TEMPUS

xT CDx

TECHNICAL INFORMATION

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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)

Contraindications

There are no known contraindications.

Limitations

- For in vitro diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The acceptable preparation method for xT CDx tumor specimens is formalin-fixation and paraffin-embedding (FFPE). Other preparations have not been evaluated.
- The test is designed to report out somatic variants and is not intended to report germline variants. xT CDx sequences tumor and patient-matched normal samples to allow personalized subtraction of germline variants from tumor sequencing results.
- xT CDx requires a minimum tumor percentage of 20% for detection of variants, with tumor content enrichment recommended for specimens with tumor percentage lower than 20%. This assay may not detect variants if the proportion of tumor cells in the sample is less than 20%. xT CDx requires a minimum tumor percentage of 30% in order to determine MSI status.
- 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.
- A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
- The clinical validity of the device to guide MSI-related treatment decisions has not been established. MSI status is based on genome-wide analysis of 239 microsatellite loci and is not based on the 5 or 7 MSI loci described in current clinical practice guidelines. The threshold for MSI-H/MSS was determined by analytical concordance to comparator assays (IHC and PCR) using multiple cancer types. An MSI result of Equivocal indicates that microsatellite instability status of MSI-H or MSS could not be determined.
- Performance of xT CDx has not been established for detection of insertions or deletions larger than 25 base pairs.
- xT CDx is only approved for use with Tempus pre-qualified Illumina NovaSeq 6000 instruments.
- The test is intended to be performed on specific serial number-controlled instruments by Tempus Labs, Inc.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.

Test Principle

The CDx Assay (xT CDx) is a single site next generation sequencing (NGS) assay. The assay includes reagents, software, instruments, and procedures for testing DNA extracted from formalin-fixed, paraffin embedded (FFPE) tumor specimens and matched normal saliva or blood specimens. The assay employs DNA extraction methods from routinely obtained FFPE tissue samples and matched normal saliva or blood samples. Extracted DNA undergoes whole-genome shotgun library construction and hybridization-based capture of specified regions from 648 cancer-related genes (including intronic overhangs and selected promoter regions), and 239 loci for MSI. Refer to Table 1 for a complete list of genes included in xT CDx. Using the IlluminaNovaSeq 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), insertions, and deletions in coding and noncoding genomic regions targeted by the assay. Additionally, MSI status is reported based on a genomic signature.

