May 12, 2026

Datar Cancer Genetics Private Limited
% Dr Abdel Halim
Global Quality and Regulatory Services
10 Scenic Way
Monroe, New Jersey 08831

Re: K260235
Trade/Device Name: CellDx-Tissue
Regulation Number: 21 CFR 866.6080
Regulation Name: Next Generation Sequencing Based Tumor Profiling Assay
Regulatory Class: Class II
Product Code: PZM
Dated: January 24, 2026
Received: January 26, 2026

Dear Abdel Halim:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality Management System Regulation (QMSR) (21 CFR Part 820), which includes, but is not limited to, ISO 13485 clause 7.3 (Design controls), ISO 13484 clause 8.3 (Nonconforming product), and ISO 13485 clause 8.5 (Corrective and preventative action). Please note that regardless of whether a change requires premarket review, the QMSR requires device manufacturers to review and approve changes to device design and production (ISO 13485 clause 7.3 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the Quality Management System Regulation (QMSR) (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak-fragale -S

Živana Težak, Ph.D.
Branch Chief
Division of Molecular Genetics and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K260235

Device Name
CellDx - Tissue

Indications for Use *(Describe)*
The CellDx-Tissue is a qualitative in vitro diagnostic (IVD) test that uses next-generation sequencing of DNA and RNA isolated from formalin-fixed paraffine-embedded (FFPE) tumor tissue from patients previously diagnosed with solid malignant neoplasm to detect tumor gene alterations in a broad multi-gene panel. The test is intended to provide tumor mutation profiling information on somatic mutations, including single nucleotide variants (SNVs), insertions and deletions (indels), one gene amplification, and three fusions.

Information provided by the CellDx-Tissue test is intended for use by qualified healthcare professionals in accordance with professional guidelines in oncology. Results from CellDx-Tissue are not intended to be conclusive or prescriptive for the labeled use of any specific therapeutic product. CellDx-Tissue is a single-site assay performed at Datar Cancer Genetics (DCG).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary for CellDx-Tissue

1. Introduction

1.1 Applicant

Datar Cancer Genetics Pvt Ltd

F-8, D Road, Ambad, Nasik - 422 010, Maharashtra, India.

E-mail: regulatory@datarpgx.com

1.2 Proprietary and Established Names:

CellDx-Tissue

1.3 510(k) Number:

K260235

1.4 Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PZM	Class II	21 CFR 866.6080 – Next Generation Sequencing Based Tumor Profiling Assay	Pathology

1.5 Submission/Device Overview

1.5.1 Purpose for Submission:

New Device

1.5.2 Measurand (quantity intended to be measured):

Somatic single nucleotide variants (SNVs), insertions and deletions (Indels), Copy Number Variant (CNV), and select gene fusions, in genomic DNA and RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue.

1.5.3 Type of Test

Next-Generation Sequencing Based Tumor Profiling Test

1.5.4 Legally Marketed Primary Predicate Device

The legally marketed primary predicate device is MSK-IMPACT (DEN170058).

1.5.5 Regulatory Basis Statement

This 510(k) Summary is submitted in accordance with the requirements of 21 CFR 807.92. The information presented herein is consistent with the United States Food and Drug Administration's (FDA) expectations for NGS–based tumor profiling tests regulated under 21 CFR 866.6080 and is intended to provide a concise description of the device, indications for use, technological characteristics, performance data, and the basis for substantial equivalence to predicate devices.

2. Device Description

2.1 Indications for Use

The CellDx-Tissue is a qualitative in vitro diagnostic (IVD) test that uses next-generation sequencing of DNA and RNA isolated from formalin-fixed paraffine-embedded (FFPE) tumor tissue from patients previously diagnosed with solid malignant neoplasm to detect tumor gene alterations in a broad multi-gene panel. The test is intended to provide tumor mutation profiling information on somatic mutations, including single nucleotide variants (SNVs), insertions and deletions (indels), one gene amplification, and three fusions.

Information provided by the CellDx-Tissue test is intended for use by qualified healthcare professionals in accordance with professional guidelines in oncology. Results from CellDx-Tissue are not intended to be conclusive or prescriptive for the labeled use of any specific therapeutic product. CellDx-Tissue is a single-site assay performed at Datar Cancer Genetics (DCG).

Special Conditions for Use Statement(s)

For prescription use. For in vitro diagnostic use.

Special Instrument Requirements

Thermo Fisher Ion GeneStudio S5 Prime NGS Systems (qualified by Datar Cancer Genetics)

2.2 Test Principle

CellDx-Tissue utilizes an amplicon-based sequencing approach to detect a broad range of clinically significant somatic alterations, including SNVs, InDels, and one gene amplification in tumor tissue DNA (ttDNA), and three gene fusions in tumor tissue RNA (ttRNA). The test requires FFPE tumor tissue specimen having a surface area of more than 125 mm^2, a thickness of at least 3 mm, and at least 20% histopathologically assessed tumor content. Specimens with lower tumor content may be enriched by macro-dissection. The assay involves target enrichment and deep sequencing of specific regions across a comprehensive panel of 517 genes, including oncogenes, tumor suppressor genes, and other clinically relevant genes. DNA and RNA primers are designed to target all coding exons, selected introns and targeted RNA sequences including chimeric fusion reads. Sequence libraries are prepared using DNA and RNA through a multiplex polymerase chain reaction (PCR) amplification step to enrich the target sequences. The target sequences are tagged with unique barcode oligonucleotides for individual sample identification and adaptor oligonucleotides that facilitate anchoring to the sequencing platform. These target sequences are then clonally amplified on microscopic beads using emulsion PCR before sequencing. Multiple barcoded sequence libraries are subsequently pooled and loaded onto a semiconductor sequencing chip. The resulting sequence reads are then aligned to the reference human genome (hg19), to identify gene variants.

2.3 Process Components

CellDx-Tissue utilizes Ion GeneStudio S5 Prime NGS System, qualified by Datar Cancer Genetics (DCG). CellDx-Tissue utilizes COTS kits and reagents, qualified by DCG. CellDx-Tissue uses positive control (PC), negative control (NC), and no-template control (NTC). PC (qualified by DCG) consists of a mixture of well-characterized cell line DNA with known somatic variants at defined VAF, and RNA with defined fusions. NCs (qualified by DCG) are derived from genomic DNA and RNA of a known wild-type (WT) material. The NTC is nuclease free water. PC, NC and NTC are included in each assay run (batch level). CellDx-Tissue uses reference databases and sequences for data analysis.

2.4 Test Workflow

2.4.1 Sample Preparation

CellDx-Tissue requires genomic DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The tumor volume and minimum tumor content needed to obtain sufficient DNA and RNA for testing to achieve the stated performance are indicated in Table 1 below. The tumor content of the FFPE specimen is determined by hematoxylin-eosin (HE) staining. If the histopathologist assessed tumor content is less than 20%, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue.

Table 1. Specimen Handling and Processing FFPE Tissue

Tissue Type	Formalin- fixed, paraffin- embedded (FFPE) tumor tissue blocks.
Specimen Volume	≥3 mm thick, total surface area, > 125mm^2
Tumor Content	≥20%
Macrodissection Requirements	If the minimum tumor content is less than 20%, macrodissection will be done to enrich neoplastic content

Limitations	Low (<20%) tumor content, Inadequate tissue volume, Long-term storage degradation.
Storage	Room temperature

2.4.2 Nucleic Acid Extraction

DNA extraction is performed using the QIAamp DNA FFPE Advanced UNG Kit (qualified by DCG) as per the manufacturer's instructions for use. The procedure includes Uracil-N-Glycosylase (UNG) treatment to remove deaminated cytosine residues and an RNase A digestion to enhance the purity of the extracted DNA. The yield of extracted DNA is evaluated by a real-time PCR using RNase P TaqMan® assay. At least 20 ng DNA is required for library synthesis. RNA extraction is performed using the Recoverall total Nucleic acid isolation kit as per the manufacturer's IFU. The extraction procedure includes a DNase digestion step to eliminate contaminating DNA. The yield of extracted RNA is determined using the fluorometric Qubit™ RNA High Sensitivity (HS) Assay, based on a standard curve generated from manufacturer-provided calibration standards. At least 20 ng RNA is required for reverse transcription (cDNA synthesis) and library synthesis.
The assay has been validated with extracted DNA and RNA stored at -20°C and -80°C, respectively for up to 28 days.

2.4.3 Library Synthesis

DNA libraries are synthesized using the Oncomine DNA kit (qualified by DCG). The assay employs an amplicon-based targeted enrichment workflow, in which predefined genomic regions are amplified and converted into sequencing-ready libraries through adapter ligation and barcode incorporation. This approach enables multiplexed sequencing across the 517-gene panel. Library preparation entails the targeted amplification of genomic regions, partial digestion of primer sequences,

ligation of barcode adapters, and subsequent clean-up and amplification to produce sequencing-competent libraries.

RNA libraries are synthesized using the Oncomine RNA Kit (qualified by DCG). The assay employs an amplicon-based targeted RNA sequencing workflow, in which input RNA is reverse-transcribed to cDNA and targeted regions are selectively amplified to generate sequencing-ready libraries. The workflow includes cDNA synthesis, targeted amplification, adapter ligation with unique barcodes, and subsequent clean-up steps to prepare multiplexed libraries suitable for NGS.

DNA and RNA libraries are quantified using the quantitation kit, which employs a real-time PCR–based method to measure the concentration of adapter-ligated libraries prior to sequencing. Library concentration results are reviewed against predefined quality acceptance criteria, and all quantitation outputs are retained as part of routine quality assurance and traceability.

2.4.4 Template Preparation and Chip Loading:

Template preparation and chip loading are performed on the Ion Chef™ System (qualified by DCG) using the manufacturer's automated workflow for emulsion PCR (ePCR), enrichment, and chip loading. Quantified libraries meeting predefined quality criteria are pooled and loaded onto the instrument along with the required reagents and consumables supplied in the template preparation kit. The Ion Chef System (qualified by DCG) performs clonal amplification of library molecules on beads through ePCR, followed by automated enrichment to isolate beads containing amplified templates. The instrument then loads the enriched, template-positive beads onto an Ion 550™ sequencing chip (qualified by DCG), producing a sequencing-ready chip for downstream processing on the compatible semiconductor sequencing platform (qualified by Datar Cancer Genetics). Instrument checks and onboard

controls ensure workflow integrity, and template-prepared chips proceed directly to sequencing.

2.4.5 Sequencing

Sequencing is performed on the Ion GeneStudio™ S5 Prime System (qualified by DCG) using the manufacturer's semiconductor sequencing workflow. The sequencing chip generated during template preparation is loaded into the instrument along with the required sequencing reagents once all automated system checks confirm acceptable setup. A predefined run plan specifies the assay configuration, sample assignments, and chip parameters. After sequencing is initiated, the system performs automated nucleotide flows and signal detection to generate base-level data across all loaded libraries. Sequencing data are accepted only when run-level QC metrics and control results meet validated acceptance criteria.

2.4.6 Sequencing Data Analysis

a. **Data Management**

Sample tracking, data processing, and archival of sequencing data for DCG2401 are managed through a Laboratory Information Management System (LIMS) integrated with a validated bioinformatics pipeline operating on a high-performance server environment. The system tracks and archives run-associated metadata including barcode identifiers, sequencing run identifiers, sample accession numbers, specimen source, library batch identifiers, and assay modality (DNA or RNA). Key functions include tracking sample status across all stages of data analysis, logging analysis iterations applied to each sample, recording software, algorithm, and database versions used for analysis, and archiving pipeline output files (BAM and VCF) together with sequencing run statistics (e.g., total reads generated, mean depth of

coverage, uniformity, and on-target rates). All annotation and population databases are maintained as access-restricted, version-locked static copies in a controlled repository. Any update to software, databases, or internal scripts is managed under a formal change-control process requiring documented impact assessment, regression testing using predefined golden datasets, QA/RA approval, and release prior to clinical use.

b. **Signal Processing, Base Calling, Read Alignment, BAM Generation, and Coverage Statistics**

Primary data processing is performed using Torrent Suite Software (qualified by DCG). During sequencing, raw semiconductor flow signals are converted into nucleotide bases through signal processing and base calling algorithms. Reads are assigned to individual samples through barcode classification, followed by trimming of barcode and adapter sequences and quality filtering. Filtered reads are aligned to the human reference genome (hg19 / GRCh37.p5) using platform-validated alignment algorithms. Aligned reads are written to Binary Alignment Map (BAM) files, which serve as the input for downstream variant calling and copy number analysis. The software also generates coverage statistics including mean depth of coverage, base coverage uniformity, and percentage of reads on target for each sample.

c. **Read Alignment Quality Check**

Read alignment metrics are used to assess sequencing and library quality. Reads are aligned to hg19, and mismatches arising from biological variation (true variants) or technical sequencing errors are recorded. Alignment performance is summarized using the Alignment Quality (AQ) score derived from a Phred-scaled $-10\log10$ transformation. The manufacturer default setting of AQ17, corresponding to a base accuracy of 98% (2% error rate), is applied. AQ17 allows longer effective read

lengths while accommodating mismatches expected in variant-rich tumor samples. Decreases in AQ are expected in samples with higher mutational burden and are evaluated in the context of overall run and sample QC metrics.

d. **Run-Level and Sample-Level Quality Control Checks**

i. Run-Level QC

A sequencing run is considered valid only if all of the following criteria are met:

1. Total sequencing output ≥ 40 million reads per run
2. Acceptable alignment quality (AQ17)

Runs failing any criterion are invalidated, investigated, and repeated following troubleshooting and corrective actions.

ii. Sample-Level QC

At the sample level, sequencing performance is evaluated using:

1. DNA libraries: mean target depth ≥ 500× and ≥ 90% of target amplicons with ≥ 100 reads
2. RNA libraries: ≥ 500,000 total fusion-mapped reads per run and ≥ 100,000 reads per RNA pool
3. Contamination score < 0.120

Samples failing QC due to specimen quality, extraction, or library preparation may be re-sequenced, re-prepared, or re-extracted according to predefined decision rules.

e. **Mutation Calling: Single Nucleotide Variants (SNVs) and Insertions/Deletions (Indels)**

i. Analysis of Positive and Negative Controls

Positive and negative controls are analyzed using the same coverage requirements as patient samples (mean depth ≥ 500× and ≥ 100× coverage across ≥ 90% of target regions). Expected variants must be detected in positive controls within validated variant allele frequency (VAF) ranges, and no reportable variants may be detected in negative controls.

ii. Filters on Sample Coverage

Tumor samples must achieve a minimum mean sequencing depth of ≥ 500× to be eligible for variant calling and reporting.

iii. Filtering for High-Confidence Mutations

Raw SNV and indel calls are subjected to locked filtering thresholds to ensure only high-confidence somatic variants are reported. Variants are classified as:

(1) Hotspot variants: VAF ≥ 2%, , total depth ≥ 40, mutant reads ≥ 8, strand bias ≤ 0.9

(2) Non-hotspot variants: VAF ≥ 5%, total depth ≥ 40, mutant reads ≥ 10, strand bias ≤ 0.85

Variants are annotated using AMP/ASCO/CAP somatic guidelines, HGVS nomenclature, and major public databases (e.g., ClinVar, gnomAD, dbSNP, 1000 Genomes). Population variants with a frequency ≥ 5% in any gnomAD or 1000 Genomes subpopulation are excluded. Filtering thresholds are fixed for clinical use, and any modification requires formal change-control and re-validation.

f. **Mutation Annotation**

Variants with functional consequences including missense, nonsense, frameshift, in-frame insertions/deletions, splice-site, and splice-region alterations are retained. Annotation incorporates gene context, predicted functional impact, and clinical relevance. Common polymorphisms present in population databases or internal normal datasets are excluded from somatic reporting.

g. **Tumor Purity Analysis**

Tumor purity of the sequenced specimen is estimated bioinformatically using allele frequencies of informative variants and sequencing read distributions. This estimate may differ from the pathologist-reported tumor percentage due to macrodissection or sampling effects. When discrepancies or software limitations are identified, tumor purity is manually reviewed using pathologist estimates and driver mutation VAFs to ensure accurate interpretation of variant calls.

h. **Copy Number Analysis**

Copy number variation analysis is performed for *ERBB2* using a variability-corrected informatics baseline derived from normal samples. Amplicon coverage log2 ratios are normalized and adjusted for tumor cellularity to estimate copy number and confidence intervals. *ERBB2* amplification is reported as Positive when the observed copy number is ≥ 8.5. Copy numbers ≥ 4 and < 8.5 are reported as Indeterminate / Low-Level Amplification (below the LoD).

i. **Fusion Analysis**

RNA-based fusion detection is validated for *ALK, RET*, and *ROS1*. Fusions are identified using split-read and discordant read-pair evidence. Clinically reportable fusions are reported only when ≥ 500 fusion-supporting reads are detected and validated against internal positive controls. Fusion calls rely exclusively on RNA-level evidence.

2.4.7 Results Reporting

The CellDx-Tissue assay report provides structured information on detected genomic alterations, level of clinical significance and their clinical implications. The detected genomic alterations are categorized into Variants with Evidence of Clinical Significance, or Variants with Potential Clinical Significance. The CellDx-Tissue does not report mutations in 217 regions among 13473 interrogated target regions due to low coverage and high GC content.

