4 and Table 5, respectively. Note that CDx variants are presented simply as variants, which are defined as the list of mutations detected for clinical use (i.e., by the *Praxis* and *therascreen* assays), which includes no insertions or deletions, and does not distinguish between SNVs and MNVs. For comparison with OM1, negative CDx variant positions were defined as those positions at which a unique CDx variant was detected in at least one tested sample, by OM1. (For comparison with the *Praxis* and *therascreen* assays that is presented in Section X, negative CDx variant positions were defined as all positions at which those assays could detect a CDx variant.) The reported variants from xT CDx were compared with results of OM1 and used to calculate the PPA and NPA. Of the 31 CDx variants positively identified by OM1, 31 were identified by xT CDx, yielding a PPA of 100% (95% CI: 88.8-100.0%). Of the 649 CDx variants identified as negative by OM1, 648 were identified as negative by xT CDx, yielding a NPA of 99.8% (95% CI: 99.1-100.0%).

CDx Accuracy (KRAS)

The detection of KRAS mutations by xT CDx was compared with results obtained using an externally validated NGS assay as an orthogonal method (OM1), using a total set of 69 patient-matched tumor and normal CRC samples, sequenced with xT CDx. The reported KRAS CDx variants from xT CDx were compared to results of OM1 and used to calculate the PPA and NPA. Of the 30 CDx variants in KRAS that were positively identified by OM1, 30 were identified by xT CDx, yielding a PPA of 100% (95% CI: 88.43-100.0%). Of the 591 CDx variants in KRAS identified as negative by OM1, 590 were identified as negative by xT CDx, yielding a NPA of 99.83% (95% CI: 99.06-100.0%).

Table 4. Positive and negative percent agreement of KRAS mutation results in CRC samples from the xT CDx Tumor Profiling Accuracy Study (n = 69) compared to OM1

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)*	NPA (95%CI)*
KRAS Exon 2	c.34G>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34G>A	2	0	0	67	69	100.0 (15.81-100)	100.0 (94.64-100)
	c.34G>C	1	0	0	68	69	100.0 (2.50-100)	100.0 (94.72-100)
	c.34_35GG>TT	0	0	0	69	69	NE	100.0 (94.79-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)*	NPA (95%CI)*
	c.34_35GG>AA	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34_36GGT>TGG	0	0	0	69	69	NE	100.0 (94.79-100)
	c.35G>A	12	0	1	56	69	100.0 (73.54-100)	98.2 (90.61-100)
	c.35G>T	5	0	0	64	69	100.0 (47.82-100)	100.0 (94.40-100)
	c.35G>C	2	0	0	67	69	100.0 (15.81-100)	100.0 (94.64-100)
	c.37G>T	1	0	0	68	69	100.0 (2.50-100)	100.0 (94.72-100)
	c.37G>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38G>A	3	0	0	66	69	100.0 (29.24-100)	100.0 (94.56-100)
	c.38_39GC>TT	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38_39GC>AA	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38_39GC>AT	0	0	0	69	69	NE	100.0 (94.79-100)
KRAS Exon 3	c.175G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.176C>G	0	0	0	69	69	NE	100.0 (94.79-100)
	c.181C>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.181C>G	0	0	0	69	69	NE	100.0 (94.79-100)
	c.182A>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.182A>G	0	0	0	69	69	NE	100.0 (94.79-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)*	NPA (95%CI)*
	c.183A>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.183A>T	3	0	0	66	69	100.0 (29.24-100)	100.0 (94.56-100)
KRAS Exon 4	c.351A>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.351A>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.436G>A	1	0	0	68	69	100.0 (2.50-100)	100.0 (94.72-100)
	c.436G>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.437C>T	0	0	0	69	69	NE	100.0 (94.79-100)

NE = not estimable
*CI = Confidence Interval, calculated using the Clopper-Pearson Exact Method

In addition, the detection of KRAS mutations by xT CDx was also assessed by comparison with results obtained using 2 additional validated orthogonal methods (FDA approved *Praxis* and *therascreen* assays). A total set of 190 patient-matched tumor and normal CRC samples was sequenced with xT CDx and *Praxis*, and a total set of 250 patient-matched tumor and normal CRC samples was sequenced with xT CDx and *therascreen*. The reported KRAS CDx variants from xT CDx were compared to results of *Praxis* and *therascreen* and used to calculate the PPA and NPA. Of the 75 CDx variants in KRAS that were positively identified by *Praxis*, 74 were identified by xT CDx, yielding a PPA of 98.67% (95% CI: 92.79-99.97%); of the 88 CDx variants in KRAS positively identified by *therascreen*, 87 were identified by xT CDx, yielding a PPA of 98.86% (95% CI: 93.83-99.97%). Of the 3276 CDx variants in KRAS identified as negative by *Praxis*, 3276 were identified as negative by xT CDx, yielding a NPA of 100.0% (95% CI: 99.89-100%); of the 1134 CDx variants in KRAS identified as negative by *therascreen*, 1134 were identified as negative by xT CDx, yielding a NPA of 100.0% 95% CI: 99.68-100%). These data are summarized and presented in the tables for the CDx concordance captured in the CDx Clinical Validation presented in Section X. Summary of Primary Clinical Studies, Table 63 and Table 69.

CDx Accuracy (NRAS)

The detection of NRAS mutations by xT CDx was compared to results obtained using an externally validated NGS assay as an orthogonal method (OM1). A total set of 69 patient-

matched tumor and normal CRC samples was sequenced with OM1 and xT CDx. The reported NRAS CDx variants from xT CDx were compared to results of OM1 and used to calculate the PPA and NPA. Of the 1 CDx variant in NRAS that was positively identified by OM1, 1 was identified by xT CDx, yielding a PPA of 100% (95% CI: 2.5-100.0%). Of the 68 CDx variants in NRAS identified as negative by the OM1, 68 were identified as negative by xT CDx, yielding a NPA of 100% (95% CI: 94.72-100.0%).

The following CDx accuracy Table 5 reflects results from CRC samples that were also tested as a part of the tumor profiling accuracy study compared to OM1, and summarizes the results for NRAS, showing all CDx variants by exon and variant.

Table 5. Positive and negative percent agreement of NRAS mutation results in CRC samples from the xT CDx Tumor Profiling Accuracy Study (n = 69) compared to OM1

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)*	NPA (95%CI)*
NRAS Exon 2	c.34G>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34G>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34_35GG>TT	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34_35GG>AA	0	0	0	69	69	NE	100.0 (94.79-100)
	c.34_36GGT>TGG	0	0	0	69	69	NE	100.0 (94.79-100)
	c.35G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.35G>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.35G>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.37G>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.37G>C	0	0	0	69	69	NE	100.0 (94.79-100)

	c.38G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38_39GC>TT	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38_39GC>AA	0	0	0	69	69	NE	100.0 (94.79-100)
	c.38_39GC>AT	0	0	0	69	69	NE	100.0 (94.79-100)
NRAS Exon 3	c.175G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.176C>G	0	0	0	69	69	NE	100.0 (94.79-100)
	c.181C>A	1	0	0	68	69	100.0 (2.50-100)	100.0 (94.72-100)
	c.181C>G	0	0	0	69	69	NE	100.0 (94.79-100)
	c.182A>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.182A>G	0	0	0	69	69	NE	100.0 (94.79-100)
	c.183A>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.183A>T	0	0	0	69	69	NE	100.0 (94.79-100)
NRAS Exon 4	c.351A>C	0	0	0	69	69	NE	100.0 (94.79-100)
	c.351A>T	0	0	0	69	69	NE	100.0 (94.79-100)
	c.436G>A	0	0	0	69	69	NE	100.0 (94.79-100)
	c.436G>C	0	0	0	69	69	NE	100.0 (94.79-100)

	c.437C>T	0	0	0	69	69	NE	100.0 (94.79-100)

NE = not estimable
*CI = Confidence Interval, calculated using the Clopper-Pearson Exact Method

The detection of NRAS mutations by xT CDx was also assessed by comparison to results obtained using an additional validated orthogonal method (FDA approved *Praxis*). A total set of 190 patient-matched tumor and normal CRC samples was sequenced with xT CDx and *Praxis*. The reported NRAS variants from xT CDx were compared to results of *Praxis* and used to calculate the PPA and NPA. Of the 9 CDx variants in NRAS that were positively identified by *Praxis*, 9 were identified by xT CDx, yielding a PPA of 100.0% 95% CI: 66.37-100%). Of the 5124 CDx variants in NRAS identified as negative by *Praxis*, 5124 were identified as negative by xT CDx, yielding a NPA of 100.0% (95% CI: 99.93-100%). These data are summarized and presented in the tables for the CDx concordance captured in the CDx Clinical Validation presented in Section X. Summary of Primary Clinical Studies, Table 63.

ii. Tumor Profiling Accuracy

The detection of mutations by xT CDx was assessed by comparison with results obtained using an externally validated NGS assay as an orthogonal method (OM1). A total set of 416 patient-matched tumor and normal samples representing 31 cancer types was sequenced with xT CDx (Table 6). The reported variants from xT CDx were compared with results of OM1 and used to calculate the PPA and NPA. Of the 1232 variants positively identified by OM1, 971, 18, 58, and 174 were identified as SNVs, MNVs, insertions and deletions respectively by xT CDx, observed in 373 exons across 84 genes, yielding an overall PPA of 99.1% (95% CI: 98.4-99.6%). Of the 415,000 variants identified as negative by OM1, 19, 3, 17 and 41 were identified as SNV, MNV, insertion and deletion variants respectively by xT CDx, yielding an overall NPA of 100% (95% CI: 100-100%).

Table 6. Distribution of cancer type among samples

Cancer Type	Count
Colorectal Cancer	69
Breast Cancer	44
Ovarian Cancer	38
Glioblastoma	34
Non-Small Cell Lung Cancer	29
Endometrial Cancer	26
Clear Cell Renal Cell Carcinoma	22

Cancer Type	Count
Bladder Cancer	18
Melanoma	17
Pancreatic Cancer	14
Thyroid Cancer	12
Low Grade Glioma	12
Sarcoma	10
Tumor of Unknown Origin	8
Meningioma	7
Prostate Cancer	7
Gastrointestinal Stromal Tumor	7
Endocrine Tumor	6
Gastric Cancer	5
Head and Neck Squamous Cell Carcinoma	4
Kidney Cancer	3
Brain Cancer	3
Small Cell Lung Cancer	3
Biliary Cancer	3
Cervical Cancer	3
Esophageal Cancer	3
Oropharyngeal Cancer	2
Liver Cancer	2
Head and Neck Cancer	2
Mesothelioma	2
Adrenal Cancer	1

The xT CDx tumor profiling analytical studies included insertions and deletions up to and greater than 25 bps in size. In clinical use, xT CDx will report insertions and deletions up to 25 bps. Concordance of variants was evaluated in both hotspot and non-hotspot regions. Differences in the number of reportable variants between the two assays were expected as a result of pipeline-specific variant filtering or germline variant classifications. In particular, the orthogonal method evaluates tumor samples followed by germline mutation filtering, whereas xT CDx sequences tumor and patient-matched

normal samples to allow personalized subtraction of germline variants from tumor sequencing results. Across all samples evaluated, a total of 148 variants reported as somatic by the orthogonal method were identified in germline samples by xT CDx (Table 7).

Table 7. Germline variants that would be subtracted by xT CDx but were classified as somatic by the orthogonal method

Type	Number of Variants
Substitutions	139
INDELs	9
All Short Variants	148

These were included as an output of xT CDx for the purposes of this analytical concordance study. A summary of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) is provided in Table 8, below, for SNVs, MNVs, insertions and deletions. Accuracy by gene and variant type is provided in Table 9.

Table 8. Concordance summary for short variants

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All Variants	1028	1221	80	11	414920	99.1% [98.4%, 99.6%]	100.0% [100.0%, 100.0%]
All SNVs	736	971	19	8	297042	99.2% [98.4%, 99.6%]	100.0% [100.0%, 100.0%]
All MNVs	22	18	3	1	8881	94.7% [74.0%, 99.9%]	100.0% [99.9%, 100.0%]
All Insertions	71	58	17	2	28656	96.7% [88.5%, 99.6%]	100.0% [100.0%, 100.0%]
All Deletions	199	174	41	0	80341	100.0% [97.9%, 100.0%]	100.0% [100.0%, 100.0%]

Table 9. Accuracy by gene and variant type

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
ABL1	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
AKT1	1	5	100.0% [47.8%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
ALK	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
APC	10	67	100.0% [94.6%, 100.0%]	100.0% [100.0%, 100.0%]	0	-	-	4	75.0% [19.4%, 99.4%]	100.0% [99.8%, 100.0%]	24	100.0% [85.8%, 100.0%]	100.0% [100.0%, 100.0%]
ATM	24	33	100.0% [89.4%, 100.0%]	100.0% [100.0%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]
BAP1	9	13	100.0% [75.3%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
BRAF	2	37	100.0% [90.5%, 100.0%]	100.0% [99.9%, 100.0%]	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
BRCA1	4	7	100.0% [59.0%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]
BRCA2	7	14	92.9% [66.1%, 99.8%]	100.0% [99.9%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
CBL	2	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-
CCNE1	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
CD274	1	2	100.0% [15.8%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
CDH1	10	10	100.0% [69.2%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
CDK4	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
CDKN2A	2	11	72.7% [39.0%, 94.0%]	100.0% [99.9%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	4	100.0% [39.8%, 100.0%]	99.9% [99.7%, 100.0%]
CHEK2	1	0	-	-	0	-	-	0	-	-	2	100.0% [15.8%, 100.0%]	100.0% [99.1%, 100.0%]
CTNNB1	2	16	100.0% [79.4%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	0	-	-
DDR2	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
DNMT3A	3	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
EGFR	9	9	100.0% [66.4%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	99.8% [98.6%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
ERBB2	6	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	2	100.0% [15.8%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-
ERBB3	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
ERBB4	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
ESR1	3	7	100.0% [59.0%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
EZH2	1	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
FBXW7	6	20	100.0% [83.2%, 100.0%]	100.0% [100.0%, 100.0%]	0	-	-	0	-	-	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
FGFR1	4	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
FGFR2	2	4	100.0% [39.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
FGFR3	4	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
FGFR4	5	7	100.0% [59.0%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	0	-	-
FLT3	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
GATA2	2	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	0	-	-	0	-	-	0	-	-
GATA3	5	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
GNA11	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
GNAS	1	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	0	-	-	0	-	-	0	-	-
HRAS	1	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	0	-	-	0	-	-	0	-	-
IDH1	1	13	100.0% [75.3%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
IDH2	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
JAK1	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
JAK2	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
JAK3	2	4	100.0% [39.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
KDR	3	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
KIT	6	7	100.0% [59.0%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	2	50.0% [1.3%, 98.7%]	100.0% [99.5%, 100.0%]	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
KRAS	3	73	100.0% [95.1%, 100.0%]	100.0% [99.9%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-
MAP2K1	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
MAX	1	0	-	-	0	-	-	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
MDM2	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
MED12	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-
MET	3	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
MSH2	5	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
MTOR	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
MYCL	1	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
MYD88	2	3	100.0% [29.2%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
NF1	32	30	100.0% [88.4%, 100.0%]	100.0% [100.0%, 100.0%]	1	0.0% [0.0%, 97.5%]	100.0% [99.1%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	14	100.0% [76.8%, 100.0%]	100.0% [99.9%, 100.0%]
NF2	7	8	100.0% [63.1%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]
NFE2L2	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
NOTCH1	17	19	100.0% [82.4%, 100.0%]	100.0% [99.9%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]
NRAS	2	7	100.0% [59.0%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
PDGFRA	5	5	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
PIK3CA	8	75	97.3% [90.7%, 99.7%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
PIK3R1	10	9	100.0% [66.4%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	12	100.0% [73.5%, 100.0%]	100.0% [99.9%, 100.0%]
PPP2R1A	2	4	100.0% [39.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
PTCH1	9	13	100.0% [75.3%, 100.0%]	100.0% [99.9%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
PTEN	9	46	100.0% [92.3%, 100.0%]	100.0% [100.0%, 100.0%]	0	-	-	6	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]	19	100.0% [82.4%, 100.0%]	100.0% [99.9%, 100.0%]
PTPN11	3	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
RAF1	1	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
RB1	16	15	100.0% [78.2%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	9	100.0% [66.4%, 100.0%]	100.0% [99.9%, 100.0%]
RPS6KB1	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
SF3B1	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
SMAD4	7	18	100.0% [81.5%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
SMARCB1	2	4	100.0% [39.8%, 100.0%]	100.0% [99.7%, 100.0%]	0	-	-	0	-	-	0	-	-
SMO	3	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	0	-	-	0	-	-
SPOP	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
STK11	7	8	87.5% [47.3%, 99.7%]	100.0% [99.9%, 100.0%]	0	-	-	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
TERT	1	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-
TET2	6	20	100.0% [83.2%, 100.0%]	100.0% [100.0%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	3	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
TP53	8	205	99.5% [97.3%, 100.0%]	100.0% [100.0%, 100.0%]	8	100.0% [63.1%, 100.0%]	100.0% [99.9%, 100.0%]	9	100.0% [66.4%, 100.0%]	100.0% [99.9%, 100.0%]	24	100.0% [85.8%, 100.0%]	100.0% [100.0%, 100.0%]
TSC1	8	8	100.0% [63.1%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-

Gene	Total Exons	SNVs			MNVs			Insertions			Deletions		
		# of SNVs	SNV PPA [95% CI]	SNV NPA [95% CI]	# of MNVs	MNV PPA [95% CI]	MNV NPA [95% CI]	# of Ins	Ins PPA [95% CI]	Ins NPA [95% CI]	# of Del	Del PPA [95% CI]	Del NPA [95% CI]
TSC2	11	12	100.0% [73.5%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	0	-	-	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
U2AF1	1	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-	0	-	-	0	-	-
VHL	3	9	100.0% [66.4%, 100.0%]	100.0% [99.9%, 100.0%]	0	-	-	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	12	100.0% [73.5%, 100.0%]	100.0% [99.9%, 100.0%]
WT1	4	4	100.0% [39.8%, 100.0%]	100.0% [99.8%, 100.0%]	0	-	-	1	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]	0	-	-
ZNF217	2	2	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]	0	-	-	0	-	-	0	-	-

Accuracy – SNVs, MNVs, and INDELs

A breakdown of the Accuracy for SNVs, MNVs, and INDEL results by variant type, level and insertion/deletion size is provided in Table 10.

Table 10. Concordance breakdown for short variants inclusive of substitutions and INDELs

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All Samples	1028	1221	80	11	414920	99.1% [98.4%, 99.6%]	100.0% [100.0%, 100.0%]
All SNVs	736	971	19	8	297042	99.2% [98.4%, 99.6%]	100.0% [100.0%, 100.0%]
Level 1 SNVs	10	31	1	0	648	100.0% [88.8%, 100.0%]	99.8% [99.1%, 100.0%]
Level 2 SNVs	128	209	1	1	27649	98.1% [95.3%, 99.5%]	100.0% [100.0%, 100.0%]

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
Level 3 SNVs	336	419	17	2	144384	99.5% [98.3%, 99.9%]	100.0% [100.0%, 100.0%]
All MNVs	22	18	3	1	8881	94.7% [74.0%, 99.9%]	100.0% [99.9%, 100.0%]
Level 1 MNVs	0	0	0	0	0	N/A	N/A
Level 2 MNVs	6	4	2	0	1508	100.0% [39.8%, 100.0%]	99.9% [99.5%, 100.0%]
Level 3 MNVs	11	9	1	1	5353	90.0% [55.5%, 99.7%]	100.0% [99.9%, 100.0%]
All Insertions	71	58	17	2	28656	96.7% [88.5%, 99.6%]	99.9% [99.9%, 100.0%]
Level 2 Insertions	20	19	2	0	2902	100.0% [82.4%, 100.0%]	99.9% [99.8%, 100.0%]
Level 3 Insertions	51	37	15	2	24946	94.9% [82.7%, 99.4%]	99.9% [99.9%, 100.0%]
Insertions, 1-5bp	60	47	16	2	24214	95.9% [86.0%, 99.5%]	99.9% [99.9%, 100.0%]
Insertions, 6-10bp	5	5	0	0	2020	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]
Insertions, 11-15bp	3	4	0	0	1211	100.0% [39.8%, 100.0%]	100.0% [99.7%, 100.0%]
Insertions, 16-20bp	1	1	0	0	404	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
Insertions, 21-25bp	0	0	0	0	0	N/A	N/A
Insertions, >25bp	2	1	1	0	807	100.0% [2.5%, 100.0%]	99.9% [99.3%, 100.0%]
All Deletions	199	174	41	0	80341	100.0% [97.9%,	99.9% [99.9%, 100.0%]

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
						100.0%]	
Level 2 Deletions	23	22	2	0	5558	100.0% [84.6%, 100.0%]	100.0% [99.9%, 100.0%]
Level 3 Deletions	154	128	39	0	65087	100.0% [97.2%, 100.0%]	99.9% [99.9%, 100.0%]
Deletions, 1-5bp	155	134	35	0	62573	100.0% [97.3%, 100.0%]	99.9% [99.9%, 100.0%]
Deletions, 6-10bp	18	19	1	0	7269	100.0% [82.4%, 100.0%]	100.0% [99.9%, 100.0%]
Deletions, 11-15bp	6	5	1	0	2423	100.0% [47.8%, 100.0%]	100.0% [99.8%, 100.0%]
Deletions, 16-20bp	6	6	0	0	2424	100.0% [54.1%, 100.0%]	100.0% [99.8%, 100.0%]
Deletions, 21-25bp	3	3	0	0	1212	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
Deletions, >25bp	11	7	4	0	4440	100.0% [59.0%, 100.0%]	99.9% [99.8%, 100.0%]

Accuracy – Hotspot Concordance

For hotspot concordance analysis with the orthogonal method, reported variants in hotspot regions overlapping with orthogonal method targeted regions were analyzed. From the 416 analyzed study samples, 164 samples had at least 1 reported variant in an overlapping hotspot region. The intersection of the defined hotspot regions of xT CDx and the orthogonal method targeted regions included 214 total base pairs, in which 192 reported variants were evaluated, including 50 unique SNVs, 3 unique MNVs, 2 unique insertions and 3 unique deletions. A summary of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) is provided in Table 11, below, for SNVs, MNVs, insertions and deletions.

Table 11. Concordance summary for short variants in hotspot regions

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All Variants	58	188	2	2	23298	98.9% [96.2%, 99.9%]	100.0% [100.0%, 100.0%]
All SNVs	50	180	2	2	20066	98.9% [96.1%, 99.9%]	100.0% [100.0%, 100.0%]
All MNVs	3	3	0	0	1212	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
All Insertions	2	2	0	0	808	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
All Deletions	3	3	0	0	1212	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]

A breakdown of the Accuracy for SNVs, MNVs, and INDELs in hotspot regions by variant type, level (i.e., variants with evidence of clinical significance/Level 2, and variants with potential clinical significance/Level 3) and insertion/deletion size is provided in Table 12.

Table 12. Concordance breakdown for short variants inclusive of substitutions and INDELs relative to orthogonal method for Level 2 and Level 3 variants

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All Variants	58	188	2	2	23298	98.9% [96.2%, 99.9%]	100.0% [100.0%, 100.0%]
All SNVs	50	180	2	2	20066	98.9% [96.1%, 99.9%]	100.0% [100.0%, 100.0%]
Level 1 SNVs	10	31	1	0	648	100.0% [88.8%, 100.0%]	99.8% [99.1%, 100.0%]
Level 2 SNVs	36	98	0	1	5468	99.0% [94.5%, 100.0%]	100.0% [99.9%, 100.0%]
Level 3 SNVs	26	51	1	1	13950	98.1% [89.7%, 100.0%]	100.0% [100.0%, 100.0%]

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All MNVs	3	3	0	0	1212	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
Level 1 MNVs	0	0	0	0	0	N/A	N/A
Level 2 MNVs	2	2	0	0	293	100.0% [15.8%, 100.0%]	100.0% [98.7%, 100.0%]
Level 3 MNVs	1	1	0	0	919	100.0% [2.5%, 100.0%]	100.0% [99.6%, 100.0%]
All Insertions	2	2	0	0	808	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
Level 2 Insertions	0	0	0	0	126	N/A	100.0% [97.1%, 100.0%]
Level 3 Insertions	1	1	0	0	278	100.0% [2.5%, 100.0%]	100.0% [98.7%, 100.0%]
Insertion, 1-5bp	1	1	0	0	404	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
Insertion, 6-10bp	1	1	0	0	404	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]
Insertion, 11-15bp	0	0	0	0	0	N/A	N/A
Insertion, 16-20bp	0	0	0	0	0	N/A	N/A
Insertion, 21-25bp	0	0	0	0	0	N/A	N/A
Insertion, >25bp	0	0	0	0	0	N/A	N/A
All Deletions	3	3	0	0	1212	100.0% [29.2%, 100.0%]	100.0% [99.7%, 100.0%]
Level 2 Deletions	1	1	0	0	114	100.0% [2.5%,	100.0% [96.8%, 100.0%]

Variant Type	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA [Exact 95% CI]	NPA [Exact 95% CI]
						100.0%]	
Level 3 Deletions	0	0	0	0	290	N/A	100.0% [98.7%, 100.0%]
Deletion, 1-5bp	2	2	0	0	808	100.0% [15.8%, 100.0%]	100.0% [99.5%, 100.0%]
Deletion, 6-10bp	0	0	0	0	0	N/A	N/A
Deletion, 11-15bp	0	0	0	0	0	N/A	N/A
Deletion, 16-20bp	0	0	0	0	0	N/A	N/A
Deletion, 21-25bp	0	0	0	0	0	N/A	N/A
Deletion, >25bp	1	1	0	0	404	100.0% [2.5%, 100.0%]	100.0% [99.1%, 100.0%]

Accuracy - MSI

The detection of MSI status by xT CDx was assessed by comparison with results obtained using a validated orthogonal method (IHC staining of MLH1, MSH2, MSH6 and PMS2). A total set of 316 patient-matched tumor and normal samples representing 30 cancer types were sequenced with xT CDx. The reported MSI status from xT CDx was compared with results of the IHC staining [MSI High (MSI-H)/deficient mismatch repair (dMMR) versus non-MSI-H/proficient mismatch repair] and used to calculate the PPA and NPA for MSI. Of the 117 samples identified as positive by IHC testing (Abnormal IHC, loss), 110 were identified as MSI-H by xT CDx, yielding a PPA of 94.0% (95% CI: 88-98%). Of the 199 samples identified as negative by IHC testing (Normal IHC, intact), 195 were identified as MSS by xT CDx, yielding a NPA of 98% (95% CI: 95-99%). Results of MSI concordance testing are provided in Table 13, below.