Table 1. xT CDx Gene List

ABCB1	*BUB1B*	*CYLD*	*FANCF*	*GRM3*	*INPP4B*	*MPL*	*PIAS4*	*RPL5*	*TBC1D12*	*CUL3*
ABCC3	*C11orf65*	*CYP1B1*	*FANCG*	*GSTP1*	*IRF1*	*MRE11*	*PIK3C2B*	*RPS15*	*TBL1XR1*	*CUL4A*
ABL1	*C3orf70*	*CYP2D6*	*FANCI*	*H19*	*IRF2*	*MS4A1*	*PIK3CA*	*RPS6KB1*	*TBX3*	*CUL4B*
ABL2	*C8orf34*	*CYP3A5*	*FANCL*	*H3F3A*	*IRF4*	*MSH2*	*PIK3CB*	*RPTOR*	*TCF3*	*CYSLTR2*
ABRAXAS1	*CALR*	*DAXX*	*FANCM*	*HAS3*	*IRS2*	*MSH3*	*PIK3CD*	*RSF1*	*TCF7L2*	*EIF1AX*
ACTA2	*CARD11*	*DDB2*	*FAS*	*HAVCR2*	*ITPKB*	*MSH6*	*PIK3CG*	*RUNX1*	*TCL1A*	*FUS*
ACVR1B	*CASP8*	*DDR2*	*FAT1*	*HDAC1*	*JAK1*	*MTAP*	*PIK3R1*	*RUNX1T1*	*TERT**	*GABRA6*
AJUBA	*CASR*	*DDX3X*	*FBXO11*	*HDAC2*	*JAK2*	*MTHFR*	*PIK3R2*	*RXRA*	*TET2*	*GLI2*
AKT1	*CBFB*	*DICER1*	*FBXW7*	*HDAC4*	*JAK3*	*MTOR*	*PIM1*	*SCG5*	*TGFBR2*	*HOXA11*
AKT2	*CBL*	*DIRC2*	*FCGR2A*	*HGF*	*JUN*	*MTRR*	*PLCG2*	*SDHA*	*TIGIT*	*HSD11B2*
AKT3	*CBLB*	*DIS3*	*FCGR3A*	*HIF1A*	*KAT6A*	*MUTYH*	*PML*	*SDHAF2*	*TMEM127*	*HSD3B1*
ALK	*CBLC*	*DIS3L2*	*FDPS*	*HIST1H1E*	*KDM5A*	*MYB*	*PMS1*	*SDHB*	*TMEM173*	*HSD3B2*
AMER1	*CBR3*	*DKC1*	*FGF1*	*HIST1H3B*	*KDM5C*	*MYC*	*PMS2*	*SDHC*	*TMPRSS2*	*KDM5D*
APC	*CCDC6*	*DNM2*	*FGF10*	*HIST1H4E*	*KDM6A*	*MYCL*	*POLD1*	*SDHD*	*TNF*	*KLF4*
APLNR	*CCND1*	*DNMT3A*	*FGF14*	*HLA-A*	*KDR*	*MYCN*	*POLE*	*SEC23B*	*TNFAIP3*	*L2HGDH*
APOB	*CCND2*	*DOT1L*	*FGF2*	*HLA-B*	*KEAP1*	*MYD88*	*POLH*	*SEMA3C*	*TNFRSF14*	*LATS1*
AR	*CCND3*	*DPYD*	*FGF23*	*HLA-C*	*KEL*	*MYH11*	*POT1*	*SETBP1*	*TNFRSF17*	*LCK*
ARAF	*CCNE1*	*DYNC2H1*	*FGF3*	*HLA-DMA*	*KIF1B*	*NBN*	*POU2F2*	*SETD2*	*TNFRSF9*	*MAGI2*
ARHGAP26	*CD19*	*EBF1*	*FGF4*	*HLA-DMB*	*KIT*	*NCOR1*	*PPARG*	*SF3B1*	*TOP1*	*MN1*
ARHGAP35	*CD22*	*ECT2L*	*FGF5*	*HLA-DOA*	*KLHL6*	*NCOR2*	*PPP1R15A*	*SGK1*	*TOP2A*	*MTHFD2*
ARID1A	*CD274*	*EGF*	*FGF6*	*HLA-DOB*	*KLLN*	*NF1*	*PPP2R1A*	*SH2B3*	*TP53*	*NOTCH4*
ARID1B	*CD40*	*EGFR*	*FGF7*	*HLA-DPA1*	*KMT2A*	*NF2*	*PPP2R2A*	*SLC26A3*	*TP63*	*OLIG2*
ARID2	*CD70*	*EGLN1*	*FGF8*	*HLA-DPB1*	*KMT2B*	*NFE2L2*	*PPP6C*	*SLC47A2*	*TPM1*	*PHGDH*
ARID5B	*CD79A*	*ELF3*	*FGF9*	*HLA-DPB2*	*KMT2C*	*NFKBIA*	*PRCC*	*SLIT2*	*TPMT*	*PHLPP1*
ASNS	*CD79B*	*ELOC*	*FGFR1*	*HLA-DQA1*	*KMT2D*	*NHP2*	*PRDM1*	*SLX4*	*TRAF3*	*PHLPP2*
ASXL1	*CDC73*	*EMSY*	*FGFR2*	*HLA-DQA2*	*KRAS*	*NKX2-1*	*PREX2*	*SMAD2*	*TSC1*	*PLCG1*
ATIC	*CDH1*	*ENG*	*FGFR3*	*HLA-DQB1*	*LAG3*	*NOP10*	*PRKAR1A*	*SMAD3*	*TSC2*	*POLQ*
ATM	*CDK12*	*EP300*	*FGFR4*	*HLA-DQB2*	*LDLR*	*NOTCH1*	*PRKN*	*SMAD4*	*TSHR*	*PPARA*
ATP7B	*CDK4*	*EPCAM*	*FH*	*HLA-DRA*	*LEF1*	*NOTCH2*	*PRSS1*	*SMARCA1*	*TUSC3*	*PPARD*
ATR	*CDK6*	*EPHA2*	*FHIT*	*HLA-DRB1*	*LMNA*	*NOTCH3*	*PTCH1*	*SMARCA4*	*TYMS*	*PPM1D*
ATRX	*CDK8*	*EPHA7*	*FLCN*	*HLA-DRB5*	*LMO1*	*NPM1*	*PTCH2*	*SMARCB1*	*U2AF1*	*PRKDC*
AURKA	*CDKN1A*	*EPHB1*	*FLT1*	*HLA-DRB6*	*LRP1B*	*NQO1*	*PTEN*	*SMARCE1*	*UBE2T*	*PTPRT*