2.4.8 Quality Metrics

A multi-tier QC framework evaluates data integrity at the run, specimen, and variant levels. Results are reported only when all applicable criteria are met.

a. Run-level metrics: evaluate overall sequencing performance, including instrument function, reagent performance, data yield, and controls.
b. Specimen level metrics: include sequencing quality, library performance, and coverage sufficiency.
c. Variant (analyte) level metrics include parameters such as locus-level coverage and read support.

Table 2. Quality Control Metrics and Assay Cut-Offs

Quality Metrics	Frequency	Acceptance Criteria
Specimen	Specimen	Labels intact and Legible, Test Requisition Form completely filled, consent signed, No loss of or damage to specimen, Number of FFPE blocks matches Test Requisition Form, FFPE specimen identifiers match Test Requisition Form and LIMS entry
Specimen	Specimen	>125mm^2 surface area; ≥3mm thick Tumor content ≥20% (Macro-dissect if <20%)

Quality Metrics	Frequency	Acceptance Criteria
DNA, RNA	Specimen	DNA yield >20 ng; RNA yield >20 ng
Library Yield	Specimen	>100 pM
Total Data Output	Run	≥ 40 million reads.
Positive Control (PC)	Run (batch)	DNA: All variants detected within expected VAF ranges RNA: All 6 fusions detected.
Negative Control (NC)	Run (batch)	DNA: None of the 890 hotspot mutations should be detected RNA: No fusions detected.
Base Quality (AQ Score)	Run	AQ17 (98% accuracy)
Mean Target Coverage (DNA)	Specimen	≥ 500x.
Coverage Uniformity	Specimen	≥ 90% of target regions with ≥ 100x coverage.
Contamination Score	Specimen	≤ 0.120
Mean Target Coverage (RNA)	Specimen	Total mapped fusion reads ≥ 500,000 Pool 1, Pool 2 (each): ≥100,000 reads
SNVs and Indels Calling	Variant	Mutation coverage (total depth) ≥ 40 Number of mutant reads (variant read count) ≥ 8 / ≥ 10** Variant Allele Fraction (VAF) ≥ 0.02 / ≥ 0.05** Strand bias < 0.9 / < 0.85** (Hotspot / Non-hotspot**)
ERBB2 Amplification	Variant	CN ≥8.5 is Positive CN ≥4 to <8.5 is Indeterminate / Low Level Amplification (below LoD) CN <4 is Negative
ALK, RET, ROS1 Fusions	Variant	Positive where supporting read counts are ≥500
Test Failure Criteria	Specimen	DNA mean coverage < 500x, or Coverage uniformity < 90%, or Contamination score >0.120, or Fusion mapped reads <500,000, or Pool reads <100,000
Reprocessed specimen	Specimen	All above QC criteria met.
Report	Specimen	Patient ID matches LIMS entry Accreditation Logos match standard template Signing authority signatures present

2.5 Determination of Assay Thresholds

2.5.1 Requirements on Exon Coverage

A power analysis was conducted to estimate the minimum sequencing depth (total number of reads) needed to detect a mutation with a true underlying variant allele fraction (VAF) of 0.02 or greater, for varying levels of statistical power (0.8 to 0.99), assuming a fixed alpha (Type I error rate) of 0.05. The 95% confidence interval (CI)

ranges of observed VAF as a function of sequencing depth were also calculated. This study showed that when a mutation is present at 0.1 VAF, the 95% CI with a sequencing depth of 500X is expected to fall between 0.075 and 0.13. When the overall coverage is 100X, the 95% CI for a mutation at 10% VAF is estimated to fall between 0.05 and 0.176.

To confirm these estimates, empirical data was obtained to measure the range of observed VAF for expected VAF, utilizing DNA from 20 formalin-fixed, paraffin-embedded (FFPE) normal tissue specimens from unrelated individuals. Equimolar parts of the DNA from these specimens were mixed to create 5 secondary specimen pools with a range of genetic variants (SNPs) having expected frequencies as low as 2%. A total of 863 common SNPs were considered for this study.

A boxplot, as shown in Figure 2, illustrates the observed variant frequencies for these common SNPs genotyped in the pooled normal samples, binned by their true underlying variant frequency. The empirical data demonstrated a strong correlation between expected and observed VAF (Pearson's r = 0.99), with a slope of 0.99 and an intercept of 0.013. For SNPs with a true underlying variant fraction of 0.1, the observed variant fraction ranged from 0.069 to 0.138 when the mean coverage of the specimen was 1151X. This range is consistent with the theoretical statistical assessment for a depth of 500X (0.075 to 0.13). This data supports using 0.05 as the lower limit for reporting mutations detected with a true underlying frequency of 10%.

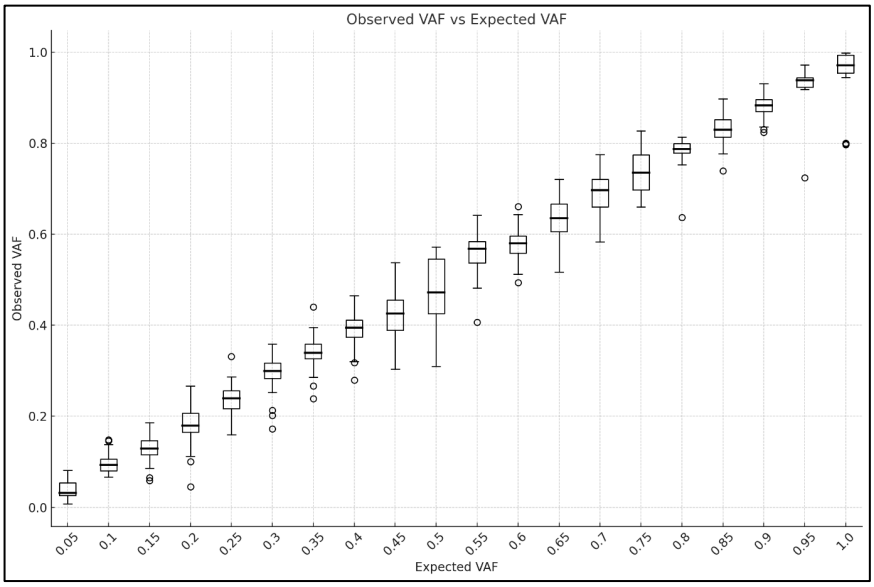
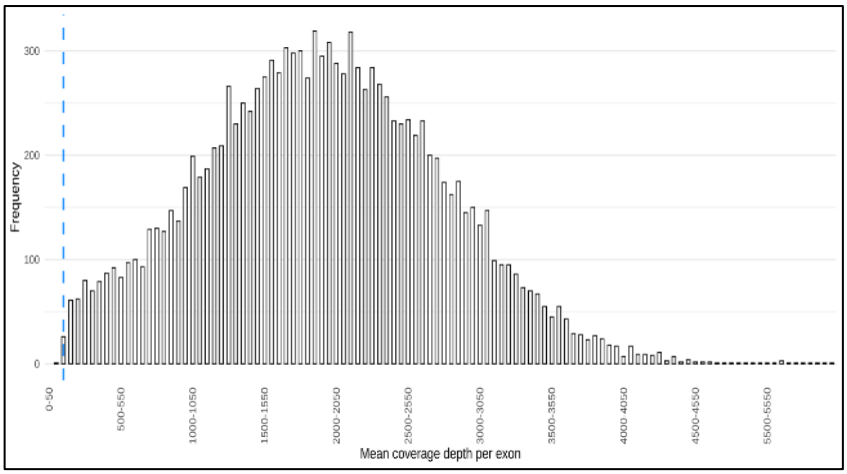


Figure 2. Observed vs. Expected Variant Allele Frequency for the CellDx-Tissue Assay

2.5.2 Requirements on Sample Coverage

Twenty FFPE normal (diploid) specimens were profiled using CellDx-Tissue to generate summary statistics across all targeted exons. The mean coverage across all amplicons at optimal depth was 2087X (Range: 1420X-3369X; SD=481X). The percentage of amplicons with >100X coverage was 99.3% (Range: 98.0-99.9%, SD=0.5%). When mapped to the exon level, the mean coverage across all targeted exons was 2087X (Range:1420X-3371X; SD=482X). Summary statistics were also computed on coverage values per exon normalized by per-sample coverage. Exons with consistently low coverage (median normalized coverage < 0.05), such as 217 amplicon regions covering 157 exons, were excluded from SNV/indel reporting, primarily due to high GC content, with the exception of two TERT promoter amplicons where variant calling parameters were relaxed with medical justification.

Sequence coverage was further evaluated to establish minimum criteria for the analysis and reporting of variants. A 100X minimum coverage threshold per exon is required, based on power calculations, to call mutations with a true underlying mutation frequency of 10% or greater, with 95% power at an alpha level of 0.05. Figure 3 shows the distribution of mean and median coverage values for targeted regions of CellDx-Tissue using high coverage samples, with a dashed line indicating 100X coverage.



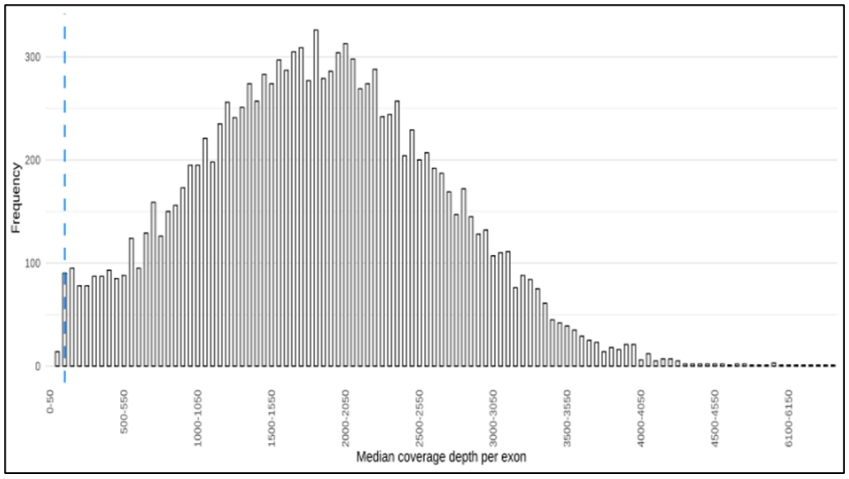
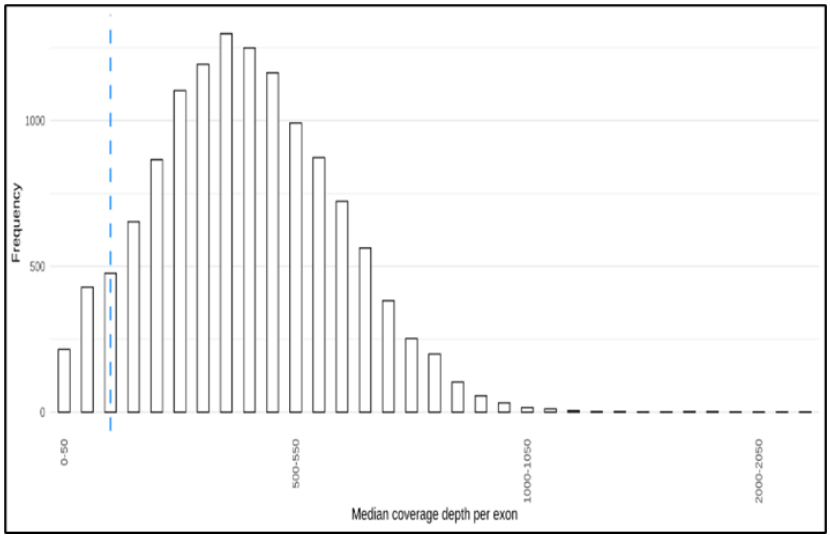


Figure 3. Distribution of mean and median coverage values for targeted regions of CellDx-Tissue using high coverage samples. (Dashed blue line indicates 100X coverage.)

A second set of 20 samples was evaluated at lower sequencing depth. The mean coverage across all amplicons was 471X (Range: 284X-663X; SD=101X). The percentage of amplicons with >100X coverage was 92.6% (Range: 86.6-96.2%, SD=2.4%). When mapped to the exon level, the mean coverage across all targeted exons was 471X (Range: 284X-662X; SD=101X). With the same remaining exons across all genes, 94.1% (Range: 88.0-97.7%; SD=2.42%) were sequenced to a depth of 100X or greater. The distribution of these mean and median coverage values for targeted exons is shown in Figure 4.



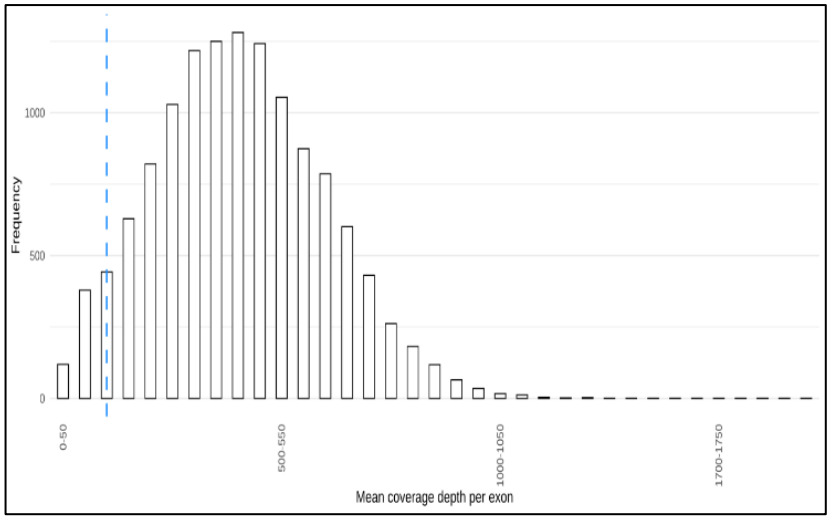


Figure 4. Distribution of mean and median coverage values for targeted regions of CellDx-Tissue using lower coverage samples. (Dashed blue line indicates 100X coverage.)

Based on these two studies, the threshold of ≥90% of amplicons achieving 100X coverage was established as a critical quality metric for the CellDx-Tissue assay.

2.5.3 Requirements on Variant Calling Thresholds

Variant calling parameters such as sequence coverage (Alternate Allele Observation, AO), variant coverage (Coverage Depth, DP) and strand bias (SB) were assessed as filters for specificity while maintaining the ability to detect true positive calls. Thresholds were established to ensure specificity is maintained at targeted VAF levels for reporting, particularly at 0.02 and 0.05 for hotspot and non-hotspot categories, respectively.

The cutoffs for AO, DP, and SB for hotspot variants and non-hotspot variants were established using two key development studies. The first study was designed to select the optimal parameter cutoff combination utilizing a dataset of 20 CellDx-Tissue runs using a Reference Standard. This involved evaluating multiple cutoffs for each of the three parameters, generating a total of 7650 combinations. A list of 3723 positive variants and 4630 negative variants was used to evaluate the performance of each parameter combination, with the goal of achieving a Positive Predictive Value (PPV) greater than 96%. Figure 4 illustrates the relationship between these parameters and PPV, highlighting the selected cutoff combination: AO = 8, DP = 40, SB = 0.90 for hotspot variants; and AO = 10, DP = 40, SB = 0.85 for non-hotspot variants.

The second study aimed to determine the optimal cutoff for Coverage Depth (DP) and minimum variant read count. This study used 10 non-cancer FFPE samples and evaluated a total of 902 hotspot variants and 6418 non-hotspot variants at different Coverage Depth cutoffs. The lowest Coverage Depth (DP) that effectively filtered out >99.7% of noise variants were selected, corresponding to a minimum of 8 reads for hotspot variants and 10 reads for non-hotspot variants

2.6 Tumor-Only vs. Matched-Normal Germline Filtering

A bridging study was conducted to demonstrate analytical equivalence between the CellDx-Tissue tumor-only (T/O) germline filtering approach and a matched tumor–normal (T/N) reference method, as required under 21 CFR 866.6080(b)(1)(iv). The study utilized 52 prospectively selected tumor specimens with matched peripheral blood, representing the following solid tumor types – bladder / urinary track (n = 1), bowel (n = 10), breast (n = 13), cervix (n = 2), esophagus / stomach (n = 1), head and neck (n = 3), liver (n = 1), lung (n = 6), ovary / fallopian tube (n = 5), pancreas (n = 3), peritoneum (n = 1), prostate (n = 1), testis (n = 1), thymus (n = 1), uterus (n = 2), and unknown primary (n = 1), as well as diverse ethnic representation (65% Asian, 27% White/Caucasian, 6% African American and 2% unspecified ethnicity from the US). The T/O pipeline employed an in-silico filtering strategy that excluded variants with population frequency ≥5% in gnomAD (ver. 2.1.1) or 1000 genome frequency (ver. Phase3v5b) databases, while the reference method used variants called using torrent variant caller (TVC, ver 5.18) with total white blood cell (WBC) genomic DNA (gDNA) as the germline control. Clinically actionable variants were defined as those with ClinVar (ver. 20250323) pathogenic/likely pathogenic classification or OncoKB (ver. 4.27) Level 1/2 oncogenic annotation with population frequency ≤0.1%. A total of 345 clinically actionable variants were evaluated, including germline pathogenic alterations in *BRCA2, CHEK2, ATM, MSH2, PALB2,* and *RAD51D.* The study demonstrated 100% PPA (345/345; 95% CI: 98.9%, 100%) and 100% NPA (2,745/2,745; 95% CI: 99.9%, 100%), with zero false negatives (FN) for actionable germline variants. Twenty-one (6.1%) actionable variants had VAF between 0.02 - 0.05, confirming assay sensitivity near the LoD. This analysis provides robust statistical assurance that the T/O filtering strategy is analytically equivalent to

matched T/N subtraction. The study confirms that the CellDx-Tissue assay does not introduce FN risk for clinically significant germline variants.