Table 13. Concordance summary for MSI status relative to IHC

	Normal IHC	Abnormal IHC
xT CDx MSI Stable (MSS)	195	7
xT CDx MSI High (MSI-H)	4	110

The distribution of the solid tumor specimens included in the study, separated by CRC/EC (colorectal cancer/endometrial cancer) and non-CRC/non-EC cancer types and MSI results is provided in Table 14.

Table 14. Distribution of solid tumor specimens, separated by CRC/EC and Non-CRC/Non-EC

Cancer Type	Number of samples	Abnormal IHC	Normal IHC
CRC/EC	108	75	33
Non-CRC/EC	208	42	166
Total	316	117	199

The MSI accuracy cohort included 108 unique CRC/EC and 208 unique non-CRC and non-EC tumor-normal paired samples across 30 tumor types. Analysis of these specimens separated by CRC/EC and non-CRC/non-EC, as well as overall, is provided in Tables 15-18 below.

Table 15. MSI accuracy in CRC/EC specimens relative to the orthogonal method

	xT CDx MSI status		
IHC status	Stable	Equivocal	High
Normal	32	0	1
Abnormal	3	0	72

Table 16. MSI accuracy in non-CRC/non-EC specimens relative to the orthogonal method

	xT CDx MSI status		
IHC status	Stable	Equivocal	High
Normal	163	0	3
Abnormal	3	1	38

Table 17. MSI accuracy in all specimens relative to the orthogonal method

	xT CDx MSI status		
IHC status	Stable	Equivocal	High
Normal	195	0	4
Abnormal	6	1	110

Table 18. Agreement for MSI status overall and by cohort relative to the orthogonal method

Cohort	OPA [Exact 95% CI]	PPA [Exact 95% CI]	NPA [Exact 95% CI]
All	96.5% [94%, 98%]	94.0% [88%, 98%]	98.0% [95%, 99%]
CRC/EC	96.3% [91%, 99%]	96.0% [89%, 99%]	97.0% [84%, 100%]
non-CRC/non-EC	96.6% [93%, 99%]	90.5.8% [77%, 97%]	98.2% [95%, 100%]

Note: Equivocal results were considered to be discordant with the orthogonal method for calculation of agreement.

Accuracy – QC pass rate for Accuracy of Short Variants

Table 19 details the pass rate at each QC step of the device for the specimens analyzed to characterize the accuracy for SNVs, MNVs, and INDELs, following exclusion of samples with insufficient extracted material. QC1 is not detailed here since QC1 is an extraction metric and these samples were previously extracted. A total of 429 samples were successfully sequenced and passed QC4; 13 samples failed QC4 due to the reasons detailed in Table 20.

Table 19. QC pass rate for Accuracy of Short Variants

Quality Control	FFPE Libraries	Blood Libraries	Saliva Libraries
QC2	442/445 passed	361/361 passed	84/84 passed
QC3	442/442 passed	361/361 passed	84/84 passed
Tumor-normal matched pairs passing QC3	442/442 passed		
QC4*	429/442 passed		

*QC4 metric is derived from collection of data using FFPE-blood (n=359) or FFPE-saliva (n=83) matched samples, where the QC4 "pass" status is assigned to the tumor-normal matched pair.

Table 20. QC4 failure explanation

Failure Reason	Number of Samples
Low normal unique read count; low normal total read count	1
Tumor contamination (somatic variants found in germline)	9
High tumor PCR duplication rate	2
Low tumor unique read count	1

Among the 429 samples that passed xT CDx QC, 6 samples failed OM1 QC. Six additional samples were removed because of unavailable results or inappropriate sample types, and one was removed because it had no variants called by either xT CDx or OM1. The final intersection of the QC-passing samples with OM1 and xT CDx resulted in a total of 416 samples, which were the set of samples analyzed for this study.

Data obtained from sequencing samples with xT CDx for the Accuracy of Short Variants study were re-analyzed for hotspot regions. Table 19 above has the summary of the QC pass rates for the samples processed with xT CDx. All 416 QC-passing samples evaluated in the Accuracy of Short Variants study were evaluated for hotspot regions.

Accuracy – QC pass rate of MSI study

A total of 440 specimens were analyzed for this study. Data obtained from sequencing 396 samples for Accuracy for SNVs, MNVs, and INDELs were analyzed for this study. Data from 8 colorectal cancer specimens from the determination of LoD for MSI study were also analyzed to increase the number of colorectal cancer specimens included in the study. From these 404 samples, there were 293 samples that also had data for MSI status from the OM, which is an IHC method. Of these 293 samples, 282 passed QC4.

An additional 36 non-CRC/non-EC specimens were included based on MSI-H status as determined by IHC.

Table 21 details the pass rate at each QC step of xT CDx for the 36 additional MSI-H non-CRC/non-EC specimens specifically processed for this study. QC1 is not detailed here since these samples were previously extracted. With xT CDx, 34 samples were sequenced and passed QC4.

Table 21. QC pass rate for Accuracy for Additional non-CRC/non-EC specimens in the MSI study

Quality Control	FFPE Libraries	Blood Libraries	Saliva Libraries
QC2	35/36 passed	29/30 passed	6/6 passed
QC3	35/35 passed	29/29 passed	6/6 passed
Tumor-normal matched pairs passing QC3	34/34 passed		
QC4*	34/34 passed		

*QC4 metric is derived from collection of data using FFPE-blood (n=29) or FFPE-saliva (n=6) matched samples, where the QC4 "pass" status is assigned to the tumor-normal matched pair.

Overall, the comparison between the MSI status determined by xT CDx and IHC included 316 samples from 30 different tumor types.

iii. Wild-Type Accuracy

The data originally generated in Accuracy of Short Variants was re-analyzed to assess wild type (WT) accuracy. 100 SNV positions and 100 INDEL positions were randomly selected (pre-specified) without knowledge of concordance, from the list of variants reported by the OM1 and in regions reportable by xT CDx, based on having at least one sample with a non-WT call in the OM1 dataset. Additionally, all 8 MNV positions that were present in both xT CDx and OM1 were selected. The resulting list of 208 positions represented 45 genes.

Each class of mutations (SNV, MNV, insertion, deletion) was evaluated within the specimens which had at least one selected mutation of that class. For example, for the 100 chosen SNVs, 165 samples contained at least one of the chosen SNVs and those were the 165 samples evaluated. In total, 224 specimens which produced results from xT CDx and OM1 and had at least one mutation (non-WT) in one of these 208 pre-specified positions were included in this analysis. See Table 22 for detailed information.

Table 22. WT accuracy by variant type; and by insertion and deletion size compared to OM1

	xT CDx^{WT}/ OM1^{WT}	xT CDx^{+}/ OM1^{WT}	xT CDx^{WT}/ OM1^{+}	xT CDx^{+}/ OM1^{+}	OPA [95% CI]	NPA [95% CI]	PPA [95% CI]
All Variants	25537	3	3	325	100.0% [99.9%, 100.0%]	100.0% [100.0%, 100.0%]	99.1% [97.4%, 99.8%]
All SNVs	19918	2	2	208	100.0% [99.9%, 100.0%]	100.0% [100.0%, 100.0%]	99.0% [96.6%, 99.9%]
SNVs - Level 2	3331	0	0	53	100.0% [99.9%, 100.0%]	100.0% [99.9%, 100.0%]	100.0% [93.3%, 100.0%]
SNVs - Level 3	16587	2	2	151	100.0% [99.9%, 100.0%]	100.0% [100.0%, 100.0%]	98.7% [95.4%, 99.8%]
All MNVs	90	1	0	9	99.0% [94.6%, 100.0%]	100.0% [96.0%, 100.0%]	90.0% [55.5%, 99.7%]
All Insertions	1329	0	1	38	99.9% [99.6%, 100.0%]	99.9% [99.6%, 100.0%]	100.0% [90.7%, 100.0%]
Insertions, 0-5bp	1219	0	1	34	99.9% [99.6%, 100.0%]	99.9% [99.5%, 100.0%]	100.0% [89.7%, 100.0%]
Insertions, 5-10bp	37	0	0	1	100.0% [90.7%, 100.0%]	100.0% [90.5%, 100.0%]	100.0% [2.5%, 100.0%]
Insertions, 10-15bp	73	0	0	3	100.0% [95.3%, 100.0%]	100.0% [95.1%, 100.0%]	100.0% [29.2%, 100.0%]
Insertions, 15-20bp	0	0	0	0	N/A	N/A	N/A

	xT CDx^{WT}/ OM1^{WT}	xT CDx^{+}/ OM1^{WT}	xT CDx^{WT}/ OM1^{+}	xT CDx^{+}/ OM1^{+}	OPA [95% CI]	NPA [95% CI]	PPA [95% CI]
Insertions, 20-25bp	0	0	0	0	N/A	N/A	N/A
All Deletions	4200	0	0	70	100.0% [99.9%, 100.0%]	100.0% [99.9%, 100.0%]	100.0% [94.9%, 100.0%]
Deletions 0-5bp	3600	0	0	60	100.0% [99.9%, 100.0%]	100.0% [99.9%, 100.0%]	100.0% [94.0%, 100.0%]
Deletions 5-10bp	300	0	0	5	100.0% [98.8%, 100.0%]	100.0% [98.8%, 100.0%]	100.0% [47.8%, 100.0%]
Deletions 10-15bp	60	0	0	1	100.0% [94.1%, 100.0%]	100.0% [94.0%, 100.0%]	100.0% [2.5%, 100.0%]
Deletions 15-10bp	0	0	0	0	N/A	N/A	N/A
Deletions 20-25bp	0	0	0	0	N/A	N/A	N/A

3. Precision

a. Precision in Well-Characterized Material

The panel-wide precision/reproducibility of xT CDx was assessed for detecting variants in well-characterized reference material by repeated measurement of NA12878, a nucleic acid (NA) extracted from the GM12878 cell line. Precision was evaluated across 22 replicates which were processed over multiple library preparations and days (n=17), hybridization capture batches (n=8), and sequencing flow cells (n=8).

A total of 2673 variants were called across all 22 replicates, and 2624 of these variants were in the Genome in a Bottle (GIAB)[1] high confidence dataset. Table 23 shows the Coefficient of Variation (CV) distribution for all 2673 variants analyzed. In the study, 95.5% of samples had a CV below 10%. Across all samples, the mean CV of the variant allele frequency (VAF) was 3.7% +/- 3.9%. Mean %CV by zygosity of the variant, as declared in the GIAB variant call file (VCF) and type variant is presented in Table 23.

Table 23. Distribution of variants by %CV in well-characterized reference material

	CV < 10%	10% '.' CV < 15%	15% '.' CV < 20%	20% < CV

[1] Zook, J. M. et al. Extensive sequencing of seven human genomes to characterize benchmark reference materials. Sci. Data 3:160025 doi: 10.1038/sdata.2016.25 (2016)

Number of Variants	2552	73	24	24
Percent of Variants	95.5%	2.70%	0.90%	0.90%

Table 24. Mean percent coefficient of variation (%CV) by zygosity declared in the GIAB VCF and type of variant for well-characterized reference material

Zygosity	SNVs and INDELs	SNVs Only	Insertions Only	Deletions Only
All[1] %CV)	3.7% +/- 3.8%	3.5% +/- 3.5%	7.0% +/- 6.3%	7.7% +/- 6.7%
Number of Total (All) Variants	2673	2525	78	70
Homozygous Only (%CV)	0.23% +/- 0.72%	0.14% +/- 0.39%	2.2% +/- 2.4%	1.2% +/-1.6%
Number of HomozygousVariants	938	889	30	19
Heterozygous Only (%CV)	5.3% +/- 3.2%	5.3% +/- 3.1%	7.9% +/- 5.5%	7.9% +/- 5.8%
Number Heterozygous of Variants	1686	1635	22	29

[1] Homozygous, Heterozygous, and missing (from GIAB VCF)

The VAF of each variant was measured in each replicate using xT CDx. For each variant, the %CV (standard deviation of VAFs / mean VAF) was calculated across replicates. Table 23 presents the distribution of the %CVs of the VAFs measured by xT CDx in the 22 replicates of the NA12878 sample.

The mean %CV for all variants of a given type SNVs and INDELs) based on zygosity was calculated as described for Table 23 (for example, for SNVs only: sum of %CVs for all SNV variants / total number of SNV variants), and is displayed in Table 24.

b. Panel-wide Precision in Clinical Specimens

Panel-wide precision of xT CDx in clinical specimens was based on repeated measurement of 49 patient specimens representing 23 different tumor types (including

melanoma, CRC, glioblastoma, and lung cancer). Replicates (n=5-10) of each specimen were measured across 3 non-consecutive days, with multiple operators, reagent lots, instruments. A total of 317 replicates contributed to the evaluation of precision. The specimens and tumor types included in the study from 49 patients are shown in Table 25.

Table 25. Number of clinical specimens per tumor type in the panel-wide precision study

Tumor Type	Number of Specimens
Basal Cell Carcinoma	1
Bladder Cancer	6
Breast Cancer	4
Colorectal Cancer	5
Endocrine Tumor	2
Endometrial Cancer	4
Esophageal Cancer	1
Gastric Cancer	1
Head and Neck Cancer	2
Liver Cancer	1
Melanoma	2
Meningioma	1
Non-Small Cell Lung Cancer	4
Ovarian Cancer	1
Prostate Cancer	1
Skin Cancer	2
Tumor of Unknown Origin	4
Adrenal Cancer	1
Cervical Cancer	1
Head and Neck Squamous Cell Carcinoma	1

Pancreatic Cancer	1
Sarcoma	2
Small Cell Lung Cancer	1
All	49

Among the specimens evaluated, there were 289 total variants represented by 151 SNVs, 9 MNVs, 26 insertions and 103 deletions. The overall positive call rate across all precision conditions (days, operators, reagents lots, and instruments), for all specimens and replicates was 94.5%, and 97.0% for variants with a VAF ≥ 15%. Results are shown in Table 26.

Table 26. Precision by variant type and VAF

Variant Type	VAF threshold (%)	Total variants	Mean VAF Range	Positive/Total Calls	Positive call rate (2-sided 95% CI)
SNV	≥0	151	3.8-84.343	911/944	96.5% (95.1,97.6)
	≥5	150	5.388-84.343	907/939	96.6% (95.2,97.7)
	≥10	132	10.418-84.343	841/849	99.1% (98.2,99.6)
	≥15	110	15.067-84.343	718/726	98.9% (97.8,99.5)
MNV	≥0	9	12.657-58.597	61/61	100.0% (94.1,100)
	≥5	9	12.657-58.597	61/61	100.0% (94.1,100)
	≥10	9	12.657-58.597	61/61	100.0% (94.1,100)
	≥15	6	15.124-58.597	35/35	100.0% (90.0,100)
Insertion	≥0	26	11.25-61.114	153/165	92.7% (87.6,96.2)
	≥5	26	11.25-61.114	153/165	92.7% (87.6,96.2)
	≥10	26	11.25-61.114	153/165	92.7% (87.6,96.2)
	≥15	23	15.187-61.114	139/145	95.9% (91.2,98.5)
Deletion	≥0	103	10.054-94.976	683/744	91.8% (89.6,93.7)
	≥5	103	10.054-94.976	683/744	91.8% (89.6,93.7)
	≥10	103	10.054-94.976	683/744	91.8% (89.6,93.7)

Variant Type	VAF threshold (%)	Total variants	Mean VAF Range	Positive/Total Calls	Positive call rate (2-sided 95% CI)
	≥15	91	15.123-94.976	646/679	95.1% (93.2,96.6)
All	≥0	289	3.8-94.976	1808/1914	94.5% (93.3,95.4)
	≥5	288	5.388-94.976	1804/1909	94.5% (93.4,95.5)
	≥10	270	10.054-94.976	1738/1819	95.5% (94.5,96.4)
	≥15	230	15.067-94.976	1538/1585	97.0% (96.1,97.8)

The positive call rates for individual sequence mutations assessed for panel-wide precision, along with the VAF range, mean, median, SD, and %CV are presented in Table 27. Variants are listed by specimen, and variant type is identified in each case.

Table 27. Panel-wide precision by specimen and variant for all replicates

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
747	ARID2 exon_15	Deletion	c.3432delC p.Ile1145fs	37.27-42.006	38.869	38.1	1.863	4.80%	4/4	100.0% (39.8,100)
	TP53 exon_4	SNV	c.396G>T p.Lys132Asn	34.351-37.248	35.587	35.375	1.147	3.20%	4/4	100.0% (39.8,100)
	NF1 exon_34	Deletion	c.4487_4490delTTTC p.Leu1496fs	22.36-30.667	27.215	27.917	3.125	11.50%	4/4	100.0% (39.8,100)
	RASA1 exon_1	Deletion	c.124delC p.Leu42fs	31.394-38.0	34.607	34.517	2.406	7.00%	4/4	100.0% (39.8,100)
723	GRIN2A exon_12	SNV	c.3042G>A p.Trp1014*	34.119-36.909	35.311	35.052	0.909	2.60%	5/5	100.0% (47.8,100)
	FANCA exon_33	SNV	c.3208C>T p.Gln1070*	34.48-36.863	35.316	35.292	0.849	2.40%	5/5	100.0% (47.8,100)
	TERT exon_1	SNV	c.-124C>T .	29.961-36.253	33.31	33.083	2.079	6.20%	5/5	100.0% (47.8,100)
	BRAF exon_15	SNV	c.1799T>A p.Val600Glu	48.048-52.455	49.439	49.057	1.571	3.20%	5/5	100.0% (47.8,100)
	PPP6C exon_8	SNV	c.901C>T p.Arg301Cys	40.278-44.345	42.187	42.652	1.481	3.50%	5/5	100.0% (47.8,100)
870	ARID1A exon_12	Deletion	c.3216delA p.Lys1072fs	14.669-16.861	15.735	15.913	0.771	4.90%	5/5	100.0% (47.8,100)
	ARID1A exon_20	Insertion	c.5548dupG p.Asp1850fs	31.944-34.5	33.382	34.04	1.038	3.10%	5/5	100.0% (47.8,100)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
	JAK1 exon_18	Deletion	c.2580delA p.Lys860fs	26.143-32.815	29.82	29.821	2.144	7.20%	5/5	100.0% (47.8,100)
	PTEN exon_8	Insertion	c.1007dupA p.Tyr336fs	39.341-41.935	40.747	40.816	0.85	2.10%	5/5	100.0% (47.8,100)
	CTCF exon_1	Insertion	c.610dupA p.Thr204fs	16.179-21.977	18.43	18.522	2.114	11.50%	5/5	100.0% (47.8,100)
	SMAD4 exon_1	Deletion	c.94_95delAG p.Ser32fs	11.172-13.109	12.09	12.062	0.625	5.20%	5/5	100.0% (47.8,100)
	LRP1B exon_3	SNV	c.214G>T p.Glu72*	24.583-29.289	27.199	28.215	2.132	7.80%	5/5	100.0% (47.8,100)
	NFE2L2 exon_2	SNV	c.100C>G p.Arg34Gly	28.923-32.333	30.556	30.769	1.214	4.00%	5/5	100.0% (47.8,100)
	PIK3R1 exon_11	Deletion	c.1214_1216delTAA p.Ile405del	31.087-33.521	32.315	32.221	1.046	3.20%	5/5	100.0% (47.8,100)
	PIK3R1 exon_14	Deletion	c.1623_1625delTAG p.Ser541del	10.008-13.544	11.867	12.103	1.234	10.40%	5/5	100.0% (47.8,100)
	CUX1 exon_19	Deletion	c.2764delC p.Leu922fs	16.052-19.589	18.141	19.102	1.448	8.00%	5/5	100.0% (47.8,100)
874	ERRFI1 exon_3	Deletion	c.855delC p.Arg286fs	39.908-46.468	42.222	41.026	2.447	5.80%	5/5	100.0% (47.8,100)
874	ERRFI1 exon_3	Deletion	c.296delC p.Pro99fs	21.444-27.672	24.547	24.55	2.082	8.50%	5/5	100.0% (47.8,100)
874	ARID1A exon_20	Insertion	c.5548dupG p.Asp1850fs	39.385-51.883	45.416	44.62	4.107	9.00%	5/5	100.0% (47.8,100)
874	ARID1A exon_20	Deletion	c.5548delG p.Asp1850fs	46.027-52.893	47.881	46.4	2.597	5.40%	5/5	100.0% (47.8,100)
874	PTEN exon_5	SNV	c.388C>G p.Arg130Gly	60.433-70.545	64.421	63.462	3.695	5.70%	5/5	100.0% (47.8,100)
874	FGFR2 exon_8	SNV	c.755C>G p.Ser252Trp	28.893-33.026	30.694	30.488	1.409	4.60%	5/5	100.0% (47.8,100)
874	KMT2D exon_28	SNV	c.6019G>T p.Glu2007*	27.251-33.794	31.501	31.951	2.279	7.20%	5/5	100.0% (47.8,100)
874	PTPN11 exon_3	SNV	c.179G>T p.Gly60Val	25.402-33.234	30.172	30	2.843	9.40%	5/5	100.0% (47.8,100)
874	SMARCA4 exon_18	SNV	c.2644G>A p.Glu882Lys	5.37-7.52	6.445	6.445	1.075	16.70%	2/5	40.0% (5.3,85.3)
874	EP300 exon_28	Insertion	c.4511dupT p.Glu1505fs	11.25-11.25	11.25	11.25	0	0.00%	1/5	20.0% (0.5,71.6)
874	PIK3CA exon_20	SNV	c.3140A>G p.His1047Arg	25.481-32.738	30.59	32.313	2.772	9.10%	5/5	100.0% (47.8,100)
874	MSH3 exon_15	Deletion	c.2216delA p.Asn739fs	10.119-10.119	10.119	10.119	0	0.00%	1/5	20.0% (0.5,71.6)
874	HDAC2	Deletion	c.1375delA	32.486-34.807	33.419	32.71	1.022	3.10%	5/5	100.0%