AURKB	*CDKN1B*	*EPHB2*	*FLT3*	*HLA-E*	*LYN*	*NRAS*	*PTPN11*	*SMC1A*	*UGT1A1*	*RHEB*
AXIN1	*CDKN1C*	*EPOR*	*FLT4*	*HLA-F*	*LZTR1*	*NRG1*	*PTPN13*	*SMC3*	*UGT1A9*	*RRM1*
AXIN2	*CDKN2A*	*ERBB2*	*FNTB*	*HLA-G*	*MAD2L2*	*NSD1*	*PTPN22*	*SMO*	*UMPS*	*SHH*
AXL	*CDKN2B*	*ERBB3*	*FOXA1*	*HNF1A*	*MAF*	*NSD2*	*PTPRD*	*SOCS1*	*VEGFA*	*SLC9A3R1*
B2M	*CDKN2C*	*ERBB4*	*FOXL2*	*HNF1B*	*MAFB*	*NT5C2*	*QKI*	*SOD2*	*VHL*	*SYNE1*
BAP1	*CEBPA*	*ERCC1*	*FOXO1*	*HOXB13*	*MALT1*	*NTHL1*	*RAC1*	*SOX10*	*VSIR*	*TFE3*
BARD1	*CEP57*	*ERCC2*	*FOXO3*	*HRAS*	*MAP2K1*	*NTRK1*	*RAD21*	*SOX2*	*WEE1*	*TFEB*
BCL10	*CFTR*	*ERCC3*	*FOXP1*	*HSP90AA1*	*MAP2K2*	*NTRK2*	*RAD50*	*SOX9*	*WRN*	*TFEC*
BCL11B	*CHD2*	*ERCC4*	*FOXQ1*	*HSPH1*	*MAP2K4*	*NTRK3*	*RAD51*	*SPEN*	*WT1*	*TGFBR1*
BCL2	*CHD4*	*ERCC5*	*FRS2*	*IDH1*	*MAP3K1*	*NUDT15*	*RAD51B*	*SPINK1*	*XPA*	*TRAF7*
BCL2L1	*CHEK1*	*ERCC6*	*FUBP1*	*IDH2*	*MAP3K7*	*NUP98*	*RAD51C*	*SPOP*	*XPC*	*VEGFB*
BCL2L11	*CHEK2*	*ERG*	*G6PD*	*IDO1*	*MAPK1*	*P2RY8*	*RAD51D*	*SPRED1*	*XPO1*	*WNK1*
BCL6	*CIC*	*ERRFI1*	*GALNT12*	*IFIT1*	*MAX*	*PAK1*	*RAD54L*	*SRC*	*XRCC1*	*WNK2*
BCL7A	*CIITA*	*ESR1*	*GATA1*	*IFIT2*	*MC1R*	*PALB2*	*RAF1*	*SRSF2*	*XRCC2*	*ZMYM3*
BCLAF1	*CKS1B*	*ETS1*	*GATA2*	*IFIT3*	*MCL1*	*PALLD*	*RANBP2*	*STAG2*	*XRCC3*	*AGO1*
BCOR	*CREBBP*	*ETS2*	*GATA3*	*IFNAR1*	*MDM2*	*PAX3*	*RARA*	*STAT3*	*YEATS4*	*TARBP2*
BCORL1	*CRKL*	*ETV1*	*GATA4*	*IFNAR2*	*MDM4*	*PAX5*	*RASA1*	*STAT4*	*ZFHX3*	
BCR	*CRLF2*	*ETV4*	*GATA6*	*IFNGR1*	*MED12*	*PAX7*	*RB1*	*STAT5A*	*ZNF217*	
BIRC3	*CSF1R*	*ETV5*	*GEN1*	*IFNGR2*	*MEF2B*	*PAX8*	*RBM10*	*STAT5B*	*ZNF471*	
BLM	*CSF3R*	*ETV6*	*GLI1*	*IFNL3*	*MEN1*	*PBRM1*	*RECQL4*	*STAT6*	*ZNF620*	
BMPR1A	*CTC1*	*EWSR1*	*GNA11*	*IKBKE*	*MET*	*PCBP1*	*RET*	*STK11*	*ZNF750*	

**promoter region also sequenced*

Summary and Explanation

xT CDx is a companion diagnostic (CDx) test for two therapeutic indications. Information generated by this test is an aid in the identification of patients who are most likely to benefit from the specific therapeutic products identified in the indications for use. In addition to use as a CDx, xT CDx identifies cancer-relevant alterations in genes identified in Table 1 that may inform patient management in accordance with professional guidelines.

xT CDx uses DNA extracted from FFPE tumor tissue, and from patient-matched normal blood or saliva tissue, to perform whole-genome shotgun library construction and hybridization-based capture followed by uniform and deep sequencing on Illumina NovaSeq 6000 sequencers qualified by Tempus. Following the sequencing of both the tumor specimen and the patient-matched normal sample, custom software is used to accurately identify somatic variants in the tumor by filtering out germline variants identified from a patient's normal DNA.

This allows identification of tumor-specific genomic biomarkers, including substitutions (single nucleotide variants, SNVs and multi-nucleotide variants, MNVs), insertion and deletion variants (INDELs); and microsatellite instability (MSI). The output of xT CDx includes information derived from the FDA-recognized content of OncoKB®, Memorial Sloan Kettering

Cancer Center's precision oncology knowledge base (https://www.oncokb.org). xT CDx results are presented in three categories:

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

Level 2: Cancer Mutations with Evidence of Clinical Significance

Level 3: Cancer Mutations with Potential Clinical Significance

The xT CDx Assay includes four critical checks conducted across the assay workflow to closely monitor assay performance and ensure that only high-quality data are generated and used for biomarker detection. These checks operate at each step of the assay as follows:

1. DNA Extraction (QC1)
2. Library Preparation (QC2)
3. Hybridization Capture (QC3)
4. Sequencing (QC4)

Test Kit Contents

The xT CDx Assay includes specimen collection and shipping kits for each specimen type used with the assay. These kits include specimen preparation instructions, shipping instructions, and a return shipping label.

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Tempus Labs Laboratory.

Sample Collection and Test Ordering

To order the xT CDx Assay, a test requisition form must be fully completed and signed by an ordering physician or authorized medical professional. Specimen preparation and mailing instructions are provided in the Specimen Kit.

For more detailed information, including Performance Characteristics, please find the FDA Summary of Safety and Effectiveness Data at: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P210011.

