EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Invitae Common Hereditary Cancers Panel
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN210011

B Applicant

Invitae Corporation

C Proprietary and Established Names

Invitae Common Hereditary Cancers Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVU	Class II	21 CFR 866.6095– High throughput DNA sequencing for hereditary cancer predisposition assessment test system	Pathology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Invitae Common Hereditary Cancers Panel

B Measurand:

Germline substitutions, small insertion and deletion alterations and copy number variants (CNV) in a panel of 47 targeted genes

C Type of Test:

Next generation sequencing based cancer-related germline mutation profiling

III Indications for Use:

A Indication(s) for Use:

The Invitae Common Hereditary Cancers Panel is a qualitative high-throughput sequencing based in vitro diagnostic test system intended for analysis of germline human genomic DNA extracted from whole blood for detection of substitutions, small insertion and deletion alterations and copy number variants (CNV) in a panel of targeted genes.

This test system is intended to provide information for use by qualified health care professionals, in accordance with professional guidelines, for hereditary cancer predisposition assessment and to aid in identifying hereditary genetic variants potentially associated with a diagnosed cancer.

The test is not intended for cancer screening or prenatal testing. Results are intended to be interpreted within the context of additional laboratory results, family history, and clinical findings.

The test is a single-site assay performed at Invitae Corporation.

B Special Conditions for Use Statement(s):

For Prescription Use Only
For in vitro diagnostic use

C Special Instrument Requirements:

Illumina NovaSeq 6000 system (qualified by Invitae)

IV Device/System Characteristics:

A Device Description:

The Invitae Common Hereditary Cancers Panel uses hybridization-based capture, next-generation sequencing (NGS), and a custom-built bioinformatics pipeline to compare all positions in targeted regions of 47 genes to a reference sequence and identify variants, including single nucleotide variants (SNVs), insertions and deletions (Indels), and copy number variants (CNVs).

Sequence analysis covers clinically important regions of each gene, including coding exons and 10 to 20 base pairs of adjacent intronic sequence on either side of the coding exons in the transcript listed in **Table 1**. Genes of "high clinical significance" are defined as those for which the test result(s) may lead to prophylactic screening, confirmatory procedures, or treatment that may incur morbidity or mortality to the patient and are shown in bold text. In addition, the

analysis covers the select non-coding variants specifically defined in the table. Any variants that fall outside these regions are not analyzed.

Table 1. Variant Type Reporting per Gene

Gene*	SNV/Indel Analysis	CNV Analysis	Notes
APC	YES	YES	The 1B promoter region is covered by both SNV/Indel and CNV analysis. The 1A promoter region is covered by CNV analysis. SNV/Indel analysis for exon 5 is limited to cds +/-10 bp.
ATM	YES	YES	SNV/Indel analysis for exons 6, 24, 43 includes only cds +/- 10 bp.
AXIN2	YES	YES	
BARD1	YES	YES	
BMPR1A	YES	YES	CNV analysis covers the promoter region.
BRCA1	YES	YES	SNV/Indel analysis includes +/- 20 base pairs of adjacent intronic sequence.
BRCA2	YES	YES	SNV/Indel analysis includes +/- 20 base pairs of adjacent intronic sequence.
BRIP1	YES	YES	
CDH1	YES	YES	
CDK4	YES	YES	
CDKN2A	YES	YES	
CHEK2	YES	YES	
CTNNA1	YES	YES	
DICER1	YES	YES	SNV/Indel analysis for exon 22 includes only cds +/- 10 bp.
EPCAM	NO	YES	SNV/Indel analysis is not offered for this gene (CNV analysis only).
GREM1	NO	YES	Promoter region duplication testing only.
HOXB13	YES	YES	
KIT	YES	YES	
MEN1	YES	YES	SNV/Indel analysis for exon 2 is limited to cds +/-10 bp.
MLH1	YES	YES	CNV analysis covers the promoter region. SNV/Indel analysis for exon 12 is limited to cds +/-10 bp.
MSH2	YES	YES	Analysis includes the exon 1-7 inversion (Boland mutation). SNV/Indel analysis for exons 2, 5 includes only cds +/- 10 bp.
MSH3	YES	YES	SNV/Indel analysis of the repeat region of exon 1 (5:79950697-79950765) is not offered