3. Substantial Equivalence Information

The legally marketed predicate device is MSK-IMPACT (DEN170058).

Table 3. Comparison of Subject Device (CellDx-Tissue) and Predicate Device (MSK-IMPACT; DEN170058)

	Predicate Device: MSK-IMPACT (DEN170058)	**Subject Device: CellDx-Tissue**
Similarities		
Indications For Use	The MSK-IMPACT assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded tumor tissue matched with normal specimens from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. The test is intended to provide information on somatic mutations (point mutations and small insertions and deletions) and microsatellite instability for use by qualified health care professionals in accordance with professional guidelines and is not conclusive or prescriptive for labeled use of any specific therapeutic product. MSK-IMPACT is a single-site assay performed at Memorial Sloan Kettering Cancer Center.	CellDx-Tissue is a qualitative in vitro diagnostic (IVD) test that uses targeted next-generation sequencing (NGS) of DNA and RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue, from patients previously diagnosed with solid malignant neoplasms to detect tumor gene alterations in a broad multi-gene panel. The test is intended to provide information on somatic mutations including single nucleotide variants (SNVs), small insertions and deletions (InDels), one gene amplification, and three fusions.. Information provided by the CellDx-Tissue test is intended for use by qualified healthcare professionals in accordance with professional guidelines in oncology. Results from CellDx-Tissue are not intended to be conclusive or prescriptive for the labeled use of any specific therapeutic product. CellDx-Tissue is a single-site assay performed at Datar Cancer Genetics (DCG).
Target Population	Patients with solid malignant neoplasms	Same

	Predicate Device: **MSK-IMPACT (DEN170058)**	**Subject Device:** **CellDx-Tissue**
Assay cut-off	MSK-IMPACT does not report mutations below 2% for known hotspot mutations and 5% for non- hotspot mutations.	Same
Laboratory / Test Environment	Single-site assay	Same
Result Report Format	Results are reported under one of these two categories: • "Variants with Evidence of Clinical Significance" or • "Variants with Potential Clinical Significance."	Same
Specimen Types	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue matched with normal specimens from patients with solid malignant neoplasms	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from patients with solid malignant neoplasms
Clinical Evidence Curation	• Uses OncoKB, knowledge base • Classification criteria were developed by MSK to communicate the level of clinical evidence available for individual mutations in the test report.	Variant calls are organized into Variant with Evidence of Clinical Significance or Variant with Potential Clinical Significance depending on the designated cancer type.
Differences		
Minimum Tumor Content	10% (20% preferred; 25% for MSI)	>20%
Analyte	DNA	DNA, RNA
Reportable Variants	SNVs, InDels, Microsatellite Instability (MSI)	SNVs, InDels, *ERBB2* amplification, *ALK, RET,* and *ROS1* fusions.
Input Nucleic Acids	50 ng – 250 ng	DNA: 20 ng – 50 ng RNA: 20 ng – 50 ng
Sequencing Instrument	Illumina HiSeq™ 2500 Sequencer	Thermo Fisher Ion GeneStudio™ S5 Prime System
Target Enrichment	Hybrid Capture	Amplicon
Sequencing Chemistry	Sequencing by synthesis (fluorescent detection)	Sequencing by synthesis (hydrogen ions detection)

	Predicate Device: **MSK-IMPACT (DEN170058)**	**Subject Device:** **CellDx-Tissue**
Genes on Panel	468 (6,357 exons)	517 (6,597 exons)
Blacklist	73 exons	157 exons
Control	• Positive control • Negative control • No template control (NTC) • Matched Normal	• Positive control • Negative control • No template control (NTC)
Germline Filtering	Matched normal specimen analysis	Computational, population database filtering.
Coverage Requirements	• ≥ 200x mean; • 100x for ≥98% target regions	• ≥ 500x; • 100x for ≥90% target regions
Variant calling Thresholds	**Hotspot:** • Mutant reads (AD) ≥ 8, • Mutation coverage (DP) ≥ 20 • Mutation frequency (VAF) ≥ 0.02 **Non-Hotspot** • Mutant reads (AD) ≥ 10, • Mutation coverage (DP) ≥ 20 • Mutation frequency (VAF) ≥ 0.05	**Hotspot:** • Mutant reads (AD) ≥ 8, • Mutation coverage (DP) ≥ 40 • Strand bias (SB) < 0.9 • Mutation frequency (VAF) ≥ 0.02 **Non-Hotspot** • Mutant reads (AD) ≥ 10, • Mutation coverage (DP) ≥ 40 • Strand bias (SB) < 0.85 • Mutation frequency (VAF) ≥ 0.05
Contamination QC	% heterozygous sites at fingerprint SNPs < 55%; Average MAF at homozygous fingerprint SNPs < 2%	Estimated contamination score < 0.120, Contamination score estimated from the signal derived from reference reads at homozygous alternate sites.
Criteria for calling test failure	If a sample presents with mean coverage across all exons < 50x and no mutations are detected due to the low overall coverage, the test is deemed "failed" for the sample.	If a sample presents with mean coverage across all exons < 500x for DNA and < 500,000 reads for RNA, the test is deemed "failed" for the sample.

4. Performance Testing Summary

4.1 Invalid Rates

Multiple factors can influence the overall robustness and performance of complex molecular tests, including pre-analytical factors and overall sample quality. If key in-

process or automated data quality metrics are not met, CellDx-Tissue supports repeating samples through the workflow once. Performance throughout the verification and validation of the device was tracked, and a summary of the rates for first pass (no repeat) and overall pass (allowing for a single repeat) are presented below. Data were aggregated for clinical cases from >25 tumor types. The data shows that the performance across tumor types is supportive of a pan-tumor profiling.

Table 4. Acceptability Rates

Clinical FFPE Samples	Acceptability Rate (n/N)	(2-sided 95% CI)
First Pass	81.8% (2190/2676)	80.33%–83.25%
After Repeat Test	93.1% (2492/2676)	92.10%–94.02%

Table 5. Comparability of Tumor Invalid Rates

Organ	Pre-analytical	Pre-run	Post-Run		Invalid Rate (n/N) (%)
	Low Tumor Purity[1]	Low Nucleic Acid Yield[2]	Library Failure[3]	Data QC Failure	
Adrenal Gland	0	0	0	0	(0/2) 0%
Ampulla of Vater	1	0	0	0	(1/33) 3%
Biliary Tract	0	0	0	0	(0/7) 0%
Bladder/UrinaryTract	0	0	0	2	(2/20) 10%
Bone	0	0	0	0	(0/2) 0%
Bowel	6	4	2	35	(47/479) 10%
Breast	3	5	2	3	(13/317) 4%
Cervix	0	0	0	0	(0/41) 0%
CNS/Brain	0	1	1	3	(5/67) 7%
Esophagus/Stomach	0	0	0	9	(9/115) 8%
Head and Neck	12	3	0	17	(32/220) 15%
Kidney	0	0	0	0	(0/18) 0%
Liver	0	0	0	0	(0/12) 0%
Lung	2	3	0	38	(43/695) 6%
Ovary/Fallopian Tube	0	1	0	3	(4/73) 5%

Organ	Pre-analytical	Pre-run	Post-Run		Invalid Rate (n/N) (%)
	Low Tumor Purity[1]	Low Nucleic Acid Yield[2]	Library Failure[3]	Data QC Failure	
Ovary/Fallopian Tube, Uterus	0	0	0	0	(0/3) 0%
Pancreas	2	0	0	2	(4/27) 15%
Peritoneum	0	0	0	0	(0/2) 0%
Pleura	0	1	0	1	(2/2) 100%
Prostate	0	0	0	6	(6/217) 3%
Skin	0	0	5	7	(12/51) 24%
Soft Tissue	1	0	0	0	(1/18) 6%
Testis	0	0	0	1	(1/2) 50%
Thymus	0	0	0	0	(0/4) 0%
Thyroid	0	0	0	0	(0/88) 0%
Unknown Primary	0	0	0	0	(0/2) 0%
Uterus	0	0	0	2	(2/155) 1%
Vulva/Vagina	0	0	0	0	(0/4) 0%
Total	**27**	**18**	**10**	**129**	**(184/2676) 6.9%**

[1]<20%; [2]<20 ng; [3]No amplification; n:Total Invalids; N: Total Specimens

4.2 Precision

The precision study utilized 17 FFPE clinical specimens that covered a broad spectrum of VAF, copy number (CN), and fusion read counts (FRC) to challenge assay capabilities across diverse specimen characteristics. A factorial design was employed that evaluated impact of multiple operators, multiple sequencing instruments, multiple reagent lots, and multiple days to generate up to 24 to 48 replicates per variant.

a. **Panel-Wide Precision:**

Precision was accessed for each variant across all replicates. The positive call rates (PCR) and negative call rates (NCR) were calculated along with the two-sided 95% confidence intervals.

Table 6 summarizes the positive call rate (PCR) and negative call rate (NCR) stratified by mutation type (SNV, insertions, and deletions) and variant allele

frequency (VAF). An overall PCR of 98.69% across all samples and replicates (2871/2909; 95% CI: 98.21% – 99.05%; Average VAF range: 2.0 – 85.52), with an increase in PCR at higher VAFs observed, and an overall NCR of 100% (533539/533539; 95% CI: 99.99, 100.00).

The positive call rates for selected individual sequence mutations assessed in the precision study, along with the VAF range, mean SD, and percent CV per variant are presented in Appendix D. A total of 48 SNVs and 15 indels (3 insertions, 15 deletions) are provided.

Table 6: Panel-wide Precision Positive (PCR) and Negative (NCR) Call Rates

Variant Type	VAF Level	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth / FSR (range)	Mean Loci Depth Range
All	AF≥0	63	98.69 (2871/2909)	100 (533539/533539)	2.00 - 85.52	46 - 1455	440 - 1984
	AF≥2.0	63	98.69 (2871/2909)	100 (533539/533539)	2.00 - 85.52	46 - 1455	440 - 1984
	AF≥5.0	60	98.99 (2758/2786)	100 (533539/533539)	3.20 - 85.52	46 - 1455	440 - 1978
	AF≥10.0	53	99.23 (2451/2470)	100 (450769/450769)	7.43 - 85.52	82 - 1455	440 - 1978
	AF≥15.0	48	99.15 (2211/2230)	100 (450769/450769)	15.44 - 85.52	142 - 1455	440 - 1978
Variants with Evidence of Clinical Significance	AF≥0	10	99.56 (455/457)	100 (365377/365377)	2.26 - 79.57	74 - 1455	604 - 1978
	AF≥2.0	10	99.56 (455/457)	100 (365377/365377)	2.26 - 79.57	74 - 1455	604 - 1978
	AF≥5.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
	AF≥10.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
	AF≥15.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
Hotspot	AF≥0	24	97.99 (1073/1095)	100 (409829/409829)	2.00 - 60.77	46 - 1092	737 - 1984
	AF≥2.0	24	97.99 (1073/1095)	100 (409829/409829)	2.00 - 60.77	46 - 1092	737 - 1984
	AF≥5.0	21	98.77 (960/972)	100 (409829/409829)	3.20 - 60.77	46 - 1092	737 - 1978
	AF≥10.0	17	99.25 (791/797)	100 (409829/409829)	15.44 - 60.77	214 - 1092	810 - 1978

Variant Type	VAF Level	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth / FSR (range)	Mean Loci Depth Range
	AF≥15.0	17	99.25 (791/797)	100 (409829/409829)	15.44 - 60.77	214 - 1092	810 - 1978
Non-Hotspot	AF≥0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF≥2.0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF≥5.0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF≥10.0	36	99.22 (1660/1673)	100 (324435/324435)	7.43 - 85.52	82 - 1455	440 - 1977
	AF≥15.0	31	99.09 (1420/1433)	100 (324435/324435)	17.53 - 85.52	142 - 1455	440 - 1977
SNVs	AF≥0	48	99.13 (2172/2191)	100 (449879/449879)	2.00 - 79.57	46 - 1455	449 - 1984
	AF≥2.0	48	99.13 (2172/2191)	100 (449879/449879)	2.00 - 79.57	46 - 1455	449 - 1984
	AF≥5.0	45	99.56 (2059/2068)	100 (449879/449879)	3.20 - 79.57	46 - 1455	449 - 1978
	AF≥10.0	41	99.84 (1891/1894)	100 (408049/408049)	10.33 - 79.57	101 - 1455	449 - 1978
	AF≥15.0	39	99.83 (1795/1798)	100 (408049/408049)	15.44 - 79.57	142 - 1455	449 - 1978
Insertions	AF≥0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF≥2.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF≥5.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF≥10.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF≥15.0	1	100 (48/48)	100 (42720/42720)	35.92 - 46.99	571 - 571	1375 - 1375
Deletions	AF≥0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF≥2.0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF≥5.0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF≥10.0	9	96.30 (416/432)	100 (170736/170736)	9.28 - 85.52	108 - 578	440 - 1699
	AF≥15.0	8	95.83 (368/384)	100 (170736/170736)	15.64 - 85.52	269 - 578	440 - 1699

Variant Type	VAF Level	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth / FSR (range)	Mean Loci Depth Range
ERBB2 Amplification		1	100% (120/120)	-	N/A	N/A	N/A
ALK Fusion		1	100% 45/45	-	N/A	7121-30568	N/A
ROS1 Fusion		1	95.0% 38/40	-	N/A	504-2328	N/A
RET Fusion		1	100% 46/46	-	N/A	740-175619	N/A

N/A: Not applicable; FSR: fusion supporting reads

Precision for selected SNVs and InDels was assessed by evaluating the mean VAF, standard deviation (SD), coefficient of variation (%CV), and positive call rate (PCR).