Instruments

xT CDx uses Illumina NovaSeq 6000 Sequencers qualified by Tempus, high throughput sequencing systems employing sequencing-by-synthesis chemistry.

Performance Characteristics

Performance characteristics were established using DNA derived from a wide range of FFPE tissue types along with patient-matched normal (blood or saliva) specimens. Studies included CDx variants and cancer types as well as a broad

range of representative alteration types, including substitutions (SNVs, MNVs) and INDELs (insertions, deletions) in various genomic contexts across a number of genes. Analysis of the genomic signature for MSI was also performed.

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 interlibrary 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 >98% of exons sequenced with a read depth ≥150x. The interlibrary mean coverage for all targeted hotspots ranged from 564x to 1557x (mean of 1042x). The coverage of target regions supports calling of variants by xT CDx at a VAF as low as 3% for substitutions and 5% for INDELs at hotspots, and 5% for substitutions and 10% for INDELs at non-hotspots.

2. Accuracy

The detection of alterations by xT CDx was compared to results of an externally validated orthogonal method (OM). Overall, there were 114 overlapping genes between the two assays. The comparison between SNVs, MNVs, insertions, and deletions detected by xT CDx and the OM included 416 samples representing 31 different tumor types. The distribution of tumor types is provided in Table 2, below.

Table 2. Distribution of Cancer Types for Characterization of Tumor Profiling Accuracy

Cancer Type	Number of Samples
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
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

Cancer Type	Number of Samples
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

Concordance was evaluated in both hotspot and non-hotspot regions. PPA and NPA were determined for each variant type to assess the accuracy of xT CDx tumor profiling. 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 OM only evaluates tumor samples, 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 OM were identified as germline variants by xT CDx (Table 3). However, because the OM is unable to distinguish germline from somatic variants 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 4, below, for substitutions and INDELs.

Table 3. 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

Table 4. Concordance for Short Variants (Substitutions and INDELs) Relative to the Orthogonal Method (OM)

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%]

For hotspot concordance analysis with the OM, reported variants in hotspot regions overlapping with OM 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 OM targeted regions included 214 total Base Pairs. In hotspots, a total of 192 reported variants from both assays were evaluated, including 187 substitutions (50 unique SNVs, 3 unique MNVs) across 10 genes, and 5 INDELs (2 unique insertions and 3 unique deletions) across 4 genes. The total variant counts of each classification across all study samples were used to calculate the PPA and NPA for Substitutions and INDELS within hotspot regions as metrics to evaluate the accuracy of the device (Table 5).

Table 5. Concordance Summary for Short Variants (Substitutions and INDELs) within Hotspot Regions Relative to the Orthogonal Method

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%]

The detection of specific KRAS and NRAS CDx variants in the 69 colorectal cancer samples tested with the OM was evaluated. Of the 31 CDx variants identified by the OM, 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 the OM, 648 were identified as negative by xT CDx, yielding a NPA of 99.8% (95% CI: 99.1-100.0%).

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 distribution of tumor types is provided in Table 6, below.

Table 6. Distribution of Cancer Types for Characterization of MSI Accuracy

Cancer Type	Number of samples	Abnormal IHC Number of MSI-H (by IHC)	Normal IHCNumber of MSS (by IHC)
CRC/EC*	108	75	33
non-CRC/non-EC**	208	42	166
Total	316	117	199

* colorectal or endometrial cancer
** non-colorectal, non-endometrial cancer

The reported MSI status from xT CDx was compared with results of IHC staining and used to calculate the PPA and NPA for MSI. 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%) Results of MSI concordance testing are provided in Tables 7 and 8, below.

Table 7. MSI Concordance Between xT CDx and IHC

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

Table 8. Agreement for MSI Status Overall and by Cancer Type

Cancer Type	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%]

* colorectal or endometrial cancer
** non-colorectal, non-endometrial cancer

3. Precision

3.1 PRECISION IN WELL-CHARACTERIZED MATERIAL

The panel-wide precision/reproducibility of xT CDx was assessed for detecting SNVs and INDELs 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 preparation 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 9 shows the Coefficient of Variation (CV) distribution for all 2673 variants analyzed. 95.5% of samples had a CV below 10%. Across all samples, the mean CV was 3.7% +/- 3.9%. Table 10 shows Mean %CV by zygosity of the variant, as declared in the GIAB variant call file (VCF) and type variant.

Table 9. Distribution of Variants by %CV in Well-Characterized Reference Material

	CV < 10%	10% ≤ CV < 15%	15% ≤ CV < 20%	20% < CV
Number of Variants	2552	73	24	24
Percent of Variants	95.5%	2.7%	0.9%	0.9%

Table 10. 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 (%CV)	SNVs Only (%CV)	INDELs Only (%CV)
All[1]	3.7% +/- 3.8%	3.5% +/- 3.5%	7.3% +/- 6.5%
Homozygous Only	0.23% +/- 0.72%	0.14% +/- 0.39%	1.8% +/- 2.1%
Heterozygous Only	5.3% +/- 3.2%	5.3% +/- 3.1%	7.9% +/- 5.6%

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

3.2 PANEL-WIDE PRECISION IN CLINICAL SPECIMENS

Panel-wide precision 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, and instruments. A total of 317 replicates contributed to the evaluation of precision. The distribution of tumor types is provided in Table 11, below.