Gene*	SNV/Indel Analysis	CNV Analysis	Notes
MSH6	YES	YES	SNV/Indel analysis for exons 7, 10 includes only cds +/- 10 bp.
MUTYH	YES	YES	
NBN	YES	YES	
NF1	YES	YES	SNV/Indel analysis for exons 2, 7, 25, 41, 48 includes only cds +/- 10 bp.
NTHL1	YES	YES	
PALB2	YES	YES	
PDGFRA	YES	YES	
PMS2	YES	YES	SNV/Indel analysis for exon 7 includes only cds +/- 10 bp.
POLD1	YES	YES	SNV/Indel analysis for exon 22 includes only cds +/- 10 bp.
POLE	YES	YES	
PTEN	YES	NO	CNV analysis is not offered for this gene. SNV/Indel analysis for exons 8 includes only cds +/- 10 bp.
RAD50	YES	YES	
RAD51C	YES	YES	
RAD51D	YES	YES	
SDHA	YES	NO	CNV analysis is not offered for this gene. SNV/Indel analysis is not offered for exon 14. SNV/Indel analysis for exons 6-8 includes only cds +/- 10 bp.
SDHB	YES	YES	
SDHC	YES	NO	CNV analysis is not offered for this gene. SNV/Indel analysis for exons 2, 6 includes only cds +/- 10 bp.
SDHD	YES	YES	
SMAD4	YES	YES	
SMARCA4	YES	YES	
STK11	YES	YES	
TP53	YES	YES	CNV analysis covers the promoter region.
TSC1	YES	YES	SNV/Indel analysis for exon 21 includes only cds +/- 10 bp.
TSC2	YES	YES	
VHL	YES	YES	

*Genes of high clinical significance are defined as those for which the test result(s) may lead to prophylactic screening, confirmatory procedures or treatment that may incur morbidity or mortality to the patient and are shown in bold text.

Identified variants are assessed by clinical professionals using currently available literature and data from public genetic variant databases. Variants are assigned a score, calculated according to an algorithm that weights the available clinical evidence. Possible outcomes include the following, which are based on joint ACMG/AMP Committee guidelines:

- Benign (not reported) – strong evidence of not being disease or risk causing
- Likely benign (not reported) – some evidence of not being disease or risk causing
- Likely pathogenic – some evidence in favor of causing disease or increasing risk
- Pathogenic – strong evidence of causing disease or increasing disease risk
- Variant of Uncertain Significance - insufficient information to classify in one of the other four categories

Variants are reported using HGVS nomenclature and the human reference genome GRCh37.

B Principle of Operation

Materials and Equipment

The reagents, consumables, and instruments needed to perform the Invitae Common Hereditary Cancers Panel test are used exclusively at, and qualified by the Invitae Corporation clinical laboratory (1400 16th Street, San Francisco, CA 94103). Instrumentation includes integrated, automated systems that incorporate liquid handling instruments, incubators, thermal cyclers, sonicators, and centrifuges, as well as Illumina NovaSeq 6000 sequencers. Reagents include extraction reagents, custom-made molecular barcode (TAFT) plates, buffers, PCR Cleanup Mag Beads, ER/AT Mix, Ligase Mix, PCR Master Mix, custom baits and blockers, PCR primer mix, streptavidin beads, and sequencing reagent kits.

Specimen Collection and Preparation

The test includes a specimen collection kit, which is sent to ordering physicians. The shipping kit contains the following components:

- Plastic whole blood collection tube with K2EDTA
- Patient information card with IB code sticker
- Test requisition form
- Biohazard bag and absorbent pouch, for submission of specimen
- Handling instructions for shipping the specimen

The healthcare provider is directed to collect a 3 mL whole blood specimen, label the tube in the space provided with the patient's full name and at least one additional unique identifier, then ship the specimen to the Sponsor's laboratory at room temperature. When the specimen is received by the Sponsor, the accessioning operators inspect it for the following acceptance criteria:

- Minimum testing volume of 1.5 mL
- No evidence of clotting

- No evidence of leaks
- Specimen collection date <90 days from receipt of the kit
- Paperwork matching tube label

Samples are processed as soon as they are received.