Table 7: Precision for Selected SNVs/Indels

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
AMER1 Exon 2	c.2185G>T p.E729*	0.48-0.77	0.554-0.773	0.641	0.037, 5.8	48/48	100 (92.6, 100)
APC Exon 16	c.2138C>G p.S713*	0.66-1.09	0.109-0.196	0.148	0.020, 13.8	48/48	100 (92.6, 100)
APC Exon 16	c.4348C>T p.R1450*	0.94-1.68	0.186-0.286	0.233	0.023, 10.1	48/48	100 (92.6, 100)
APC Exon 9	c.847C>T p.R283*	0.66-1.41	0.154-0.277	0.234	0.023, 9.9	48/48	100 (92.6, 100)
ASXL2 Exon 3	c.143G>A p.S48N	0.82-1.51	0.274-0.351	0.313	0.016, 5.2	48/48	100 (92.6, 100)
ATM Exon 47	c.6908delA p.K2303Rfs*7	0.84-2.28	0.287-0.381	0.324	0.020, 6.3	42/48	88 (75.3, 94.1)
ATM Exon 8	c.1009C>T p.R337C	0.55-1.19	0.256-0.392	0.326	0.028, 8.6	48/48	100 (92.6, 100)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
AXIN1 Exon 11	c.2463-14C>T	1.23-1.93	0.436-0.533	0.491	0.017, 3.6	48/48	100 (92.6, 100)
B2M Exon 1	c.3G>A	0.79-1.56	0.034-0.086	0.057	0.012, 21.3	47/48	98 (89.1, 99.6)
B2M Exon 1	c.43_44delCT p.L15Ffs*41	0.69-1.45	0.035-0.102	0.063	0.012, 19.8	48/48	100 (92.6, 100)
B2M Exon 2	c.244_247del TTCT p.F82Ifs*20	0.69-1.45	0.052-0.107	0.068	0.011, 16	45/48	94 (83.2, 97.9)
B2M Exon 2	c.142_145del TCTG p.S48Gfs*12	0.94-1.73	0.274-0.363	0.322	0.017, 5.4	48/48	100 (92.6, 100)
B2M Exon 2	c.68-1G>A	0.94-1.73	0.281-0.361	0.323	0.020, 6.1	48/48	100 (92.6, 100)
BRAF Exon 15	c.1799_1800delTGinsAA p.V600E	0.39-0.83	0.359-0.47	0.415	0.023, 5.5	48/48	100 (92.6, 100)
BRCA2 Exon 11	c.4588A>T p.K1530*	0.36-0.78	0.413-0.546	0.481	0.030, 6.3	46/46	100 (92.3, 100)
BRCA2 Exon 20	c.8581A>T p.R2861*	1.20-2.00	0.738-0.796	0.772	0.013, 1.7	48/48	100 (92.6, 100)
CIC Exon 14	c.3216_3217delGT p.S1073Tfs*77	0.27-0.776	0.702-0.855	0.773	0.035, 4.6	48/48	100 (92.6, 100)
CUL3 Exon 8	c.1162_1163delCT p.S389Ifs*4	0.37-1.35	0.093-0.182	0.132	0.020, 15.2	48/48	100 (92.6, 100)
DNMT3A Exon 23	c.2701delC p.L901Sfs*5	0.78-1.25	0.05-0.13	0.072	0.014, 19.4	46/48	96 (86.0, 98.8)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
DNMT3A Exon 6	c.541C>T p.R181C	0.05-0.68	0.227-0.55	0.331	0.086, 26	31/31	100 (89.0, 100)
EGFR Exon 19	c.2236_2250delGAATTAAGAGAAGCA p.E746_A750del	0.62-1.55	0.201-0.333	0.237	0.023, 9.6	48/48	100 (92.6, 100)
ERBB2 Exon 19	c.2305G>T p.D769Y	0.76-1.64	0.265-0.407	0.342	0.032, 9.4	46/46	100 (92.3, 100)
ERBB3 Exon 23	c.2783A>G p.E928G	1.07-2.55	0.024-0.042	0.03	0.004, 12.9	44/46	96 (84.6, 99.0)
ERCC2 Exon 21	c.1975C>A p.H659N	0.08-0.47	0.262-0.412	0.352	0.030, 8.6	48/48	100 (92.6, 100)
ERCC4 Exon 8	c.1811+1G>A	0.86-1.36	0.269-0.382	0.331	0.024, 7.3	48/48	100 (92.6, 100)
ETV6 Exon 4	c.427_428delCA p.Q143Afs*10	0.48-1.27	0.27-0.367	0.322	0.023, 7.3	48/48	100 (92.6, 100)
FANCA Exon 24	c.2222+7G>A	0.32-0.83	0.387-0.585	0.49	0.041, 8.4	48/48	100 (92.6, 100)
FANCA Exon 38	c.3828+1G>C	0.25-0.75	0.686-0.795	0.75	0.020, 2.7	48/48	100 (92.6, 100)
FAT1 Exon 26	c.13002A>C p.T4334T	1.16-1.92	0.175-0.26	0.208	0.018, 8.7	48/48	100 (92.6, 100)
FUBP1 Exon 14	c.1213C>T p.Q405*	0.49-1.25	0.265-0.351	0.31	0.023, 7.3	48/48	100 (92.6, 100)
IDH1 Exon 4	c.395G>A p.R132H	1.60-2.19	0.414-0.503	0.458	0.019, 4.2	48/48	100 (92.6, 100)
KEAP1 Exon 4	c.1531+8G>C	0.88-1.52	0.454-0.565	0.514	0.026, 5.1	48/48	100 (92.6, 100)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
KMT2A Exon 3	c.1142delA p.K381Rfs*19	1.34-5.37	0.296-0.384	0.34	0.018, 5.4	42/48	88 (75.3, 94.1)
KMT2D Exon 32	c.7061delC p.P2354Lfs*30	0.78-1.55	0.156-0.243	0.208	0.019, 9	45/48	94 (83.2, 97.9)
KMT2D Exon 49	c.14710C>T p.R4904*	0.85-1.71	0.3-0.373	0.328	0.016, 4.9	48/48	100 (92.6, 100)
KRAS Exon 2	c.35G>C p.G12A	1.03-2.52	0.023-0.061	0.039	0.008, 20.4	44/46	96 (85.5, 98.8)
KRAS Exon 2	c.34G>T p.G12C	1.32-2.88	0.028-0.069	0.044	0.011, 25.8	25/31	81 (63.7, 90.8)
KRAS Exon 2	c.38G>A p.G13D	1.32-2.88	0.281-0.354	0.307	0.018, 6	31/31	100 (89.0, 100)
KRAS Exon 2	c.35G>T p.G12V	0.96-1.86	0.327-0.404	0.364	0.017, 4.6	48/48	100 (92.6, 100)
MAX Exon 4	c.179G>A p.R60Q	0.86-2.06	0.286-0.369	0.325	0.021, 6.4	48/48	100 (92.6, 100)
MLH3 Exon 3	c.3367C>T p.Q1123*	1.34-2.07	0.415-0.573	0.489	0.039, 8	31/31	100 (89.0, 100)
MRE11 Exon 14	c.1563+1G>A	0.64-1.76	0.427-0.546	0.488	0.026, 5.4	48/48	100 (92.6, 100)
NBN Exon 2	c.171+4T>C	0.76-1.44	0.419-0.538	0.474	0.025, 5.3	46/46	100 (92.3, 100)
NOTCH1 Exon 26	c.4777C>A p.L1593M	0.10-2.37	0.295-0.484	0.361	0.037, 10.4	48/48	100 (92.6, 100)
NOTCH3 Exon 4	c.348_349delCT p.D116Efs*45	0.71-1.90	0.328-0.46	0.388	0.028, 7.3	47/48	98 (89.1, 99.6)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
PIK3CA Exon 21	c.3140A>G p.H1047R	0.92-1.80	0.038-0.082	0.062	0.012, 19.5	26/31	84 (67.4, 92.9)
PIK3CA Exon 21	c.3073A>G p.T1025A	0.93-1.58	0.194-0.273	0.233	0.016, 6.7	48/48	100 (92.6, 100)
PIK3CA Exon 10	c.1624G>A p.E542K	0.25-1.60	0.502-0.608	0.564	0.018, 3.2	48/48	100 (92.6, 100)
POLD1 Exon 17	c.2066G>A p.R689Q	0.10-1.01	0.253-0.356	0.297	0.028, 9.5	48/48	100 (92.6, 100)
PPM1D Exon 6	c.1281G>A p.W427*	0.62-1.02	0.245-0.359	0.303	0.026, 8.4	48/48	100 (92.6, 100)
PTEN Exon 5	c.389G>A p.R130Q	0.71-1.09	0.032-0.115	0.063	0.014, 22.7	48/48	100 (92.6, 100)
PTEN Exon 7	c.741_742insA p.P248Tfs*5	0.48-0.77	0.074-0.149	0.109	0.017, 15.9	48/48	100 (92.6, 100)
RAD51B Exon 6	c.453-7C>T	0.65-1.29	0.514-0.645	0.573	0.027, 4.7	48/48	100 (92.6, 100)
RBM10 Exon 13	c.1444-2A>C	0.69-1.26	0.193-0.351	0.297	0.036, 12	31/31	100 (89.0, 100)
RECQL4 Exon 10	c.1704+6G>T	2.00-3.67	0.274-0.364	0.318	0.020, 6.4	48/48	100 (92.6, 100)
SETD2 Exon 20	c.7432-10C>T	0.92-1.31	0.103-0.205	0.144	0.020, 14.1	48/48	100 (92.6, 100)
SOX9 Exon 3	c.1280_1281dup p.Y428Tfs*43	0.40-1.27	0.096-0.145	0.117	0.010, 8.8	48/48	100 (92.6, 100)
TET2 Exon 3	c.1771C>T p.Q591*	0.42-1.16	0.051-0.084	0.07	0.008, 11.7	47/48	98 (89.1, 99.6)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage range	VAF range	VAF mean	VAF (SD), VAF (%CV)	Positive /Total Calls	PCR% (95% CI)
TGFBR2 Exon 6	c.1411G>A p.D471N	0.62-1.33	0.297-0.366	0.334	0.014, 4.3	48/48	100 (92.6, 100)
TP53 Exon 6	c.614A>G p.Y205C	0.40-1.08	0.209-0.3	0.253	0.019, 7.5	48/48	100 (92.6, 100)
TP53 Exon 7	c.742C>T p.R248W	0.59-1.14	0.437-0.537	0.492	0.022, 4.5	48/48	100 (92.6, 100)
TP53 Exon 5	c.536A>G p.H179R	1.10-2.45	0.301-0.563	0.507	0.037, 7.3	45/48	94 (83.2, 97.9)
TSC2 Exon 27	c.3131+2T>A	0.68-2.98	0.299-0.394	0.344	0.023, 6.6	48/48	100 (92.6, 100

b. **Per-Specimen Precision for SNVs and Indels:**

Precision was assessed at the specimen level by evaluating the PCR and NCR for all detected mutations within each sample, along with the 95% CI.

Table 8: Per-Specimen Precision

Specimen	Cancer Type	Total Unique mutations[1]	PCR per Mutation	PCR %, n/N, (95% CI)	NCR %, n/N, (95% CI)
Sample 1	Ovary / Fallopian Tube	4	45/48 for 1[3] 48/48 for 3	98.43 189/192 (95.5, 99.5)	100 42672/42672, (99.99,100)
Sample 2	Thyroid	1	48/48 for 1	100 48/48, (92.6, 100)	100 42672/42672, (99.99,100)
Sample 3	Prostate	1	48/48 for 1	100 48/48, (92.6, 100)	100, 42672/42672, (99.99,100)
Sample 4	Bowel	6	25/31 for 1[2] 26/31 for 1[2] 31/31 for 4[2]	94.09, 175/186, (88.0, 95.5)	100, 42672/42672, (99.99,100)

Specimen	Cancer Type	Total Unique mutations[1]	PCR per Mutation	PCR %, n/N, (95% CI)	NCR %, n/N, (95% CI)
Sample 5	Lung	1	46/48 for 1^3	95.83, 46/48, (85.8, 99.5)	100, 42720/42720, (100, 100)
Sample 6	Bowel	16	42/48 for 2^3 45/48 for 1^3 48/48 for 13	98.04, 753/768, (96.8, 98.8)	99.99, 42522/42528, (99.97,99.99)
Sample 7	Lung	1	47/48 for 1	97.91, 47/48, (89.1, 99.6)	99.91, 42634/42672, (99.88,99.94)
Sample 8	Prostate	2	46/46 for 2^2	100, 92/92, (96.0, 100)	100, 40940/40940, (99.99,100)
Sample 9	Lung	6	48/48 for 6	100, 288/288, (98.7, 100)	100, 42623/42624, (99.99,100)
Sample 10	Bowel	12	45/48 for 1^3 47/48 for 1^3 48/48 for 10	99.3, 572/576, (98.7, 100)	100, 42432/42432, (99.99,100)
Sample 11	CNS / Brain	4	48/48 for 4	100, 192/192, (98.0, 100)	99.94, 42646/42672, (99.91,99.96)
Sample 12	Bowel	3	44/46 for $1^{2,3}$ 44/46 for 1^2 46/46 for 1^2	97.1, 134/138, (98.0, 100)	100, 40801/40802, (99.99,100)
Sample 13	Breast	6	47/48 for 1^3 48/48 for 5	99.65, 287/288, (98.1, 99.9)	100, 42623/42624, (99.99,100)

[1]Detected across 48 replicates; [*]Reduction in number of replicates due to QC failure of data, **Variant with VAF at LOD; PCR: Positive Call Rate; NCR: Negative Call Rate

c. **Analysis of Source of Variants**

The Average Positive Agreement (APA) and Average Negative Agreement (ANA) metrics were assessed to analyze the imprecision caused by different sources of

variance. Data analysis is presented stratified by variant type and presented for overall, inter-instrument, inter-operator, and inter-day.

Table 9. Precision Metrics: Analysis of Variation Sources

Alteration Type	Metric	Overall %, (95% CI)	Inter-Instrument %, (95% CI)	Inter-Operator %, (95% CI)	Inter-Day %, (95% CI)
Variants with Evidence of Clinical Significance	**APA**	99.56 (98.42, 99.88)	99.56 (95.19, 100)	99.57 (98.34, 100)	99.57 (96.74, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Hotspot	**APA**	97.99 (96.98, 98.67)	97.94 (95.27, 99.44)	97.95 (96.21, 98.74)	97.99 (95.31, 98.99)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Non-Hotspot	**APA**	99.12 (98.57, 99.46)	99.11 (97.62, 99.82)	99.11 (98.27, 99.55)	99.12 (97.76, 99.66)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
SNVs	**APA**	99.13 (98.65, 99.44)	99.11 (97.61, 99.72)	99.12 (98.33, 99.50)	99.13 (97.88, 99.61)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (All)	**APA**	100 (97.40, 100)	100 (86.20, 100)	100 (94.93, 100)	100 (90.36, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (1 – 5 bp)	**APA**	100 (97.40, 100)	100 (86.20, 100)	100 (94.93, 100)	100 (90.36, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (5 – 10 bp)	**APA**	-	-	-	-
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (11 – 30 bp)	**APA**	-	-	-	-
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (All)	**APA**	96.69 (94.89, 97.87)	96.69 (89.77, 98.37)	96.69 (93.71, 98.10)	96.69 (92.13, 98.51)

Alteration Type	Metric	Overall %, (95% CI)	Inter-Instrument %, (95% CI)	Inter-Operator %, (95% CI)	Inter-Day %, (95% CI)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (1 – 5 bp)	**APA**	96.39 (94.43, 97.68)	96.38 (88.89, 98.22)	96.38 (93.14, 97.92)	96.39 (91.44, 98.37)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (5 – 10 bp)	**APA**	-	-	-	-
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (11 – 30 bp)	**APA**	100 (92.59, 100)	100 (67.56, 100)	100 (86.20, 100)	100 (75.75, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
ALK Fusion	**APA**	100 (92.13, 100)	100 (60.97, 100)	100 (79.61, 100)	100 (74.12, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)
RET Fusion	**APA**	100 (92.29, 100)	100 (64.57, 100)	100 (79.61, 100)	100 (75.75, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)
ROS1 Fusion	**APA**	95.00 (83.50, 98.62)	91.67 (43.65, 96.99)	95.83 (77.19, 100)	95.00 (72.25, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)
ERBB2 Amplification	**APA**	100 (92.59, 100)	100 (67.56, 100)	100 (86.20, 100)	100 (75.75, 100)
	ANA	100 (99.25, 100)	100 (95.68, 100)	100 (98.51, 100)	100 (97.06, 100)

-: Not available / applicable

d. **Precision for *ERBB2* amplification**

The precision for *ERBB2* amplification was evaluated using 15 specimens across the primary and supplemental studies, of which 4 had confirmed *ERBB2* amplification and 11 were *ERBB2* amplification negative. The *ERBB2* amplification-positive specimens include one specimen evaluated in the primary

precision study and three additional specimens evaluated in a supplemental precision study. The CellDx-Tissue reports an ERBB2 amplification when the observed copy number for the gene is determined by the test to be 8.5 copies or more. For all positive specimens (n = 4), the mean copy number (CN) at each input level and pooled across input levels are presented below. 100% PCR with consistent CN and low CV were observed at both input levels with these performance metrics meeting predefined acceptance criteria.

Table 10: PCR for *ERBB2* Amplification.

Specimen	Cancer type	Tumor Purity	Input (ng)	Mean CN	CN CV (%)	PCR %, (n/N), (95% CI)
Sample 1	Breast	58%	10	16.3	11.0	100, (24/24), (85.7,100)
			20	16.4	7.0	100, (24/24), (85.7,100)
			(10, 20)	16.4	8.8	100, (48/48), (92.6,100)
Sample 2	Bowel	21%	10	8.06	6.3	100, (12/12), (75.8, 100)
			20	7.82	4.9	100, (12/12), (75.8, 100)
			(10, 20)	7.94	5.8	100, (24/24), (86.2, 100)
Sample 3	Esophagus	49%	10	15.57	10.2	100, (12/12), (75.8, 100)
			20	15.34	7.7	100, (12/12), (75.8, 100)
			(10, 20)	15.46	8.9	100, (24/24), (86.2, 100)
Sample 4	Ovarian / Fallopian Tube	55%	10	14.54	7.1	100, (12/12), (75.8, 100)
			20	14.02	7.0	100, (12/12), (75.8, 100)
			(10, 20)	14.28	7.2	100, (24/24), (86.2, 100)

All *ERBB2* amplification-negative specimens (n = 11), representing various cancer types and tumor purities, demonstrated 100% NCR with well-controlled CI and confirmed the assay's high specificity. The mean observed CNs ranged from 1.28-2.73 and were below the amplification threshold. Variations in SD and CV (3.1% - 16.3%) reflected the inherent variability in low-CN measurements.

Table 11: *ERBB2* Precision Metrics for Negative Call Rate.