[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)

Table 11. Distribution of Cancer Types for Characterization of Panel-Wide Precision

Cancer Type	Number of Samples
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, reagent lots, and instruments) for all specimens and replicates was 94.5%, and 97.0% for variants with a VAF ≥15%. Results are provided in Table 12.

Table 12. Precision by Variant Type and Variant Allele Fraction (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)
	≥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)

3.3 PRECISION FOR DETERMINATION OF MSI STATUS

All 49 unique specimens and 317 replicates were evaluated for MSI precision. Of these, 46/49 (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.

3.4 PRECISION FOR KRAS AND NRAS DETECTION

Precision of detection of alterations associated with CDx claims was evaluated independently of panel-wide precision. Intra-run (run on same plate under same conditions) and inter-run (run on different plates under different conditions)

conditions were assessed and compared across multiple instruments, reagent lots, days, and operators. 18 different CDx variants across all relevant exons of each CDx gene were included in the study. Included variants are provided in Table 13.

Table 13. Variants evaluated for Precision of KRAS and NRAS Detection

Gene	Variant	Number of Specimens
KRAS	p.Gly12Ser	1
KRAS	p.Gly12Arg	1
KRAS	p.Gly12Ala	1
KRAS	p.Gly12Cys	2
KRAS	p.Gly12Asp	5
KRAS	p.Gly12Val	1
KRAS	p.Gly13Asp	1
KRAS	p.Gly13Cys	1
KRAS	p.Ala59Thr	1
KRAS	p.GlyGln60GlyLys	1
KRAS	p.Gln61Arg	1
KRAS	p.Ala146Pro	1
KRAS	p.Ala146Thr	1
NRAS	p.Gly12Val	2
NRAS	p.Gly13Arg	1
NRAS	p.Gln61Leu	1
NRAS	p.Gln61His	1
NRAS	p.Ala146Val*	1
N/A	wild type	4

* evaluated using a cell line, all other variants were evaluated in clinical specimens

522 total replicates across 26 unique CRC samples, and 24 replicates from one cell line, were evaluated; one clinical sample included two variants. 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). Precision results by variant are shown in Table 14, a summary of results by gene is shown in Table 15.

Table 14. Precision for KRAS and NRAS Detection by Exon and Variant

Gene	Exon	Variant	n	True Positive	False Negative	% Correct Call	95% CI
KRAS	2	All KRAS Exon 2	242	241	1	99.6	(97.7, 100.0)
	2	p.Gly12Ala	18	18	0	100	(81.5, 100)

Gene	Exon	Variant	n	True Positive	False Negative	% Correct Call	95% CI
	2	p.Gly12Arg	19	19	0	100	(82.4, 100)
	2	p.Gly12Asp	102	101	1	99	(94.7, 100.0)
	2	p.Gly12Cys	43	43	0	100	(91.8, 100)
	2	p.Gly12Ser	23	23	0	100	(85.2, 100)
	2	p.Gly12Val	22	22	0	100	(84.6, 100)
	2	p.Gly13Asp	15	15	0	100	(78.2, 100)
	3	All KRAS Exon 3	60	60	0	100	(94.0, 100)
	3	p.Ala59Thr	19	19	0	100	(82.4, 100)
	3	p.Gln61Arg	19	19	0	100	(82.4, 100)
	3	p.GlyGln60GlyLys	22	22	0	100	(84.6, 100)
	4	All KRAS Exon 4	39	39	0	100	(91.0, 100)
	4	p.Ala146Pro	20	20	0	100	(83.2, 100)
	4	p.Ala146Thr	19	19	0	100	(82.4, 100)
NRAS	2	All NRAS Exon 2	56	56	0	100	(93.6, 100)
	2	p.Gly12Val	39	39	0	100	(91.0, 100)
	2	p.Gly13Arg	17	17	0	100	(80.5, 100)
	3	All NRAS Exon 3	37	37	0	100	(90.5, 100)
	3	p.Gln61His	17	17	0	100	(80.5, 100)
	3	p.Gln61Leu	20	20	0	100	(83.2, 100)
	4	All NRAS Exon 4	24	24	0	100	(85.8, 100)
	4	p.Ala146Val	24	24	0	100	(85.8, 100)

Table 15. Positive and Negative Percent Agreement for CDx Biomarkers by Gene and Overall

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)**

4. Analytical Sensitivity

4.1 TUMOR PURITY

The minimum tumor purity for detection of CDx variants was determined by evaluating 31 CRC FFPE specimens (and patient-matched normal tissue) with known CDx biomarkers, ranging in tumor purity from 5% to 50%. All CDx biomarkers

were concordant between xT CDx and results of orthogonal testing for all tumor purities at or above 10%. Macrodissection (enrichment for tumor content) of specimens below 10% tumor purity enabled successful detection of the CDx biomarkers in all samples. The minimum recommended tumor purity for detection of CDx variants is 20%, with macrodissection required for specimens with tumor purity lower than 20%.