DNA Extraction, Normalization and Shearing

Genomic DNA is extracted from whole blood specimens per protocol using automated extraction and extraction kit qualified by Invitae. Unique molecular tags (TAFTs, Tagged Amplicons For Tracking) are appended to a region of non-clinical significance and processed with the samples from the time of extraction throughout the wet lab process. This allows sample identification to be maintained when samples are processed multiplexed. Extracted DNA is quantified and normalized to 23ng/uL to create batches for assay processing. The DNA is then sonically sheared into consistently sized pieces. Following DNA shearing, the DNA goes through a series of small enzyme-mediated steps that repair the sheared ends of the DNA sample and attach specific oligonucleotides to the ends of the sample. These oligonucleotides are specific for primers that are used in subsequent steps to anneal to them. This includes end-repair, A-tailing, adapter ligation, and dual-indexing of each sample.

DNA Processing and Library Preparation

The specific fragments that contain targeted regions for the assay (i.e., the exonic regions for all 47 genes that are sequenced in this test) can be isolated from the remaining gDNA in the sample. Library production is fully automated end-to-end with integrated robotic systems; the system can create approximately 1,600 libraries every 8 hours with no human intervention. Libraries are pooled by row to create 8 pools of 12 libraries each.

Hybridization

Following library preparation, the barcoded fragments are mixed, and the relevant DNA segments annealed to biotinylated probes for magnetic bead capture. The probes hybridize to the patient DNA samples and these fragments are separated from the rest of the genomic DNA using a magnet that interacts with ferromagnetic beads attached to avidin bound to biotin, which enables all non-bound DNA to be washed away. The beads are manufactured by a third-party vendor per the Sponsor's specification. Both ends of the fragments are sequenced, the sequence of the links is deconvoluted using the molecular barcode to identify which sample each fragment was derived from, and the sequence is aligned to the reference.

Sequencing

The system uses Illumina's NovaSeq, a high throughput sequencing system that employs Sequencing-by-Synthesis chemistry, to perform paired-end reads of 150 bases in length and dual-index barcode reads of 8-nucleotides on the adapters. Equimolar mixing of hybridization reactions forms sequencing pools that allow each library to be sequenced with a minimum of

4,000,000 clusters on the Illumina flow cell. The system's average read depth (i.e., coverage) is 450x; any region with coverage below 50x is flagged as "low" and manually reviewed in the bioinformatics pipeline. Any region below 20x coverage is flagged as "very low," and the sample is failed and either re-run or a new sample is requested.

Sequence Alignment/Mapping and Variant Calling

Following sequencing, raw BCL files are demultiplexed, producing individual fastq files for each sample, then mapped to the reference sequence, producing BAM files. The Invitae bioinformatics software then identifies "Active Regions", i.e., regions that may contain a putative variant, and different variant types are then called by the software.

Variant Interpretation and Review

Variant calls are annotated based on evidence from published literature, public databases, prediction programs, and an internal curated variants database. Regions with low read depth, split reads, or low CNV quality scores are flagged for manual review. All other variants are processed through a combination of automated processes and manual review. For all variants that have been seen previously, the most recent interpretation and associated evidence is automatically placed in the report. For novel variants, variants that have not been seen recently, and variants that have new clinical evidence available, the bioinformatics software has tools to aid the genetics professional in variant interpretation/classification. Variants are assigned one of the following classifications, which are based on joint ACMG/AMP Committee guidelines:

- Pathogenic (Odds >99:1 in favor of causing disease or increasing disease risk)
- Likely Pathogenic (Odds >9:1 but <99:1 in favor of causing disease or increasing risk)
- Variant of Uncertain (Unknown) Significance (VUS) – Insufficient information to classify in one of the other four categories
- Likely Benign (Odds >9:1 but <99:1 in favor of not being disease or risk causing)
- Benign (Odds >99:1 in favor of not being disease or risk causing)

Refer to Section VI. C for more information on the interpretation and curation process.