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
	exon_12		p.Thr459fs							(47.8,100)
874	KMT2C exon_38	Deletion	c.8390delA p.Lys2797fs	25.065-27.583	26.626	26.928	0.947	3.60%	4/5	80.0% (28.4,99.5)
1016	B2M exon_1	SNV	c.1A>G p.Met1?	43.956-53.915	48.015	47.676	3.49	7.30%	5/5	100.0% (47.8,100)
1016	GRIN2A exon_12	SNV	c.3453C>A p.Tyr1151*	27.551-34.673	31.367	33.14	3.077	9.80%	5/5	100.0% (47.8,100)
1016	TP53 exon_4	SNV	c.473G>T p.Arg158Leu	71.429-77.601	74.286	74.173	2.015	2.70%	5/5	100.0% (47.8,100)
1016	STK11 exon_1	Deletion	c.70_71delAC p.Thr24fs	63.972-69.595	67.261	67.535	1.985	3.00%	5/5	100.0% (47.8,100)
1016	FAT1 exon_14	Deletion	c.10004delT p.Phe3335fs	20.605-26.309	24.884	25.922	2.17	8.70%	5/5	100.0% (47.8,100)
1160	KRAS exon_1	SNV	c.35G>A p.Gly12Asp	24.027-28.624	26.93	27.233	1.554	5.80%	5/5	100.0% (47.8,100)
1160	TP53 exon_7	SNV	c.818G>A p.Arg273His	42.857-46.858	44.988	45.258	1.597	3.60%	5/5	100.0% (47.8,100)
1162	ERRFI1 exon_3	Deletion	c.383delC p.Pro128fs	15.815-20.748	18.521	18.475	1.8	9.70%	5/5	100.0% (47.8,100)
1162	ARID1A exon_2	Deletion	c.1337_1338delCT p.Ser446fs	13.956-17.547	15.859	15.902	1.169	7.40%	5/5	100.0% (47.8,100)
1162	ARID1A exon_18	SNV	c.4381C>T p.Arg1461*	14.745-18.51	16.753	16.849	1.369	8.20%	5/5	100.0% (47.8,100)
1162	KRAS exon_1	SNV	c.34G>A p.Gly12Ser	3.526-4.401	3.8	3.637	0.351	9.20%	4/5	80.0% (28.4,99.5)
1162	KMT2D exon_31	Deletion	c.7306delT p.Cys2436fs	13.934-17.845	15.832	15.757	1.331	8.40%	5/5	100.0% (47.8,100)
1162	BRCA2 exon_10	Deletion	c.5351delA p.Asn1784fs	12.532-15.332	14.02	13.919	0.91	6.50%	5/5	100.0% (47.8,100)
1162	RB1 exon_19	Deletion	c.1959delA p.Val654fs	12.228-21.272	15.921	15.472	2.964	18.60%	5/5	100.0% (47.8,100)
1162	CHD2 exon_28	Deletion	c.3734delA p.Lys1245fs	18.182-24.63	21.369	21.296	2.633	12.30%	3/5	60.0% (14.7,94.7)
1162	FBXO11 exon_29	Deletion	c.3261delC p.Phe1088fs	10.054-10.054	10.054	10.054	0	0.00%	1/5	20.0% (0.5,71.6)
1162	LRP1B intron_26	SNV	c.4335-1G>T .	9.972-12.287	11.381	11.779	0.844	7.40%	5/5	100.0% (47.8,100)
1162	SETD2 exon_8	Insertion	c.4953dupT p.Thr1652fs	12.62-17.53	15.677	16.017	1.635	10.40%	5/5	100.0% (47.8,100)
1162	HDAC2 exon_1	Deletion	c.33delA p.Val12fs	23.432-23.432	23.432	23.432	0	0.00%	1/5	20.0% (0.5,71.6)
1162	ARID1B exon_21	SNV	c.5428C>T p.Gln1810*	25.658-30.134	28.15	28.324	1.5	5.30%	5/5	100.0% (47.8,100)
1162	CUX1 intron_14	SNV	c.1158+1G>A .	10.877-14.723	13.735	14.417	1.452	10.60%	5/5	100.0% (47.8,100)
1164	PTEN exon_6	Deletion	c.623delG p.Gly208fs	51.89-60.588	57.124	57.047	3.067	5.40%	5/5	100.0% (47.8,100)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
1164	KRAS exon_3	SNV	c.436G>A p.Ala146Thr	55.501-65.068	60.351	60.407	3.191	5.30%	5/5	100.0% (47.8,100)
1164	SDHA exon_6	Deletion	c.667delG p.Asp223fs	29.343-29.343	29.343	29.343	0	0.00%	1/5	20.0% (0.5,71.6)
1164	APC exon_18	Deletion	c.4192delA p.Ser1398fs	58.567-63.434	60.946	61.679	1.758	2.90%	5/5	100.0% (47.8,100)
1166	ARID2 exon_14	SNV	c.1825C>T p.Gln609*	5.664-7.034	6.516	6.849	0.607	9.30%	3/5	60.0% (14.7,94.7)
1166	ZFHX3 exon_8	SNV	c.8077C>T p.Arg2693*	11.4-18.596	15.367	15.039	2.509	16.30%	5/5	100.0% (47.8,100)
1166	TP53 exon_7	SNV	c.832C>T p.Pro278Ser	16.667-20.543	18.621	18.202	1.547	8.30%	5/5	100.0% (47.8,100)
1166	TP53 exon_5	SNV	c.637C>T p.Arg213*	12.373-20.19	15.623	14.645	2.646	16.90%	5/5	100.0% (47.8,100)
1166	ASXL1 exon_14	SNV	c.4067G>A p.Trp1356*	9.98-12.558	11.129	10.893	0.974	8.80%	5/5	100.0% (47.8,100)
1166	TERT exon_1	SNV	c.-146C>T .	17.857-27.429	22.567	22.581	3.114	13.80%	5/5	100.0% (47.8,100)
1166	PTCH1 exon_10	SNV	c.895C>T p.Gln299*	32.749-38.653	35.658	36.264	2.318	6.50%	5/5	100.0% (47.8,100)
1020	FOXA1 exon_2	Insertion	c.794_795dupGC p.Phe266fs	37.54-47.324	43.59	45.388	3.745	8.60%	5/5	100.0%(47.8,100)
1020	ZFHX3 exon_8	Deletion	c.3968-27_3977delGTTTGATTTTTTTTTTTTTTTTAACAGAAACCTCAGAinsC p.Glu1323_Glu1326delins???	35.714-46.032	40.252	38.71	3.559	8.80%	5/5	100.0% (47.8,100)
854	ERBB3 exon_3	SNV	c.310G>T p.Val104Leu	19.575-22.33	20.473	19.864	1.037	5.10%	5/5	100.0% (47.8,100)
854	BRCA2 exon_10	SNV	c.6398C>G p.Ser2133*	7.307-9.859	8.659	8.735	1.129	13.00%	4/5	80.0% (28.4,99.5)
854	ERBB2 exon_9	SNV	c.929C>A p.Ser310Tyr	8.532-10.545	9.099	8.815	0.743	8.20%	5/5	100.0% (47.8,100)
854	SF3B1 exon_19	SNV	c.2704G>A p.Glu902Lys	29.828-44.593	38.724	40.143	5.143	13.30%	5/5	100.0% (47.8,100)
854	MAPK1 exon_7	SNV	c.964G>A p.Glu322Lys	11.419-12.919	11.876	11.592	0.549	4.60%	5/5	100.0% (47.8,100)
854	EP300 exon_31	SNV	c.7000C>T p.Gln2334*	12.428-15.559	14.215	14.388	1.016	7.10%	5/5	100.0% (47.8,100)
854	RHOA exon_1	SNV	c.50G>A p.Gly17Glu	9.322-12.766	10.95	10.742	1.149	10.50%	5/5	100.0% (47.8,100)
854	FAT1 exon_18	SNV	c.10825C>T p.Gln3609*	19.213-24.622	21.261	20.951	2.04	9.60%	5/5	100.0% (47.8,100)
854	TERT exon_1	SNV	c.-124C>T .	15.079-22.907	17.353	16.071	2.903	16.70%	5/5	100.0% (47.8,100)
854	CDKN2A	SNV	c.322G>C	18.129-24.164	21.012	21.189	2.341	11.10%	5/5	100.0%

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
	exon_3		p.Asp108His							(47.8,100)
854	KDM6A exon_18	Insertion	c.2370dupT p.Asn791fs	31.398-38.631	35.265	34.711	2.509	7.10%	5/5	100.0% (47.8,100)
854	STAG2 exon_23	SNV	c.2401C>T p.Gln801*	33.015-35.092	33.777	33.439	0.806	2.40%	5/5	100.0% (47.8,100)
1168	KMT2D exon_1	SNV	c.49+1G>C .	33.436-37.238	35.048	34.05	1.689	4.80%	5/5	100.0% (47.8,100)
1168	TP53 exon_3	Deletion	c.243_244delAC p.Pro82fs	48.276-60.45	53.165	50.883	4.79	9.00%	5/5	100.0% (47.8,100)
1168	KMT2C exon_38	Insertion	c.8369dupT p.Ser2791fs	14.052-19.301	16.849	16.064	1.988	11.80%	5/5	100.0% (47.8,100)
1022	TP53 exon_7	SNV	c.817C>T p.Arg273Cys	53.476-60.043	56.712	57.56	2.489	4.40%	5/5	100.0% (47.8,100)
1022	APC exon_18	SNV	c.3682C>T p.Gln1228*	47.505-50.829	49.397	50.455	1.499	3.00%	5/5	100.0% (47.8,100)
1024	TP53 exon_6	SNV	c.743G>A p.Arg248Gln	82.517-85.911	83.335	82.604	1.306	1.60%	5/5	100.0% (47.8,100)
1172	ARID1A exon_20	SNV	c.6562C>T p.Gln2188*	29.692-32.698	31.455	31.853	1.086	3.50%	5/5	100.0% (47.8,100)
1172	C11orf65 exon_10	SNV	c.8929G>T p.Glu2977*	5.035-9.109	6.492	5.333	1.854	28.60%	3/5	60.0% (14.7,94.7)
1172	RB1 exon_7	SNV	c.608-1G>A .	26.939-32.157	29.597	29.742	1.668	5.60%	5/5	100.0% (47.8,100)
1172	MLH3 exon_8	SNV	c.3987+1G>A .	10.891-13.093	12.339	12.621	0.784	6.40%	5/5	100.0% (47.8,100)
1172	GPS2 exon_7	SNV	c.718C>T p.Gln240*	20.228-29.176	23.088	22.414	3.217	13.90%	5/5	100.0% (47.8,100)
1172	LRP1B exon_70	SNV	c.10784C>A p.Ser3595*	26.629-36.884	31.152	29.447	3.842	12.30%	5/5	100.0% (47.8,100)
1172	FAT1 exon_8	SNV	c.4701C>A p.Tyr1567*	7.068-9.102	7.923	8.034	0.703	8.90%	5/5	100.0% (47.8,100)
1172	TERT exon_1	SNV	c.-124C>T .	25.065-30.783	28.028	28.125	2.097	7.50%	5/5	100.0% (47.8,100)
1172	RAC1 exon_2	SNV	c.85C>T p.Pro29Ser	8.23-12.963	10.601	10.259	1.633	15.40%	5/5	100.0% (47.8,100)
1172	CUX1 exon_12	SNV	c.874G>T p.Glu292*	7.878-9.322	8.558	8.316	0.605	7.10%	5/5	100.0% (47.8,100)
1026	SPEN exon_6	SNV	c.1244-1G>C .	14.921-20.606	17.904	17.56	2.246	12.50%	5/5	100.0% (47.8,100)
1026	PTEN exon_8	Deletion	c.955_958delACTT p.Thr319fs	26.953-32.115	29.628	29.293	1.946	6.60%	5/5	100.0% (47.8,100)
1026	TP53 exon_6	MNV	p.R248H	19.298-26.852	23.611	23.83	2.893	12.30%	5/5	100.0% (47.8,100)
1026	TP53 exon_5	SNV	c.638G>A p.Arg213Gln	11.373-17.11	14.129	14.537	2.215	15.70%	5/5	100.0% (47.8,100)
1026	MAP3K1 exon_9	SNV	c.1639C>T p.Gln547*	24.948-35.714	29.497	28.228	4.025	13.60%	5/5	100.0% (47.8,100)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
1026	ZMYM3 exon_1	SNV	c.161C>A p.Ser54*	32.487-36.877	34.508	34	1.792	5.20%	5/5	100.0% (47.8,100)
1012	ARID2 exon_12	SNV	c.1499-1G>A .	5.423-6.808	6.115	6.115	0.693	11.30%	2/5	40.0% (5.3,85.3)
1012	KMT2D exon_39	Insertion	c.12219dupA p.Gln4074fs	13.605-18.124	15.187	14.645	1.658	10.90%	5/5	100.0% (47.8,100)
1012	ZFHX3 exon_8	Deletion	c.5774delG p.Gly1925fs	10.122-13.479	11.611	11.384	1.116	9.60%	5/5	100.0% (47.8,100)
1012	TP53 exon_11	SNV	c.1039G>A p.Ala347Thr	21.875-25.494	23.647	24.021	1.234	5.20%	5/5	100.0% (47.8,100)
1012	MAP2K4 exon_1	Insertion	c.69dupC p.Val24fs	16.304-19.372	18.287	18.737	1.18	6.50%	4/5	80.0% (28.4,99.5)
1012	NF1 exon_30	Deletion	c.4076delC p.Pro1359fs	57.048-65.84	61.381	59.77	3.28	5.30%	5/5	100.0% (47.8,100)
1012	CIC exon_21	Deletion	c.7517delC p.Pro2506fs	15.331-20.513	17.69	17.578	1.937	10.90%	5/5	100.0% (47.8,100)
1012	ASXL1 exon_14	Deletion	c.1934delG p.Gly645fs	23.457-30.861	27.525	28.288	2.54	9.20%	5/5	100.0% (47.8,100)
1012	PIK3CA exon_9	SNV	c.1624G>A p.Glu542Lys	9.455-12.744	11.307	11.414	1.086	9.60%	5/5	100.0% (47.8,100)
1012	PIK3CA exon_20	SNV	c.3129G>A p.Met1043Ile	7.057-10.366	8.668	8.654	1.05	12.10%	5/5	100.0% (47.8,100)
1012	PIK3CA exon_20	SNV	c.3130A>T p.Asn1044Tyr	11.56-13.941	13.015	13.18	0.907	7.00%	5/5	100.0% (47.8,100)
1012	MSH3 exon_7	Deletion	c.1148delA p.Lys383fs	21.373-24.788	22.752	22.581	1.165	5.10%	5/5	100.0% (47.8,100)
1028	KMT2D exon_32	SNV	c.8086C>T p.Gln2696*	34.312-39.523	36.249	35.34	1.904	5.30%	5/5	100.0% (47.8,100)
1028	BRAF exon_15	Insertion	c.1797_1799dupAGT p.Val600dup	27.356-33.109	30.406	30.818	1.889	6.20%	5/5	100.0% (47.8,100)
1030	RB1 exon_8	Deletion	c.820_823delATTGinsT p.Ile274_Glu275delinsTer	61.011-72.034	66.825	67.832	4.055	6.10%	5/5	100.0% (47.8,100)
1030	TP53 exon_4	SNV	c.438G>A p.Trp146*	65.289-73.549	69.588	69.605	2.623	3.80%	5/5	100.0% (47.8,100)
1030	PIK3CA exon_9	SNV	c.1633G>A p.Glu545Lys	83.301-85.482	84.343	84.502	0.776	0.90%	5/5	100.0% (47.8,100)
1032	CREBBP exon_4	SNV	c.1142C>A p.Ser381*	5.228-6.788	6.038	6.098	0.638	10.60%	3/5	60.0% (14.7,94.7)
1032	XPO1 exon_14	SNV	c.1711G>A p.Glu571Lys	5.31-7.125	6.219	6.223	0.741	11.90%	3/5	60.0% (14.7,94.7)
1032	TERT intron_1	SNV	c.-57A>C .	5.915-10.652	7.473	7.129	1.728	23.10%	5/5	100.0% (47.8,100)
1032	NOTCH1 exon_34	SNV	c.7400C>A p.Ser2467*	5.102-6.367	5.711	5.688	0.469	8.20%	4/5	80.0% (28.4,99.5)
1032	NOTCH1 exon_27	SNV	c.5033T>C p.Leu1678Pro	5.729-5.729	5.729	5.729	0	0.00%	1/5	20.0% (0.5,71.6)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
1034	TERT exon_1	SNV	c.-124C>T .	14.634-20.988	16.969	15.287	2.854	16.80%	3/3	100.0% (29.2,100)
1158	NRAS exon_2	SNV	c.182A>G p.Gln61Arg	22.955-25.482	24.218	24.26	0.824	3.40%	5/5	100.0% (47.8,100)
1158	TP53 exon_10	SNV	c.1024C>T p.Leu342Leu	61.491-61.491	61.491	61.491	0	0.00%	1/5	20.0% (0.5,71.6)
1158	TP53 exon_6	SNV	c.772G>T p.Glu258*	30.986-38.381	33.07	32.148	2.731	8.30%	5/5	100.0% (47.8,100)
1158	APC exon_7	SNV	c.601G>T p.Glu201*	11.357-14.286	12.696	12.539	1.034	8.10%	5/5	100.0% (47.8,100)
1158	APC exon_18	SNV	c.3880C>T p.Gln1294*	24.635-27.564	26.03	25.667	1.126	4.30%	5/5	100.0% (47.8,100)
1036	TP53 exon_4	SNV	c.377A>G p.Tyr126Cys	13.508-20.0	15.682	15.257	2.341	14.90%	5/5	100.0% (47.8,100)
1038	ARID1A exon_3	MNV	p.SQ587*	16.616-20.081	18.545	19.227	1.385	7.50%	5/5	100.0% (47.8,100)
1038	NOTCH2 exon_30	Deletion	c.5226_5227delGC p.Gln1743fs	11.094-16.526	13.196	13.094	1.835	13.90%	5/5	100.0% (47.8,100)
1038	KMT2D exon_28	MNV	p.NQ1999*	15.047-18.944	17.102	16.716	1.477	8.60%	5/5	100.0% (47.8,100)
1038	PHLPP2 exon_15	SNV	c.2351G>A p.Trp784*	13.69-21.938	18.475	18.898	2.8	15.20%	5/5	100.0% (47.8,100)
1038	TP53 exon_7	SNV	c.856G>A p.Glu286Lys	36.98-41.866	39.223	39.566	1.775	4.50%	5/5	100.0% (47.8,100)
1038	TP53 exon_7	SNV	c.817C>T p.Arg273Cys	17.346-19.075	18.404	18.701	0.622	3.40%	5/5	100.0% (47.8,100)
1038	LDLR exon_7	SNV	c.1033C>T p.Gln345*	11.327-14.355	13.203	13.426	1.105	8.40%	5/5	100.0% (47.8,100)
1038	MLH1 exon_14	SNV	c.1609C>T p.Gln537*	15.196-19.331	17.043	17.143	1.425	8.40%	5/5	100.0% (47.8,100)
1038	SETD2 exon_17	SNV	c.7185G>A p.Trp2395*	15.049-19.222	17.287	17.119	1.395	8.10%	5/5	100.0% (47.8,100)
1038	BAP1 exon_1	MNV	p.W5*	13.717-16.475	15.124	14.793	1.001	6.60%	5/5	100.0% (47.8,100)
1038	ATR exon_1	SNV	c.3G>A p.Met1?	17.001-20.161	18.72	18.634	1.185	6.30%	5/5	100.0% (47.8,100)
1038	FAT1 exon_9	Deletion	c.5238_5240delCACinsTA p.Thr1747fs	16.343-21.48	18.937	20.031	2.148	11.30%	5/5	100.0% (47.8,100)
1038	TERT exon_1	SNV	c.-146C>T .	26.829-31.964	28.708	28.498	1.798	6.30%	5/5	100.0% (47.8,100)
1038	NOTCH1 intron_23	SNV	c.3902-1G>A .	17.945-22.283	19.386	19.051	1.506	7.80%	5/5	100.0% (47.8,100)
1038	KDM6A exon_12	SNV	c.985C>T p.Gln329*	30.576-42.887	36.348	36.253	3.989	11.00%	5/5	100.0% (47.8,100)
1040	ARID1A exon_20	Insertion	c.5548dupG p.Asp1850fs	12.762-15.429	14.229	14.351	0.942	6.60%	5/5	100.0% (47.8,100)
1040	HNF1A exon_4	Deletion	c.864delG p.Pro291fs	18.957-20.845	20.197	20.255	0.667	3.30%	5/5	100.0% (47.8,100)

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1040	RB1 exon_19	Deletion	c.1959delA p.Val654fs	14.461-18.052	16.39	16.71	1.185	7.20%	5/5	100.0% (47.8,100)
1040	CHD2 exon_5	Deletion	c.522delA p.Val175fs	14.094-17.631	15.982	16.216	1.447	9.10%	5/5	100.0% (47.8,100)
1040	ZFHX3 exon_8	Deletion	c.6347delC p.Pro2116fs	10.14-10.833	10.404	10.239	0.306	2.90%	3/5	60.0% (14.7,94.7)
1040	FANCA exon_42	Deletion	c.4124_4125delCA p.Thr1375fs	14.696-16.763	16.059	16.261	0.735	4.60%	5/5	100.0% (47.8,100)
1040	BRCA1 exon_8	Insertion	c.668dupA p.Ala224fs	13.043-19.236	15.65	15.244	2.15	13.70%	5/5	100.0% (47.8,100)
1040	SMAD4 intron_6	SNV	c.905-2A>G .	11.033-14.11	12.712	12.706	1.009	7.90%	5/5	100.0% (47.8,100)
1040	NOTCH3 exon_33	Insertion	c.6102dupC p.Gly2035fs	12.546-18.44	15.744	15.641	1.919	12.20%	5/5	100.0% (47.8,100)
1040	CUX1 exon_25	Deletion	c.4237delC p.Leu1413fs	10.526-10.526	10.526	10.526	0	0.00%	1/5	20.0% (0.5,71.6)
1040	PTPRD intron_23	SNV	c.3876-1G>T .	13.305-14.869	13.925	13.889	0.577	4.10%	5/5	100.0% (47.8,100)
1040	BCOR exon_13	Deletion	c.4862delC p.Pro1621fs	10.357-13.306	12.164	12.828	1.293	10.60%	3/5	60.0% (14.7,94.7)
1040	BCOR intron_5	SNV	c.3239-2A>G .	5.049-5.728	5.388	5.388	0.34	6.30%	2/5	40.0% (5.3,85.3)
1042	KMT2D exon_39	SNV	c.13279G>T p.Gly4427*	6.667-8.493	7.611	7.649	0.622	8.20%	5/5	100.0% (47.8,100)
1042	TP53 exon_5	SNV	c.659A>C p.Tyr220Ser	6.148-8.923	7.578	7.216	1.016	13.40%	5/5	100.0% (47.8,100)
1042	CDKN2A exon_3	SNV	c.322G>T p.Asp108Tyr	11.45-16.6	14.108	15.011	1.94	13.70%	5/5	100.0% (47.8,100)
1044	MTOR exon_42	SNV	c.5930C>T p.Thr1977Ile	5.353-7.475	6.185	5.957	0.834	13.50%	4/5	80.0% (28.4,99.5)
1044	KMT2D exon_34	Deletion	c.8675delG p.Gly2892fs	10.811-10.93	10.87	10.87	0.06	0.50%	2/5	40.0% (5.3,85.3)
1044	TP53 exon_5	SNV	c.659A>G p.Tyr220Cys	13.22-19.245	16.493	17.552	2.484	15.10%	5/5	100.0% (47.8,100)
1044	PBRM1 exon_10	Deletion	c.1042delG p.Ala348fs	12.857-17.536	15.123	15.366	1.939	12.80%	5/5	100.0% (47.8,100)
1044	FAT1 exon_1	SNV	c.1582G>T p.Glu528*	13.022-17.371	14.686	13.567	1.751	11.90%	5/5	100.0% (47.8,100)
1046	ZNF750 exon_1	Deletion	c.640delC p.Leu214fs	46.459-57.932	51.136	50	3.835	7.50%	5/5	100.0% (47.8,100)
1046	PIK3CA exon_9	SNV	c.1633G>A p.Glu545Lys	12.368-17.769	16.039	16.515	1.901	11.90%	5/5	100.0% (47.8,100)
1046	FAT1 exon_22	Insertion	c.12119dupG p.Cys4040fs	55.538-66.288	61.092	60.901	3.443	5.60%	5/5	100.0% (47.8,100)
1046	KMT2C exon_38	MNV	p.SQ2631*	56.598-62.615	58.597	57.227	2.217	3.80%	5/5	100.0% (47.8,100)
1046	KMT2C exon_22	SNV	c.3454G>T p.Glu1152*	58.427-64.557	60.855	60.223	2.063	3.40%	5/5	100.0% (47.8,100)

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1048	TP53 exon_6	SNV	c.733G>C p.Gly245Arg	9.036-13.393	10.501	10.12	1.551	14.80%	5/5	100.0% (47.8,100)
1252	KRAS exon_1	SNV	c.35G>T p.Gly12Val	11.872-15.824	13.612	13.625	1.223	9.00%	8/8	100.0% (63.1,100)
1252	RNF43 exon_4	MNV	p.QQ152*	10.659-14.349	12.657	12.749	1.388	11.00%	8/8	100.0% (63.1,100)
1254	BRCA2 exon_10	SNV	c.3895G>T p.Glu1299*	23.98-31.855	28.764	29.944	2.663	9.30%	10/10	100.0% (69.2,100)
1254	MLH3 exon_1	Deletion	c.1985_1989delTAAGT p.Leu662fs	10.312-17.439	15.365	16.102	2.229	14.50%	10/10	100.0% (69.2,100)
1254	CUX1 exon_7	Deletion	c.543_555delTGACTTTGCAGAA p.Asn181fs	15.666-31.579	22.198	21.712	5.37	24.20%	10/10	100.0% (69.2,100)
1254	ATRX exon_17	Deletion	c.4749_4752delGAAA p.Lys1583fs	53.32-62.914	57.24	56.895	2.714	4.70%	10/10	100.0% (69.2,100)
1256	TP53 exon_7	SNV	c.818G>A p.Arg273His	57.602-61.954	59.324	59.402	1.359	2.30%	8/8	100.0% (63.1,100)
1256	SMARCA4 intron_15	Deletion	c.2439-11_2444delTGTTCCATCAGAACGCT p.Thr814_Leu815del	11.636-24.167	17.604	17.073	4.308	24.50%	7/8	87.5% (47.3,99.7)
1256	NF2 intron_12	SNV	c.1340+1G>T .	38.974-44.49	41.961	42.172	1.988	4.70%	8/8	100.0% (63.1,100)
1258	TP53 exon_6	SNV	c.722C>T p.Ser241Phe	35.44-44.428	39.045	38.672	2.792	7.20%	10/10	100.0% (69.2,100)
1258	GATA6 exon_1	Deletion	c.524_540delCGGCGGCAGCAGCCGCG p.Ala175fs	12.195-40.959	24.31	24.99	8.02	33.00%	10/10	100.0% (69.2,100)
1258	TERT exon_1	SNV	c.-124C>T .	21.585-29.091	26.327	26.641	1.989	7.60%	10/10	100.0% (69.2,100)
1260	KMT2D exon_10	Insertion	c.2657dupC p.Gly887fs	13.6-19.97	16.819	17.258	1.835	10.90%	9/9	100.0% (66.4,100)
1260	TP53 exon_7	SNV	c.818G>A p.Arg273His	38.903-48.431	45.042	46.56	3.417	7.60%	9/9	100.0% (66.4,100)
1260	NOTCH1 exon_34	Deletion	c.7314_7332delGAGCCAGGCAGACGTGCAG p.Ser2439fs	30.508-50.655	40.296	42.496	7.062	17.50%	9/9	100.0% (66.4,100)
1262	ATM exon_53	SNV	c.7909C>T p.Gln2637*	11.405-15.53	13.052	12.373	1.392	10.70%	9/9	100.0% (66.4,100)
1262	RB1 exon_1	Deletion	c.121B137+5delGACCTGCCTCTCGTCAGGTGAG p.Asp41fs	14.103-38.983	27.501	29.412	8.666	31.50%	7/9	77.8% (40.0,97.2)
1262	TP53 exon_6	SNV	c.743G>A p.Arg248Gln	68.338-77.197	73.288	73.713	2.475	3.40%	9/9	100.0% (66.4,100)
1262	TERT exon_1	SNV	c.-124C>T .	36.609-47.28	42.896	44.186	3.455	8.10%	9/9	100.0% (66.4,100)
1264	TP53 exon_6	SNV	c.711G>T p.Met237Ile	66.509-76.111	70.245	69.347	2.964	4.20%	10/10	100.0% (69.2,100)