4.2 DNA INPUT AND LIMITS OF DETECTION (LOD)

The minimum DNA input needed to detect CDx biomarkers was determined by testing 2 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 50 ng and 100 ng. 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 16.

Table 16. 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*

Additional samples were evaluated for the assay gene panel to determine the minimum DNA input and LOD for short variants (substitutions and INDELs) and for determination of MSI status. The minimum DNA inputs of 50 ng and 100 ng for short variants were established using 3 tumor-normal paired specimens at five dilution levels per specimen, with each replicate measured in duplicate.

The LOD for short variants was then assessed using minimal acceptable DNA inputs for processing 12 tumor-normal paired samples, representing 8 tumor types, each containing at least one known variant. Tumor DNA including 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 replicates of 17 tumor-normal paired samples diluted to achieve expected VAFs for the tested variants at or around the target LOD for each variant type (5% for substitutions and 10% for INDELs; 3% for hotspot substitutions and 5% for hotspot INDELs). The results of the gene panel LOD confirmation for short variants is summarized in Table 17.

Table 17. Summary of Variant Detection Near LoD Allele Fraction

Variant Type	Tested VAF	Positive Call Rate
Substitution	5%	97.5% (79/81)

Variant Type	Tested VAF	Positive Call Rate
Substitution (hotspot)	3%	100% (10/10)
INDEL	10%	100% (87/87)
INDEL (hotspot)	5%	100% (23/23)

Preliminary MSI LOD determination was evaluated in 22 CRC FFPE specimens known to be MSI-H based on orthogonal method testing. 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 around the expected tumor purity LOD (30%). Positive agreement of xT CDx MSI-H status was 94.6% (142/150 replicates identified as MSI-H) for samples diluted to achieve a tumor purity at or around 30%.

4.3 LIMIT OF BLANK

The LOB of was established by assessing the frequency of false-positive identification of CDx and tumor profiling biomarkers in 23 FFPE tumors (with patient-matched normal specimens) known to be wild-type for KRAS and NRAS. Specimens were evaluated with 4 or 5 replicate measures per specimen based on tissue availability. No false-positive variants were detected at a VAF threshold of 3% in 102 replicates of these samples, confirming the LOB. 22 replicates of well-characterized material were evaluated for false positive results at any reportable position; no false positives were detected.

5. Reagent Lot Interchangeability

Reagent lot interchangeability was assessed for CDx variants by testing 4 CRC samples containing alterations in the KRAS or NRAS gene over 63 replicates using multiple reagent lots in 3, 5, and 8 combinations for library preparation, hybridization capture, and sequencing reagents, respectively, across all tested specimens. No effect of interchanging reagents lots was observed for variant detection for KRAS and NRAS CDx biomarkers. In addition, variant detection across the entire gene panel was assessed in 375 replicates across 52 specimens representing a broad diversity of tumor types 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.

6. Stability

6.1 REAGENT STABILITY

The stability of reagents used in the library preparation, hybridization capture, and sequencing steps for xT CDx were evaluated using 3 lots of reagents for each assay step, tested at defined time points. Results support the stability of library preparation and hybridization capture reagents up to 7 months and sequencing reagents up to 5 months.

6.2 SAMPLE STABILITY

6.2.1 EXTRACTED DNA

Stability of DNA was evaluated using specimens extracted with the Tempus xT LDT assay. Samples from 468 unique clinical tumor specimens and 454 unique clinical normal specimens from 33 different tissues of origin were evaluated. DNA specimens evaluated were stored at -80°C for either 91-180 days or >210 days. More than 99% of the specimens that had been stored for longer than 9 months were successfully used to generate libraries with xT CDx. Based on this data, DNA stored in accordance with internal procedures can be considered stable for up to 9 months.

6.2.2 FFPE SLIDES

FFPE slide stability study was assessed prospectively and by analysis of previously prepared aged slides. For prospective analysis, results were analyzed from 5 tumor specimens across 4 cancer types with slides stored at room temperature for 0 days, 15 days, or 30 days, and then processed with xT CDx. 15 variants were detected at all 3 timepoints tested, as summarized in Table 18.

Table 18. Variants Detected in Tumor Specimens at Each Timepoint

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 existed in the T= 15 time point which was below LOD in the T=0 timepoint. The T=15 sample had a VAF of 3.5% and the T=0 sample had a VAF of 2.9%*

Analysis of previously prepared aged slides involved analysis of slides from 124 tumor specimens representing 23 tumor types. Slides were stored for varying durations at room temperature prior to DNA extraction. Stability was assessed by the number of specimens meeting minimum DNA yield criteria for xT CDx; results are summarized in Table 19.

Table 19. Evaluation of FFPE Slides at QC1 Based on Length of Storage

Months since Slide Preparation	Number of Specimens Evaluated	Number of Specimens with ≥50 ng 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%)**

6.2.3 FFPE BLOCKS

The stability of FFPE blocks was established by studying 349 FFPE blocks of tumor specimens stored at room temperature for 1-7 years by evaluating DNA extraction yield. The blocks were grouped into 5 age groups based on duration of storage since block preparation. More than 95% of the blocks in each age group produced 3x the minimum DNA yield of 50 ng needed for the device when processed under standard conditions. Results are summarized in table 20.