Controls

a) No Template Control (NTC): A NTC is processed as a negative control through the DNA extraction process and post-extraction DNA quantification process. NTC is checked for position and post-extraction DNA quantification, to ensure the plate is being processed in the correct orientation and that the plate is not contaminated. The NTC is not included in the test steps post DNA quantification.
b) Positive control: A reference cell line sample is processed as a positive control from DNA extraction through sequencing. The positive control is checked for quality metrics such as library concentration, sequence coverage, and gap rates. Failure of the positive control to meet the pre-defined quality metrics will result in a plate failure.

Result Reporting

The Invitae Common Hereditary Cancers Panel test results are for professional use only; the final clinical reports generated from the assay summarize the clinical findings for the ordering physician. Test reports are generated and reviewed by PhD level scientists, genetic counselors, as well as licensed, board-certified clinical molecular geneticists or licensed, board-certified molecular pathologists before signing out.

C Instrument Description Information

1. Instrument Name:

   Illumina NovaSeq 6000 system (qualified by Invitae)

2. Specimen Identification:

   Whole blood

3. Specimen Sampling and Handling:

   Refer to Section IV. B, Specimen Collection and Preparation

4. Calibration:

   Not applicable

5. Quality Control:

Quality metrics are evaluated throughout the bench workflow, including library preparation, hybridization, and sequencing, to verify that samples have appropriate DNA concentrations at key points and that the sequencing run generates reads with adequate quality and depth. Failure to meet the prespecified quality thresholds results in manual review and possible resequencing. Post-sequencing quality metrics are assessed at the sample and batch level to determine sample and batch quality (**Table 2)**. Samples are held for review whenever the value for a metric exceeds the thresholds in the QC Service. Batch quality is determined by reviewing the number of samples that failed for an individual given metric and a batch is held when the number of individual failures exceeds a predetermined threshold for number of failures. Samples and/or batches with holds are reviewed by trained professionals for quality according to Standard Operating Procedure (SOP) documents.

Table 2. Post-Sequencing Sample and Batch QC Metrics

Metric	Description	Sample Threshold	Batch Threshold (# failed samples)
Mean target coverage	The mean depth of coverage (i.e., number of reads) over a target region	≥300x	≤21
Very low coverage	Number of targeted bases (assay-wide) with <20x depth of coverage	≤400	≤42
Percent Selected Bases	The fraction of bases in aligned reads that map directly to or within a fixed interval containing the baited region.	≥68%	≤21
Pass Filter and High Quality aligned read base calling error rate	The fraction of bases that mismatch the genomic reference sequence in reads that pass quality filters and align with mapping quality Phred score of 20 or higher.	≤0.01	≤21
AT dropout (bias)	A measure of how undercovered regions with ≤50% GC content are relative to the mean. A higher score implies that a higher percentage of total reads that should have mapped to regions with ≤50% GC content mapped elsewhere.	≤6%	≤10
GC dropout (bias)	A measure of how undercovered regions with ≥50% GC content are relative to the mean. A higher score implies that a higher percentage of total reads that should have mapped to regions with ≥50% content mapped elsewhere	≤15%	≤21
Median insert size	The median size of sections of DNA being sequenced	200 to 320 bp	≤21
Percent duplication	Percent of paired and unpaired reads marked as duplicates	≤0.2%	≤21
Contamination estimate	A maximum likelihood estimate of the percentage of the DNA in a sample that is from a foreign source.	***	***
Consensus variant rate	The fraction of processed loci that are classified as variant loci.	0.0006 to 0.0029	≤21
Consensus heterozygous/homozygous ratio	The ratio of heterozygous to homozygous variant loci.	1 to 5.8	≤21
Consensus heterozygous rate	The fraction of processed loci that are classified as heterozygous variant loci.	≤0.0015	≤21
Sample Goodness-of-fit to a Baseline sample set	A per-sample measure of how well the observed data at each CNV target are described by the mean model of predicted sample read counts as a	***	***

Metric	Description	Sample Threshold	Batch Threshold (# failed samples)
	function of the read counts for a set of baseline samples.		
CNVitae Q-score <35	Called copy-number error probability p-value represented as a Phred-scaled quality score.	***	***
TAFT (molecular barcode) - minimum expected counts	The number of reads that align to the molecular barcode sequence	***	***
TAFT - proportion of classified	The proportion of reads that align to the correct molecular barcode sequence	***	***
CNVitae_XY_ploidy_match	Whether the X and Y chromosome count observed through sequencing match the biological sex reported by the provider or patient.	***	***

*** Pre-specified threshold values provided. Data not shown

In addition, based on per variant QC metrics, any variant whose data does not reflect a minimum level of confidence that the variant was correctly called is assigned a "FILTER" flag. The criteria for SNV and Indel variants and CNVs are detailed in **Table 3** and **Table 4** below, respectively.