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1264	LRP1B exon_46	SNV	c.7638C>A p.Tyr2546*	21.879-31.397	25.999	25.647	2.785	10.70%	10/10	100.0% (69.2,100)
1264	PIK3R1 exon_17	Deletion	c.2115delG p.Gln705fs	31.961-43.426	38.559	38.541	3.429	8.90%	10/10	100.0% (69.2,100)
1264	CDKN2A exon_3	Deletion	c.158_166delTGATGGGCAinsG p.Met53fs	17.647-55.645	39.65	40.233	11.292	28.50%	10/10	100.0% (69.2,100)
1264	RBM10 exon_14	Deletion	c.1878B1888+7delCAGCCAGTACCGTGAGTA p.Ser627fs	15.254-35.294	23.462	20.833	6.8	29.00%	8/10	80.0% (44.4,97.5)
1266	KRAS exon_2	MNV	p.Q61L	9.351-14.943	12.752	12.583	1.468	11.50%	10/10	100.0% (69.2,100)
1266	TP53 exon_6	SNV	c.742C>T p.Arg248Trp	28.613-35.451	32.424	31.982	1.801	5.60%	10/10	100.0% (69.2,100)
1266	SF3B1 exon_16	SNV	c.2098A>G p.Lys700Glu	22.93-28.285	25.923	26.546	1.775	6.80%	10/10	100.0% (69.2,100)
1268	RB1 exon_8	Deletion	c.796_797delCA p.Gln266fs	93.633-96.606	94.976	94.999	0.94	1.00%	10/10	100.0% (69.2,100)
1268	TP53 exon_3	Deletion	c.340_363delTTGCATTCTGGGACAGCCAAGTCTinsG p.Leu114fs	67.5-89.474	79.516	79.131	5.736	7.20%	10/10	100.0% (69.2,100)
1268	EP300 exon_6	Deletion	c.1369_1376delAGTCAGAT p.Ser457fs	19.466-36.364	29.803	30.269	4.927	16.50%	10/10	100.0% (69.2,100)
1270	KMT2A exon_3	SNV	c.2584C>T p.Arg862*	29.713-38.149	34.455	34.265	2.635	7.60%	8/8	100.0% (63.1,100)
1270	NF1 exon_27	SNV	c.3520C>T p.Gln1174*	20.493-24.934	22.552	22.549	1.217	5.40%	8/8	100.0% (63.1,100)
1270	NF1 exon_41	SNV	c.6121G>T p.Gly2041*	8.708-11.125	10.418	10.788	0.746	7.20%	8/8	100.0% (63.1,100)
1270	SETD2 exon_3	Insertion	c.2220_2221insGCAG p.Lys741fs	16.129-20.189	18.2	18.484	1.594	8.80%	8/8	100.0% (63.1,100)
1270	KDR exon_23	SNV	c.3095G>A p.Arg1032Gln	16.414-21.03	18.66	18.544	1.204	6.50%	8/8	100.0% (63.1,100)
1270	FBXW7 exon_12	MNV	p.G619C	10.099-18.576	13.696	12.783	2.642	19.30%	8/8	100.0% (63.1,100)
1270	FBXW7 intron_6	SNV	c.862-1G>T .	11.809-18.679	16.502	17.518	2.226	13.50%	8/8	100.0% (63.1,100)
1270	TERT exon_1	SNV	c.-124C>T .	10.27-19.549	14.233	13.903	2.51	17.60%	8/8	100.0% (63.1,100)
1272	ARID1A exon_4	Deletion	c.1890_1900delGAGCCTTCAGT p.Ser631fs	34.649-56.545	43.2	42.739	6.503	15.10%	10/10	100.0% (69.2,100)
1272	ZFHX3 intron_4	SNV	c.3530-2A>C .	26.694-35.567	31.837	32.88	2.721	8.50%	10/10	100.0% (69.2,100)
1272	SMARCA4 exon_16	Insertion	c.2445_2458dupGTCCAACTGGGCGT p.Tyr820fs	13.025-25.743	19.094	19.429	3.934	20.60%	10/10	100.0% (69.2,100)
1272	MAPK1	SNV	c.241G>A	18.526-29.762	25.145	25.009	3.088	12.30%	10/10	100.0%

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
	exon_2		p.Glu81Lys							(69.2,100)
1272	FOXP1 exon_6	SNV	c.424C>T p.Gln142*	27.215-38.395	32.716	32.197	2.826	8.60%	10/10	100.0% (69.2,100)
1272	FAT1 exon_9	SNV	c.8176C>T p.Arg2726*	32.166-42.602	37.126	37.075	2.676	7.20%	10/10	100.0% (69.2,100)
1272	TERT intron_1	SNV	c.-57A>C .	20.984-29.517	25.321	25.553	2.227	8.80%	10/10	100.0% (69.2,100)
1272	KMT2C exon_38	SNV	c.9103C>T p.Gln3035*	36.992-47.378	43.231	43.727	3.279	7.60%	10/10	100.0% (69.2,100)
1274	ARID1A exon_12	Deletion	c.3281delA p.Lys1094fs	21.379-28.468	25.393	25.182	2.361	9.30%	8/8	100.0% (63.1,100)
1274	ARID1A exon_20	Deletion	c.5693delC p.Pro1898fs	22.937-29.643	26.349	25.862	1.984	7.50%	8/8	100.0% (63.1,100)
1274	JAK1 exon_8	Deletion	c.1289delC p.Pro430fs	22.849-28.796	27.205	27.67	1.827	6.70%	8/8	100.0% (63.1,100)
1274	JAK1 exon_7	Deletion	c.1016delA p.Asn339fs	21.881-29.43	24.712	23.821	2.605	10.50%	8/8	100.0% (63.1,100)
1274	NOTCH2 exon_2	SNV	c.145G>T p.Gly49*	9.906-25.455	15.067	12.455	6.11	40.50%	4/8	50.0% (15.7,84.3)
1274	PTEN exon_2	Deletion	c.97_99delATT p.Ile33del	18.912-24.91	22.258	22.293	1.638	7.40%	8/8	100.0% (63.1,100)
1274	PTEN exon_5	SNV	c.302T>C p.Ile101Thr	22.272-28.331	24.388	23.918	1.969	8.10%	8/8	100.0% (63.1,100)
1274	PTEN exon_5	SNV	c.377C>A p.Ala126Asp	21.797-27.186	24.325	24.27	1.48	6.10%	8/8	100.0% (63.1,100)
1274	FGFR2 exon_11	SNV	c.1147T>C p.Cys383Arg	21.658-30.106	25.485	25.441	2.481	9.70%	8/8	100.0% (63.1,100)
1274	KMT2D exon_31	Deletion	c.7061delC p.Pro2354fs	24.902-28.969	27.498	27.907	1.405	5.10%	8/8	100.0% (63.1,100)
1274	CHD2 exon_5	Deletion	c.522delA p.Val175fs	23.995-29.501	27.664	28.586	2.134	7.70%	8/8	100.0% (63.1,100)
1274	ZFHX3 exon_8	Deletion	c.5677_5678delAG p.Arg1893fs	23.096-29.921	26.654	26.772	2.149	8.10%	8/8	100.0% (63.1,100)
1274	NF1 exon_16	Deletion	c.1841delA p.Asn614fs	19.949-26.03	23.198	23.952	2.03	8.80%	8/8	100.0% (63.1,100)
1274	NF1 exon_17	Deletion	c.1882delT p.Tyr628fs	24.231-30.47	26.562	26.296	1.966	7.40%	8/8	100.0% (63.1,100)
1274	CIC exon_16	Deletion	c.6470delC p.Pro2157fs	24.359-30.642	27.235	26.957	1.867	6.90%	8/8	100.0% (63.1,100)
1274	MSH3 exon_7	Deletion	c.1148delA p.Lys383fs	12.816-17.48	15.602	16.373	1.647	10.60%	8/8	100.0% (63.1,100)
1274	RAD50 exon_13	Deletion	c.2165delA p.Lys722fs	27.894-32.242	29.75	29.87	1.473	5.00%	6/8	75.0% (34.9,96.8)
1274	ARID1B exon_19	SNV	c.4198C>T p.Gln1400*	21.429-28.0	25.496	25.871	2.038	8.00%	8/8	100.0% (63.1,100)
1274	AMER1 exon_1	Deletion	c.1637delT p.Leu546fs	20.956-30.784	27.557	28.896	2.893	10.50%	8/8	100.0% (63.1,100)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
1276	PTEN exon_7	Deletion	c.801+1B801+23delGTTTGTACTTTACTTTCATTGGG .	11.915-28.995	21.576	22.158	5.255	24.40%	10/10	100.0% (69.2,100)
1276	TP53 exon_6	Deletion	c.723delC p.Cys242fs	50.685-60.43	54.402	53.438	3.149	5.80%	10/10	100.0% (69.2,100)
1280	NF2 intron_4	Deletion	c.448-5_464delTCCAGTATGGTGACTACGACCC p.Tyr150fs	18.395-40.698	30.094	29.829	7.595	25.20%	10/10	100.0% (69.2,100)
1282	ARID1A exon_18	Deletion	c.4925delC p.Pro1642fs	29.508-35.417	33.206	33.429	1.582	4.80%	10/10	100.0% (69.2,100)
1282	ARID1A exon_20	Deletion	c.5548delG p.Asp1850fs	24.204-30.821	26.806	26.7	1.974	7.40%	10/10	100.0% (69.2,100)
1282	FUBP1 intron_13	SNV	c.1104+2T>G .	27.425-33.666	30.736	30.86	1.529	5.00%	10/10	100.0% (69.2,100)
1282	FUBP1 exon_2	Deletion	c.133delA p.Ile45fs	10.541-25.342	20.455	21.339	4.809	23.50%	10/10	100.0% (69.2,100)
1282	FUBP1 exon_2	Deletion	c.121-3_129delTAGATTGCAGCAinsAGATTGCTGC p.Ile41_Ala43delinsLeuLeu???	16.822-16.822	16.822	16.822	0	0.00%	1/10	10.0% (0.3,44.5)
1282	FUBP1 exon_2	Deletion	c.121-3_129delTAGATTGCAGCAinsAGATTGGAGC p.Ile41_Ala43delinsLeuGlu???	10.398-10.398	10.398	10.398	0	0.00%	1/10	10.0% (0.3,44.5)
1282	FUBP1 exon_2	Deletion	c.121-3_129delTAGATTGCAGCAinsAGATTGCAGC p.Ile41_Ala43delinsLeuGln???	10.373-21.622	15.631	15.457	3.57	22.80%	8/10	80.0% (44.4,97.5)
1282	H3F3A exon_1	SNV	c.104G>A p.Gly35Glu	29.898-38.328	32.612	32.175	2.423	7.40%	10/10	100.0% (69.2,100)
1282	TCF7L2 exon_18	Deletion	c.1403delA p.Lys468fs	30.872-40.275	35.297	35.446	2.583	7.30%	10/10	100.0% (69.2,100)
1282	KRAS exon_1	SNV	c.38G>A p.Gly13Asp	28.289-32.999	30.598	30.696	1.288	4.20%	10/10	100.0% (69.2,100)
1282	KMT2D exon_10	Deletion	c.2657delC p.Pro886fs	30.52-37.113	33.425	33.392	1.86	5.60%	10/10	100.0% (69.2,100)
1282	ACVR1B exon_1	Deletion	c.85delG p.Val29fs	28.922-42.194	35.641	35.524	3.477	9.80%	10/10	100.0% (69.2,100)
1282	ACVR1B exon_9	SNV	c.1453C>T p.Arg485*	29.842-34.314	32.354	32.422	1.441	4.50%	10/10	100.0% (69.2,100)
1282	BRCA2 exon_10	Deletion	c.3708delA p.Ala1237fs	28.143-35.651	31.061	30.488	1.94	6.20%	10/10	100.0% (69.2,100)
1282	BCL11B	Deletion	c.1742delG	26.364-37.707	33.213	32.873	3.324	10.00%	10/10	100.0%

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
	exon_4		p.Gly581fs							(69.2,100)
1282	CTCF intron_9	SNV	c.1999+2T>C .	29.335-34.155	32.116	32.615	1.445	4.50%	10/10	100.0% (69.2,100)
1282	CDH1 exon_3	Deletion	c.377delC p.Pro126fs	30.576-36.972	33.608	33.777	2.172	6.50%	10/10	100.0% (69.2,100)
1282	ZFHX3 exon_9	Deletion	c.10215delC p.Ala3407fs	27.58-37.607	31.824	31.552	2.758	8.70%	10/10	100.0% (69.2,100)
1282	ASXL1 exon_14	Deletion	c.1934delG p.Gly645fs	32.063-39.767	35.912	36.168	2.458	6.80%	10/10	100.0% (69.2,100)
1282	MLH1 exon_12	Insertion	c.1127_1131dupATAAG p.Val378fs	24.911-50.442	38.437	41.302	9.411	24.50%	10/10	100.0% (69.2,100)
1282	CTNNB1 exon_2	SNV	c.121A>G p.Thr41Ala	63.387-71.575	65.768	65.13	2.131	3.20%	10/10	100.0% (69.2,100)
1282	ATR exon_17	Deletion	c.3402delT p.Phe1134fs	29.827-38.162	34.123	33.454	2.436	7.10%	10/10	100.0% (69.2,100)
1282	PIK3CA exon_20	SNV	c.3141T>A p.His1047Gln	29.117-35.633	32.128	32.16	2.072	6.50%	10/10	100.0% (69.2,100)
1282	KIT exon_2	Insertion	c.120_123dupTCCA p.Gly42fs	29.116-34.097	31.6	31.237	1.732	5.50%	10/10	100.0% (69.2,100)
1282	PIK3R1 exon_1	Deletion	c.95_96delTG p.Val32fs	29.952-33.896	31.742	31.636	1.152	3.60%	10/10	100.0% (69.2,100)
1282	PIK3R1 exon_12	Deletion	c.1344delA p.Lys448fs	23.109-33.598	30.68	31.511	2.828	9.20%	10/10	100.0% (69.2,100)
1282	RAD50 exon_13	Deletion	c.2165delA p.Lys722fs	25.963-31.481	29.242	29.501	1.678	5.70%	7/10	70.0% (34.8,93.3)
1282	CFTR exon_7	Insertion	c.850dupA p.Met284fs	10.336-12.818	11.421	11.142	0.947	8.30%	8/10	80.0% (44.4,97.5)
1282	KMT2C exon_38	Deletion	c.8390delA p.Lys2797fs	32.756-37.786	35.365	35.27	1.544	4.40%	10/10	100.0% (69.2,100)
1282	MTAP exon_3	Deletion	c.155delA p.Asn52fs	32.675-40.573	37.18	37.79	2.602	7.00%	10/10	100.0% (69.2,100)
1284	TP53 exon_11	SNV	c.1024C>T p.Arg342*	59.052-67.481	62.677	62.418	2.927	4.70%	8/8	100.0% (63.1,100)
1284	EGFR exon_22	Insertion	c.2311_2319dupAACCCCAC p.Asn771_His773dup	39.571-46.787	42.958	42.62	2.324	5.40%	8/8	100.0% (63.1,100)
1284	CDKN2A intron_2	SNV	c.151-1G>A .	25.0-36.449	28.418	28	3.274	11.50%	8/8	100.0% (63.1,100)
1284	CDKN2A exon_2	Insertion	c.49_50insTG p.Ala17fs	26.761-36.923	32.415	32.596	3.61	11.10%	8/8	100.0% (63.1,100)
1286	KMT2D exon_39	SNV	c.12649C>T p.Gln4217*	71.92-76.074	73.991	74.163	1.056	1.40%	10/10	100.0% (69.2,100)
1286	CREBBP exon_13	SNV	c.2311C>T p.Gln771*	48.592-53.771	51.571	51.908	1.883	3.70%	10/10	100.0% (69.2,100)
1286	GRIN2A exon_12	SNV	c.2738C>G p.Ser913*	21.588-25.19	23.326	23.208	0.982	4.20%	10/10	100.0% (69.2,100)
1286	PIK3CA exon_7	MNV	p.ED453KN	12.523-21.96	17.74	17.768	2.656	15.00%	10/10	100.0% (69.2,100)

Sample	Gene Exon	Variant Type	Mutation (cDNA/Protein Changes)	VAF Range	VAF Mean	VAF Median	VAF (SD)	VAF (%CV)	Positive/Total Calls	Positive Call Rate (95% CI)
1286	KMT2C exon_35	SNV	c.5135C>G p.Ser1712*	51.613-57.58	54.985	55.039	1.876	3.40%	10/10	100.0% (69.2,100)
1278	RB1 exon_17	SNV	c.1654C>T p.Arg552*	74.898-83.721	79.443	79.896	2.477	3.10%	8/8	100.0% (63.1,100)
1278	TP53 exon_11	Deletion	c.1014_1024delCGAGATGTTCC p.Phe338fs	42.437-57.759	49.27	48.288	5.228	10.60%	8/8	100.0% (63.1,100)
1278	FBXO11 exon_25	Insertion	c.3939_3957dupTCAAAAGGGACATAGAAAA p.Ala1320fs	51.852-67.123	61.114	64.368	6.645	10.90%	3/8	37.5% (8.5,75.5)
1278	ASXL1 exon_14	Deletion	c.1934delG p.Gly645fs	72.954-81.395	75.736	75.218	2.584	3.40%	8/8	100.0% (63.1,100)
1278	CTNNB1 exon_2	SNV	c.94G>A p.Asp32Asn	71.077-81.346	76.666	75.949	3.267	4.30%	8/8	100.0% (63.1,100)
1278	RAD50 exon_13	Deletion	c.2165delA p.Lys722fs	26.818-33.155	29.825	29.283	1.898	6.40%	7/8	87.5% (47.3,99.7)
1278	ARID1B exon_2	SNV	c.1543-1G>T .	70.098-82.867	78.125	78.929	3.449	4.40%	8/8	100.0% (63.1,100)
1288	TP53 intron_5	SNV	c.673-2A>G .	28.117-34.708	31.57	31.727	2.636	8.30%	4/4	100.0% (39.8,100)
1288	ERBB2 exon_21	Insertion	c.2313_2324dupATACGTGATGGC p.Ala775_Gly776insTyrValMetAla	24.665-36.798	29.711	28.691	4.856	16.30%	4/4	100.0% (39.8,100)

??? - complete protein change not provided

* - stop codon

c. Precision for KRAS and NRAS Detection in CRC specimens

A total of 26 FFPE colorectal cancer tumor specimens and their matched normal specimens were processed with 24 replicates each for this study in order to evaluate precision in all 7 alleles represented by the *therascreen* assay and in all exons represented by the *Praxis* assay. Additionally, the well characterized HEC-59 cell line was sequenced in order to calculate precision for rare NRAS exon 4 variants. Table 28 details the variants and variant types for each tested specimen.

Table 28. Summary of samples analyzed in Precision for KRAS and NRAS Detection

FFPE Tumor specimen	KRAS/NRAS Variant	Variant Type
22-A67610	NRAS p.Gly12Val	SNV
22-A76888	NRAS p.Gly12Val	SNV
22-A76889	NRAS p.Gly13Arg	SNV
22-A67639	NRAS p.Gln61Leu	SNV

FFPE Tumor specimen	KRAS/NRAS Variant	Variant Type
22-A67732	NRAS p.Gln61His	SNV
22-A67798	KRAS p.GlyGln60GlyLys	MNV
22-A76886	KRAS p.Gly12Ser	SNV
22-A67772	KRAS p.Gly12Arg	SNV
22-A76885	KRAS p.Gly12Ala	SNV
22-A67392	KRAS p.Gln61Arg	SNV
20-A45621	KRAS p.Gly12Cys	SNV
20-A45628	KRAS p.Gly12Cys	SNV
20-A45622	KRAS p.Gly12Asp	SNV
20-A45623	KRAS p.Gly12Asp	SNV
20-A45627	KRAS p.Gly12Asp	SNV
20-A45629	KRAS p.Gly12Asp	SNV
20-A45970	KRAS p.Gly12Asp	SNV
20-A45971	KRAS p.Gly12Val	SNV
22-A77158	KRAS p.Gly13Asp	SNV
20-A45624	KRAS p.Gly13Cys	SNV
22-A76887	KRAS p.Ala59Thr	SNV
22-A67610	KRAS p.Ala146Pro	SNV
20-A45973	KRAS p.Ala146Thr	SNV
HEC-59*	NRAS p.Ala146Val	SNV
20-A45630	wild type	N/A
20-A45631	wild type	N/A
20-A45632	wild type	N/A
20-A45972	wild type	N/A

* cell line

Intra-run (run on same plate under same conditions) and inter-run (run on different plates under different conditions) conditions were assessed and compared for xT CDx across multiple instruments, reagent lots, days, and operators. Fourteen (14) different CDx variants across all relevant exons of each CDx gene were included in the study. Five hundred twenty-two (522) total replicates across 26 unique CRC samples were evaluated. The overall positive call rate was 99.8% and 25 of the 26 samples had a positive call rate

of 100%. No false positive results were observed across all potential CDx biomarker positions and all replicates (> 28,000 positions), and 1 false negative variant was detected. Results are shown in Table 29. Detailed results for each of the relevant 56 CDx variants across all 522 replicates are in Table 30 (KRAS) and Table 31 (NRAS). Exon-level concordance for KRAS and NRAS is summarized in Table 32.