Table 20. DNA Yield from aged FFPE Blocks

Age Group	Year of Block Preparation	Number of Specimens	Mean DNA Yield	% Samples ≥150 ng 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%

6.2.4 BLOOD AND BUFFY COAT STABILITY

Stability of blood and buffy coat samples used as the source of matched normal specimens in xT CDx was established by collecting blood samples from 6 healthy volunteers. Buffy coat stability was determined by separation of buffy coat from blood upon receipt of a specimen, with storage of the buffy coat fraction at –20°C for 0, 15, 30, and 60 days, followed by DNA extraction and processing through xT CDx. Blood stability was determined by 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. Concordance was evaluated by comparing results at each time point to results from the day 0 time point. For both blood and buffy coat, somatic variant concordance by matching with a randomly selected tumor specimen was 100% and germline concordance was >99% at each time point evaluated. These results establish storage of whole blood at room temperature for up to 20 days, and storage of the buffy coat fraction at –20°C for up to 60 days.

7. Tissue Comparability

A large-scale retrospective analysis was conducted using 6,373 unique tumor specimens across 34 cancer types in order to establish the comparability of assay performance across tumor tissue types. The dataset for analysis consisted of routine clinical samples analyzed using the Tempus xT LDT assay, from 06/06/2020 to 10/05/2020. Approximately 89% of samples were matched to blood and 11% of samples were matched to saliva. xT CDx includes four 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 QC checks are as follows: DNA Extraction (QC1), Library Preparation (QC2), Hybridization Capture

(QC3), and Sequencing (QC4). The pass rate for each of these QC steps for each cancer type is summarized in Table 21. More than 91% of specimens passed the check at each assay step regardless of cancer type, demonstrating that assay performance of xT CDx is independent of tissue type.

Table 21. Pass Rate at Each Assay 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
Head and Neck Squamous Cell Carcinoma	100.0%	100.0%	96.4%	98.2%	111
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

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

8. Interference

The robustness of the Tempus xT CDx Assay process was assessed while evaluating human FFPE samples in the presence of exogenous and endogenous interfering samples. 22 FFPE specimens representing 13 different tumor types and their matched normal specimens were evaluated. The addition of interfering substances including xylene, ethanol, melanin, and proteinase K, each at two concentrations, was evaluated to determine if they were impactful to xT CDx and the results were compared to the control (no interference) condition. 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%. Results are presented in Table 22.

Table 22. Interference Study Summary

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

Analysis of all four substances on MSI determination showed 100% concordance for MSI calling under all conditions except for 93.3% concordance for MSS samples tested at 0.05 mg/mL of Proteinase K. Interference of necrotic tissue was evaluated across 348 CRC specimens with necrotic tissue percentage ranging from <5% to >50%. Equivalent invalid rates were observed at all necrotic tissue levels evaluated, and only a single clinically discordant result was observed in the dataset, in a sample with <5% necrotic tissue.

9. Guardbanding

Guardbanding studies were performed to evaluate the performance of xT CDx and the impact of process variation with regard to the measurement of DNA input at various stages of 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 were evaluated in duplicate at 6-8 input levels representing inputs below the minimum and above the maximum recommended input at each assay step. Each of the three guardbanding experiments demonstrated reliable and robust performance at DNA input levels above and below the range. Results are summarized in Table 23.

Table 23. 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 – 1.5 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

Process	Input Level	# of Samples Passing QC
Seq	1x minimum	31/31
Seq	1x maximum	26/26
Seq	1.25x maximum	26/26
Seq	1.5x maximum	32/32

10. Cross-Contamination

10.1 CARRYOVER / CROSS-CONTAMINATION

DNA sample carryover (between plates) and cross-contamination (within plates) during the library preparation and hybridization capture steps of the xT CDx Assay 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.

10.2 INDEX CROSS-CONTAMINATION

xT CDx uses unique dual index adaptors 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 ranged from $5.85x10^{-5}$ to $6.42x10^{-9}$, with an average probability across all analyzed flowcells of $1.35x10^{-5}$.

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 24.

Table 24. 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 cancer and specimen types. The dataset for analysis consisted of routine clinical samples analyzed using the Tempus xT LDT assay from 06/01/2020 to 12/08/2020. The samples were subjected to pre-specified retrospective analysis based on thresholds for success at each assay step. Results are presented in Table 25. Of the 4628 tumor-normal paired samples evaluated, 4122 (89.1%) were successfully processed across all steps of the assay.