Table 3. FILTER Flag Criteria for SNVs/Indels

Metric	Description	Acceptance Criteria
QUAL (quality) score	Phred-scaled quality score measures the error probability of the called variant.	< 30
QD (quality by depth) score if variant length is ≤3 bp	The QD is the QUAL score normalized by allele depth (AD) for a variant.	< 2.0
Allele balance if variant length is ≤7 bp	In a location where a variant has been detected, the proportion of reads that indicate a variant	< 0.15
Repeat Unit Wobble Filter	The detected variant is in a region with a high amount of variability between reads with respect to the number of short tandem repeats (STRs - short repeated sequences of DNA)	True
Variant is on curated "block list" of known artifacts	The detected variant has been repeatedly identified in the past as being a result of the sequencing process or something other than an actual variant.	True
Variant was removed following manual review of data	Used in specific cases which are selected using additional criteria	True

Table 4. FILTER Flag Criteria for CNVs

Metric	Description	Acceptance Criteria
Excluded Target	Targets are excluded due to very low counts, as well as inability to confidently call copy number (at that target) in too many of the baseline samples. Baseline samples are used to establish what the expected profile of a sample with a normal number of copies of a target region looks like.	***
BaselineCNV	High proportion of copy number variants were called in the baseline OR there was a failure to confidently call copy number in the baseline at this target.	***
CNVLowQual: Quality score	The quality score is derived from the likelihood that the call is normal for copy number normal events and at least part of the called segment is a deletion/duplication for deletion/duplication events. Failing values indicate that there remains significant uncertainty in the call.	***
Variant is on curated "block list" of known artifacts	The detected variant has been repeatedly identified in the past as being a result of the sequencing process or something other than an actual variant.	True
Variant was removed following manual review of data	Used in specific cases which are selected using additional criteria	True

*** Pre-specified threshold values provided. Data not shown

Variants with a FILTER flag are later discarded. All other variants are assigned one of three flags (WARN, LIGHTWARN or CONFIDENT), which are later used to guide whether manual inspection may be needed.

V Standards/Guidance Documents Referenced:

- Considerations for Design, Development, and Analytical Validation of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Intended to Aid in the Diagnosis of Suspected Germline Diseases
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices
- General Principles of Software Validation
- Recommended Content Format of Non-Clinical Bench Performance Testing Information in Premarket Submissions
- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices
- Statistical Guidance on Reporting Results from Studies

- CLSI standard EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures (3rd ed)
- CLSI standard EP07 Interference Testing in Clinical Chemistry (3rd ed)
- CLSI standard EP37 Supplemental Tables for Interference Testing in Clinical Chemistry

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the Invitae Common Hereditary Cancers Panel was evaluated with two sets of clinical samples. The first set included 25 samples, with a total of 2,047 SNVs across 40 genes (including 11 genes of high clinical significance), 33 Insertions across 7 genes (including 2 genes of high clinical significance), 38 Deletions across 5 genes (including 1 gene of high clinical significance), 5 CNV duplications across 4 genes (including 1 gene of high clinical significance). and 16 CNV deletions across 12 genes (including 5 genes of high clinical significance). The second set included 18 samples enriched for Indels and CNVs, with a total of 19 Insertions across 4 genes (including 1 genes of high clinical significance), 12 Deletions across 7 genes (including 1 gene of high clinical significance), 7 CNV duplications across 6 genes (including 4 genes of high clinical significance) and 16 CNV deletions across 6 genes (including 5 genes of high clinical significance). Each sample was tested with 14 replicates across 3 different operators, 3 different sequencers, and 3 different sequencing reagent lots. The assay runs were performed on 3 different, non-consecutive days. The repeatability of the assay was assessed by analyzing the concordance between sample replicates within the same run. The reproducibility of the assay was assessed by analyzing the concordance between sample replicates across runs, overall and across sequencing reagent lots, sequencing operators, and sequencers.