Table 29. Positive and negative percent agreement for KRAS and NRAS precision

Gene	TP	FP	TN	FN	Total	PPA (95% CI)	NPA (95% CI)
KRAS	340	0	14275	1	14616	99.7 (98.4, 100.0)	100.0 (100.0, 100)
NRAS	117	0	14499	0	14616	100.0 (96.9, 100)	100.0 (100.0, 100)
Total	457	0	28774	1	29232	99.8 (98.8, 100.0)	100.0 (100.0, 100)

Table 30. Positive and negative percent agreement for KRAS

Exon	Amino Acid	Mutation	TP	FP	TN	FN	Total	PPA (95% CI)	NPA (95% CI)
KRAS Exon 2	p.Gly13Val	c.38_39GC>TT	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Glu	c.38_39GC>AA	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Asp	c.38_39GC>AT	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Asp	c.38G>A	15	0	507	0	522	100.0 (78.2, 100)	100.0 (99.3, 100)
	p.Gly13Cys	c.37G>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Arg	c.37G>C	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Trp	c.34_36GGT>TGG	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Ala	c.35G>C	18	0	504	0	522	100.0 (81.5, 100)	100.0 (99.3, 100)
	p.Gly12Asp	c.35G>A	101	0	420	1	522	99.02 (94.7, 100.0)	100.0 (99.1, 100)
	p.Gly12Phe	c.34_35GG>TT	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Asn	c.34_35GG	0	0	522	0	522	-	100.0 (99.3,

Exon	Amino Acid	Mutation	TP	FP	TN	FN	Total	PPA (95% CI)	NPA (95% CI)
		>AA							100)
	p.Gly12Val	c.35G>T	22	0	500	0	522	100.0 (84.6, 100)	100.0 (99.3, 100)
	p.Gly12Ser	c.34G>A	23	0	499	0	522	100.0 (85.2, 100)	100.0 (99.3, 100)
	p.Gly12Cys	c.34G>T	43	0	479	0	522	100.0 (91.8, 100)	100.0 (99.2, 100)
	p.Gly12Arg	c.34G>C	19	0	503	0	522	100.0 (82.4, 100)	100.0 (99.3, 100)
KRAS Exon 3	p.Gln61His	c.183A>C	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61His	c.183A>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61Leu	c.182A>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61Arg	c.182A>G	19	0	503	0	522	100.0 (82.4, 100)	100.0 (99.3, 100)
	p.Gln61Lys	c.181C>A	22	0	500	0	522	100.0 (84.6, 100)	100.0 (99.3, 100)
	p.Gln61Glu	c.181C>G	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala59Gly	c.176C>G	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala59Thr	c.175G>A	19	0	503	0	522	100.0 (82.4, 100)	100.0 (99.3, 100)
KRAS Exon 4	p.Ala146Val	c.437C>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala146Thr	c.436G>A	19	0	503	0	522	100.0 (82.4, 100)	100.0 (99.3, 100)
	p.Ala146Pro	c.436G>C	20	0	502	0	522	100.0 (83.2, 100)	100.0 (99.3, 100)
	p.Lys117Asn	c.351A>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Lys117Asn	c.351A>C	0	0	522	0	522	-	100.0 (99.3, 100)

Table 31. Positive and negative percent agreement for NRAS

Exon	Amino Acid	Mutation	TP	FP	TN	FN	Total	PPA (95% CI)	NPA (95% CI)
NRAS Exon 2	p.Gly13Glu	c.38_39GT>AG	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Glu	c.38_39GT>AA	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Asp	c.38G>A	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Val	c.38G>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Cys	c.37G>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly13Arg	c.37G>C	17	0	505	0	522	100.0 (80.5, 100)	100.0 (99.3, 100)
	p.Gly12Trp	c.34_36GGT>TGG	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Ala	c.35G>C	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Val	c.35G>T	39	0	483	0	522	100.0 (91.0, 100)	100.0 (99.2, 100)
	p.Gly12Asp	c.35G>A	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Phe	c.34_35GG>TT	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Asn	c.34_35GG>AA	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Ser	c.34G>A	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Cys	c.34G>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gly12Arg	c.34G>C	0	0	522	0	522	-	100.0 (99.3, 100)
NRAS Exon 3	p.Gln61His	c.183A>T	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61His	c.183A>C	17	0	505	0	522	100.0 (80.5, 100)	100.0 (99.3, 100)
	p.Gln61Arg	c.182A>G	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61Leu	c.182A>T	20	0	502	0	522	100.0 (83.2, 100)	100.0 (99.3, 100)

Exon	Amino Acid	Mutation	TP	FP	TN	FN	Total	PPA (95% CI)	NPA (95% CI)
	p.Gln61Lys	c.181C>A	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Gln61Glu	c.181C>G	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala59Gly	c.176C>G	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala59Thr	c.175G>A	0	0	522	0	522	-	100.0 (99.3, 100)
NRAS Exon 4	p.Ala146Val	c.437C>T	24	0	498	0	522	100.0 (85.8, 100)	100.0 (99.3, 100)
	p.Ala146Thr	c.436G>A	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Ala146Pro	c.436G>C	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Lys117Asn	c.351G>C	0	0	522	0	522	-	100.0 (99.3, 100)
	p.Lys117Asn	c.351G>T	0	0	522	0	522	-	100.0 (99.3, 100)

Table 32. Precision results by variant, for all KRAS and NRAS variants

Gene	Exon	Variant	n	True Positive	False Negative	% Correct Call	95% CI
KRAS	All KRAS Exons		341	340	1	99.7	(98.4, 100.0)
	2	All KRAS Exon 2	242	241	1	99.6	(97.7, 100.0)
	2	p.Gly12Ala	18	18	0	100.0	(81.5, 100)
	2	p.Gly12Arg	19	19	0	100.0	(82.4, 100)
	2	p.Gly12Asp	102	101	1	99.0	(94.7, 100.0)
	2	p.Gly12Cys	43	43	0	100.0	(91.8, 100)
	2	p.Gly12Ser	23	23	0	100.0	(85.2, 100)
	2	p.Gly12Val	22	22	0	100.0	(84.6, 100)
	2	p.Gly13Asp	15	15	0	100.0	(78.2, 100)
	3	All KRAS Exon 3	60	60	0	100.0	(94.0, 100)
	3	p.Ala59Thr	19	19	0	100.0	(82.4, 100)
	3	p.Gln61Arg	19	19	0	100.0	(82.4, 100)

Gene	Exon	Variant	n	True Positive	False Negative	% Correct Call	95% CI
	3	p.GlyGln60GlyLys	22	22	0	100.0	(84.6, 100)
	4	All KRAS Exon 4	39	39	0	100.0	(91.0, 100)
	4	p.Ala146Pro	20	20	0	100.0	(83.2, 100)
	4	p.Ala146Thr	19	19	0	100.0	(82.4, 100)
NRAS	All NRAS Exons		117	117	0	100.0	(96.9, 100)
	2	All NRAS Exon 2	56	56	0	100.0	(93.6, 100)
	2	p.Gly12Val	39	39	0	100.0	(91.0, 100)
	2	p.Gly13Arg	17	17	0	100.0	(80.5, 100)
	3	All NRAS Exon 3	37	37	0	100.0	(90.5, 100)
	3	p.Gln61His	17	17	0	100.0	(80.5, 100)
	3	p.Gln61Leu	20	20	0	100.0	(83.2, 100)
	4	All NRAS Exon 4	24	24	0	100.0	(85.8, 100)
	4	p.Ala146Val	24	24	0	100.0	(85.8, 100)

d. MSI Precision

Precision for detection of MSI status was evaluated across 49 unique specimens and 317 replicates with a range of MSI scores. Mean, range and SD for percent unstable loci are shown in Table 33. xT CDx converts percent of unstable loci into probability scores for MSI status based on trained models and evaluates probability scores against established thresholds. A total of 46/49 tested specimens (94%) showed a positive call rate for MSI of 100% across all replicates. The other 3 specimens each had 80% concordance across 5 replicates due to 4 MSS and 1 MSI-H call in each case.

Table 33. MSI Precision by specimen

Cancer type	Expected MSI status	Total replicates	Percent Unstable Loci			Agreement
			mean	range	SD	
Melanoma	stable	5	3.468	1.316-5.532	1.531	5/5 (100.0%)

Cancer type	Expected MSI status	Total replicates	Percent Unstable Loci			Agreement
			mean	range	SD	
Tumor of Unknown Origin	stable	4	8.694	5.439-11.345	2.581	4/4 (100.0%)
Bladder Cancer	stable	5	2.853	1.293-4.184	1.148	5/5 (100.0%)
Endometrial Cancer	high	5	43.386	41.026-45.607	1.973	5/5 (100.0%)
Endometrial Cancer	high	5	64.999	63.866-67.699	1.57	5/5 (100.0%)
Endocrine Tumor	high	5	25.9	24.681-26.778	0.79	5/5 (100.0%)
Endocrine Tumor	stable	5	8.434	7.203-9.664	1.74	5/5 (100.0%)
Meningioma	stable	5	3.96	2.51-4.641	0.871	5/5 (100.0%)
Prostate Cancer	stable	5	7.527	5.462-8.824	1.489	5/5 (100.0%)
Colorectal Cancer	stable	5	7.207	5.907-8.787	1.068	5/5 (100.0%)
Endometrial Cancer	stable	5	10.021	7.522-13.136	2.045	5/5 (100.0%)
Breast Cancer	stable	5	5.12	3.004-7.531	2.102	5/5 (100.0%)
Head and Neck Cancer	stable	5	2.955	0.844-7.203	2.476	5/5 (100.0%)
Breast Cancer	stable	5	ND	ND	ND	5/5 (100.0%)
Head and Neck Cancer	stable	5	3.106	0.84-6.303	2.173	5/5 (100.0%)
Liver Cancer	stable	3	5.042	2.929-7.595	2.364	3/3 (100.0%)
Non-Small Cell Lung Cancer	stable	5	3.966	1.255-7.66	2.349	5/5 (100.0%)
Skin Cancer	stable	5	2.353	0.837-3.814	1.107	5/5 (100.0%)
Gastric Cancer	high	5	72.836	71.967-73.64	0.676	5/5 (100.0%)
Non-Small Cell Lung Cancer	stable	5	2.189	0.837-5.021	1.656	5/5 (100.0%)
Tumor of Unknown Origin	stable	5	2.115	1.255-4.184	1.206	5/5 (100.0%)
Skin Cancer	stable	5	3.139	2.51-4.274	0.713	5/5 (100.0%)
Ovarian Cancer	stable	5	3.547	1.277-5.042	1.591	5/5 (100.0%)
Colorectal Cancer	stable	5	6.274	1.299-23.377	9.58	4/5 (80.0%)
Colorectal Cancer	stable	5	8.748	1.702-25.641	9.707	4/5 (80.0%)

Cancer type	Expected MSI status	Total replicates	Percent Unstable Loci			Agreement
			mean	range	SD	
Esophageal Cancer	high	5	67.283	66.387-68.908	1.023	5/5 (100.0%)
Colorectal Cancer	stable	5	8.852	4.184-24.153	8.574	4/5 (80.0%)
Basal Cell Carcinoma	stable	5	4.373	2.941-6.723	1.669	5/5 100.0%)
Bladder Cancer	stable	5	5.794	2.929-7.95	1.979	5/5 (100.0%)
Bladder Cancer	stable	5	3.368	2.092-4.681	0.959	5/5 (100.0%)
Pancreatic Cancer	stable	8	3.466	1.261-5.462	1.398	8/8 (100.0%)
Sarcoma	stable	10	9.163	5.858-12.552	1.927	10/10 100.0%)
Tumor of Unknown Origin	stable	8	5.515	3.361-7.143	1.374	8/8 (100.0%)
Bladder Cancer	stable	10	3.808	1.674-6.276	1.725	10/10 100.0%)
Breast Cancer	high	9	20.941	18.41-23.529	1.544	9/9 (100.0%)
Bladder Cancer	stable	9	8.926	7.531-11.297	2.064	9/9 (100.0%)
Non-Small Cell Lung Cancer	stable	10	6.402	3.361-9.664	2.068	10/10 100.0%)
Tumor of Unknown Origin	stable	10	3.222	2.092-4.184	0.685	10/10 100.0%)
Small Cell Lung Cancer	stable	10	3.572	2.51-5.508	1.052	10/10 100.0%)
Melanoma	stable	8	3.818	2.092-5.439	1.213	8/8 (100.0%)
Bladder Cancer	stable	10	8.494	5.439-10.879	2.002	10/10 100.0%)
Endometrial Cancer	high	8	57.561	56.904-59.414	0.931	8/8 (100.0%)
Breast Cancer	stable	10	8.201	6.276-10.879	1.744	10/10 100.0%)
Adrenal Cancer	stable	8	ND	ND	ND	8/8 (100.0%)
Sarcoma	stable	10	ND	ND	ND	10/10 100.0%)
Colorectal Cancer	high	10	74.18	73.109-74.79	0.526	10/10 100.0%)

Cancer type	Expected MSI status	Total replicates	Percent Unstable Loci			Agreement
			mean	range	SD	
Head and Neck Squamous Cell Carcinoma	stable	8	8.308	6.276-13.808	2.788	8/8 100.0%)
Cervical Cancer	stable	10	5.904	4.184-7.531	1.228	10/10 100.0%)
Non-Small Cell Lung Cancer	stable	4	5.137	4.603-5.462	0.413	4/4 (100.0%)

e. Reagent Lot Interchangeability

Reagent lot interchangeability was assessed by testing 4 CRC samples in duplicate using multiple reagent lots in 3, 5 and 8 combinations for library construction, hybrid capture, and sequencing reagents respectively. A total of 63 replicates were processed and no effect of interchanging reagent lots was observed for variant detection for CDx biomarkers. 100% positive concordance for KRAS and NRAS CDx biomarkers was observed across all replicates and all reagent lot combinations. In addition, variant detection across the entire gene panel was assessed independently in 375 replicates of 52 specimens representing a broad diversity of tumor types that was sequenced with multiple reagent lots. Results showed 97.8% positive agreement (2294/2345) and 100% negative agreement for substitutions and INDELs, and 96.9% positive agreement and 96.2% negative agreement for MSI.

4. Analytical Sensitivity: Limit of Blank (LoB)

The LoB was established by assessing the frequency of false-positive identification of KRAS and NRAS CDx biomarker variants in 23 FFPE tumors (with patient-matched normal specimens) representing 15 tumor types and known to be wild-type for KRAS and NRAS. These specimens were evaluated with 4 or 5 replicate measures per specimen based on tissue availability. A total of 102 tumor-normal sample pairs were evaluated. No false-positive CDx variants were detected at a VAF threshold of 3% in these samples; and no false positive Level 2 variants were identified, establishing the LoB.
False positive Level 2 and Level 3 variants were also evaluated in 22 replicates of well-characterized reference material and no false positives were observed.

5. Analytical Sensitivity: Limits of Detection (LoD)

a. Tumor Purity

The minimum tumor purity needed as input into xT CDx was determined by evaluating 31 CRC FFPE specimens ranging from 5% tumor purity to 50% tumor purity with known CDx biomarkers, along with patient-matched normal specimens. All CDx biomarkers were concordant between xT CDx and results of orthogonal testing for all tumor purities greater than or equal to 10%. Without macrodissection, xT CDx failed to identify one CDx biomarker in a specimen with 5% tumor purity; however, the biomarker was successfully detected after the tumor content was enriched with macrodissection. The results support a minimum tumor purity specification of 20% for xT CDx detection of variants, with macrodissection recommended for specimens with tumor purity lower than 20%.

b. DNA Input for CDx variants

The minimum DNA input needed to detect CDx biomarkers was determined by testing two CRC FFPE tumor specimens (with patient-matched normal specimens) with a previously detected KRAS variant (p.G12D) at six different DNA mass inputs (37.5 ng, 50 ng, 62.5 ng, 75 ng, 100 ng, 125 ng), with each input level tested in duplicate, for a total of 12 replicates per specimen. The LoD for CDx biomarker VAF was then assessed by testing minimal acceptable DNA inputs of 50ng and 100ng. The samples used were as follows (Table 34):

Table 34. Samples used in the study of DNA Input for CDx variants

Sample ID	Tumor Type	Matched Normal Sample
1152	Colorectal Cancer	Blood
1136	Colorectal Cancer	Blood

The KRAS variant was detected in all samples indicating that 50ng and 100ng are acceptable minimum DNA mass inputs for detection of CDx variants by xT CDx.

c. *DNA Input for SNVs, MNVs, Insertions, and Deletions*

The minimum DNA input needed to detect short variants (SNVS, MNVs, INDELs) was determined by testing two tumor FFPE specimens with matched normal saliva specimens and one tumor FFPE specimen with matched normal blood. Each specimen was processed with two replicates with each of five different DNA mass inputs (5ng, 50ng, 100ng, 300ng, 600ng) into library preparation for a total of 10 replicates per specimen The tumor FFPE specimens were also evaluated by orthogonal testing using OM1 with standard DNA mass input. The samples used were as follows (Table 35):

Table 35. Samples used in the study of DNA Input for short variants

Sample ID	Tumor Type	Matched Normal Sample
203	Non-Small Cell Lung Cancer	Blood
231	Glioblastoma	Saliva
651	Endometrial Cancer	Saliva

At the recommended DNA input levels ranging from 50ng to 300ng, 15 out of 17 replicates (88.2%) had 100% concordance between variants detected by xT CDx and OM1. A single variant (position chr5:67589583, variant PIK3R1 p.Glu451_Tyr452dup) showed low coverage for 7 replicates at all DNA input levels and was not consistently detected. Aside from this variant, all other variants were successfully detected in all specimens, indicating that 50ng and 100ng DNA mass input are acceptable minimum DNA mass inputs for detection of short variants by xT CDx.

d. LoD for CDx Variants

DNA from 2 CRC FFPE specimens with previously detected CDx biomarkers were serially diluted with DNA isolated from a known wild-type FFPE specimen to achieve expected VAF as follows: undiluted, 15%, 5%, 2.5%, 1.25%, and 0.63%. For each specimen, at each DNA input level, 2 replicates of each undiluted sample were processed and analyzed, and 20 replicates were processed and analyzed at each subsequent dilution level. A total of 198 tumor-normal paired replicates passed all QC metrics and were used for determination of LoD, with results provided in Table 36.

Table 36. Summary of LoD for CDx variants

DNA Input	LoD VAF % (Hit Rate)*	LoD VAF % (Probit)**
50 ng	2.41%	2.25%
100 ng	3.61%	2.30%

*LoD calculations for CDx variants were based on the hit rate approach, as there were less than three dilution levels between 10-90%. LoD from the hit rate approach was defined as the lowest level with 95% hit rate.
**LoD calculations for the CDx variants based on the probit approach with 95% probability of detection

Studies were also conducted to establish LoD panel-wide for short variants (substitutions and INDELs) and for determination of MSI status.

e. ***LoD for SNVs, MNVs, Insertions, and Deletions***

Samples used in to establish LoD for SNVs, MNVs, insertions and deletions included 17 clinical samples, 11 matched with blood and six matched with saliva, and represented the following nine different tumor types: pancreatic, bladder, endocrine, endometrial, colorectal, prostate, non-small cell lung, gastrointestinal stromal, and glioblastoma.

The LoD for variants was then assessed using the minimal acceptable DNA inputs of 50ng and 100ng for processing 12 tumor-normal paired samples representing eight tumor types. Tumor DNA with known variants was serially diluted with tumor DNA known to be wild type for those variants to generate a range of expected mutation allele frequencies. This dilution series was used to establish a preliminary LoD, which was subsequently confirmed by testing additional replicates of each specimen diluted to achieve expected VAFs at or around the target LoD for xT CDx (5% for substitutions and 10% for INDELs; 3% for hotspot substitutions and 5% for hotspot INDELs). The results of the LoD determination for short variants is summarized in Table 37 and Table 38.

Table 37. Summary of variant detection near LoD VAF

Variant Type	Tested VAF	Positive Call Rate
Substitution	5%	97.5% (79/81)
Substitution (hotspot)	3%	100% (10/10)
Insertion	10%	100% (49/49)
Insertion (hotspot)	5%	100% (23/23)
Deletion	10%	100% (38/38)

Table 38. Analytical sensitivity (LoD VAF) for SNVs, MNVs and INDELs

Variant Type	Gene	Mutation	DNA Input	Range DPt	Range ADtt	Range VAF	Positive Call Rate
SNV	GNAS	p.R201C	100ng	616-808	29-39	4.185%-5.125%	100.0% (5/5)
SNV	JAK2	p.K642*	100ng	322-624	9-21	2.564%-4.380%	100.0% (5/5)

SNV	PIK3CA	p.E545K	100ng	549-866	9-38	1.639%-6.368%	100.0% (5/5)
SNV	BRCA2	p.V941G	100ng	648-872	59-80	8.819%-9.650%	100.0% (5/5)
SNV	ESR1	p.R256Q	100ng	978-1282	30-42	2.804%-3.276%	100.0% (4/4)
SNV	PTEN	p.N49I	100ng	163-380	3-9	1.705%-2.368%	75.0% (3/4)
SNV	BRCA2	p.S1262*	100ng	210-456	11-29	3.679%-7.214%	100.0% (5/5)
SNV	BRCA1	p.M1?	100ng	311-496	15-27	3.024%-8.257%	100.0% (4/4)
MNV	BRCA2	p.EE3342*	100ng	487-740	17-32	2.738%-4.324%	100.0% (4/4)
SNV	GNAS	p.R201C	50ng	714-782	46-71	6.389%-9.384%	100.0% (5/5)
SNV	JAK2	p.K642*	50ng	574-736	21-43	3.659%-5.931%	100.0% (5/5)
SNV	PIK3CA	p.E545K	50ng	538-725	24-50	4.223%-8.013%	100.0% (5/5)
SNV	BRCA2	p.V941G	50ng	543-760	50-77	7.669%-12.439%	100.0% (5/5)
SNV	ESR1	p.R256Q	50ng	513-610	23-41	3.770%-6.856%	100.0% (5/5)
SNV	PTEN	p.N49I	50ng	284-431	5-11	1.160%-3.521%	80.0% (4/5)
SNV	BRCA2	p.S1262*	50ng	210-576	3-38	1.429%-6.597%	100.0% (5/5)
SNV	BRCA1	p.M1?	50ng	358-542	25-43	5.682%-10.056%	100.0% (5/5)
MNV	BRCA2	p.EE3342*	50ng	645-789	51-61	6.464%-8.997%	100.0% (5/5)
SNV	GNAS	p.T55A	50ng	440-596	6-15	1.364%-2.708%	100.0% (5/5)

Deletion	PTEN	p.T319fs	100ng	213-619	12-61	4.734%-11.031%	100.0% (8/8)
Deletion	NF1	p.L1543_P1553delinsIIM	100ng	407-456	29-40	6.921%-8.830%	100.0% (5/5)
Deletion	BRCA2	p.T2214fs	100ng	138-477	27-97	15.888%-21.296%	100.0% (5/5)
Deletion	TP53	p.P27fs	100ng	178-230	25-41	12.255%-19.431%	100.0% (5/5)
Insertion	ESR1	p.N232fs	100ng	891-1211	39-51	3.277%-4.489%	100.0% (4/4)
Insertion	EGFR	p.A767_V769dup	100ng	944-1469	163-287	15.929%-21.129%	100.0% (8/8)
Insertion	TP53	p.C182_S183insCC	100ng	522-801	38-67	5.241%-9.167%	100.0% (5/5)
Insertion	ERBB2	p.A775_G776insYVMA	100ng	460-563	22-38	4.618%-7.739%	100.0% (5/5)
Insertion	TP53	p.L252_T253insNII	100ng	406-555	13-25	2.342%-5.219%	100.0% (5/5)
Insertion	EGFR	p.S768_D770dup	100ng	678-1014	81-98	8.679%-13.227%	100.0% (5/5)
Insertion	KIT	p.A502_Y503dup	100ng	446-594	35-62	7.128%-10.438%	100.0% (5/5)
Deletion	PTEN	p.T319fs	50ng	519-667	55-78	9.970%-11.694%	100.0% (5/5)
Deletion	TP53	p.P27fs	50ng	173-238	32-57	17.582%-23.950%	100.0% (5/5)
Insertion	ESR1	p.N232fs	50ng	561-644	29-49	4.785%-7.609%	100.0% (5/5)

Insertion	EGFR	p.A767_V769dup	50ng	754-1006	145-214	18.905%-22.132%	100.0% (5/5)
Deletion	BRCA2	p.N1287fs	50ng	248-419	7-18	1.772%-4.327%	100.0% (5/5)
Insertion	TP53	p.C182_S183insCC	50ng	472-823	23-80	4.873%-9.831%	100.0% (5/5)
Insertion	ERBB2	p.A775_G776insYVMA	50ng	452-581	55-71	11.071%-13.100%	100.0% (5/5)
Insertion	TP53	p.L252_T253insNII	50ng	493-649	18-37	3.651%-6.789%	100.0% (5/5)
Insertion	EGFR	p.S768_D770dup	50ng	654-766	60-77	8.915%-10.505%	100.0% (5/5)
Insertion	KIT	p.A502_Y503dup	50ng	524-635	45-69	7.741%-10.866%	100.0% (5/5)

t DP = coverage depth tt AD = number of variant reads

f. LoD for MSI

Preliminary MSI LoD determination was evaluated in 22 CRC FFPE specimens known to be MSI-H based on orthogonal testing. 11 samples were matched to blood, 1 was matched to saliva and 10 were matched to normal FFPE as the normal specimen type. Each tumor specimen was diluted using its matched normal specimen to generate 3 dilution levels simulating tumor purities ranging from 10% to 40%. Specimens were evaluated with minimum DNA mass input into library preparation to identify the minimum tumor purity at which MSI status could be detected. This dilution series was used to establish a preliminary LoD, which was subsequently confirmed in an independent study by testing 5 additional replicates of each specimen at or near the target tumor purity (30%). Positive agreement of xT CDx MSI-H status was 94.6% (142/150 replicates identified as MSI-H) for these samples, establishing a minimum tumor purity of 30% in order to determine MSI status.

6. Tissue Comparability

A large-scale retrospective analysis was conducted using 6373 unique tumor specimens across 34 cancer types in order to establish the comparability of assay performance across tumor tissue types. The dataset for analysis using xT CDx consisted of routine clinical

samples processed using the Tempus xT LDT from June 6, 2020 to October 5, 2020. Approximately 89% of samples were matched to blood and 11% of samples were matched to saliva. xT CDx includes four critical QC checks conducted across the assay workflow to closely monitor performance at each step and ensure that only high-quality data are generated and used for variant detection. The quality control checks are as follows: DNA Extraction (QC1), Library Preparation (QC2), Hybridization Capture (QC3), and Sequencing (QC4). The pass rate for each of these quality control steps for each cancer type is summarized in Table 39. More than 91% of specimens passed each QC step regardless of cancer type, demonstrating that assay performance of xT CDx is independent of tissue type.

Table 39. Pass rate at each QC step across cancer types

Cancer Type	DNA Extraction Pass Rate	Library Preparation Pass Rate	Hybridization Capture Pass Rate	Sequencing Pass Rate	Total Samples
Adrenal Cancer	100.0%	100.0%	93.3%	100.0%	15
Biliary Cancer	99.5%	99.5%	96.7%	99.5%	184
Bladder Cancer	99.6%	100.0%	97.7%	99.6%	259
Brain Cancer	100.0%	100.0%	100.0%	100.0%	22
Breast Cancer	99.8%	99.7%	97.3%	99.1%	639
Cervical Cancer	100.0%	100.0%	95.9%	100.0%	49
CRC	100.0%	99.8%	97.8%	98.6%	808
Endocrine Tumor	100.0%	100.0%	94.7%	100.0%	95
Endometrial Cancer	100.0%	100.0%	97.8%	98.9%	184
Esophageal Cancer	99.3%	100.0%	95.9%	99.3%	148
Gastric Cancer	100.0%	100.0%	98.2%	99.1%	109
Gastrointestinal Stromal Tumor	100.0%	100.0%	96.4%	96.4%	28
Glioblastoma	100.0%	100.0%	99.4%	100.0%	163
Head and Neck Cancer	100.0%	100.0%	97.5%	100.0%	40

Cancer Type	DNA Extraction Pass Rate	Library Preparation Pass Rate	Hybridization Capture Pass Rate	Sequencing Pass Rate	Total Samples
Head and Neck Squamous Cell Carcinoma	100.0%	100.0%	96.4%	98.2%	111
Heme Other	100.0%	100.0%	100.0%	91.7%	12
Kidney Cancer	99.3%	100.0%	95.9%	100.0%	58
Liver Cancer	100.0%	100.0%	95.0%	100.0%	40
Low Grade Glioma	100.0%	100.0%	100.0%	100.0%	34
Melanoma	99.4%	100.0%	98.8%	98.2%	164
Meningioma	100.0%	100.0%	93.3%	100.0%	45
Mesothelioma	100.0%	100.0%	95.2%	100.0%	21
Non-Small Cell Lung Cancer	99.6%	99.6%	97.3%	98.9%	851
Oropharyngeal Cancer	100.0%	100.0%	100.0%	98.0%	49
Ovarian Cancer	100.0%	100.0%	98.2%	100.0%	326
Pancreatic Cancer	99.3%	99.8%	97.7%	99.1%	432
Peritoneal Cancer	100.0%	100.0%	100.0%	100.0%	10
Prostate Cancer	99.2%	99.4%	96.4%	98.0%	511
Sarcoma	99.7%	99.7%	97.5%	98.1%	317
Skin Cancer	100.0%	100.0%	96.0%	100.0%	50
Small Cell Lung Cancer	100.0%	100.0%	100.0%	100.0%	64
Testicular cancer	100.0%	100.0%	100.0%	100.0%	18
Thyroid Cancer	100.0%	100.0%	98.8%	97.6%	85

Cancer Type	DNA Extraction Pass Rate	Library Preparation Pass Rate	Hybridization Capture Pass Rate	Sequencing Pass Rate	Total Samples
Tumor of Unknown Origin	100.0%	99.4%	97.9%	99.1%	332

7. Interference

The robustness of xT CDx was assessed while evaluating FFPE tumor specimens in the presence of exogenous and endogenous interfering substances. 22 FFPE specimens representing 13 different tumor types, and their matched normal specimens, were evaluated. The addition of potentially interfering substances including xylene, ethanol, melanin, and proteinase K (ProK), each at two concentrations, was evaluated to determine if these impacted detection of CDx variants, SNVs, MNVs, insertions, deletions, or MSI status. Results in the presence of potentially interfering substances were compared to control (no potential interferent) conditions. 274 data points were analyzed across the four interfering substances, which were considered non-interfering if the positive agreement for variant detection in the presence and absence of that substance was >90%. Across all samples, ≥95% PPA and 100% NPA was observed for each potential interferent compared to control conditions (Table 40).