Specimen	Cancer Type	Tumor Purity	Input (ng)	Mean CN	CN CV (%)	NCR %, (n/N), (95% CI)
Sample 1	CNS	92%	10	1.98	5.1	100, (24/24), (86.2, 100)
			20	1.98	4.6	100, (24/24), (86.2, 100)
			(10, 20)	1.98	4.8	100, (48/48), (92.6, 100)
Sample 2	Bowel	37%	10	1.34	15.2	100, (24/24), (86.2, 100)
			20	1.32	14.9	100, (24/24), (86.2, 100)
			(10, 20)	1.33	14.9	100, (48/48), (92.6, 100)
Sample 3	Lung	28%	10	1.97	8.8	100, (24/24), (86.2, 100)
			20	1.93	8.8	100, (24/24), (86.2, 100)
			(10, 20)	1.95	8.8	100, (48/48), (92.6, 100)
Sample 4	Prostate	22%	10	1.79	18.6	100, (24/24), (86.2, 100)
			20	1.75	13.7	100, (22/22), (85.1, 100)
			(10, 20)	1.77	16.3	100, (46/46), (91.3, 100)
Sample 5	Lung	20%	10	1.28	15.1	100, (24/24), (86.2, 100)
			20	1.28	16.4	100, (24/24), (86.2, 100)
			(10, 20)	1.28	15.6	100, (48/48), (92.6, 100)
Sample 6	Bowel	29%	10	1.54	5.7	100, (24/24), (86.2, 100)
			20	1.54	10.3	100, (24/24), (86.2, 100)
			(10, 20)	1.54	8.3	100, (48/48), (92.6, 100)
Sample 7	Lung	45%	10	1.58	11.8	100, (24/24), (86.2, 100)
			20	1.62	9.2	100, (24/24), (86.2, 100)
			(10, 20)	1.60	10.5	100, (48/48), (92.6, 100)
Sample 8	Bowel	39%	10	2.87	24.2	100, (7/7), (59.0, 100)
			20	2.70	8.0	100, (24/24), (86.2, 100)
			(10, 20)	2.73	13.6	100, (31/31), (88.7, 100)
Sample 9	Prostate	36%	10	1.98	6.6	100, (24/24), (86.2, 100)
			20	2.08	5.7	100, (24/24), (86.2, 100)
			(10, 20)	2.03	6.5	100, (48/48), (92.6, 100)
Sample 10	Thyroid	22%	10	1.99	2.9	100, (24/24), (86.2, 100)

Specimen	Cancer Type	Tumor Purity	Input (ng)	Mean CN	CN CV (%)	NCR %, (n/N), (95% CI)
			20	1.99	3.3	100, (24/24), (86.2, 100)
			(10, 20)	1.99	3.1	100, (48/48), (92.6, 100)
Sample 11	Ovary	56%	10	1.52	13.7	100, (24/24), (86.2, 100)
			20	1.58	9.1	100, (24/24), (86.2, 100)
			(10, 20)	1.55	11.6	100, (48/48), (92.6, 100)

e. **Precision for Fusions**

The precision for *ALK*, *RET* and *ROS1* fusion detection was evaluated using specimens with varying tumor purities. The results consistently demonstrate high PCR across all evaluated RNA fusion genes. For *ALK* fusion, 100% PCR (45/45, 95% CI: 92.1% - 100%) was reported. For *RET* Fusion, 100% PCR (46/46, 95% CI: 92.3% - 100%) was reported. For *ROS1* fusion, 95% PCR (38/40, 95% CI: 90.8% to 100%) was reported.

Table 12: RNA Fusion Precision Summary

Fusion	Tumor Purity	LoD Level	Mean supporting reads (range)	PCR %, (n/N), (95% CI)	NCR (n/N) (95% CI)
ALK	20%	1x *	15062 (7121-30568)	100 (45/45), (92.1, 100)	100 (609/609) (99.4, 100)
RET	25%	1.8x *	24438 (740-175619)	100 (46/46), (92.3, 100)	100 (608/608) (99.4, 100)
ROS1	45%	0.8x **	1273 (504-2328)	95.0 (38/40), (90.8, 100)	100 (614/614) (99.4, 100)

** Tumor purity, ** fusion supporting reads*

f. **Lot-to-Lot Precision**

CellDx-Tissue Performance was assessed across two kit lots by determining the concordance of variant calls in FFPE specimens. The table below lists the average percent agreement (APA) and average negative agreement (ANA) used to assess inter-lot performance.

Table 13. Lot-to-Lot Precision.

Variant Type	Performance	Lot 1 vs Lot 2 (95% CI)
Variants with Evidence of Clinical Significance	APA	99.57 (98.34, 100)
	ANA	100 (100, 100)
Panel-Wide SNVs + InDels	APA	98.68 (97.97, 99.16)
	ANA	100 (100, 100)
Hotspot SNVs (including Level 2)	APA	97.95 (96.21, 98.74)
	ANA	100 (100, 100)
Non-Hotspot SNVs (including Level 2)	APA	99.11 (98.27, 99.55)
	ANA	100 (100, 100)
SNVs (Hotspot + Non-Hotspot)	APA	99.12 (98.33, 99.50)
	ANA	100 (100, 100)
Insertions	APA	100 (94.93, 100)
	ANA	100 (100, 100)
Deletions	APA	96.69 (93.71, 98.10)
	ANA	100 (100, 100)
ERBB2 Amplification	APA	100 (86.2, 100)[1] 100 (90.36, 100)[2]
	ANA	100 (98.51, 100)[1]
ALK Fusion	APA	100 (85.13, 100)
	ANA	100 (98.41, 100)
RET Fusion	APA	100 (85.69, 100)
	ANA	100 (98.41, 100)
ROS1 Fusion	APA	93.75 (76.39, 99.11)
	ANA	100 (98.41, 100)

[1]*Primary Study,* [2]*Supplemental Study*

4.3 Limit of Blank

Non-cancerous FFPE tissues were evaluated for analytical specificity to access the risk of false positives in normal tissue when detecting SNVs, indels, amplifications, and fusions using the CellDx – Tissue assay. A total of 37 normal or benign-adjacent tissue were processed across two reagent lots, multiple operators, and days. Ninety

(90) false positive events were detected for SNVs and indels for a false positive rate (FPR) of 0.000054 (95 CI: 0.000043 - 0.000066). None of the detected variants were classified as Variants with Evidence of Clinical Significance (Level 2), while four were classified as Variants with Potential Evidence of Clinical Significance (Level3). The FPR was determined to be 0 (95 CI: 0 - 3.09) for ERBB2 amplifications, and ALK, RET, and ROS1 fusion genes.

Table 14: False Positive Rates by Variant Type

Variant Type	**False Positives**	**Total Positions/ Observations**	**FPR (%), (95% CI)**
(SNVs/InDels	90	167,807,731	0.000054 (0.000043, 0.000066)
ERBB2 Amplification	0	119	0 (0, 3.09)
ALK Fusions	0	119	0 (0, 3.09)
RET Fusions	0	119	0 (0, 3.09)
ROS1 Fusions	0	119	0 (0, 3.09)

4.4 Limit of Detection

a. SNVs and Indels:

The LoD for SNVs and InDels was determined from 10 clinical FFPE tumor tissues representing seven organ types (Lung 3, Uterus 2, Prostate 1, Head and neck 1, Esophagus 1, Breast 1 and Bowel 1) and encompassing 29 SNVs, 1 insertion, and 2 deletions. Each sample was diluted to 5 levels and tested in 10 replicates per level using 2 reagent lots. The established VAF ranges for each variant type, based on analytical cutoffs of 2% (0.02 VAF) for hotspot variants, and 5% (0.05 VAF) for non-hot spot variants, for all evaluated variants are summarized below:

Table 15. Estimation of the LoD range for representative variants, including variant ID, gene, exon, mean MAF range and call rates.

Tumor Type	Mut Type	Level	Gene	AA Change	Avg DP	Avg AD	VAF Range	Mean VAF	PCR %
Lung	SNV	Level-3	*TP53*	p.R175H	1869	68	0.026-0.048	0.037	100
Lung	SNV	Level-5	*TET2*	c.3954+3T>G	1426	63	0.024-0.098	0.043	100
Lung	DEL	Level-5	*EGFR*	p.E746_A750del	1939	69	0.026-0.058	0.035	100
Breast	SNV	Level-2	*GPS2*	c.318-6C>T	1886	123	0.056-0.078	0.065	100
Breast	SNV	Level-4	*PIK3CA*	p.H1047R	1678	58	0.022-0.049	0.035	100
Uterus	SNV	Level-2	*TP53*	p.R273C	1955	75	0.024-0.053	0.038	100
Uterus	SNV	Level-2	*CTLA4*	p.A54T	2000	132	0.054-0.078	0.066	100
Uterus	SNV	Level-3	*CTNNB1*	p.G34E	1959	74	0.026-0.045	0.037	100
Head & Neck	SNV	Level-2	*NRAS*	p.A59T	2000	107	0.044-0.071	0.054	100
Head and Neck	SNV	Level-3	*DICER1*	c.3269+6C>T	1921	128	0.045-0.13	0.068	100
Head and Neck	SNV	Level-4	*TP53*	p.G262V	1885	75	0.026-0.047	0.039	100
Head and Neck	SNV	Level-3	*NF2*	p.R196*	659	48	0.059-0.104	0.067	100
Head and Neck	SNV	Level-4	*EP300*	c.3728+5G>A	1271	56	0.035-0.052	0.040	100
Head and Neck	SNV	Level-4	*FAT1*	p.R1205*	1919	118	0.05-0.078	0.061	100
Head and Neck	SNV	Level-4	*LATS1*	p.Q953*	1603	56	0.028-0.047	0.034	100
Head and Neck	SNV	Level-3	*PHF6*	p.Y301*	938	52	0.041-0.077	0.057	100
Bowel	SNV	Level-2	*TP53*	p.R273H	1925	127	0.052-0.077	0.066	100
Esophagus/Stomach	INS	Level-4	*ARID1A*	p.A1136Gfs*57	1503	92	0.042-0.088	0.062	100

Tumor Type	Mut Type	Level	Gene	AA Change	Avg DP	Avg AD	VAF Range	Mean VAF	PCR %
Esophagus/Stomach	SNV	Level-3	*TP53*	p.R209*	1924	165	0.064-0.16	0.085	100
Esophagus/Stomach	SNV	Level-3	*POT1*	p.E456*	1281	71	0.032-0.107	0.055	100
Lung	SNV	Level-1	*TP53*	p.R248W	1797	124	0.043-0.094	0.069	100
Uterus	SNV	Level-2	*FANCA*	c.2015-8C>T	1765	101	0.04-0.066	0.057	100
Uterus	SNV	Level-2	*TP53*	p.Y220C	1287	85	0.055-0.083	0.066	100
Uterus	SNV	Level-2	*PPP2R1A*	p.P179R	1708	57	0.022-0.05	0.034	100
Prostate	SNV	Level-5	*MLH1*	p.Q346H	1781	82	0.033-0.056	0.041	100
Prostate	SNV	Level-5	*AR*	p.L702H	1579	83	0.044-0.072	0.054	100
Lung	INS	Level-2	*ARID1A*	p.P1468Lfs*13	938	88	0.076-0.122	0.095	100
Lung	SNV	Level-5	*TCF7L2*	c.1162-12C>T	1720	134	0.039-0.144	0.080	100
Lung	SNV	Level-3	*TP53*	p.Y126*	1911	151	0.029-0.154	0.072	100
Lung	SNV	Level-4	*FANCE*	c.855+6T>A	1029	64	0.026-0.095	0.052	100
Lung	SNV	Level-2	*KMT2C*	p.E1333*	891	105	0.045-0.214	0.115	100
Lung	SNV	Level-3	*KMT2D*	c.840-10G>A	1861	147	0.025-0.153	0.073	100

Table 16. LoD for representative SNVs and indels.

Variant	Established VAF Range	Tumor Purity	Avg Allele Depth	Number of Variants in Clinical Cases within the Established Range
Hotspot SNVs	0.022 to 0.094	7%	57-124	10
Non-hotspot SNVs	0.024 to 0.214	4%-24%	63-105	19

Variant	Established VAF Range	Tumor Purity	Avg Allele Depth	Number of Variants in Clinical Cases within the Established Range
Insertions	0.042 to 0.088	6%	92	1
Deletions	0.026 to 0.122	4-24%	69-88	2
Indels: Homopolymer context	0.042 to 0.088	6%	92	1
InDels: Non-Homopolymer context	0.026 to 0.122	4-24%	69-88	2

Table 17: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R175H	1662	184	0.111	100 (10/10)
Level-2		1469	108	0.073	100 (10/10)
Level-3		1869	68	0.036	100 (10/10)
Level-4		1954	37	0.019	20 (2/10)
Level-5		(not detected)			0% (0/10)

Table 18: *TET2* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.3954+3T>G	1049	410	0.386	100 (10/10)
Level-2		997	275	0.281	100 (10/10)
Level-3		1349	219	0.158	100 (10/10)
Level-4		1323	104	0.08	100 (10/10)
Level-5		1426	63	0.042	100 (10/10)

Table 19: *EGFR* Deletion

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	E746_A750del	1887	486	0.256	100 (10/10)
Level-2		1678	321	0.192	100 (10/10)
Level-3		1914	209	0.108	100 (10/10)
Level-4		1896	109	0.057	100 (10/10)
Level-5		1939	69	0.035	100 (10/10)

Table 20: *GPS2* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.318-6C>T	1695	248	0.145	100 (10/10)
Level-2		1886	123	0.065	100 (10/10)
Level-3		1713	58	0.029	90 (9/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 21: *PIK3CA* c.318-6C>T SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	H1047R	1940	411	0.212	100 (10/10)
Level-2		1885	238	0.126	100 (10/10)
Level-3		1614	119	0.075	100 (10/10)
Level-4		1678	58	0.035	100 (10/10)
Level-5		(not detected)			0 (/10)

Table 22: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R273C	1928	157	0.081	100 (10/10)
Level-2		1955	75	0.038	100 (10/10)
Level-3		1920	28	0.014	20 (2/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 23: *CTLA4* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	A54T	2000	294	0.146	100 (10/10)
Level-2		2000	132	0.066	100 (10/10)
Level-3		2000	60	0.017	60 (6/10)
Level-4		2000	46	0.002	10 (1/10)
Level-5		(not detected)			0 (/10)

Table 24: *CTNNB1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	G34E	1998	381	0.19	100 (10/10)
Level-2		1999	171	0.085	100 (10/10)
Level-3		1959	74	0.037	100 (10/10)
Level-4		1983	38	0.018	50 (5/10)

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-5		1999	28	0.014	30 (3/10)

Table 25: *NRAS* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	A59T	1999	223	0.111	100 (10/10)
Level-2		2000	107	0.053	100 (10/10)
Level-3		1999	50	0.025	80 (8/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 26: *DICER1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.3269+6C>T	1961	478	0.243	100 (10/10)
Level-2		1876	238	0.126	100 (10/10)
Level-3		1921	128	0.068	100 (10/10)
Level-4		1932	62	0.025	80 (8/10)
Level-5		1571	47	0.012	40 (4/10)

Table 27: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	G262V	1950	705	0.36	100 (10/10)
Level-2		1998	350	0.175	100 (10/10)
Level-3		1965	166	0.084	100 (10/10)
Level-4		1885	75	0.039	100 (10/10)
Level-5		1769	33	0.017	20 (2/10)

Table 28: *NF2* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R196*	640	204	0.327	100 (10/10)
Level-2		637	100	0.159	100 (10/10)
Level-3		659	48	0.067	100 (10/10)
Level-4		780	40	0.03	60 (6/10)
Level-5		690	25	0.007	20 (2/10)

Table 29: *EP300* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.3728+5G>A	1126	358	0.316	100 (10/10)
Level-2		1317	205	0.154	100 (10/10)
Level-3		1145	98	0.083	100 (10/10)
Level-4		1271	56	0.039	100 (10/10)
Level-5		736	22	0.006	20 (2/10)

Table 30: *FAT1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R1205*	1998	819	0.41	100 (10/10)
Level-2		1987	464	0.233	100 (10/10)
Level-3		1927	222	0.114	100 (10/10)
Level-4		1919	118	0.061	100 (10/10)
Level-5		1707	61	0.034	90 (9/10)

Table 31: *LATS1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	Q953*	1861	406	0.217	100 (10/10)
Level-2		1755	217	0.123	100 (10/10)
Level-3		1681	114	0.068	100 (10/10)
Level-4		1603	56	0.034	100 (10/10)
Level-5		(not detected)			0 (/10)

Table 32: *PHF6* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	Y301*	798	226	0.284	100 (10/10)
Level-2		883	116	0.131	100 (10/10)
Level-3		938	52	0.056	100 (10/10)
Level-4		1134	34	0.024	80 (8/10)
Level-5		778	20	0.002	10 (1/10)

Table 33: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R273H	1834	280	0.152	100 (10/10)
Level-2		1925	127	0.065	100 (10/10)
Level-3		2000	60	0.03	80 (8/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 34: *ARID1A* Insertion

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	A1136Gfs*57	1816	728	0.4	100 (10/10)
Level-2		1649	349	0.212	100 (10/10)
Level-3		1800	201	0.111	100 (10/10)
Level-4		1503	92	0.062	100 (10/10)
Level-5		(not detected)			0 (/10)

Table 35: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R209*	1823	615	0.337	100 (10/10)
Level-2		1682	278	0.165	100 (10/10)
Level-3		1924	165	0.085	100 (10/10)
Level-4		1686	63	0.029	80 (8/10)
Level-5		1986	65	0.003	10 (1/10)

Table 36: *POT1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	E456*	1585	234	0.145	100 (10/10)
Level-2		1387	126	0.088	100 (10/10)
Level-3		1281	71	0.054	100 (10/10)
Level-4		836	31	0.023	60 (6/10)
Level-5		1144	32	0.002	10 (1/10)

Table 37: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	R248W	1797	124	0.068	100 (10/10)
Level-2		1893	51	0.026	60 (6/10)
Level-3		(not detected)			0 (/10)
Level-4		(not detected)			0 (/10)

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-5		(not detected)			0 (/10)

Table 38: *FANCA* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.2015-8C>T	1529	202	0.131	100 (10/10)
Level-2		1765	101	0.056	100 (10/10)
Level-3		1810	49	0.021	80 (8/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 39: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	Y220C	1026	152	0.15	100 (10/10)
Level-2		1287	85	0.065	100 (10/10)
Level-3		1180	36	0.031	70 (7/10)
Level-4		1087	17	0.015	20 (2/10)
Level-5		(not detected)			0 (/10)

Table 40: *PPP2R1A* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	P179R	1368	126	0.09	100 (10/10)
Level-2		1708	57	0.034	100 (10/10)
Level-3		1700	29	0.017	20 (2/10)
Level-4		1648	11	0.006	0 (/10)
Level-5		(not detected)			0 (/10)