Table 25. Summary of Invalid Rates at Each QC Step by Specimen Type

	Invalids		
Assay Step	**FFPE**	**Blood (n=4054)**	**Saliva (n=574)**
DNA Extraction	9/4628 (0.19%)	0/4054 (0.00%)	0/574 (0.00%)
Library Preparation	7/4619 (0.15%)	2/4504 (0.05%)	0/574 (0.00%)
Hybridization Capture	116/4612 (2.52%)	104/4052 (2.57%)	14/574 (2.44%)
Sequencing	223/4392 (5.08%)	48/3847 (1.25%)	5/545 (0.92%)

14. Clinical Concordance for KRAS and NRAS

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 panitumumab when mutations are detected in exons 2, 3, or 4 of *KRAS* or *NRAS* was established by evaluating 412 samples from CRC patients. Samples were not pre-screened to enrich for positive samples. All specimens were assessed for a minimum tumor percentage of 20% based on pathology review and availability of matched-normal tissue. Based on this evaluation, samples from 348 patients were included in the study. All 348 samples were sent for orthogonal testing with two FDA-approved CDx assays used as comparators: (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 comparator method. Concordance of xT CDx with the *Illumina Praxis Extended RAS Panel* (Praxis comparator device, 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 the replicate measurements). Concordance of xT CDx with the *Qiagen therascreen KRAS RGQ PCR Kit* (*therascreen* comparator device, 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 the replicate measurements). Samples used in the study were not obtained from a clinical trial, and not all samples had demographic

data available. Based on samples evaluated for concordance and with available data, the sex, age, and race were similar between the xT CDx concordance study and the clinical studies of the two comparator methods, with a more even distribution of sexes in the xT CDx concordance study relative to the clinical studies of the comparator methods. Specimen characteristics, including tumor percentage, percent necrosis, and variant allele distribution, were similar for specimens in the xT CDx concordance study and in the clinical studies for both comparator methods,

By defining the reference result as the consensus calls between two replicate measurements from each comparator methods, the 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). Results of concordance testing are summarized in Table 26 below.

Table 26. 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

Non-inferiority analysis demonstrated that the agreement between xT CDx and the *Illumina Praxis Extended RAS Panel* is non-inferior to the agreement between two replicates of that assay; and that the agreement between xT CDx and the *Qiagen Therascreen KRAS RGQ PCR Ki* is non-inferior to the agreement between two replicates of that assay.

xT CDx

PHYSICIAN INSERT
For *in vitro* Diagnostic Use

Genetic Companion Diagnostic (CDx) Test for Targeted Therapy Selection in Colorectal Cancer (CRC)

For the most current information on the association of the biomarker and therapeutic outcomes, refer to the therapeutic labels available at Drugs@FDA on the FDA website.

Tempus xT CDx Intended 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)

Warnings and Precautions

Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.

Test Limitations

- For in vitro diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The test is designed to report out somatic variants and is not intended to report germline variants. xT CDx sequences tumor and patient-matched normal samples to allow personalized subtraction of germline variants from tumor sequencing results.
- xT CDx requires a minimum tumor percentage of 20% for detection of variants, with tumor content enrichment recommended for specimens with tumor percentage lower than 20%. This assay may not detect variants if the proportion of tumor cells in the sample is less than 20%. xT CDx requires a minimum tumor percentage of 30% in order to determine MSI status.
- 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.
- A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
- The clinical validity of the device to guide MSI-related treatment decisions has not been established. MSI status is based on genome-wide analysis of 239 microsatellite loci and is not based on the 5 or 7 MSI loci described in current clinical practice guidelines. The threshold for MSI-H/MSS was determined by analytical concordance to comparator assays (IHC and PCR) using multiple cancer types. An MSI result of Equivocal indicates that microsatellite instability status of MSI-H or MSS could not be determined.
- Performance of xT CDx has not been established for detection of insertions or deletions larger than 25 base pairs.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.

Explanation of the Tiered Reporting

Genomic findings other than those listed in the Intended Use are not prescriptive or conclusive for labeled use of any specific therapeutic product. Test results should be interpreted in the context of pathological evaluation of tumors, treatment history, clinical findings, and other laboratory data. The test report includes genomic findings reported in the following levels (Table 1).[2]

[2] https://www.fda.gov/media/109050/download

Table 1. FDA Levels of Biomarkers

FDA Level of Biomarkers	Description
Level 1: Companion Diagnostics	CDx biomarkers that provide information that is essential for the safe and effective use of a corresponding therapeutic product, such as a drug. Such claims are supported by analytical validity of the test for each specific biomarker and a clinical study establishing either the link between the result of that test and patient outcomes or clinical concordance to a previously approved CDx. For Tempus xT CDx, Level 1 results are reported for CRC patients who may benefit from treatment with cetuximab due to the presence of a KRAS wild-type biomarker (the absence of mutations in codons 12 or 13) or panitumumab due to the presence of NRAS and KRAS wild-type biomarkers (the absence of mutations in exons 2, 3, or 4).
Level 2: Cancer Mutations with Evidence of Clinical Significance	Biomarkers described as cancer mutations with evidence of clinical significance enable health care professionals to use information about their patients' tumors in accordance with clinical evidence, such as clinical evidence presented in professional guidelines, as appropriate. Such claims are supported by a demonstration of analytical validity (either on the mutation itself or via a representative approach, when appropriate) and clinical validity (typically based on publicly available clinical evidence, such as professional guidelines and/or peer-reviewed publications).
Level 3: Cancer Mutations with Potential Clinical Significance	Biomarkers described as cancer mutations with potential clinical significance. These mutations may be informational or used to direct patients towards clinical trials for which they may be eligible. Such claims are supported by analytical validation, principally through a representative approach, when appropriate, and clinical or mechanistic rationale for inclusion in the panel. Such rationales would include peer-reviewed publications or in vitro pre-clinical models.