Table 40. Interference study summary

Substance (Concentration)	Replicates	TP	FN	FP	TN	PPA	PPA Confidence Interval	NPA	NPA Confidence Interval
Ethanol (5%)	46	412	7	2	9355657	98.30%	[96.6, 99.3]	100.00%	[100.0, 100.0]
Ethanol (10%)	32	277	5	3	6508291	98.20%	[95.9, 99.4]	100.00%	[100.0, 100.0]
Melanin (0.05 ug/mL)	48	360	12	3	9762489	96.80%	[94.4, 98.3]	100.00%	[100.0, 100.0]
Melanin (0.1 ug/mL)	32	239	9	3	6508325	96.40%	[93.2, 98.3]	100.00%	[100.0, 100.0]
ProK (0.03 mg/mL)	32	239	9	8	6508320	96.40%	[93.2, 98.3]	100.00%	[100.0, 100.0]
ProK (0.05 mg/mL)	19	114	6	1	3864346	95.00%	[89.4, 98.1]	100.00%	[100.0, 100.0]
Xylene 0.000025%)	39	314	7	4	7932002	97.80%	[95.6, 99.1]	100.00%	[100.0, 100.0]

Substance (Concentration)	Replicates	TP	FN	FP	TN	PPA	PPA Confidence Interval	NPA	NPA Confidence Interval
Xylene 0.000050%)	26	209	5	3	5288001	97.70%	[94.6, 99.2]	100.00%	[100.0, 100.0]

The impact of necrotic tissue was analyzed by assessing the impact of % necrosis on invalid rates and variant-level discordances. No effect of necrotic tissue percentage up to 50% was observed on invalid rates and no false positives were observed at any CDx variant position in any CRC sample, for any potentially interfering substance.

8. Guardbanding

Guardbanding studies were performed to evaluate the performance of xT CDx and the impact of process variation with regard to DNA input at various steps within the workflow. Guardbands were evaluated relative to observed and measured process variability for Library Construction (LC), Hybrid Capture (HC), and sequencing (Seq).

For each process, at least 12 unique FFPE specimens with previously detected KRAS or NRAS variants were evaluated in duplicate at 6-8 DNA input levels representing inputs below the minimum and above the maximum recommended input for each assay step. Results demonstrate reliable and robust performance of xT CDx at DNA input levels above and below the range of acceptable DNA input (i.e., the 1x minimum and 1x maximum) at each step of the assay as summarized in Table 41.

Table 41. Summary of the success rate per process and per input level

Process	Input Level	# of Samples Passing QC
LC	12.5 ng – 0.25x minimum	6/26
LC	25 ng – 0.5x minimum	20/26
LC	50 ng – 1x minimum	26/26
LC	300 ng – 1x maximum	26/26
LC	375 ng – 1.25x maximum	26/26
LC	450 ng – 1.5x maximum	26/26
HC	43.75 ng - 0.25x minimum	24/24
HC	87.5 ng – 0.5x minimum	24/24

HC	175 ng – 1x minimum	24/24
HC	250 ng – 1x maximum	24/24
HC	312 ng – 1.25x maximum	24/24
HC	375 ng – 1.5x maximum	24/24
Seq	0.25x minimum	15/15
Seq	0.5x minimum	26/26
Seq	0.8x minimum	26/26
Seq	0.9x minimum	32/32
Seq	1x minimum	31/31
Seq	1x maximum	26/26
Seq	1.25x maximum	26/26
Seq	1.5x maximum	32/32

9. Sample Carry-Over/Cross-Contamination

DNA sample carry-over (between plates) and cross-contamination (within plates) during the library preparation and hybridization capture steps of xT CDx were assessed. DNA from two FFPE specimens with unique KRAS genotypes, one with a KRAS alteration and one wild-type for KRAS, were plated in a checkerboard matrix pattern as alternating positive and negative samples run with 9 total replicates per specimen. Carryover and cross-contamination were assessed as evidence of germline mutations unique to one specimen being found in the other specimen or as evidence of the KRAS variant in the wild-type specimen. Across all replicates, the overall percent agreement of germline mutations was 100% indicating no sample carryover or cross-contamination. In addition, the KRAS variant was only detected in the specimen that was known to have a KRAS variant based on previous LDT results and was not detected in the known KRAS wild-type specimen. No carryover or cross-contamination was observed.

10. Index Carry-Over/Cross-Contamination

xT CDx uses unique dual index adapters to generate libraries; captured libraries are pooled for sequencing. Index cross-contamination based on incorrect assignment of reads between samples in a pool, as a result of read misassignment from index hopping was assessed across > 138 billion reads obtained on 22 flowcells used during xT CDx

performance characterization. The probability of read misassignment from dual index hopping was determined based on the observed rate of single index hops. The probabilities of read misassignment from dual index hopping ranged from 0.000000642% to 0.00585%, with an average probability across all analyzed flowcells of 0.00135%.

11. Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage at the base level for targeted regions included in xT CDx in 20 samples. 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. The mean coverage for CDx genes (KRAS and NRAS) was > 500x, with > 95% of reads mapping to these genes having high base quality scores of ≥30. When assessing panel-wide coverage, within-sample mean coverage for all targeted regions ranged from 508x-1218x (mean of 904.8x), with > 98% of exons with a depth of ≥ 150x and > 99% of exons with a depth of ≥ 100x.

12. DNA Extraction

DNA extraction was assessed by duplicate extraction of 124 tumor specimens representing 22 different tumor types (including melanoma, prostate, lung, GBM, breast, and bladder), using 2 extraction instruments and 3 extraction reagent lots. The average DNA yield and concordance of variant calling across all samples was evaluated. The mean yield across all 248 extractions was 5076.4 ng, significantly higher than the minimum DNA input of 50ng needed for library preparation. Variant concordance was assessed in 68 tumor specimens across 11 tumor types extracted in duplicate. Variant concordance in the duplicate samples with sufficient DNA was 97.0%, shown in Table 42.

Table 42. Somatic variant concordance observed in duplicate DNA extractions

Level 1 Variants	Level 2 Variants	Level 3 Variants	# Concordant	# Total	Overall Concordance	95% CI
1/1	29/30	193/199	223	230	97.0%	(93.8, 98.8)

13. Invalid Rates

A large-scale retrospective analysis was conducted using 4628 unique tumor-normal matched specimens across 41 cancer types in order to establish the invalid rates at each step of the xT CDx workflow for a variety of specimen types. The dataset for analysis consisted of routine clinical samples analyzed using the Tempus xT LDT assay from June 1, 2020 to December 8, 2020. The samples were subjected to pre-specified retrospective analysis based on thresholds for success at each step of the assay: DNA Extraction, Library

Preparation, Hybridization Capture, and Sequencing. Results are shown in Table 43. Of the 4628 tumor-normal paired samples evaluated, 4122 (89.1%) were successfully processed across all steps of the assay.

Table 43. xT CDx invalid rates

Tumor Type	Percent rejected at Specimen Qualification*	N (passed qualification and processed with the assay)	Percent Invalid - Assay Steps				Total Assay Invalid Rate % excluding specimen qualification ***
			DNA Extraction (tumor|normal)	Library Construction (tumor|normal)	Hybridization Capture (tumor|normal)	Sequencing **	
Adrenal Cancer	0	22	0.0|0.0	0.0|0.0	9.1|0.0	10	18.18
Basal Cell Carcinoma	15.38	4	0.0|0.0	0.0|0.0	0.0|0.0	50	50
Biliary Cancer	6.33	222	0.0|0.0	0.0|0.0	4.5|1.8	4.76	9.91
Bladder Cancer	2.47	430	0.5|0.0	0.0|0.0	2.3|0.9	5.29	8.37
Brain Cancer	1.35	44	0.0|0.0	0.0|0.0	0.0|0.0	4.55	4.55
Breast Cancer	6.88	690	0.0|0.0	0.6|0.0	2.9|2.3	6.75	11.88
Cervical Cancer	2.46	82	0.0|0.0	0.0|0.0	4.9|0.0	2.56	7.32
Chromophobe Renal Cell Carcinoma	0	12	0.0|0.0	0.0|0.0	0.0|0.0	16.67	16.67
Clear Cell Renal Cell Carcinoma	4.79	130	1.5|0.0	0.0|0.0	4.7|3.1	8.47	16.92
Colorectal Cancer	3.19	1308	0.0|0.0	0.3|0.2	2.3|3.7	5.38	11.18
Endocrine Tumor	3.48	146	0.0|0.0	0.0|0.0	6.8|4.1	3.08	13.7
Endometrial Cancer	1.8	316	0.0|0.0	0.0|0.0	2.5|2.5	3.33	8.23
Esophageal Cancer	4.11	250	0.8|0.0	0.0|0.0	4.8|2.4	6.09	13.6
Gastric Cancer	5.81	194	0.0|0.0	0.0|0.0	1.0|1.0	14.74	16.49
Gastrointestinal Stromal Tumor	3.06	40	0.0|0.0	0.0|0.0	0.0|0.0	10	10
Glioblastoma	1.28	300	0.0|0.0	0.0|0.0	0.7|0.7	6.76	8
Head and Neck Cancer	5.04	66	0.0|0.0	0.0|0.0	3.0|0.0	9.38	12.12
Head and Neck Squamous Cell Carcinoma	4.39	192	0.0|0.0	0.0|0.0	4.2|7.3	3.49	13.54
Kidney Cancer	6.47	82	0.0|0.0	0.0|0.0	4.9|0.0	5.13	9.76
Liver Cancer	2.04	52	0.0|0.0	0.0|0.0	3.8|3.8	16.67	23.08
Low Grade	2.5	64	0.0|0.0	0.0|0.0	0.0|3.1	0	3.12

Tumor Type	Percent rejected at Specimen Qualification*	N (passed qualification and processed with the assay)	Percent Invalid - Assay Steps				Total Assay Invalid Rate % excluding specimen qualification ***
			DNA Extraction (tumor|normal)	Library Construction (tumor|normal)	Hybridization Capture (tumor|normal)	Sequencing **	
Glioma							
Medulloblastoma	0	10	0.0|0.0	0.0|0.0	0.0|0.0	20	20
Melanoma	5.61	260	0.8|0.0	0.0|0.0	1.6|0.8	6.35	9.23
Meningioma	3.39	88	0.0|0.0	0.0|0.0	6.8|2.3	0	9.09
Mesothelioma	3.7	36	0.0|0.0	0.0|0.0	0.0|11.1	6.25	16.67
Neuroblastoma	6.25	18	0.0|0.0	0.0|0.0	0.0|0.0	0	0
Non-Clear Cell Renal Cell Carcinoma	0	6	0.0|0.0	0.0|0.0	0.0|0.0	0	0
Non-Small Cell Lung Cancer	6.36	1160	0.2|0.0	0.2|0.2	2.9|2.9	7.48	12.59
Oropharyngeal Cancer	2.94	82	0.0|0.0	0.0|0.0	0.0|7.3	5.26	12.2
Ovarian Cancer	3.89	564	0.0|0.0	0.0|0.0	2.1|3.5	4.48	9.22
Pancreatic Cancer	5.65	586	0.3|0.0	0.0|0.0	1.7|3.4	7.58	12.63
Peritoneal Cancer	0	20	0.0|0.0	0.0|0.0	0.0|0.0	0	0
Prostate Cancer	6.68	492	0.8|0.0	0.4|0.0	3.7|3.3	7.02	13.82
Sarcoma	4	512	0.4|0.0	0.4|0.0	1.6|0.8	4.03	7.03
Skin Cancer	2.21	84	0.0|0.0	0.0|0.0	4.8|2.4	5	9.52
Small Cell Lung Cancer	4.49	78	0.0|0.0	0.0|0.0	0.0|5.1	10.81	15.38
Testicular cancer	0	32	0.0|0.0	0.0|0.0	0.0|0.0	0	0
Thymoma	10.53	10	0.0|0.0	0.0|0.0	0.0|0.0	0	0
Thyroid Cancer	4.38	140	0.0|0.0	0.0|0.0	0.0|0.0	5.71	5.71
Tumor of Unknown Origin	8.28	426	0.0|0.0	0.0|0.0	0.9|0.9	6.64	8.37
Uveal Melanoma	9.52	4	0.0|0.0	0.0|0.0	0.0|0.0	50	50

*Rejection rates based on pathology review
**Sequencing invalid (fail) status is evaluated on the patient level, based on tumor-normal matched sample pairs
*** Total assay invalid rate is evaluated on the patient level, based on tumor-normal matched sample pairs

14. Specimen Stability Studies

a. Sample Stability – FFPE Slides

FFPE slide stability study was assessed prospectively and by analysis of previously prepared aged slides. Samples from eight specimens were included in the prospective study, two specimens were not evaluated due to failures of the normal sample (1 at QC2

and 1 at QC4) at T=0. At T=30, one sample failed at QC4 and was also not evaluated. For prospective analysis, multiple slides from the remaining 5 tumor specimens across 4 cancer types were prepared and stored at room temperature for 0 days, 15 days, or 30 days, and then processed with xT CDx. Results at each timepoint were compared to results at T=0. Across the 5 samples evaluated, all 15 variants were detected at all 3 timepoints tested as summarized in Table 44.

Table 44. Variant detection supporting FFPE slide stability

Tumor Type	T=0 Variants	T=15 Days Concordance	T=30 Days Concordance
Ovarian	3	3/3	3/3
Prostate	2	2/2	2/2
Lung	4	4*/4	4/4
Ovarian	2	2/2	2/2
Colorectal	4	4/4	4/4
Total	**15**	**100.0% (15/15)**	**100.0% (15/15)**

*A variant was detected at the T=15 timepoint with a VAF of 3.5%, above the reporting threshold of 3%; however, it was detected below the reporting threshold, at a VAF of 2.9%, at the T 0 timepoint.

Analysis of previously prepared aged slides involved analysis of slides from 124 tumor specimens across 23 tumor types, which had been stored for varying durations at room temperature. These slides were subjected to DNA extraction and grouped into 4 time brackets (< 3 months, 3-6 months, 6-18 months, 18-82 months) based on storage duration. Stability was assessed by the number of specimens meeting minimum DNA yield criteria for xT CDx; results are summarized in Table 45.

Table 45. Evaluation of FFPE slides at QC1 (DNA Extraction) based on length of storage

Months since Slide Preparation	Number of Specimens Evaluated	Number of Specimens with 50ng DNA Yield at Extraction
0-3	50	47 (94.0%)
3-6	60	58 (96.7%)
6-18	11	11 (100.0%)
18-82	3	3 (100.0%)
Total	**124**	**119 (96.0%)**

b. Sample Stability – FFPE Blocks

The stability of FFPE blocks was established using 349 FFPE blocks stored at room temperature for 1-7 years by evaluating DNA extraction yield. The blocks were grouped into 5 time brackets based on storage since block preparation. More than 95% of the blocks in each age bracket produced 3x the minimum DNA yield of 50ng needed for xT CDx, when processed under standard conditions. Results are summarized in Table 46.

Table 46. DNA yield from FFPE blocks based on year of preparation

Block Group	Year of Block Preparation	Number of Specimens	Mean QC1 Yield (ng)	%Samples 2: 150ng DNA Yield
1	2019	40	4000.5	100.0%
2	2018	22	2792.7	95.5%
3	2016-2017	117	2683.0	99.2%
4	2014-2015	125	2564.5	96.8%
5	2012-2013	45	3646.2	100.0%

c. Sample Stability - Blood and Buffy Coat

Stability of blood and buffy coat samples used as the source of matched normal specimens for xT CDx was established in this study. Normal blood samples were collected from 6 healthy volunteers and blood and buffy coat stability were determined either by: 1) separation of buffy coat from blood upon receipt of a specimen, with storage for the buffy coat fraction at –20°C for 0, 15, 30, and 60 days followed by DNA extraction and processing through xT CDx; or 2) storage of whole blood specimens at room temperature for 0, 5, 10, 15, and 20 days followed by separation of the buffy coat fraction, DNA extraction and processing through xT CDx. A randomly selected tumor specimen that included 4 known somatic variants was used as the tumor matched to all

blood samples. Concordance was evaluated by comparing results at each time point to results from T=0. For both blood and buffy coat, somatic variant concordance was 100% and germline concordance was >99% at each time point evaluated.

15. Reagent Stability Studies

a. Reagent Stability

The stability of critical reagent lots used in library preparation, and hybridization capture for xT CDx were evaluated with respect to CDx variants and short variants, in two studies. The first study consisted of 3 lots of each set of reagents evaluated at 3 timepoints, T=0, T=1 month, and T=7 months, reagents were stored under the manufacturer's recommended conditions. A second study consisted of 3 reagent sets at T=7.75 months. In the first study, five specimens (melanoma, tumor of unknown origin, endocrine tumor, non-small cell lung cancer, and thyroid cancer) were evaluated. Samples were tested with 5 replicates for each of three reagent lots across time points 0 months and 1 month. At 7 months, samples were tested with 2-5 replicates of two reagent lots. Results from each time point were compared against results from samples tested at time 0. Out of 180 replicates across all timepoints (0 months, 1 month, 7 months), 4 were excluded because of failed QC. Across 73 replicates at 1 month, 98.3% (174/177) of variants were concordant. Across 28 replicates at 7 months, 100.0% (71/71) of variants were concordant. In the second study, 5 specimens (representing head and neck squamous cell carcinoma, endometrial cancer, non-small cell lung cancer, and colorectal cancer) were evaluated. Samples were tested with 1 replicate with an orthogonal method (OM1) at time point 0 to establish a baseline. At time point 7.75 months, samples were tested with xT CDx in 5 replicates for 3 reagent lots. Results from the 7.75-month time point were compared to the results of OM1 at time 0. One (1) sample set failed due to somatic contamination of the germline sample and all 15 replicates were excluded. Of the remaining 60 replicates, 24 were excluded because of failed QC. Across 34 evaluable replicates at 7.75 months, 97.7% (172/176) of variants were concordant and 100.0% (9/9) of CDx variants were concordant. The claimed stability of library preparation and hybridization capture reagents is 7 months.

The stability of critical reagents used in sequencing for xT CDx was evaluated with respect to CDx variants and short variants. The study tested reagents at 2 timepoints, shortly after receipt and after aging at least 2 months. At each time point, 8 CRC tumor specimens were evaluated with 1-4 replicates. Two specimens were excluded from analysis due to failing the QC check using the fresh reagents. Of the remaining 6 specimens processed with 9 replicates using fresh reagents, and 15 replicates using aged reagents, 100.0% (94/94) of all short variants were concordant. The claimed stability for sequencing reagents is up to 5 months post-receipt, within manufacturer recommended expiration dates.

b. Shipping Stability

Simulated shipping of specimen collection kits was conducted under expected shipping conditions and established stability of collection kits and shipped specimens under standard conditions in accordance with product labeling.

B. Animal Studies

No animal studies were conducted using xT CDx.

C. Additional Studies

No additional studies were conducted using xT CDx.

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

A. Study Design

Clinical validity of xT CDx as a CDx used for identifying patients with CRC who may not be eligible for treatment with cetuximab when mutations are detected in *KRAS* codons 12 or 13, or to determine who may not be eligible for panitumumab when mutations are detected in exons 2, 3, or 4 of *KRAS* or *NRAS,* was established by evaluating 412 samples from CRC patients, using a non-inferiority statistical testing approach. All specimens were evaluated for a minimum tumor purity of 20% based on pathology review and availability of matched-normal tissue. Based on tumor purity and matched-normal tissue availability, samples from 351 patients were included in the study. Based on specimen availability, 348 samples were tested with xT CDx and with two FDA-approved comparator CDx assays: (1) the *Illumina Praxis Extended RAS Panel* (P160038); and, (2) the *Qiagen therascreen KRAS RGQ PCR Kit* (P110027). Orthogonal testing was conducted in duplicate for each sample, for each method.
Overall concordance between xT CDx and *Praxis* is 100.00% (190/190), and concordance between xT CDx and *therascreen* is 99.60% (249/250).

1. Clinical Inclusion and Exclusion Criteria

   The samples used for this study were purchased from specimen repositories. The requirements for these specimens were that they were surgical specimens fixed in 10% neutral buffered formalin, available as FFPE blocks (not slides), and represented tumor specimens from patients with malignant colon cancer. Specimens were not pre-screened to enrich for positive samples. Percent tumor and percent necrosis were evaluated for all specimens by Tempus' laboratory pathology services and trained pathologists based on standard clinical laboratory practices. Not all demographic data were available for all specimens.

   A total of 412 CRC samples were evaluated in the CDx Clinical Validation study. Samples without adequate matched normal tissue were excluded because they did

not meet the xT CDx requirement of having a matched normal specimen. Of the remaining 351 samples, xT CDx data were not generated for two of these samples because they were depleted and insufficient material was available for xT CDx testing. Subsequent review of the remaining samples identified one as a gastrointestinal stromal tumor (GIST), not CRC, and it was therefore not included in the study.

Of the 348 evaluable samples tested with xT CDx, 190 generated results with both xT CDx and the *Praxis* assay (to support clinical validity with respect to biomarker detection for panitumumab) and 250 generated results with both xT CDx and the *therascreen* assay (to support clinical validity with respect to biomarker detection for cetuximab). These two sets of samples were also used for evaluation of accuracy when compared to the orthogonal methods. A summary of the number of tested samples and the samples with available demographic data for comparison to each of the *Praxis* and *therascreen* assays is provided in Table 47, below.

Table 47. Availability of demographic/sample data for CDx Clinical Validation study samples

Sample/ Demographic Characteristic	Total Samples Tested with xT CDx and sent for Comparator Testing (n=348)	Samples with xT CDx and *Praxis* Results with Available Data (n=190 total samples)	Samples with xT CDx and *therascreen* Results with Available Data (n=250 total samples)
Sex	302 (86.8%)	190 (100%)	237 (94.8%)
Age	301 (86.5%)	190 (100%)	237 (94.8%)
Race	171 (49.1%)	129 (67.4%)	143 (57.2%)
Tumor Site	94 (27.0%)	31 (16.3%)	60 (24%)
TNM Stage	94 (27.0%)	31 (16.3%)	60 (24%)
Tumor Percentage	348 (100%)	190 100%)	250 (100%)
Percent Necrosis	348 (100%)	190 (100%)	250 (100%)

2. Follow-up Schedule
   None required for this study. The CDx Clinical Validation study involved retrospective testing of samples; as such, no additional patient follow-up was conducted.

3. Clinical Endpoints

None required for this study. The clinical validity was established by evaluating 412 samples from CRC patients, using a non-inferiority statistical testing approach.

B. Accountability of PMA Cohort

Tumor FFPE blocks from 412 patients diagnosed with colorectal cancer, along with matched normal FFPE blocks, when available, were purchased. Tumor blocks without matched normal blocks available were reviewed for adjacent normal tissue. After exclusion of samples lacking a matched normal or failing pathology review for minimum tumor purity for xT CDx, a total of 351 samples were processed. All 351 samples were sent out for orthogonal testing with the orthogonal devices but only 349 samples were processed with xT CDx due to exhaustion of two of the FFPE blocks. Results for one sample were not returned by the orthogonal testing facility and therefore this sample was not included in the analysis. Upon further review, results from an additional sample were excluded during analysis because the sample was determined to be a gastro-intestinal stromal tumor (GIST).

In order for the results from xT CDx to be compared for accuracy against each reference device and included in this study, samples had to meet the inclusion criteria of both xT CDx and the respective criteria for the comparator device. The inclusion criteria for the respective comparisons is as follows:

i. Tumor-normal pair passed QC4 for xT CDx and tumor QC passed two *Praxis* assay runs

ii. Tumor-normal pair passed QC4 for xT CDx and tumor QC passed two *therascreen* assay runs

For each sample that met both inclusion criteria, *KRAS/NRAS* mutations called by *Praxis*/*therascreen* results were compared to xT CDx reportable *KRAS/NRAS* variants.

Non-Inferiority margins were computed using the 'Wilson score interval with continuity correction' method according to Li, 2016 (Section 4.1: Concordance Study When Reference Standard is not available).

The Clopper-Pearson interval based on Beta distribution method with a significance level of 0.05 was used to compute two-sided confidence intervals for NPA and PPA.