Table 41: *MLH1* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	Q346H	1680	685	0.411	100 (10/10)
Level-2		1899	500	0.262	100 (10/10)
Level-3		1579	237	0.147	100 (10/10)
Level-4		1851	156	0.084	100 (10/10)
Level-5		1781	82	0.04	100 (10/10)

Table 42: *AR* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	L702H	1280	704	0.554	100 (10/10)
Level-2		1650	606	0.36	100 (10/10)
Level-3		1110	249	0.261	100 (10/10)
Level-4		1580	186	0.113	100 (10/10)
Level-5		1579	83	0.053	100 (10/10)

Table 43: *ARID1A* Deletion

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	P1468Lfs*13	983	158	0.162	100 (10/10)
Level-2		938	88	0.094	100 (10/10)
Level-3		1099	65	0.029	50 (5/10)
Level-4		(not detected)			0 (/10)
Level-5		(not detected)			0 (/10)

Table 44: *TCF7L2* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.1162-12C>T	1633	605	0.368	100 (10/10)
Level-2		1586	388	0.243	100 (10/10)
Level-3		1472	190	0.13	100 (10/10)
Level-4		1816	154	0.082	100 (10/10)
Level-5		1720	134	0.08	100 (10/10)

Table 45: *TP53* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	Y126*	1854	378	0.204	100 (10/10)
Level-2		1798	248	0.138	100 (10/10)
Level-3		1861	147	0.072	100 (10/10)
Level-4		1959	90	0.036	80 (8/10)
Level-5		1814	90	0.035	70 (7/10)

Table 46: *FANCE* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.855+6T>A	1471	260	0.174	100 (10/10)
Level-2		1244	155	0.125	100 (10/10)
Level-3		1444	118	0.082	100 (10/10)
Level-4		1029	64	0.051	100 (10/10)

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-5		1351	84	0.046	70 (7/10)

Table 47: *KMT2C* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	E1333*	876	155	0.179	100 (10/10)
Level-2		891	105	0.115	100 (10/10)
Level-3		758	76	0.06	60 (6/10)
Level-4		1147	51	0.031	70 (7/10)
Level-5		1075	57	0.028	50 (5/10)

Table 48: *KMT2D* SNV

Dilution	AA Change	Average DP	Average AD	Average VAF	PCR% (n/N)
Level-1	c.840-10G>A	1854	378.4	0.205	100 (10/10)
Level-2		1798	248	0.139	100 (10/10)
Level-3		1861	147	0.073	100 (10/10)
Level-4		1959	90	0.037	80 (8/10)
Level-5		1814	90	0.035	70 (7/10)

b. *ERBB2* Gene Amplification:

The LoD for *ERBB2* amplification was assessed using a single positive breast cancer sample. At high tumor purity levels (60% - 15%), PCRs were 100% with mean observed CN between 30.2 and 8.5. At 8% tumor purity and below, the PCRs did not meet acceptance criteria. CellDx-Tissue achieves consistent detection at CN≥8.5 (tumor purity ≥15%).

Table 49: *ERBB2* amplification detection rates across tumor purity levels, including observed copy numbers and call rates.

Dilution Level	Tumor Purity (%)	Mean Observed Copy Number (Mean ± SD)	Observed Copy Number Range	Positive Calls (n/N)	Positive Call Rate (%)
1	60	30.2 ± 0.8	29–32	10/10	100
2	30	15.0 ± 0.7	14–16	10/10	100
3	15	8.5 ± 0.2	8–11	10/10	100
4	8	5.1 ± 0.9	4–7	2/10	20

Dilution Level	Tumor Purity (%)	Mean Observed Copy Number (Mean ± SD)	Observed Copy Number Range	Positive Calls (n/N)	Positive Call Rate (%)
5	4	3.15 ± 0.1	3–3	0/10	0

c. RNA Variants (Fusions):

The LoD for fusions was evaluated using 3 FFPE specimens (all lung cancer). The *ROS1* fusion was tested across sixteen dilution levels (across the primary and supplemental studies), with tumor purity ranging from 70% to 2%. assay demonstrated consistent performance (≥95% detection rate) at tumor purities of 5% and greater. Mean read counts decreased progressively with decreasing tumor purity, ranging from 80,775 reads at 70% tumor purity to 883 reads at 3% tumor purity with no reads at 2% tumor purity. At 5% tumor purity, the minimum observed read counts were 1,605 (primary study) and 892 (supplemental study). Based on these findings, the LoD for *ROS1* is estimated to be 5% tumor purity, with a minimum read count threshold of approximately 892 reads ensuring consistent detection.

The observed read counts at 5% tumor purity in the supplemental evaluation were lower than those observed in the primary study, reflecting expected specimen-to-specimen variability in transcript abundance.

The *ALK* fusion was tested across five dilution levels, with tumor purity ranging from 20% to 6%. At the highest tumor purity (20%), the assay achieved 100% detection rates, with mean read counts of 4,868 reads. Detection rates decreased at lower tumor purities, being 80% at 15% tumor purity, and 30% at tumor purities of 11%, 8%, and 6%, respectively. Mean read counts at lower tumor purities ranged from 1,048–1,104 reads at 15% and 11% tumor purity to 931–1,014 reads at 8% and 6% tumor purity. Based on these findings, the LoD for *ALK* is estimated to be 20% tumor purity , with a minimum read count threshold of approximately 1640 reads indicating consistent detection.

The *RET* fusion was tested across five dilution levels, with tumor purity ranging from 25% to 8%. The assay achieved 100% detection rates at tumor purities of 25%–11%. At 8% tumor purity, the detection rate decreased to 86% (6/7 replicates detected). Mean read counts ranged from 11,534 reads at 25% tumor purity to 6,095 reads at 8% tumor purity. At the tumor purity level of 14%, the minimum

observed read count was >1366 reads. Based on these results, the LoD for *RET* is estimated to be 14% tumor purity, with a minimum read count threshold of approximately >1366 reads ensuring consistent detection.

Table 50: RNA fusion detection rates across dilution levels.

Gene	Dilution	Tumor Purity (%)	Fusion Read Counts (mean, Range)	PCR% (n/N)
ALK	Level-1	20	4868 (1640-8989)	100 (10/10)
	Level-2	15	1048 (603-1544)	80 (8/10)
	Level-3	11	1104 (734-1506)	30 (3/10)
	Level-4	8	1014 (642-1409)	30 (3/10)
	Level-5	6	931 (740-1049)	30 (3/10)
RET	Level-1	25	11534 (5788-19474)	100 (10/10)
	Level-2	19	7321 (3996-10730)	100 (7/7)
	Level-3	14	4282 (1366-8416)	100 (10/10)
	Level-4	11	7604 (2120-16136)	100 (7/7)
	Level-5	8	6095 (600-18193)	86 (6/7)
ROS1	Level-1	70	80775 (28692-130814)	100 (10/10)
	Level-2	53	41636 (28054-58784)	100 (10/10)
	Level-3	39	24685 (5883-39701)	100(10/10)
	Level-4	30	46067 (9422-123494)	100 (10/10)
	Level-5	22	9000 (5861-14743)	100 (10/10)
	Level-6	12	11498 (4925-33280)	100 (9/9)
	Level-7	9	5692 (1785-13236)	100 (10/10)
	Level-8	7	4393 (1015-13653)	100 (10/10)
	Level-9	5	4346 (1605-7058)	100 (10/10)

Gene	Dilution	Tumor Purity (%)	Fusion Read Counts (mean, Range)	PCR% (n/N)
*ROS1**	Level-10	20	39,875 (34,176 - 49,210)	100 (5/5)
	Level-11	10	30,333 (22,285 - 42,599)	100 (5/5)
	Level-12	7	1,853 (1,158 - 2,641)	100 (5/5)
	Level-13	5	1,599 (892 - 3,587)	100 (10/10)
	Level-14	4	942 (715 - 1,431)	70 (7/10)
	Level-15	3	883 (554 - 1,141)	30 (3/10)
	Level-16	2	-	0 (0/10)
**Supplemental Study*				

Table 51. Summary of RNA Fusion LoD

Fusion Gene	Tumor Purity Range (%)	Fusion Read Counts Range	LoD (Tumor purity %, supporting reads)
ROS1	70 - 5	80,775–4,346	5%, (>1605)
*ROS1**	20 - 2	39,875-883	5% (>892)
RET	25 - 8	11,533–6,095	14% (>1366)
ALK	20 - 6	4,868-931	20% (>1640)
**Supplemental LoD Study*			

4.5 Interference

The impact of interfering substances on the performance of the CellDx – Tissue assay was assessed by processing DNA and RNA from FFPE samples tested in the presence of each interfering substance at varying amounts. The samples were evaluated for concordance of variant call when compared to samples processed without the interfering substances. Replicates for six test cases were analyzed for six experimental and one baseline condition. Performance was evaluated across nine samples x seven conditions x five replicates. Analysis of all variant types tested (SNVs, indels, amplifications, and fusions showed no effect of exogenous and endogenous interferent for all conditions.

Table 52. Potentially Interfering Agents and their Spiking Scheme

No.	Agent / Factor	Type	Concentration Spiked	
			At Extraction	At Library Prep
1	Control	-	-	-
2	Ethanol	Exogenous	3x	5%
3	Index adapter	Exogenous	-	3x
4	Proteinase K	Exogenous	3x	0.04 mg/mL
5	Wash buffer	Exogenous	3x	5%
6	Melanin	Endogenous	0.2 mg/ mL	-
7	Hemoglobin	Endogenous	2 mg / mL	-

The assay met the predefined acceptance criteria of PCR ≥95% and NCR ≥99% for all interfering agents except for proteinase K, where PCR was 94.9% (112/118), with no missed detection of clinically actionable hotspot variants.

Table 53: Interference effects for SNVs/Indels

Interferent	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Control	100 (119/119) (96.9, 100)	100 (105196/105196) (99.99, 100)
Ethanol	99.2 (119/120) (95.4, 99.9)	100 (106080/106080) (99.99, 100)
Adaptor	98.8 (115/120) (90.6, 98.2)	100 (106080/106080) (99.99, 100)
Proteinase K	94.9 (112/118) (89.3, 97.6)	100 (104312/104312) (99.99, 100)
Wash Buffer	98.3 (118/120) (94.1, 99.5)	100 (106080/106080) (99.99, 100)
Melanin	100 (110/110) (96.6, 100)	100 (97240/97240) (99.99, 100)
Hemoglobin	98.3 (118/120) (94.1, 99.5)	100 (106080/106080) (99.99, 100)

ERBB2 amplification detection exhibited 100% PCR and 100% NCR across all tested interfering substances. There was no observed impact of any of the

potential interfering agents on copy number interpretation, confirming strong resistance to interference.

Table 54: Interference effect on ERBB2 gene amplification Call Rates

Interferent	**PCR (%) (n/N), (95% CI)**	**NCR (%) (n/N) (95% CI)**
Control	100 (10/10), (69.2, 100)	100 (34/34), (89.8, 100)
Ethanol	100 (10/10), (69.2, 100)	100 (35/35), (90.1, 100)
Adaptor	100 (10/10), (69.2, 100)	100 (35/35), (90.1, 100)
Proteinase K	100 (10/10), (69.2, 100)	100 (35/35), (90.1, 100)
Wash Buffer	100 (10/10), (69.2, 100)	100 (35/35), (90.1, 100)
Melanin	100 (10/10), (69.2, 100)	100 (30/30), (88.6, 100)
Hemoglobin	100 (10/10), (69.2, 100)	100 (35/35), (90.1, 100)

Table 55: Interference effects on *ERBB2* Gene Copy Number

Specimen (Cancer Type)	**Interferent**	**Copy Number (CN)**				
		Mean	**SD**	**CV (%)**	**MAPE (%)**	**p-value**
***ERBB2* Amplification Positive Specimen**						
Sample 1 (Bowel)	Control	45.7	8.6	18.7	-	-
	Ethanol	38.4	1.1	2.8	16.0	0.13
	Adpator	45.3	7.3	16.1	0.8	0.92
	Proteinase K	40.1	0.9	2.3	12.4	0.22
	Wash Buffer	47.6	1.3	2.8	4.1	0.68
	Melanin	60.1	0.9	1.5	31.5	0.02
	Hemoglobin	66.7	5.2	7.8	45.8	0.01
Sample 2	Control	59.2	4.0	6.7	-	-
	Ethanol	63.0	2.8	4.5	6.4	0.21

Specimen (Cancer Type)	Interferent	Copy Number (CN)				
		Mean	SD	CV (%)	MAPE (%)	p-value
(Breast)	Adpator	51.9	7.5	14.5	12.5	0.08
	Proteinase K	57.0	1.0	1.8	3.7	0.33
	Wash Buffer	64.3	5.6	8.7	8.5	0.15
	Melanin	54.3	0.7	1.2	8.4	0.07
	Hemoglobin	61.7	1.7	2.7	4.1	0.27
***ERBB2* Amplification Negative Specimen**						
Sample 3 (Lung)	Control	3.0	0.5	15.6	-	-
	Ethanol	3.0	0.2	6.4	2.0	0.79
	Adpator	2.6	0.2	8.7	10.5	0.20
	Proteinase K	2.9	0.2	6.4	1.7	0.87
	Wash Buffer	3.0	0.1	5.0	1.5	0.86
	Melanin	2.5	0.2	9.6	15.7	0.16
	Hemoglobin	2.8	0.1	3.1	4.8	0.49
Sample 4 (Cervix)	Control	3.2	0.5	16.7	-	-
	Ethanol	2.9	0.0	0.7	7.7	0.37
	Adpator	2.7	0.2	6.4	14.2	0.14
	Proteinase K	3.0	0.2	5.3	6.2	0.46
	Wash Buffer	2.7	0.1	3.7	12.9	0.19
	Melanin	2.7	0.1	3.4	14.6	0.09
	Hemoglobin	2.7	0.2	6.8	13.3	0.21
Sample 5 (Breast)	Control	2.1	0.8	35.5	-	-
	Ethanol	2.0	0.2	9.6	4.7	0.82
	Adpator	1.8	0.2	9.9	18.2	0.23
	Proteinase K	2.2	0.1	4.2	1.8	0.92
	Wash Buffer	2.2	0.2	9.4	4.0	0.85
	Melanin	2.1	0.3	12.2	2.4	0.91
	Hemoglobin	2.9	0.1	3.7	37.2	0.08
	Control	2.2	0.5	21.1	-	-

Specimen (Cancer Type)	Interferent	Copy Number (CN)				
		Mean	SD	CV (%)	MAPE (%)	p-value
Sample 6 (Skin)	Ethanol	2.0	0.2	11.9	12.2	0.50
	Adpator	1.8	0.1	4.9	20.8	0.15
	Proteinase K	2.0	0.2	11.4	11.3	0.39
	Wash Buffer	1.8	0.2	8.8	21.9	0.23
	Melanin	1.8	0.1	3.0	19.9	0.14
	Hemoglobin	2.2	0.5	21.1	-	-
Sample 7 (Lung)	Control	2.8	0.2	7.4	-	-
	Ethanol	2.5	0.3	13.8	10.4	0.11
	Adpator	2.7	0.2	8.9	1.9	0.65
	Proteinase K	2.6	0.2	7.0	7.9	0.21
	Wash Buffer	2.5	0.0	1.2	9.7	0.04
	Melanin	2.8	0.0	1.5	0.7	0.83
	Hemoglobin	3.0	0.2	7.4	7.9	0.28
Sample 8 (Lung)	Control	2.2	0.1	5.9	-	-
	Ethanol	2.0	0.1	4.2	7.4	0.05
	Adpator	2.5	0.2	8.0	15.6	0.06
	Proteinase K	2.6	0.4	15.8	20.2	0.06
	Wash Buffer	2.6	0.2	7.2	19.1	0.00
	Melanin	2.4	0.2	9.1	11.3	0.03
	Hemoglobin	2.5	0.3	10.3	13.2	0.15
Sample 9 (Lung)	Control	3.0	0.0	0.7	-	-
	Ethanol	3.0	0.0	0.8	0.1	0.62
	Adpator	3.1	0.1	2.2	4.0	0.01
	Proteinase K	2.9	0.1	2.0	2.0	0.08
	Wash Buffer	2.6	0.0	1.5	11.9	0.00
	Melanin	3.6	0.1	3.9	20.7	0.00
	Hemoglobin	2.9	0.1	2.1	2.0	0.11

RNA fusions demonstrated 100% PCR and 100% NCR across all tested interfering agents.