Results of the precision study are summarized in **Table 5** to **Table 11**. The overall PPA is 99.95% (95% CI 99.92-99.97%) for SNVs, 99.57% (95% CI 99.07-99.80%) for Indels and 99.67% (95% CI 98.80-99.91%) for CNVs. The overall NPA is >99.99% for all variant types (95% CI >99.99-100%) (**Table 5**).

Table 5. Overall Precision by Variant Type

Type	# Total Positive Variants*	# Detected Positive Variants**	# Total Negative Variants*	# Detected Negative Variants**	PPA (95% CI)	NPA (95% CI)
SNVs	28633	28619	35645541	35645506	99.95% (99.92-99.97%)	>99.99% (>99.99-100%)
Indels	1405	1399	14854187	14854180	99.57% (99.07-99.80%)	>99.99% (>99.99-100%)
Insertions	728	728	3973866	3973866	100% (99.48-100%)	100% (>99.99-100%)
Deletions	677	671	10880321	10880314	99.11% (98.08-99.59%)	>99.99% (>99.99-100%)

CNVs	602	600	467326	467323	99.67% (98.80-99.91%)	>99.99% (>99.99-100%)
CNV duplications	157	157	467326	467326	100% (97.61-100%)	100% (>99.99-100%)
CNV deletions	445	443	467326	467323	99.55% (98.38-99.88%)	>99.99% (>99.99-100%)

* #Total positive/negative variants: the number of expected positive/negative variants in all replicates of all samples, based on majority call. However, any expected variants in a region that did not pass quality metrics were not counted in this total (these would be accounted for in the no call rate).
** # Detected positive/negative variants: the total number of positive/negative variants that were detected across replicates of the study samples.

The PPAs are >99% across different zygosity (for SNVs), variant sizes (for Indels and CNVs) or genomic contexts, except for deletions of 1-5bp, for which the PPA is 97.53% (95% CI 94.72-98.86%) (**Table 6** and **Table 7)**. This was due to 6 false negative results for the same variant on the SDHA gene, which were in a low mappability/complexity region.

Table 6. Precision by Zygosity and Variant Size

Variant Type Stratified by Zygosity and Size		# Total Positive Variants*	PPA	95% CI	# Total Negative Variants*	NPA	95% CI
SNVs	Homo	10248	100%	99.96-100%	N/A	N/A	N/A**
	Hetero	17181	99.92%	99.86-99.95%	N/A	N/A	N/A
	Unknown zygosity	1204	100%	99.68-100%	N/A	N/A	N/A
Insertions	1-5 bp	588	100%	99.35-100%	3525432	100%	>99.99-100%
	6-10 bp	112	100%	96.68-100%	315417	100%	>99.99-100%
	11-20 bp	14	100%	78.47-100%	131247	100%	>99.99-100%
	21+ bp	14	100%	78.47-100%	1770	100%	>99.99-100%
Deletions	1-5 bp	243	97.53%	94.72-98.86%	9706966	>99.99%	>99.99-100%
	6-10 bp	196	100%	98.08-100%	813027	100%	>99.99-100%
	11-20 bp	196	100%	98.08-100%	359433	100%	>99.99-100%
	21+ bp	42	100%	91.62-100%	888	100%	>99.99-100%
CNV Duplications	≤ Single Exon	40	100%	91.24-100%	N/A	N/A	N/A
	2-5 Exons	70	100%	94.80-100%	N/A	N/A	N/A
	6-9 Exons	12	100%	75.75-100%	N/A	N/A	N/A
	10+ Exons	14	100%	78.47-100%	N/A	N/A	N/A