Table 54 summarizes the number of samples passing at each QC step for xT CDx. The tumor specimens were processed first so some available normal specimens were not processed if the matching tumor specimen had already failed.

Table 54. Quality control (QC) pass rate for this study

Quality Control	Tumor FFPE Samples	Normal FFPE Samples
QC1	349/349 passed	338/347 passed
QC2	311/349 passed	308/337 passed
QC3	311/311 passed	307/307 passed
Tumor and normal passed QC3	292 sample pairs	
QC4*	251/292 passed	

*QC4 numbers refer to pairs of samples (paired tumor and normal samples that had both passed all prior QC steps).

For the samples failing xT CDx at QC4, Table 55 summarizes the various metrics causing samples to be rejected. A majority of the samples (32/41) failed at QC4 due to tumor-specific somatic variants being identified in the normal sample, likely due to the presence of contaminating tumor cells in the adjacent normal tissue used as the matched normal.

Table 55. Summary of QC4 failure reasons

Fail reasons	Number of samples
Tumor Contamination (Somatic variants found in germline)	30
Tumor Contamination (Somatic variants found in germline) and high tumor PCR duplication rate	1
High Tumor PCR duplication rate	5
Normal Contamination (Tumor sample contaminated by external source)	1
Normal Contamination (Tumor sample contaminated by external source) and low fingerprint score	1
Low unique read count (tumor sample), High Tumor PCR duplication rate	1
Low on target rate (normal sample)	1
Tumor Contamination (Somatic variants found in germline), Normal Contamination	1

Fail reasons	Number of samples
(Tumor sample contaminated by external source) and low fingerprint score	

Specimens evaluated by xT CDx had the following mutation distribution:

a. WT in KRAS and NRAS - 56.57% (142/251)
b. KRAS exon 2 (G12, 13) - 34.26% (86/251)
c. KRAS exon 3 (A59, Q61) - 1.59% (4/251)
d. KRAS exon 4 (K117, A146) - 3.59% (9/251)
e. NRAS exon 2 (G12, G13) - 1.99% (5/251)
f. NRAS exon 3 (A59, Q61) - 1.99% (5/251)
g. NRAS exon 4 (K117, A146) - 0.00% (0/251)

Variants in NRAS exon 4 are rare and none of the clinical specimens used in this study were positive for CDx variants in this region. Coverage analysis for NRAS exon 4 in tested clinical specimens showed that coverage for this exon is adequate in all 251 xT CDx samples that passed QC4 (minimum coverage: 297x, maximum coverage: 4609x, median coverage: 1650x).

A total of 350 CRC samples were sent to an external certified lab for orthogonal testing using the FDA approved *Praxis* and *therascreen* CDx devices. However, the external lab did not return results for one of the samples and therefore it could not be included in the analysis. Every sample that met inclusion criteria for each comparator device was independently tested in duplicate with that device. Table 56 and Table 57 describe the observed invalid rates for *Praxis* and *therascreen* respectively. The *Praxis* device requires samples to have a minimum of 50% tumor purity and has stringent DNA qualification criteria, both of which account for the high invalid rate.

Table 56. Summary of samples tested with the *Praxis* device

	Pass	Fail QC	Total	Observed Invalid Rate
Replicate 1	229	120	349	34.38%
Replicate 2	223	126	349	36.10%

Table 57. Summary of samples tested with the *therascreen* device

	Pass	Fail QC	Total	Observed Invalid Rate

Replicate 1	349	0	349	0.00%
Replicate 2	349	0	349	0.00%

C. Study Population Demographics and Baseline Parameters

Demographic and clinical characteristics of the samples in the xT CDx clinical validity study are summarized in tabular form below in comparison to the study populations comprising the clinical validity studies for each of the comparator methods for xT CDx claims.

Tables 48 through 53 below describe patient demographic characteristics for 3 populations:

- All samples: all samples included in the study and tested with xT CDx, and with available demographic data

- Comparison samples: all samples producing results (i.e., a passing result, not an invalid result) with both xT CDx and the comparator assay and with available demographic data

- Comparator clinical validation study: study population from the clinical validity study of the comparator method; either *Praxis* (for panitumumab) or *therascreen* (for cetuximab)

The HEC-59 cell line was separately evaluated to support concordance for NRAS Exon 4 variants; however, the sample characteristics described below only include clinical specimens and therefore, the HEC-59 cell line is excluded for the purposes of describing specimen characteristics and demographics.

Tables 48 and 49 describe sex demographics.

Table 48. xT CDx comparison to *Praxis*, sex demographics for samples with available sex demographic data

Sex	All Samples with Sex Data, n=302	Comparison Samples with Sex Data, n=190	*Praxis* Clinical Validity Study*
Male	155 (51.3%)	97 (51.1%)	63.2%
Female	147 (48.7%)	93 (48.9%)	36.8%

* Includes Wild-type RAS, Mutant RAS, and Wild-type KRAS Exon 2 Mutant RAS sets from P160038 SSED Table 14

Table 49. xT CDx comparison to *therascreen*, sex demographics for samples with available sex demographic data

Sex	All Samples with Sex Data, n=302	Comparison Samples with Sex Data, n=250	*therascreen* Clinical Validity Study*
Male	155 (51.3%)	118 (49.8%)	66.2%
Female	147 (48.7%)	119 (50.2%)	33.8%

* Includes K-Ras Evaluated set from Demographic Characteristics Table in Section X.C of P110030 SSED

Tables 50 and 51 describe race demographics.

Table 50. xT CDx comparison to *Praxis*, race demographics for samples with available race demographic data

Race	All Samples with Race Data, n=171	Comparison Samples with Race Data, n=128	*Praxis* Clinical Validity Study*
White	146 (85.4%)	114 (89.1%)	91.3%
Black**	18 (10.5%)	11 (8.6%)	2.7%
Other***	7 (4.1%)	3 (2.2%)	6.0%

* Includes Wild-type RAS, Mutant RAS, and Wild-type KRAS Exon 2 Mutant RAS sets from P160038 SSED Table 14
** Includes race reported as black or African American, or black of African or Caribbean heritage
*** Includes race reported as Hispanic or Latino, Asian, Asian/pacific island, black/Asian, American Indian or Alaska native, other, or unknown

Table 51. xT CDx comparison to *therascreen*, race demographics for samples with available race demographic data

Race	All Samples with Race Data, n=171	Comparison Samples with Race Data, n=143	*therascreen* Clinical Validity Study*
White	146 (85.4%)	125 87.4%)	91.4%
Black**	18 (10.5%)	14 (9.8%)	1.3%
Other***	7 (4.1%)	4 (2.8%)	7.3%

* Includes K-Ras Evaluated set from Demographic Characteristics Table in Section X.C of P110030 SSED
** Includes race reported as black or African American, or black of African or Caribbean heritage
*** Includes race reported as Hispanic or Latino, Asian, Asian/pacific island, black/Asian, American Indian or Alaska native, other, or unknown

Table 52 describes age demographics.

Table 52. xT CDx comparison to *Praxis* and *therascreen*, age demographics for samples with available age demographic data

	All Samples with Age Data, n=301	Comparison to *Praxis* with Age Data, n=190	*Praxis* Clinical Validity Study*	Comparison to *therascreen* with Age Data, n=237	*therascreen* Clinical Validity Study**
< 65	137 (45.5%)	92 (48.4%)	ND	112 (47.3%)	58.5%
2: 5	164 (54.5%)	98 (51.6%)	ND	125 (52.7%)	41.5%
Mean	65.8	64.6	61.3	65.1	ND
Median	66	65	ND	65	ND
Min - Max	22 - 98	22 - 98	ND	22 - 98	28.6- 88.1

* Includes Wild-type RAS, Mutant RAS, and Wild-type KRAS Exon 2 Mutant RAS sets from P160038 SSED Table 14; mean age was calculated as a weighted average of mean age in each set
** Includes K-Ras Evaluated set from Demographic Characteristics Table in Section X.C of P110030 SSED; mean age was not available, mean across all samples in this set could not be determined from available data

Table 53 describes available sample characteristics of tumor percentage and percent necrosis for study samples.

Table 53. Summary tumor percentage and necrosis for xT CDx's CDx Accuracy study

	All Samples, n=348	Comparison to *Praxis*, n=190	Comparison to *therascreen*, n=250
Tumor Percentage Mean	44.1%	51.7%	47.4%
Tumor Percentage Median	40%	50%	50%
Tumor Percentage Range*	5%-90%	5%-90%	5%-90%
Necrosis < 5%	254 (73.0%)	134 (70.5%)	182 (72.8%)
Necrosis 5-20%	81 (23.3%)	48 (25.3%)	59 (23.6%)
Necrosis > 20-50%	11 (3.2%)	7 (3.7%)	8 (3.2%)
Necrosis > 50%	2 (0.6%)	1 (0.5%)	1 (0.4%)

* Specimens with a tumor percentage below the minimum required tumor percentage for detection of CDx variants 20% were macrodissected to enrich tumor content

D. Safety and Effectiveness Results

Concordance Study for KRAS and NRAS Wild Type in CRC

Results of concordance testing are summarized in Table 58, below.

Concordance of xT CDx with the *Illumina Praxis Extended RAS Panel* (PCD) was evaluated using a total of 190 samples; those that passed all xT CDx quality control metrics and with two successful measurements with the comparator (PCD1 and PCD2 denote replicate measurements with this comparator). Concordance of xT CDx with the *Qiagen therascreen KRAS RGQ PCR Kit* (TCD) was evaluated using a total of 250 samples; those that passed all xT CDx quality control metrics and with two successful measurements with the comparator (TCD1 and TCD2 denote replicate measurements with this comparator).

Overall concordance between xT CDx and the *Illumina Praxis Extended RAS Panel* was 100.00% (190/190), and overall concordance between xT CDx and the *Qiagen therascreen KRAS RGQ PCR Kit* was 99.60% (249/250).

Table 58. Concordance of CDx variant calling with comparator methods

	PCD1+		**PCD1-**		**TCD1+**		**TCD1-**	
	PCD2+	**PCD2 -**	**PCD2 +**	**PCD2 -**	**TCD2+**	**TCD2 -**	**TCD2 +**	**TCD2 -**
xT CDx+	82	0	0	0	87	0	0	0
xT CDx-	0	0	0	108	1	0	0	162

Praxis Extended RAS Panel

Concordance of xT CDx with *Praxis* was evaluated using a total of 190 samples (specimens that passed xT CDx QC and had two successful *Praxis* runs). By defining the reference standard as the consensus calls between *Praxis* replicate 1 (PCD1) and replicate 2 (PCD2), xT CDx achieved a PPA of 100.00% (82/82) (95% CI [95.6, 100.00]) and NPA of 100.00% (108/108) (95% CI [96.64,100.00]) as summarized in Table 59. PPA and NPA for every pairwise comparison are summarized in Table 61.

Table 59. Summary of sample-level concordance data using agreement between PCD1 and PCD2 (*Praxis*) as the reference.

	PCD1+/PCD2+	**PCD1-/PCD2-**
xT CDx+	82*	0

xT CDx-	0	108

*One sample had three variants reported by *Praxis* (KRAS p.Gly12Ser, NRAS p.Gly12Asp and NRAS p.Gly13Arg). All of them were detected by xT CDx, but KRAS p.Gly12Ser was not flagged as a reportable variant due to being called at a low VAF (2.26%). However, both xT CDx and *Praxis* reported the same clinical result (i.e., that the patient is not wild-type for KRAS or NRAS), which would contraindicate for the associated therapy. Therefore, for the purpose of establishing clinical validity this sample was considered as biomarker concordant.

Concordance analysis showed that the upper bounds of 95% one-sided Confidence Interval (CI) were below 5% for all four Non-inferiority (NI) hypothesis tests sPPA1: 0.000, 95% CI [-0.045, 0.045]; sPPA2 0.000, 95% CI [-0.045, 0.045]; sNPA1: 0.000, 95% CI [-0.034, 0.034]; sNPA2: 0.000, 95% CI [-0.034, 0.034]). Concordance results are summarized in Table 60 below. Of note, the confidence intervals were calculated using the Wilson score method and the correlation is ignored due to 100% agreement between PCD1 and PCD2 (*Praxis*).

Table 60. Concordance Table with PCD1, PCD2 (*Praxis*), and xT CDx results with eligible samples

	PCD1+			**PCD1-**		
	PCD2+	PCD2-	Total	PCD2+	PCD2-	Total
xT CDx+	82	0	82	0	0	82
xT CDx-	0	0	0	0	108	108

Table 61. Concordance table with PCD1, PCD2 (*Praxis*), and xT CDx results with eligible samples

	% Agreement
PPA_{P1P2}	100.00%
NPA_{P1P2}	100.00%
PPA_{P2P1}	100.00%
NPA_{P2P1}	100.00%
$PPA_{P1xTCDx}$	100.00%
$NPA_{P1xTCDx}$	100.00%
$PPA_{P2xTCDx}$	100.00%
$NPA_{P2xTCDx}$	100.00%

PPA_{P1P2} is the PPA between *Praxis* replicate 1 (PCD1) and *Praxis* replicate 2 (PCD2) conditional on PCD1.

NPA_{P1P2} is the NPA between PCD1 and PCD2 conditional on PCD1.
PPA_{P2P1} is the PPA between PCD1 and PCD2 conditional on PCD2.
NPA_{P2P1} is the NPA between PCD1 and PCD2 conditional on PCD2.
$PPA_{P1xTCDx}$ is the PPA between PCD1 and xT CDxconditional on PCD1.
$NPA_{P1xTCDx}$ is the NPA between PCD1 and xT CDx conditional on PCD1.
$PPA_{P2xTCDx}$ is the PPA between PCD1 and xT CDx conditional on PCD2.
$NPA_{P2xTCDx}$ is the NPA between PCD2 and xT CDx conditional on PCD2.

Overall positive and negative agreement was calculated using xT CDx and *Praxis* results for comparison. PPA and NPA calculated at the gene level and variant level for each sample are shown in Table 62 and Table 63.

Table 62. *Praxis* mutation-level positive and negative agreement by gene

Gene	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95% CI)
KRAS/All	74	1*	0	3276	3351	98.67 (92.79-99.97)	100.0 (99.89-100)
NRAS/All	9	0	0	5124	5133	100.0 (66.37-100)	100.0 (99.93-100)

*A false negative variant was present in a sample which had three variants detected by *Praxis* (KRAS c.34G>A, NRAS c.35G>A and NRAS c.37G>C). All were detected by xT CDx, but KRAS c.34G>A was not reportable by xT CDx due to its low VAF (2.26%), which was below the CDx variant reporting threshold of 3%. However, for this specimen, both xT CDx and *Praxis* detected and reported two NRAS variants, and therefore generated the same clinical result, i.e., the patient is not wild-type for KRAS or NRAS and would therefore not receive the associated therapy. Therefore, although there was a discrepancy at the variant level in this specimen, for the purpose of establishing clinical validity this sample is considered as biomarker concordant.

Table 63. *Praxis* mutation level positive and negative agreement in KRAS and NRAS

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95%CI)
KRAS Exon 2	c.34G>T	4	0	0	188	192	100.0 (39.76-100)	100.0 (98.06-100)
	c.34G>A	3	1*	0	188	192	75.0 (19.41-99.37)	100.0 (98.06-100)
	c.34G>C	2	0	0	190	192	100.0 (15.81-100)	100.0 (98.08-100)
	c.34_35GG>TT	0	0	0	192	192	NE	100.0 (98.1-100)
	c.34_35GG>AA	0	0	0	192	192	NE	100.0 (98.1-100)
	c.34_36GGT>TGG	0	0	0	192	192	NE	100.0 (98.1-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95%CI)
	c.35G>A	29	0	0	163	192	100.0 (88.06-100)	100.0 (97.76-100)
	c.35G>T	11	0	0	181	192	100.0 (71.51-100)	100.0 (97.98-100)
	c.35G>C	3	0	0	189	192	100.0 (29.24-100)	100.0 (98.07-100)
	c.37G>T	0	0	0	192	192	NE	100.0 (98.1-100)
	c.37G>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38G>A	13	0	0	179	192	100.0 (75.29-100)	100.0 (97.96-100)
	c.38_39GC>TT	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38_39GC>AA	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38_39GC>AT	0	0	0	192	192	NE	100.0 (98.1-100)
KRAS Exon 3	c.175G>A	0	0	0	192	192	NE	100.0 (98.1-100)
	c.176C>G	0	0	0	192	192	NE	100.0 (98.1-100)
	c.181C>A	0	0	0	192	192	NE	100.0 (98.1-100)
	c.181C>G	0	0	0	192	192	NE	100.0 (98.1-100)
	c.182A>T	0	0	0	192	192	NE	100.0 (98.1-100)
	c.182A>G	0	0	0	192	192	NE	100.0 (98.1-100)
	c.183A>C	2	0	0	190	192	100.0 (15.81-100)	100.0 (98.08-100)
	c.183A>T	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
KRAS Exon 4	c.351A>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.351A>T	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.436G>A	4	0	0	188	192	100.0 (39.76-100)	100.0 (98.06-100)
	c.436G>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.437C>T	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.34G>T	0	0	0	192	192	NE	100.0 (98.1-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95%CI)
NRAS Exon 2	c.34G>A	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.34G>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.34_35GG>TT	0	0	0	192	192	NE	100.0 (98.1-100)
	c.34_35GG>AA	0	0	0	192	192	NE	100.0 (98.1-100)
	c.34_36GGT>TGG	0	0	0	192	192	NE	100.0 (98.1-100)
	c.35G>A	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.35G>T	0	0	0	192	192	NE	100.0 (98.1-100)
	c.35G>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.37G>T	0	0	0	192	192	NE	100.0 (98.1-100)
	c.37G>C	2	0	0	190	192	100.0 (15.81-100)	100.0 (98.08-100)
	c.38G>A	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38_39GC>TT	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38_39GC>AA	0	0	0	192	192	NE	100.0 (98.1-100)
	c.38_39GC>AT	0	0	0	192	192	NE	100.0 (98.1-100)
NRAS Exon 3	c.175G>A	0	0	0	192	192	NE	100.0 (98.1-100)
	c.176C>G	0	0	0	192	192	NE	100.0 (98.1-100)
	c.181C>A	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.181C>G	0	0	0	192	192	NE	100.0 (98.1-100)
	c.182A>T	1	0	0	191	192	100.0 (2.5-100)	100.0 (98.09-100)
	c.182A>G	3	0	0	189	192	100.0 (29.24-100)	100.0 (98.07-100)
	c.183A>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.183A>T	0	0	0	192	192	NE	100.0 (98.1-100)
NRAS Exon 4	c.351A>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.351A>T	0	0	0	192	192	NE	100.0 (98.1-100)
	c.436G>A	0	0	0	192	192	NE	100.0 (98.1-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95%CI)
	c.436G>C	0	0	0	192	192	NE	100.0 (98.1-100)
	c.437C>T	0	0	0	192	192	NE	100.0 (98.1-100)

*A false negative variant was present in a sample which had three variants detected by *Praxis* (KRAS c.34G>A, NRAS c.35G>A and NRAS c.37G>C). All were detected by xT CDx, but KRAS c.34G>A was not reportable by xT CDx due to its low VAF (2.26%), which was below the CDx variant reporting threshold of 3%. However, for this specimen, both xT CDx and *Praxis* detected and reported two NRAS variants, and therefore generated the same clinical result; i.e., the patient is not wild-type for KRAS or NRAS and would therefore not receive the associated therapy. Therefore, although there was a discrepancy at the variant level in this specimen, for the purpose of establishing clinical validity this sample is considered as biomarker concordant.

The total allelic calls resulting from the xT CDx and *Praxis* Extended RAS Panel Devices are represented as a confusion table in Table 64. As described in footnotes to Tables 59, 62 and 63, one specimen contained three RAS variants (1 KRAS variant and 2 NRAS variants), therefore, the total count shown in the matrix is greater than the number of samples.

Table 64. Confusion table of *Praxis* and xT CDx results

		Praxis																			
		KRAS Exon 2							KRAS Exon 3		KRAS Exon 4			NRAS Exon 2			NRAS Exon 3				
		c.34 G>T	c.34 G>A	c.34 G>C	c.35 G>A	c.35 G>T	c.35 G>C	c.38 G>A	c.183 A>C	c.183 A>T	c.35 A>T	c.436 G>A	c.437 C >T	c.34 G>A	c.35G >A	c.37 G>C	c.181 C >A	c.182 A>T	c.182 A>G	Negative	Total
KRAS Exon 2	c.34 G>T	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	c.34 G>A	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	4
	c.34 G>C	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	c.35 G>A	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
	c.35 G>T	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
	c.35 G>C	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
	c.38 G>A	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	13
KRAS Exon 3	c.183 A>C	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
	c.183 A>T	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1

Praxis																						
		KRAS Exon 2							KRAS Exon 3		KRAS Exon 4			NRAS Exon 2			NRAS Exon 3				
KRAS Exon 4	c.351 A>T	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
	c.436 G>A	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4
	c.437 C>T	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
NRAS Exon 2	c.34 G>A	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
	c.35 G>A	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	0	0	1
	c.37 G>C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2*	0	0	0	0	2
NRAS Exon 3	c.181 C>A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
	c.182 A>T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	c.182 A>G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3
	Negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	108	108
	Total	4	3	2	29	11	3	13	2	1	1	4	1	1	1	2	1	1	3	109	192

*Label indicates one subject with more than one mutation tested

therascreen KRAS RGQ PCR kit

Concordance of xT CDx with *therascreen* was evaluated using a total of 250 samples. By defining the reference standard as the consensus calls between *therascreen* replicate 1 (TCD1) and replicate 2 (TCD2), xT CDx achieved a PPA of 98.86% (87/88) (95% CI [93.83, 99.97]) and NPA of 100.00% (162/162) (95% CI [97.75, 100.00]) as summarized in Table 65 below. PPA and NPA for every pairwise comparison are summarized in Table 67.

Table 65. Summary of concordance data using agreement between TCD1 and TCD2 (*therascreen*) as the reference

	TCD1+/TCD2+	TCD1-/TCD2-
xT CDx+	87 *	0
xT CDx-	1	162

*One sample had a KRAS variant c.34_36GGT>TGGp.G12W detected by xT CDx, whereas *therascreen* reported KRAS variant c.34G>Tp.G12C. Manual inspection of the pileup verified a consistent call by xT CDx. Discrepancy between xT CDx and *therascreen* results is likely due to a difference in the methodology used by these two devices. *therascreen* uses a PCR-based method that looks at specific nucleotide bases and is therefore not able to identify cases of multiple nucleotides changes in a single codon. However, in each case the sample was determined to be biomarker positive by both devices, yielding the same clinical conclusion and thus the results were considered concordant.

Concordance analysis showed that the upper bounds of 95% one-sided Confidence Interval (CI) were below 7% for all four NI hypothesis tests (sPPA1: 0.011, 95% CI [-0.031, 0.062]; sPPA2 0.011, 95% CI [-0.031, 0.062]; sNPA1: 0.000, 95% CI [-0.023, 0.023]; sNPA2: 0.000, 95% CI [-0.023, 0.023]). Concordance results are summarized in Table 66 below.

Table 66. Concordance table with TCD1, TCD2 (*therascreen*), and xT CDx results with eligible samples

	TCD1+			**TCD1-**		
	TCD2+	TCD2-	Total	TCD2+	TCD2-	Total
xT CDx+	87*	0	87	0	0	0
xT CDx-	1	0	0	0	162	163

Table 67. Concordance table with TCD1, TCD2 (*therascreen*), and xT CDx results with eligible samples

	% Agreement
PPA_{T1T2}	100.00%
NPA_{T1T2}	100.00%
PPA_{T2T1}	100.00%
NPA_{T2T1}	100.00%
$PPA_{T1xTCDx}$	98.86%
$NPA_{T1xTCDx}$	100.00%
$PPA_{T2xTCDx}$	98.86%
$NPA_{T2xTCDx}$	100.00%

PPA_{T1T2} is the PPA between *therascreen* replicate 1 (TCD1) and *therascreen* replicate 2 (TCD2) conditional on TCD1.

NPA_{T1T2} is the NPA between TCD1 and TCD2 conditional on TCD1.
$PPAT_{2T1}$ is the PPA between TCD1 and TCD2 conditional on TCD2.
NPA_{T2T1} is the NPA between TCD1 and TCD2 conditional on TCD2.
$PPA_{T1xTCDx}$ is the PPA between TCD1 and xT CDxconditional on TCD1.
$NPAT_{1xTCDx}$ is the NPA between TCD1 and xT CDx conditional on TCD1.
$PPAT_{2xTCDx}$ is the PPA between TCD1 and xT CDx conditional on TCD2.
$NPAT_{2xTCDx}$ is the NPA between TCD2 and xT CDx conditional on TCD2.

Overall positive and negative agreement was calculated using xT CDx and *therascreen* results for comparison. PPA and NPA calculated at the mutation and gene level for each sample are shown in Table 68 and Table 69.