Table 56: Interference testing results for RNA

Interferent	PCR (%) (n/N), (95% CI)	NCR (%) (n/N) , (95% CI)
Control	100 (15/15), (78.2, 100)	100 (29/29), (88.1, 100)
Ethanol	100 (15/15) , (78.2, 100)	100 (30/30), (88.4, 100)
Adapter	100 (15/15) , (78.2, 100)	100 (30/30) , (88.4, 100)
Proteinase K	100 (15/15) , (78.2, 100)	100 (29/29) , (88.1, 100)
Wash Buffer	100 (15/15) , (78.2, 100)	100 (30/30) , (88.4, 100)
Melanin	100 (15/15) , (78.2, 100)	100 (30/30) , (88.4, 100)
Hemoglobin	100 (15/15) , (78.2, 100)	100 (30/30) , (88.4, 100)

Secondary studies were conducted to evaluate the assay's performance across necrotic content of specimens and tumor block age. To determine the impact of necrosis, the specimens (n = 378) were stratified into 4 level of necrotic content: 0–10%, 10–20%, 20–30%, and >30%. For DNA libraries (n = 378), invalid rates were 1.78% (5/281) for 0-5% necrosis, 0% (0/72) for 5-20% necrosis and 10.52 % (2/19) for 21-40 % necrosis. No failures were observed for 5-20% (0/72) or >41% necrosis (range 41-80%) (0/6). For RNA libraries (n = 377): Invalid rates were 1.42% (4/281) for 0-5% necrosis and 1.4% (1/71) for 5-20% necrosis. No failures were observed for 21-40% (0/19) or >41% necrosis (range 41-80%) (0/6). To determine the impact of tumor block age, the specimens (n = 378) were stratified into 3 age levels: less than 2 years, 2 -4 years, and 4 years or older (range 4-9 years). For DNA Libraries (n = 378), the invalid rate was 0.8% (2/248) for specimens <2 years, 6.1% (5/82) for specimens at 2-4 years. No DNA library failures were observed in specimens stored for ≥4 years (0/48). For RNA Libraries (n = 377) the invalid rate followed a similar trend, with 0.8% (2/245) failure in specimens <2 years old, 3.6% (3/83) for 2-4 years old specimen. No RNA library failures were observed in the ≥4-year group (0/49).

Table 57: Concordance of Overall Variant Detection by Necrotic Tissue Content for SNVs and InDels (Level 2 and Level 3)

Necrotic Content (%)	Sample Count	PPA (%) (n/N) (95% CI)	NPA (%) (n/N) (95% CI)
0 to 5	86	97.8 (135/138) (93.8, 99.3)	99.6 (13140/13197) (99.4, 99.7)
> 5 to 20	28	100 (39/39) (91.0, 100)	99.2 (4268/4303) (98.9, 99.4)
21 to 40	8	100 (13/13) (77.2, 100)	99.8 (1224/1227) (99.3, 99.9)
41 to 53	1	100 (2/2) (34.2, 100)	99.3 (152/153) (96.4, 99.9)
Overall	**123**	**98.4 (189/192) (95.5, 99.5)**	**99.5 (18784/18880) (99.4, 99.6)**

Table 58: Necrotic Tissue Pass Rates for DNA and RNA

Cancer Types	Sample Count	Necrotic Content (%)	QC Pass Rate (n/N)
DNA			
23	281	0 to 5	98.22 (276/281)
15	72	5 to 20	100 (72/72)
12	19	21 to 40	89.47 (17/19)
4	6	41 to 80	100 (6/6)
RNA			
23	281	0 to 5	98.58 (277/281)
15	71	5 to 20	98.59 (70/71)
12	19	21 to 40	100 (19/19)
4	6	41 to 80	100 (6/6)

4.6 Cross-Contamination and Carryover

The CellDx-Tissue assay incorporates a quantitative metric, CONTAMINATION_SCORE, designed to estimate the proportion of foreign reads. This study established and validated the effectiveness of this metric. Using a dataset of 178 samples (20 negative controls and 158 tumor samples spiked with various proportions of wild-type DNA), a CONTAMINATION_SCORE cutoff of ≥0.120 was selected. This cutoff

yielded a high sensitivity of 99.3% (95% CI: 96.5%–99.9%) and a specificity of 100% (95% CI: 83.9%–100%) for detecting contaminated samples.

Cross-contamination and sample carryover were assessed by evaluating the false positive rate in 24 FFPE samples. Twelve of the 24 FFPE samples had known positive variants, and the remaining samples were known negative samples. All FFPE samples were assessed across two batched to test for contamination within and between runs. The samples were processed in a checkerboard layout during library synthesis. In the first batch, 24 libraries and 12 libraries underwent a single DNA and RNA sequencing, respectively. The second batch contained 12 DNA libraries and six RNA libraires with known negative samples were processed after the first batch. No positive variant results were observed in the known negative samples tested.

4.7 Input DNA and RNA

The assay's robustness was evaluated through an input study assessing its performance across varying DNA and RNA input levels. Six FFPE tumor specimens spanning multiple variant and organ types were processed for DNA and RNA extraction, followed by library synthesis at seven input levels (2.5 ng, 5 ng, 10 ng, 20 ng, 30 ng, 40 ng, 50 ng) across 12 replicates. 3 FFPE specimens were used for the DNA input study (SNVs, indels, *ERBB2* amplification), generating 252 DNA libraries. All FFPE specimens (3 fusion-positive and 3 fusion-negative) were utilized for the RNA input study. QC metrics demonstrated improved depth and consistency at DNA input levels ≥10 ng, while lower levels (2.5 ng and 5 ng) reported higher QC failures. Invalid DNA library rates were higher at 5 ng and 2.5 ng.

For SNVs and InDels, higher PCR was observed at and above 10 ng input DNA. Variants with <0.1 VAF exhibited higher CV below 10 ng. The NCR was ≥99.9% at all input levels.

Table 59: Call Rates (PCR, NCR) of SNVs and InDels Across Input Levels.

DNA Input	PCR %, (n/N), 95% CI	NCR %, (n/N), 95% CI
2.5 ng	85.8, (151/176), (77.8, 88.5)	99.9, (45812769/45813184), (99.9, 100)
5 ng	80.1, (137/171), (69.4, 81.8)	99.9, (38654612/38654874) , (99.9, 100)
10 ng	97.8, (175/179), (93.7, 98.8)	99.9, (50108021/51539832) , (99.9, 100)

DNA Input	PCR %, (n/N), 95% CI	NCR %, (n/N), 95% CI
20 ng	97.2, (174/179), (92.9, 98.5)	99.9, (50108137/50108170) , (99.9, 100)
30 ng	97.8, (175/179), (93.7, 98.8)	99.9, (50108155/50108170) , (99.9, 100)
40 ng	98.3, (176/179), (94.4, 99.1)	99.9, (50108167/50108170) , (99.9, 100)
50 ng	100, (180/180), (97.9, 100)	99.9, (51539823/51539832) , (99.9, 100)

For *ERBB2* amplification, mean CN ranged from 7.6 at 50 ng to 9.1 at 2.5 ng, with CV ≤15% at ≥21% tumor purity. The PCR for *ERBB2* amplification was 100% at input levels ≥10 ng. The mean CN values were consistent across input levels, with no significant deviation from the reference level (20 ng).

Table 60: Mean Observed Copy Number (CN) for *ERBB2* at Each Input Level

DNA Input Level (ng)	Tumor Purity	Mean CN (Range)	SD	%CV	PCR%, (n/N), (95% CI)
2.5	21 %	9.1 (8–11)	±1.0	11.5	92, (11/12), (61.5, 99.8)
5	21 %	9.0 (8–10)	±0.7	7.4	83, (10/12), (51.6, 97.9)
10	21 %	7.4 (6–9)	±0.8	10.7	100, (12/12), (73.5, 100)
20 (Reference)	21 %	7.5 (6–9)	±0.9	12.1	100, (12/12), (73.5, 100)
30	21 %	8.3 (7–10)	±1.1	13.7	100, (12/12), (73.5, 100)
40	21 %	7.1 (6–8)	±0.5	7.6	92, (11/12), (61.5, 99)
50	21 %	7.6 (7–9)	±0.9	11.8	100, (12/12), (73.5, 100)

For *ROS1* fusion 100% PCR was achieved at ≥10 ng input, with mean supporting read counts increasing from 776 at 10 ng to 137,465 at 50 ng. For *RET* fusion 100% PCR was achieved ≥10 ng, with mean supporting read counts ranging from 636 at 10 ng to 4771 at 50 ng. For *ALK* fusion, 100% PCR was achieved at ≥5 ng input, with mean supporting read counts increasing from 9,711 at 5 ng to 29,524 at 50 ng. These findings support input levels ≥10 ng (and the nominal ≥20 ng RNA input) for reliable detection of all three fusions.

Table 61: Mean Read Counts for %PCR RNA Fusions Across Input Levels

Fusion	Tumor purity	RNA Input (ng)	Mean Read Counts	%PCR (n/N), (95% CI)
ROS1	29%	2.5	0	0, (0/12), (0-24.3)
	29%	5	549	75, (9/12) , (46.8-91.1)
	29%	10	776	100, (12/12) , (75.7-100)
	29%	20	1491	100, (12/12) , (75.7-100)
	29%	30	2665	100, (12/12) , (75.7-100)
	29%	40	4569	100, (12/12) , (75.7-100)
	29%	50	137465	100, (12/12) , (75.7-100)
RET	73%	2.5	0	0, (0/12) , (0-24.3)
	73%	5	527	33.3, (4/12) , (13.8-60.9)
	73%	10	636	100, (12/12) , (75.7-100)
	73%	20	943	100, (12/12) , (75.7-100)
	73%	30	1421	100, (12/12) , (75.7-100)
	73%	40	2115	100, (12/12) , (75.7-100)
	73%	50	4771	100, (12/12) , (75.7-100)
ALK	29%	2.5	7358	33, (4/12) , (13.8-60.9)
	29%	5	9711	100, (12/12) , (75.7-100)
	29%	10	12428	100, (12/12) , (75.7-100)
	29%	20	14842	100, (12/12) , (75.7-100)
	29%	30	16876	100, (12/12) , (75.7-100)
	29%	40	19954	100, (12/12) , (75.7-100)
	29%	50	29524	100, (12/12) , (75.7-100)

4.8 Method Comparison (Accuracy)

Concordance of variant calls, gene fusions, and ERBB2 detection, were compared between the appropriate comparator method and CellDx-Tissue assay. SNVs, insertions, and deletions are compared to an externally validated orthogonal NGS method. Gene fusions and ERB2 CNV were compared to the corresponding FISH test. In total there were 252 FFPE samples in the accuracy study. For all analyses, the PPA

and NPA were calculated by comparing the concordance between the CellDx-Tissue assay to the appropriate comparator to evaluate the degree of concordance between the assays.

Table 62. Accuracy of the CellDx-Tissue Assay

Variant Category	Orthogonal Method	Analysis Category	PPA% (95% C1)	NPA% (95% C1)
Overall	NGS	All	98.4 (95.5, 99.5)	99.5 (99.4, 99.6)
		SNV	98.8 (95.6, 99.7)	99.7 (99.6, 99.8)
		Insertion	85.7 (48.7, 97.4)	99.9 (99.8, 99.9)
		Deletion	100 (86.2, 100)	99.9 (99.9, 99.9)
Variants with Evidence of Clinical Significance	NGS	All	98.2 (90.6, 99.7)	99.8 (99.6, 99.9)
		SNV	100 (92.6, 100)	99.8 (99.7, 99.9)
		Insertion	66.7 (20.8, 93.9)	100 (99.9, 100)
		Deletion	100 (56.6, 100)	100 (99.9, 100)
Variants with Potential Clinical Significance	NGS	All	98.5 (94.8, 99.6)	99.4 (99.3, 99.5)
		SNV	98.2 (93.8, 99.5)	99.6 (99.5, 99.7)
		Insertion	100 (51.0, 100)	99.9 (99.8, 99.9)
		Deletion	100 (83.2, 100)	99.9 (99.8, 99.9)
Hotspot	NGS	All	97.8 (92.3, 99.4)	99.9 (99.8, 99.9)
		SNV	97.8 (92.3, 99.4)	99.9 (99.8, 99.9)
		Insertion	-	100 (99.8, 100)
		Deletion	-	100 (99.8, 100)
Non-Hotspot	NGS	All	99 (94.6, 99.8)	99.6 (99.5, 99.7)
		SNV	100 (94.8, 100)	99.8 (99.7, 99.9)
		Insertion	85.7 (48.7, 97.4)	99.9 (99.8, 99.9)
		Deletion	100 (86.2, 100)	99.9 (99.9, 99.9)
ERBB2	FISH	Amplification	91.18 (77.04, 96.95)	100 (92.13, 100)
ALK	FISH	Fusion	94.10 (73.0, 99.0)	100 (89.8, 100)
RET	FISH	Fusion	100 (43.9, 100)	100 (92.7, 100)
ROS1	FISH	Fusion	91.70 (64.6, 98.5)	100 (91.0, 100)

a. Accuracy-SNVs and Indels

The CellDx-tissue accuracy study included a total of 189 unique variants in 88 exons over 41 genes. Variants included 159 SNVs, six insertions, and 24 deletions. Performance was stratified by mutation type and gene for positive percent agreement (PPA) and negative percent agreement (NPA) with two-sided 95% confidence interval (CI). Overall, the CellDx-Tissue assay yielded concordant analytical performance for variant calls across the SNVs and Indels with a PPA of 98.4% and an NPA ≥ 99.5%. The concordance between detected mutations for each category of variants (Variants with Evidence of Clinical Significance, Variants with Potential Clinical Significance, Hotspot, and Non-Hotspot) are shown in Table 63.

Table 63: Concordance Summary for Small DNA Variants by Variant Type and Clinical Significance

Variant Category	Analysis Category	PPA%, (n/N) (95% CI)	NPA%, (n/N) (95% CI)
Overall	All	98.40 (189/192) (95.5, 99.5)	99.50 (18784/18880) (99.4, 99.6)
	SNV	98.80 (159/161) (95.6, 99.7)	99.70 (18851/18911) (99.6, 99.8)
	Insertions	85.70 (6/7) (48.7, 97.4)	99.90 (19046/19065) (99.8, 99.9)
	Deletions	100 (24/24) (86.2, 100)	99.90 (19031/19048) (99.9, 99.9)
Variants with Evidence of Clinical Significance	All	98.20 (55/56) (90.6, 99.7)	99.80 (4405/4414) (99.6, 99.9)
	SNV	100 (48/48) (92.6, 100)	99.80 (4415/4422) (99.7, 99.9)
	Insertions	66.70 (2/3) (20.8, 93.9)	100 (4466/4467) (99.9, 100)
	Deletions	100 (5/5) (56.6, 100)	100 (4464/4465) (99.9, 100)
Variants with Potential Clinical Significance	All	98.50 (134/136) (94.8, 99.6)	99.40 (14379/14466) (99.3, 99.5)
	SNV	98.20 (111/113) (93.8, 99.5)	99.60 (14436/14489) (99.5, 99.7)
	Insertions	100 (4/4) (51.0, 100)	99.90 (14580/14598) (99.8, 99.9)
	Deletions	100 (19/19) (83.2, 100)	99.90 (14567/14583) (99.8, 99.9)
Hotspot	All	97.80 (89/91) (92.3, 99.4)	99.90 (18959/18981) (99.8, 99.9)
	SNV	97.80 (89/91) (92.3, 99.4)	99.90 (18959/18981) (99.8, 99.9)
	Insertions	-	100 (19072/19072) (99.8, 100)

Variant Category	Analysis Category	PPA%, (n/N) (95% CI)	NPA%, (n/N) (95% CI)
	Deletions	-	100 (19072/19072) (99.8, 100)
Non-Hotspot	All	99.00 (100/101) (94.6, 99.8)	99.60 (18897/18971) (99.5, 99.7)
	SNV	100 (70/70) (94.8, 100)	99.80 (18964/19002) (99.7, 99.9)
	Insertions	85.70 (6/7) (48.7, 97.4)	99.90 (19046/19065) (99.8, 99.9)
	Deletions	100 (24/24) (86.2, 100)	99.90 (19031/19048) (99.9, 99.9)
Insertions	1 – 5 bp	80.00 (4/5) (37.6, 96.4)	99.90 (19049/19067) (99.9, 99.9)
	6 – 10 bp	100 (1/1) (20.7, 100)	100 (19070/19071) (100, 100)
	11 – 20 bp	100 (1/1) (20.7, 100)	100 (19071/19071) (100, 100)
Deletions	1 – 5 bp	100 (19/19) (83.2, 100)	99.90 (19038/19053) (99.9, 100)
	6 – 10 bp	-	100 (19071/19072) (100, 100)
	11 – 20 bp	100 (3/3) (43.9, 100)	100 (19069/19069) (100, 100)
	21 – 30 bp	100 (2/2) (34.2, 100)	100 (19069/19070) (100, 100)

Two-sided CIs were estimated using the Wilson method.

b. Wild Type Accuracy

For assessing the accuracy of wild-type calls, evaluation of clinically relevant positions was performed by observation of the NPA of Level 2 small DNA variants. The NPA was 99.8% (4416/4423) (99.7%, 99.9%) for SNVs, 100% (4469/4469) (99.9%, 100%) for MNVs, 100% (4469/4469) (99.9%, 100%) for Insertions, and 99.98% (4464/4465) (99.9%, 100%) for Deletions.

Table 64: Wild Type Accuracy - NPA for Level 2 Small DNA Variants

Variant Category	NPA% (n/N) 95% CI
SNVs	99.80 (4415/4422) (99.7, 99.9)
Insertions	99.98 (4466/4467) (99.9, 100)
Deletions	99.98 (4464/4465) (99.9, 100)

c. Accuracy-*ERBB2* gene amplification

In total, 83 different FFPE samples representing 11 different tumor types, including Breast, Bowel, Esophagus/Stomach, Lung, Head and Neck,

Ovary/Fallopian Tube, Bladder/Urinary Tract, Uterus, Biliary Tract, Bone and Prostate were analyzed for concordance between FISH status and CellDx-Tissue ERBB2 status. Four samples that had a positive ERBB2 FISH result were classified as Indeterminate by the CellDx-Tissue (below assay's limit of detection for ERBB2) and were excluded from analysis (see Table 66). Three samples had a positive ERBB2 FISH result but were negative for ERBB2 in the CellDx-Tissue assay. Of these three samples, one had a FISH score of 2.02 (borderline) and history of HER2-negtive IHC findings. The remaining two discordant samples had a FISH score of 2.84 and 2.94, with a history of HER2-negative IHC reported. The PPA and NPA values for ERBB2 amplification reflected the across borderline and non-borderline samples (Table 66). In non-borderline cases (excluding all cases of a FISH ratio 1.5 – 2.5), a PPA of 93.55% (95% CI: 79.28%, 98.21%) and an NPA of 100% (95% CI: 89.85%, 100%) (Table 65) was observed.