Table 68. *therascreen* gene level positive and negative agreement in KRAS

Gene/Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95% CI)
KRAS/All	87	1	0	1134	1222	98.86 (93.83-99.97)	100.0 (99.68-100)

Table 69. *therascreen* mutation level positive and negative agreement in KRAS

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95% CI)
KRAS Exon 2	c.34G>T	6	0	0	244	250	100.0 (54.07-100)	100.0 (98.5-100)
	c.34G>A	4	0	0	246	250	100.0 (39.76-100)	100.0 (98.51-100)
	c.34G>C	2	0	0	248	250	100.0 (15.81-100)	100.0 (98.52-100)
	c.34_35GG>TT	0	0	0	250	250	NE	100.0 (98.54-100)
	c.34_35GG>AA	0	0	0	250	250	NE	100.0 (98.54-100)
	c.34_36GGT>TGG	1*	0	0	249	250	100.0 (2.5-100)	100.0 (98.53-100)
	c.35G>A	35	0	0	215	250	100.0 (90.0-100)	100.0 (98.3-100)
	c.35G>T	18	1	0	231	250	94.74 (73.97-99.87)	100.0 (98.42-100)
	c.35G>C	3	0	0	247	250	100.0 (29.24-100)	100.0 (98.52-100)
	c.37G>T	0	0	0	250	250	NE	100.0 (98.54-100)
	c.37G>C	0	0	0	250	250	NE	100.0 (98.54-100)
	c.38G>A	18	0	0	232	250	100.0 (81.47-100)	100.0 (98.42-100)

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95% CI)
	c.38_39GC>TT	0	0	0	250	250	NE	100.0 (98.54-100)
	c.38_39GC>AA	0	0	0	250	250	NE	100.0 (98.54-100)
	c.38_39GC>AT	0	0	0	250	250	NE	100.0 (98.54-100)
KRAS Exon 3	c.175G>A	0	0	0	250	250	NE	100.0 (98.54-100)
	c.176C>G	0	0	0	250	250	NE	100.0 (98.54-100)
	c.181C>A	0	0	0	250	250	NE	100.0 (98.54-100)
	c.181C>G	0	0	0	250	250	NE	100.0 (98.54-100)
	c.182A>T	0	0	0	250	250	NE	100.0 (98.54-100)
	c.182A>G	0	0	0	250	250	NE	100.0 (98.54-100)
	c.183A>C	0	0	0	250	250	NE	100.0 (98.54-100)
	c.183A>T	0	0	0	250	250	NE	100.0 (98.54-100)
KRAS Exon 4	c.351A>C	0	0	0	250	250	NE	100.0 (98.54-100)
	c.351A>T	0	0	0	250	250	NE	100.0 (98.54-100)
	c.436G>A	0	0	0	250	250	NE	100.0 (98.54-100)
	c.436G>C	0	0	0	250	250	NE	100.0 (98.54-100)
	c.437C>T	0	0	0	250	250	NE	100.0 (98.54-100)

*One sample had a KRAS variant detected by xT CDx as c.34_36GGT>TGG; however, the *therascreen* assay is not able to detect or report on variants at positions c.35 and c.36 and therefore only reported c.34G>T. Both xT CDx and *therascreen* identified the identical nucleic acid alteration (a G to T transversion at position c.34) and therefore this result is considered concordant.

The total allelic calls resulting from the xT CDx and *therascreen* KRAS RGQ PCR kit Devices are represented as a confusion matrix in Table 70. Note that the KRAS variant detected by xT CDx as c.34_36GGT>TGG is reported as concordant with the KRAS variant detected by the *therascreen* assay as c.34G>T. The *therascreen* assay is not able to detect or report on nucleic acid variants at positions c.35 and c.36 and therefore only reported c.34G>T. However, both xT CDx and *therascreen* identified the identical nucleic acid alteration (a G to T transversion at position c.34); and therefore this result is considered concordant.

Table 70. Confusion table of *therascreen* and xT CDx results

		therascreen										
		KRAS Exon 2										
			c.34 G>T	c.34 G>A	c.34 G>C	c.34_36GGT >TGG	c.35 G> A	c.35 G> T	c.35 G> C	c.38 G> A	Negative	Total
xT CDx	KRAS Exon 2	c.34G>T	6	0	0	0	0	0	0	0	0	6
		c.34G>A	0	4	0	0	0	0	0	0	0	4
		c.34G>C	0	0	2	0	0	0	0	0	0	2
		c.34_36GGT>TGG	1	0	0	0	0	0	0	0	0	1
		c.35G>A	0	0	0	0	35	0	0	0	0	35
		c.35G>T	0	0	0	0	0	18	0	0	0	19
		c.35G>C	0	0	0	0	0	0	3	0	0	3
		c.38G>A	0	0	0	0	0	0	0	18	0	18
		Negative	0	0	0	0	0	0	0	0	162	162
	Total	Total	7	4	2	0	35	18	3	18	163	250

All acceptance criteria were met for this study.

Overall concordance between xT CDx and *Praxis* is 100.00% (190/190), and concordance between xT CDx and *therascreen* KRAS RGQ PCR Kit is 99.60% (249/250).The non-inferiority analysis shows that NI margin to *Praxis* is less than 5%, and NI margin to *therascreen* is less than 7%, supporting the conclusion that the agreement between xT CDx and *Praxis* is non-inferior to the agreement

between two replicates of *Praxis* by a margin of 5%, and the agreement between xT CDx and *therascreen* KRAS RGQ PCR Kit is non-inferior to the agreement between two replicates of *therascreen* by a margin of 7%.

A higher overall invalid rate was observed for xT CDx in this study as compared to the invalid rate determined for the device in the Invalid Rate study. The higher invalid rate observed in this study can be attributed to the use of normal FFPE tissue instead of normal blood or saliva. Though matched normal FFPE tissue is not a claimed sample type for normal samples for xT CDx, the Normal Concordance study was conducted to support the use of normal FFPE tissue for variant calling in xT CDx validation studies. Matched normal blood or saliva specimens were commercially unavailable at the time these studies were conducted. Additionally, in this study 85.7% of failures before sequencing (48/56) were due to FFPE blocks purchased from the same vendor, suggesting that the method of preparation or long-term storage of these FFPE blocks may have caused a higher-than-expected invalid rate in this study.

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 of Medical Devices, 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 xT CDx using FFPE tissue or DNA extracted from FFPE tissue from a variety of cancer types. When the test is used in accordance with the directions provided, the sensitivity for detecting the tested variants is characterized based on clinical and non-clinical studies conducted for the device and described above. The clinical benefit of xT CDx in the detection of alterations listed in intended use was demonstrated in a clinical concordance study using previously approved CDx tests as the comparator methods. All studies based on a non-inferiority (NI) statistical testing approach passed the acceptance criteria specified in each study protocol. The concordance observed between xT CDx and the

approved CDx tests supports the effectiveness of xT CDx to identify patients whose tumors are positive for the alterations listed in the intended use and for which xT CDx results can be used to direct use of the associated therapeutics.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support this PMA as described above. xT CDx is an IVD test, which involves testing of DNA extracted from FFPE tumor and patient-matched normal tissue (blood or saliva). The assay can be performed using DNA extracted from an existing (archival) tumor tissue samples routinely collected as part of the diagnosis and patient care, and with either patient blood or saliva as the source of normal tissue.

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 receiving false results may undergo inappropriate treatment in regard to one of the therapies listed in the intended use and may experience adverse reactions associated with inappropriate therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

C. Benefit-Risk Determination

The probable benefit of the Tempus xT CDx device, which is a qualitative Next Generation Sequencing (NGS)-based in vitro diagnostic device intended for use in the tumor profiling of solid malignant neoplasms for substitutions (single nucleotide variants (SNVs) and multi-nucleotide variants (MNVs)) and insertion and deletion alterations (INDELs), as well as microsatellite instability (MSI), was demonstrated by a series of analytical validation studies. The analytical accuracy evaluated the detection of alterations by xT CDx was assessed by comparing it to results of a well-validated orthogonal method (OM). The comparison included 416 tumor specimens across 31 different tumor types with 1028 unique variants (736 SNVs, 22 MNVs, 71 insertions, 199 deletions) observed in 373 exons across 84 genes. The xT CDx tumor profiling analytical studies included insertions and deletions up to and greater than 25 bps in size. Concordance of variants was evaluated in both hotspot and non-hotspot regions, revealing robust performance with a PPA of 99.1% overall and an NPA of 100.0% overall. In addition, The detection of MSI status by xT CDx was assessed by comparison with results obtained using a validated OM (IHC staining of MLH1, MSH2, MSH6 and PMS2). A total set of 316 patient-matched tumor and normal samples representing 30 cancer types were sequenced with xT CDx. Of the 117 samples identified as positive by IHC testing, 110 were identified as MSI-H by xT CDx, yielding a PPA of 94.0% (95% CI: 88-98%). Of the 199 samples identified as

negative by IHC testing, 195 were identified as MSS by xT CDx, yielding a NPA of 98% (95% CI: 95-99%). The strength of this data indicates that this device has probable benefit for the tumor profiling of solid malignant neoplasms, including SNVs, MNVs, INDELS and MSI.

In addition, the probable benefit of the Tempus xT CDx as a companion diagnostic used for identifying patients with CRC who may not be eligible for treatment with cetuximab when mutations are detected in KRAS codons 12 or 13 or panitumumab when mutations are detected in exons 2, 3, or 4 of KRAS or NRAS was established a non-inferiority study, which revealed overall concordance between xT CDx and *Praxis* of 100.00% (190/190), and concordance between xT CDx and *therascreen* of 99.60% (249/250). The totality of the data supports a probable benefit for this device for tumor profiling and selection of cetuximab or panitumumab therapy for patients with eligible KRAS WT status.

For the tumor profiling claim and the companion diagnostic claims, there is probable risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. However, for tumor profiling results this test is not conclusive or prescriptive for the use of any specific therapeutic product and should not be viewed as a formal treatment recommendation. These tumor profiling results are intended to be used with professional guidelines, and do have potential risks associated with false negativity and false positivity, as well as a failure to provide results or issues with incorrect interpretation.

For the companion diagnostic, for the determination of KRAS WT status (no mutations in codon 12 and 13) for cetuximab and KRAS/NRAS WT status, (no mutation in exon 2, 3 and 4), there are attendant risks, described below. Patients who are deemed falsely not eligible for these drugs may be forgoing effective therapy, and patients who are deemed falsely eligible for these drugs may be treated with the drugs without the expected benefit.

These risks for the tumor profiling and companion diagnostic claims are partly mitigated by the analytical and clinical performance of the device respectively. Additional factors considered in determining probable risks and benefits for xT CDx included the representation of the variants in the analytical and clinical studies.

Patient perspectives were not collected as part of this PMA.

In conclusion, given the available information above, the data support that for xT CDx, 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 analytical validation and clinical concordance studies support the performance of xT CDx as an aid for the identification of cancer patients for whom the therapies listed in the Intended Use statement may be indicated.

XIV. CDRH DECISION

CDRH issued an approval order on April 28, 2023.

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

Zook, J. M. et al. Extensive sequencing of seven human genomes to characterize benchmark reference materials. Scientific Data 3:160025 (2016)

Li M. Statistical methods for clinical validation of follow-on companion diagnostic devices via an external concordance study. Statistics in Biopharmaceutical Research 8(3):1-24 (2016)

APPENDICES

Appendix 1. List of genes included in xT CDx

ABCB1	*CBFB*	*DNMT3A*	*FGFR2*	*HLA-G*	*MAGI2*	*PAK1*	*RANBP2*	*SUZ12*
ABCC3	*CBL*	*DOT1L*	*FGFR3*	*HNF1A*	*MALT1*	*PALB2*	*RARA*	*SYK*
ABL1	*CBLB*	*DPYD*	*FGFR4*	*HNF1B*	*MAP2K1*	*PALLD*	*RASA1*	*SYNE1*
ABL2	*CBLC*	*DYNC2H1*	*FH*	*HOXA11*	*MAP2K2*	*PAX3*	*RB1*	*TAF1*
ABRAXAS1	*CBR3*	*EBF1*	*FHIT*	*HOXB13*	*MAP2K4*	*PAX5*	*RBM10*	*TANC1*
ACTA2	*CCDC6*	*ECT2L*	*FLCN*	*HRAS*	*MAP3K1*	*PAX7*	*RECQL4*	*TAP1*
ACVR1 (ALK2)	*CCND1*	*EGF*	*FLT1*	*HSD11B2*	*MAP3K7*	*PAX8*	*RET*	*TAP2*
ACVR1B	*CCND2*	*EGFR*	*FLT3*	*HSD3B1*	*MAPK1*	*PBRM1*	*RHEB*	*TARBP2*
AGO1	*CCND3*	*EGLN1*	*FLT4*	*HSD3B2*	*MAX*	*PCBP1*	*RHOA*	*TBC1D12*
AJUBA	*CCNE1*	*EIF1AX*	*FNTB*	*HSP90AA1*	*MC1R*	*PDCD1*	*RICTOR*	*TBL1XR1*
AKT1	*CD19*	*ELF3*	*FOXA1*	*HSPH1*	*MCL1*	*PDCD1LG2*	*RINT1*	*TBX3*
AKT2	*CD22*	*ELOC (TCEB1)*	*FOXL2*	*IDH1*	*MDM2*	*PDGFRA*	*RIT1*	*TCF3*
AKT3	*CD274 (PDL1)*	*EMSY*	*FOXO1*	*IDH2*	*MDM4*	*PDGFRB*	*RNF139*	*TCF7L2*
ALK	*CD40*	*ENG*	*FOXO3*	*IDO1*	*MED12*	*PDK1*	*RNF43*	*TCL1A*
AMER1	*CD70*	*EP300*	*FOXP1*	*IFIT1*	*MEF2B*	*PHF6*	*ROS1*	*TERT**
APC	*CD79A*	*EPCAM*	*FOXQ1*	*IFIT2*	*MEN1*	*PHGDH*	*RPL5*	*TET2*
APLNR	*CD79B*	*EPHA2*	*FRS2*	*IFIT3*	*MET*	*PHLPP1*	*RPS15*	*TFE3*
APOB	*CDC73*	*EPHA7*	*FUBP1*	*IFNAR1*	*MGMT*	*PHLPP2*	*RPS6KB1*	*TFEB*
AR	*CDH1*	*EPHB1*	*FUS*	*IFNAR2*	*MIB1*	*PHOX2B*	*RPTOR*	*TFEC*
ARAF	*CDK12*	*EPHB2*	*G6PD*	*IFNGR1*	*MITF*	*PIAS4*	*RRM1*	*TGFBR1*
ARHGAP26	*CDK4*	*EPOR*	*GABRA6*	*IFNGR2*	*MKI67*	*PIK3C2B*	*RSF1*	*TGFBR2*
ARHGAP35	*CDK6*	*ERBB2 (HER2)*	*GALNT12*	*IFNL3*	*MLH1*	*PIK3CA*	*RUNX1*	*TIGIT*
ARID1A	*CDK8*	*ERBB3*	*GATA1*	*IKBKE*	*MLH3*	*PIK3CB*	*RUNX1T1*	*TMEM127*
ARID1B	*CDKN1A*	*ERBB4*	*GATA2*	*IKZF1*	*MLLT3*	*PIK3CD*	*RXRA*	*TMEM173*
ARID2	*CDKN1B*	*ERCC1*	*GATA3*	*IL10RA*	*MN1*	*PIK3CG*	*SCG5*	*TMPRSS2*

ARID5B	*CDKN1C*	*ERCC2*	*GATA4*	*IL15*	*MPL*	*PIK3R1*	*SDHA*	*TNF*
ASNS	*CDKN2A*	*ERCC3*	*GATA6*	*IL2RA*	*MRE11*	*PIK3R2*	*SDHAF2*	*TNFAIP3*
ASPSCR1	*CDKN2B*	*ERCC4*	*GEN1*	*IL6R*	*MS4A1*	*PIM1*	*SDHB*	*TNFRSF14*
ASXL1	*CDKN2C*	*ERCC5*	*GLI1*	*IL7R*	*MSH2*	*PLCG1*	*SDHC*	*TNFRSF17*
ATIC	*CEBPA*	*ERCC6*	*GLI2*	*ING1*	*MSH3*	*PLCG2*	*SDHD*	*TNFRSF9*
ATM	*CEP57*	*ERG*	*GNA11*	*INPP4B*	*MSH6*	*PML*	*SEC23B*	*TOP1*
ATP7B	*CFTR*	*ERRFI1*	*GNA13*	*IRF1*	*MTAP*	*PMS1*	*SEMA3C*	*TOP2A*
ATR	*CHD2*	*ESR1*	*GNAQ*	*IRF2*	*MTHFD2*	*PMS2*	*SETBP1*	*TP53*
ATRX	*CHD4*	*ETS1*	*GNAS*	*IRF4*	*MTHFR*	*POLD1*	*SETD2*	*TP63*
AURKA	*CHD7*	*ETS2*	*GPC3*	*IRS2*	*MTOR*	*POLE*	*SF3B1*	*TPM1*
AURKB	*CHEK1*	*ETV1*	*GPS2*	*ITPKB*	*MTRR*	*POLH*	*SGK1*	*TPMT*
AXIN1	*CHEK2*	*ETV4*	*GREM1*	*JAK1*	*MUTYH*	*POLQ*	*SH2B3*	*TRAF3*
AXIN2	*CIC*	*ETV5*	*GRIN2A*	*JAK2*	*MYB*	*POT1*	*SHH*	*TRAF7*
AXL	*CIITA*	*ETV6*	*GRM3*	*JAK3*	*MYC*	*POU2F2*	*SLC26A3*	*TSC1*
B2M	*CKS1B*	*EWSR1*	*GSTP1*	*JUN*	*MYCL*	*PPARA*	*SLC47A2*	*TSC2*
BAP1	*CREBBP*	*EZH2*	*H19*	*KAT6A*	*MYCN*	*PPARD*	*SLC9A3R1*	*TSHR*
BARD1	*CRKL*	*FAM46C*	*H3F3A*	*KDM5A*	*MYD88*	*PPARG*	*SLIT2*	*TUSC3*
BCL10	*CRLF2*	*FANCA*	*HAS3*	*KDM5C*	*MYH11*	*PPM1D*	*SLX4*	*TYMS*
BCL11B	*CSF1R*	*FANCB*	*HAVCR2*	*KDM5D*	*NBN*	*PPP1R15A*	*SMAD2*	*U2AF1*
BCL2	*CSF3R*	*FANCC*	*HDAC1*	*KDM6A*	*NCOR1*	*PPP2R1A*	*SMAD3*	*UBE2T*
BCL2L1	*CTC1*	*FANCD2*	*HDAC2*	*KDR*	*NCOR2*	*PPP2R2A*	*SMAD4*	*UGT1A1*
BCL2L11	*CTCF*	*FANCE*	*HDAC4*	*KEAP1*	*NF1*	*PPP6C*	*SMARCA1*	*UGT1A9*
BCL6	*CTLA4*	*FANCF*	*HGF*	*KEL*	*NF2*	*PRCC*	*SMARCA4*	*UMPS*
BCL7A	*CTNNA1*	*FANCG*	*HIF1A*	*KIF1B*	*NFE2L2*	*PRDM1*	*SMARCB1*	*VEGFA*
BCLAF1	*CTNNB1*	*FANCI*	*HIST1H1E*	*KIT*	*NFKBIA*	*PREX2*	*SMARCE1*	*VEGFB*
BCOR	*CTRC*	*FANCL*	*HIST1H3B*	*KLF4*	*NHP2*	*PRKAR1A*	*SMC1A*	*VHL*
BCORL1	*CUL1*	*FANCM*	*HIST1H4E*	*KLHL6*	*NKX2-1*	*PRKDC*	*SMC3*	*VSIR*
BCR	*CUL3*	*FAS*	*HLA-A*	*KLLN*	*NOP10*	*PRKN*	*SMO*	*WEE1*
BIRC3	*CUL4A*	*FAT1*	*HLA-B*	*KMT2A*	*NOTCH1*	*PRSS1*	*SOCS1*	*WNK1*

BLM	*CUL4B*	*FBXO11*	*HLA-C*	*KMT2B*	*NOTCH2*	*PTCH1*	*SOD2*	*WNK2*
BMPR1A	*CUX1*	*FBXW7*	*HLA-DMA*	*KMT2C*	*NOTCH3*	*PTCH2*	*SOX10*	*WRN*
BRAF	*CXCR4*	*FCGR2A*	*HLA-DMB*	*KMT2D*	*NOTCH4*	*PTEN*	*SOX2*	*WT1*
BRCA1	*CYLD*	*FCGR3A*	*HLA-DOA*	*KRAS*	*NPM1*	*PTPN11*	*SOX9*	*XPA*
BRCA2	*CYP1B1*	*FDPS*	*HLA-DOB*	*L2HGDH*	*NQO1*	*PTPN13*	*SPEN*	*XPC*
BRD4	*CYP2D6*	*FGF1*	*HLA-DPA1*	*LAG3*	*NRAS*	*PTPN22*	*SPINK1*	*XPO1*
BRIP1	*CYP3A5*	*FGF10*	*HLA-DPB1*	*LATS1*	*NRG1*	*PTPRD*	*SPOP*	*XRCC1*
BTG1	*CYSLTR2*	*FGF14*	*HLA-DPB2*	*LCK*	*NSD1*	*PTPRT*	*SPRED1*	*XRCC2*
BTK	*DAXX*	*FGF2*	*HLA-DQA1*	*LDLR*	*NSD2*	*QKI*	*SRC*	*XRCC3*
BUB1B	*DDB2*	*FGF23*	*HLA-DQA2*	*LEF1*	*NT5C2*	*RAC1*	*SRSF2*	*YEATS4*
C11orf65	*DDR2*	*FGF3*	*HLA-DQB1*	*LMNA*	*NTHL1*	*RAD21*	*STAG2*	*ZFHX3*
C3orf70	*DDX3X*	*FGF4*	*HLA-DQB2*	*LMO1*	*NTRK1*	*RAD50*	*STAT3*	*ZMYM3*
C8orf34	*DICER1*	*FGF5*	*HLA-DRA*	*LRP1B*	*NTRK2*	*RAD51*	*STAT4*	*ZNF217*
CALR	*DIRC2*	*FGF6*	*HLA-DRB1*	*LYN*	*NTRK3*	*RAD51B*	*STAT5A*	*ZNF471*
CARD11	*DIS3*	*FGF7*	*HLA-DRB5*	*LZTR1*	*NUDT15*	*RAD51C*	*STAT5B*	*ZNF620*
CARM1	*DIS3L2*	*FGF8*	*HLA-DRB6*	*MAD2L2*	*NUP98*	*RAD51D*	*STAT6*	*ZNF750*
CASP8	*DKC1*	*FGF9*	*HLA-E*	*MAF*	*OLIG2*	*RAD54L*	*STK11*	*ZNRF3*
CASR	*DNM2*	*FGFR1*	*HLA-F*	*MAFB*	*P2RY8*	*RAF1*	*SUFU*	*ZRSR2*

Appendix 2: List of genes/exons with consistently low coverage in the Accuracy of Short Variants study.

Eleven autosomal exons profiled by xT CDx in the Accuracy of Short Variants study exhibited mean coverage below 150x across all samples. All of these exons are in regions known to be difficult to sequence according to the Genome in a Bottle consortium (Appendix 2 - Table). Low coverage exons were compared against the GIAB genome UnionAllDifficult bed file, which aggregates all tandem repeats, all homopolymers >6bp, all imperfect homopolymers >10bp, all difficult to map regions, all segmental duplications, GC <25% or >65%, "Bad Promoters", chrX/Y XTR and ampliconic, satellites and "OtherDifficult" regions (including regions from the T2T-consortium for GRCh38 only). Low complexity sequence regions, include homopolymers, STRs, VNTRs and other locally repetitive sequences, and low mappability regions denote regions where short read mapping can be challenging. “OtherDifficult" regions include miscellaneous difficult regions such as T cell and B cell VDJ regions that somatically recombine, chromosomes 2, 14 and 22, rare haplotype boundaries in GRCh38, and gnomAD inbreedingcoeff variants (Zook 2020).

Appendix 2 - Table. Low coverage exons with sequencing difficulty annotation

Chr	Start	End	Gene	Exon range (nucleotide positions)	All samples min	All samples mean	All samples max	All samples std	Annotation
1	23037475	23037536	EPHB2	23037475 - 23037536	0	122.0730235	795.6229508	108.1506985	GCcontent65to100,GCcontent65to100,UnionAllDifficult,LowComplexity,LowComplexity
1	120572528	120572566	NOTCH2	120572528 - 120572566	0.5	51.35172987	1017.789474	88.82429363	allOtherDifficultregions,SegmentalDuplication,lowMappabilityAll,UnionAllDifficult
1	120572528	120572610	NOTCH2	120572528 - 120572610	2.182926829	83.77764277	1157.317073	105.6587863	allOtherDifficultregions,SegmentalDuplication,lowMappabilityAll,UnionAllDifficult
Chr	Start	End	Gene	Exon range (nucleotide positions)	All samples min	All samples mean	All samples max	All samples std	Annotation
10	103535625	103535657	FGF8	103535625 - 103535657	0	132.5399074	811	104.2809873	GCcontent65to100,UnionAllDifficult

2	109363166	109363254	RANBP2	109363166 - 109363254	0	83.8682619	475.625	65.8777973	SegmentalDuplication,lowMappabilityAll,UnionAllDifficult
21	34634865	34635021	IFNAR2	34634865 - 34635021	0.5384615385	76.53522915	433.2115385	55.9671493	UnionAllDifficult
6	135502651	135502674	MYB	135502651 - 135502674	0	147.9806646	2229.695652	211.0292837	GCcontent65to100,UnionAllDifficult
7	6013029	6013173	PMS2	6013029 - 6013173	0	91.12160794	1150.131944	80.45770267	SegmentalDuplication,lowMappabilityAll,UnionAllDifficult
9	97873477	97873627	FANCC	97873477 - 97873627	0.1	16.79011746	133.1933333	15.45563374	GCcontent65to100,UnionAllDifficult
9	101867487	101867584	TGFBR1	101867487 - 101867584	1.340206186	100.027671	754.7319588	96.87888282	GCcontent65to100,UnionAllDifficult,LowComplexity
9	137218477	137218505	RXRA	137218477 - 137218505	0	115.076636	545.1785714	78.80232852	GCcontent65to100,UnionAllDifficult