Table 65: Accuracy of CellDx-Tissue for *ERBB2* Amplification against FISH, stratified by FISH score

Category	Total Cases	TP	FP	TN	FN	I/LL*	PPA% (95% CI)	NPA% (95% CI)
All Cases	83	31	0	45	3	4	91.18 (77.04, 96.95)	100 (92.13, 100)
Excluding FISH 1.8-2.2	78	31	0	42	2	3	93.94 (80.39, 98.32)	100 (91.62, 100)
Only FISH 1.8-2.2	5	0	0	3	1	1	0 (0, 79.35)	100 (43.85, 100)
Excluding FISH 1.5-2.5	68	29	0	34	2	3	93.55 (79.28, 98.21)	100 (89.85, 100)
Only FISH 1.5-2.5	15	2	0	11	1	1	66.67 (20.77, 93.85)	100 (74.12, 100)

**Indeterminate / Low-Level Amplification (below LoD).*

Table 66: Accuracy of CellDx-Tissue for *ERBB2* Amplification vs. FISH

CellDx-Tissue	FISH		
	***ERBB2* (+)**	***ERBB2* (-)**	**Total**
***ERBB2* (+) (CN ≥8.5)**	31 (TP)	0 (FP)	31

CellDx-Tissue	FISH		
	***ERBB2* (+)**	***ERBB2* (-)**	**Total**
***ERBB2* (I/LLA*)** **(CN ≥4 to < 8.5)**	4 (Excluded from PPA[#])	0 (FP)	4
***ERBB2* (-)** **(CN < 4)**	3 (FN)	45 (TN)	48
Total	38 (34 for PPA)	45	83
PPA% (n/N, 2-sided 95% CI)	91.18 (31/34; 77.04, 96.95)		
NPA% (n/N, 2-sided 95% CI)	100 (45/45; 92.13, 100)		
OPA% (n/N, 2-sided 95% CI)	96.20 (76/79; 89.42, 98.70)		

** Samples with CN ≥ 4 to <8.5 are classified as " Indeterminate / Low-Level Amplification (below LoD)" and require reflex testing with another FDA-cleared test. Per the tiered ERBB2 reporting framework, these specimens are excluded from the primary PPA calculation as they are not definitive positive calls.*

[#]4 FISH-positive samples yielded Indeterminate / Low-Level Amplification (Below LOD, CN ≥4 to <8.5) by DCG2401. Per the statistical guidance for qualitative assays (CLSI EP12-A2), Indeterminate / Low-Level Amplification (below LoD) results are excluded from the calculation of agreement statistics to reflect the performance of definitive calls.

d. *ALK* Fusions

A total of 51 FFPE samples from six tumor types, including Lung, Thyroid, Soft Tissue, Breast, Bowel and Prostate, were included in the analysis. Out of the 17 ALK positive samples, 16 were detected for ALK gene fusions using both FISH and CellDx-Tissue assay. One ALK positive sample by FISH was not reported as positive by the CellDx-Tissue assay due to borderline tumor purity. The concordance between the CellDx-Tissue assay and FISH is shown in Table 67.

Table 67: Accuracy of CellDx-Tissue for *ALK* Fusion vs. FISH

CellDx-Tissue	FISH		
	***ALK* (+)**	***ALK* (-)**	**Total**
***ALK* (+)**	16 (TP)	0 (FP)	16
***ALK* (-)**	1 (FN)	34 (TN)	35
Total	17	34	51

CellDx-Tissue	FISH		
	***ALK* (+)**	***ALK* (-)**	**Total**
PPA% (n/N, 2-sided 95% CI)	94.10 (16/17; 73.0, 99.0)		
NPA% (n/N, 2-sided 95% CI)	100 (34/34; 89.8, 100)		
OPA% (n/N, 2-sided 95% CI)	98.04 (50/51; 1.0, 27.0)		

e. *ROS1* Fusions

A total of 51 FFPE samples from six tumor types, including Lung, Thyroid, Bowel, Soft Tissue, Breast and Prostate, were included in the analysis. Out of the 12 ROS1 positive samples, 11 were detected for ROS1 gene fusions using both FISH and CellDx-Tissue assay. One ROS1 positive sample by FISH was not reported as positive by the CellDx-Tissue assay; this discordant result was confirmed by an independent NGS test and classified as a false negative. The concordance between the CellDx-Tissue assay and FISH is shown in Table 68.

Table 68: Accuracy of CellDx-Tissue for *ROS1* Fusion vs. FISH

CellDx-Tissue	FISH		
	***ROS1* (+)**	***ROS1* (-)**	**Total**
***ROS1* (+)**	11 (TP)	0 (FP)	11
***ROS1* (-)**	1 (FN)	39 (TN)	40
Total	12	39	51
PPA% (n/N, 2-sided 95% CI)	91.70 (11/12; 64.6, 98.5)		
NPA% (n/N, 2-sided 95% CI)	100 (39/39; 91.0, 100)		
OPA% (n/N, 2-sided 95% CI)	98.04 (50/51; 89.6, 99.6)		

f. *RET* Fusions

A total of 52 FFPE samples from six tumor types, including Lung, Thyroid, Bowel, Soft Tissue, Breast and Prostate, were included in the analysis. Out of the three RET positive samples, three were detected for RET gene fusions using both FISH and CellDx-Tissue assay. The concordance between the CellDx-Tissue assay and FISH is shown in Table 69

Table 69: Accuracy of CellDx-Tissue for *RET* Fusion vs. FISH

CellDx-Tissue	FISH		
	***RET* (+)**	***RET* (-)**	**Total**
***RET* (+)**	3 (TP)	0 (FP)	3
***RET* (-)**	0 (FN)	49 (TN)	49
Total	3	49	52
PPA% (n/N, 2-sided 95% CI)	100 (3/3; 43.9, 100)		
NPA% (n/N, 2-sided 95% CI)	100 (49/49; 92.7, 100)		
OPA% (n/N, 2-sided 95% CI)	100 (52/52; 93.1, 100)		

g. Invalid Results

Invalid results were infrequent across all tested biomarkers, consistently remaining within the predefined acceptance criteria for the CellDx-Tissue workflow. The occurrence of invalid outcomes varied slightly depending on the analyte type and initial sample quality, but overall rates demonstrated the inherent robustness of the assay.

Table 70: Invalid Rates and Failure Categories Observed in Accuracy Study

	DNA	RNA	Grand Total
1st Pass Valid	233	221	454
After Repeat Test Valid	10	23	33
Low Nucleic Acid Yield (<20 ng)	14	6	17
Library Failure	1	0	1
Invalid (Sequencing QC Failure)	7	5	12
Total Invalid	22	11	33
Total Attempts	265	252	517
Total Invalid (%)	8.30%	4.4%	6.38%

Note: 'Total Invalid' refers to samples that initially failed to produce a valid result in their respective category. 'Total Attempts' is the total number of samples processed for each analyte type. 'After Repeat Test Valid' indicates samples that initially failed but successfully yielded valid results upon re-processing.

4.9 Tissue Comparability

Across all the tissue types evaluated, the CellDx-Tissue assay performance consistently met the pre-defined acceptance criteria. The accuracy of the assay was retained even in tissue types (e.g,, pancreatic and hepatic) that present pre-analytical challenges such as suboptimal nucleic acid due to high nuclease activity or variable fixation practices.

To evaluate performance by variant type and tissue origin, data were stratified and analyzed for key biomarker classes, i.e., SNVs, InDels, fusions and *ERBB2* amplification.

Table 71: Assay Performance for SNVs and InDels in various Tissue types. The table indicates the distribution of false negatives (FN), false positives (FP), true negatives (TN) and true positives (TP), positive percent agreement (PPA) and negative percent agreement (NPA) along with their 95% confidence intervals (CI).

Tissue	Sample Number	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
		FN	FP	TN	TP		
Adrenal Gland	1			1410148	1	100 (20.65, 100)	100 (99.99, 100)
Biliary Tract	2		3	2820294	1	100 (20.65, 100)	99.99 (99.99, 100)
Bladder/Urinary Tract	2			2820295	3	100 (43.85,100)	100 (99.99, 100)
Bowel	25	1	29	35253643	52	98.11 (90.06, 99.67)	99.99 (99.99, 100)
Breast	26		10	36663825	39	100 (91.03, 100)	100 (99.99, 100)
Cervix	3		3	4230442	2	100 (34.24, 100)	100 (99.99, 100)
CNS/Brain	5			7050735	10	100 (72.25, 100)	100 (99.99, 100)
Esophagus/Stomach	3		2	4230442	3	100 (43.85, 100)	100 (99.99, 100)
Head and neck	6		3	8460884	7	100 (64.57, 100)	100 (99.99, 100)
Lung	15		9	21152197	29	100 (88.30, 100)	100 (99.99, 100)
Ovary/Fallopian Tube	8	1	9	11281177	5	83.33 (43.65, 96.99)	100 (99.99, 100)

Tissue	Sample Number	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
		FN	FP	TN	TP		
Pancreas	5		9	7050731	5	100 (56.55, 100)	100 (99.99, 100)
Peritoneum	1		1	1410148		NA	100 (99.99, 100)
Prostate	3		3	4230440	4	100 (51.01, 100)	100 (99.99, 100)
Skin	3	1	1	4230443	2	66.67 (20.77, 93.85)	100 (99.99, 100)
Soft Tissue	3			4230440	7	100 (64.57, 100)	100 (99.99, 100)
Testis	1			1410147	2	100 (34.24, 100)	100 (99.99, 100)
Thymus	2		4	2820292	2	100 (34.24, 100)	100 (99.99, 100)
Thyroid	3			4230444	3	100 (43.85, 100)	100 (99.99, 100)
Unknown Primary	1			1410148	1	100 (20.65, 100)	100 (99.99, 100)
Uterus	5		10	7050724	11	100 (74.12, 100)	100 (99.99, 100)
Overall	**123**	**3**	**96**	**173448039**	**189**	**98.44 (95.51, 99.47)**	**100 (99.99, 100)**

Table 72: Assay Performance for *ERBB2* gene amplification in various cancer types. The table indicates the distribution of FN, FP, TN and TP, PPA and NPA along with their 95% CI. NA: not applicable.

Tissue (N)	Variant Calls					PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP	I/LL*		
Breast (42)	1		23	18		94.74 (75.36, 99.06)	100 (85.69, 100)
Bowel (32)	1		21	8	2	88.89 (56.50, 98.01)	100 (84.54, 100)
Lung (11)			4	6	1	100 (60.97, 100)	100 (51.01, 100)
Head and Neck (7)	1		4	1	1	50 (9.45, 90.55)	100 (51.01, 100)

Tissue (N)	Variant Calls					PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP	I/LL*		
Esophagus/Stomach (6)			2	4		100 (51.01, 100)	100 (34.24, 100)
Ovary/Fallopian Tube (6)			4	2		100 (34.24, 100)	100 (51.01, 100)
Pancreas (4)			3	1		100 (20.65, 100)	100 (43.85, 100)
Uterus (4)			2	2		100 (34.24, 100)	100 (34.24, 100)
Bladder/Urinary Tract (3)			2	1		100 (20.65, 100)	100 (34.24, 100)
Prostate (3)			3			NA	100 (43.85, 100)
Biliary Tract (2)				2		100 (34.24, 100)	NA
Cervix (2)			2			NA	100 (34.24, 100)
Bone (1)			1			NA	100 (20.65, 100)
CNS/Brain (1)			1			NA	100 (20.65, 100)
Liver (1)			1			NA	100 (20.65, 100)
Thymus (1)			1			NA	100 (20.65, 100)
Overall (126)	**3**	**0**	**74**	**45**	**4**	**93.75 (83.16, 97.85)**	**100 (95.06, 100)**

**Indeterminate / Low-Level Amplification (below LoD).*

Table 73: Assay Performance for *ALK* gene fusions in various cancer types. The table indicates the distribution of FN, FP, TN and TP, PPA and NPA along with their 95% CI. NA: not applicable

Cancer Type	Variant calls				PPA (%) [95% CI]	NPA (%) [95% CI]
	FN	FP	TN	TP		
Lung (n = 64)			36	28	100 (87.94, 100)	100 (90.36, 100)

Cancer Type	Variant calls				PPA (%) [95% CI]	NPA (%) [95% CI]
	FN	FP	TN	TP		
Thyroid (n = 13)			13		NA	100 (77.19, 100)
Head and Neck (n = 6)			6		NA	100 (60.97, 100)
Breast (n = 5)			4	1	100 (20.65, 100)	100 (51.01, 100)
Bowel (n = 4)			4		NA	100 (51.01, 100)
Cervix (n = 3)			3		NA	100 (43.85, 100)
Ovary / Fallopian Tube (n = 3)			3		NA	100 (43.85, 100)
Pancreas (n = 3)			3		NA	100 (43.85, 100)
Prostate (n = 3)			3		NA	100 (43.85, 100)
Soft Tissue (n = 3)	1		2		0 (0, 79.35)	100 (34.24, 100)
Uterus (n = 3)			3		NA	100 (43.85, 100)
Esophagus / Stomach (n = 2)			2		NA	100 (34.24, 100)
Bladder / Urinary Tract (n = 1)			1		NA	100 (20.65, 100)
Liver (n = 1)			1		NA	100 (20.65, 100)
Thymus (n = 1)			1		NA	100 (20.65, 100)
Overall (n = 115)	**1**	**0**	**85**	**29**	**96.67 (83.33, 99.41)**	**100 (95.68, 100)**

Table 74: Assay Performance for RET gene fusions in various cancer types. The table indicates the distribution of FN, FP, TN and TP, PPA and NPA along with their 95% CI. NA: not applicable.

Cancer Type	Variant calls				PPA (%) [95% CI]	NPA (%) [95% CI]
	FN	FP	TN	TP		
Lung			45	6	100 (54.07, 100)	100 (92.13, 100)

Cancer Type	Variant calls				PPA (%) [95% CI]	NPA (%) [95% CI]
	FN	FP	TN	TP		
(n = 51)						
Thyroid (n = 15)			14	1	100 (20.65, 100)	100 (78.47, 100)
Head and Neck (n = 6)			6		NA	100 (60.97, 100)
Breast (n = 5)			5		NA	100 (56.55, 100)
Bowel (n = 4)			4		NA	100 (51.01, 100)
Cervix (n = 3)			3		NA	100 (43.85, 100)
Ovary/Fallopian Tube (n = 3)			3		NA	100 (43.85, 100)
Pancreas (n = 3)			3		NA	100 (43.85, 100)
Prostate (n = 3)			3		NA	100 (43.85, 100)
Soft Tissue (n = 3)			3		NA	100 (43.85, 100)
Uterus (n = 3)			3		NA	100 (43.85, 100)
Esophagus/Stomach (n = 2)			2		NA	100 (34.24, 100)
Bladder/Urinary Tract (n = 1)			1		NA	100 (20.65, 100)
Liver (n = 1)			1		NA	100 (20.65, 100)
Thymus **(n = 1)**			1		NA	100 (20.65, 100)
Overall (n = 104)	**0**	**0**	**97**	**7**	**100 (59.04, 100)**	**100 (96.26, 100)**

Table 75: Assay Performance for *ROS1* gene fusions in various cancer types. The table indicates the distribution of FN, FP, TN and TP, PPA and NPA along with their 95% CI. NA: not applicable.

Cancer Type	Variant calls				PPA (%) [95% CI]	NPA (%) [95% CI]
	FN	FP	TN	TP		
Lung (n = 59)	1		38	20	95.24 (77.33, 99.15)	100 (90.77, 100)
Thyroid (n = 13)			13		NA	100 (77.19, 100)
Head and Neck (n = 6)			6		NA	100 (60.97, 100)
Breast (n = 5)			5		NA	100 (56.55, 100)
Bowel (n = 4)			4		NA	100 (51.01, 100)
Cervix (n = 3)			3		NA	100 (43.85, 100)
Ovary/Fallopian Tube (n = 3)			3		NA	100 (43.85, 100)
Pancreas (n = 3)			3		NA	100 (43.85, 100)
Prostate (n = 3)			2	1	NA	66.67 (20.77, 93.85)
Soft Tissue (n = 3)			3		NA	100 (43.85, 100)
Uterus (n = 3)			3		NA	100 (43.85, 100)
Esophagus/Stomach (n = 2)			2		NA	100 (34.24, 100)
Bladder/Urinary Tract (n = 1)			1		NA	100 (20.65, 100)
Liver (n = 1)			1		NA	100 (20.65, 100)
Thymus (n = 1)			1		NA	100 (20.65, 100)
Overall (n = 110)	**1**	**0**	**88**	**21**	**95.45 (78.20, 99.19)**	**100 (95.82, 100)**