Variant Type Stratified by Zygosity and Size		# Total Positive Variants*	PPA	95% CI	# Total Negative Variants*	NPA	95% CI
	Entire Coding Sequence	21	100%	84.54-100%	N/A	N/A	N/A
	Other (intronic, non-coding, combination)	0	N/A	N/A	N/A	N/A	N/A
CNV Deletions	≤ Single Exon	235	99.15%	96.95-99.77%	N/A	N/A	N/A
	2-5 Exons	116	100%	96.79-100%	N/A	N/A	N/A
	6-9 Exons	61	100%	94.08-100%	N/A	N/A	N/A
	10+ Exons	33	100%	89.57-100%	N/A	N/A	N/A
	Entire Coding Sequence	0	N/A	N/A	N/A	N/A	N/A
	Other (intronic, non-coding, combination)	0	N/A	N/A	N/A	N/A	N/A

* #Total positive/negative variants: the number of expected positive/negative variants in all replicates of all samples, based on majority call. However, any expected variants in a region that did not pass quality metrics were not counted in this total (these would be accounted for in the no call rate).
** N/A: data not available or not calculated.

Table 7. Precision by Genomic Context

Variant Type Stratified by Genomic Context		# Total Positive Variants*	PPA	95% CI of PPA	# Total Negative Variants*	NPA	95% CI of NPA
High GC Content (>70%)	SNVs	504	100%	99.24-100%	118395	100%	>99.99-100%
	Insertions	0	N/A**	N/A	12297	100%	99.97-100%
	Deletions	182	100%	97.93-100%	12951	100%	99.97-100%
	CNV duplications	56	100%	93.58-100%	25223	100%	99.98-100%
	CNV deletions	126	100%	97.04-100%	25223	100%	99.98-100%
Low GC Content (<30%)	SNVs	5320	100%	99.93-100%	7191969	100%	>99.99-100%
	Insertions	14	100%	78.47-100%	946545	100%	>99.99-100%
	Deletions	168	100%	97.76-100%	3087513	100%	>99.99-100%

Variant Type Stratified by Genomic Context		# Total Positive Variants*	PPA	95% CI of PPA	# Total Negative Variants*	NPA	95% CI of NPA
	CNV duplications	76	100%	95.19-100%	129043	100%	>99.99-100%
	CNV deletions	209	100%	98.17-100%	129043	100%	>99.99-100%
Low Mappability/ Low Complexity	SNVs	9075	99.84%	99.74-99.91%	9738339	>99.99%	>99.99-100%
	Insertions	238	100%	98.41-100%	1163808	100%	>99.99-100%
	Deletions	607	99.01%	97.86-99.55%	3817896	>99.99%	>99.99-100%
	CNV duplications	43	100%	91.80-100%	28710	100%	99.99-100%
	CNV deletions	104	100%	96.44-100%	28710	100%	99.99-100%

* #Total positive/negative variants: the number of expected positive/negative variants in all replicates of all samples, based on majority call. However, any expected variants in a region that did not pass quality metrics were not counted in this total (these would be accounted for in the no call rate).
**N/A: data not available or not calculated.

Table 8. Precision by Sources of Variance and Variant Type

Variable	Average Positive Agreement (95% CI)				
	SNVs	Insertions	Deletions	CNV duplications	CNV deletions
Repeatability	99.91% (99.88-99.94%)	100% (>99.99-100%)	98.08% (97.48-99.52%)	99.83% (99.83-100%)	99.43% (98.29-99.82)
Reproducibility – Instrument-to-Instrument	99.93% (99.91-99.96%)	100% (>99.99-100%)	97.72% (96.59-99.05%)	96.69% (94.17-98.80%)	98.99% (98.06-99.68%)
Reproducibility - Lot-to-Lot	99.87% (99.83%-99.90%)	100% (>99.99-100%)	99.24% (99.16-99.85%)	98.37% (95.14-99.16%)	>99.99% 99.13-100%
Reproducibility - Operator-to-Operator	99.89% (99.85-99.92%)	100% (>99.99-100%)	99.85% (98.90-99.93%)	98.72% (96.82-100%	99.85% (99.16-100%)
Reproducibility - Run-to-Run/Day-to-Day	99.83% (99.74-99.89%)	100% (>99.99-100%)	95.17% (90.55-96.49%)	100% (95.63-100%)	100% (>99.99-100%)

Table 9. No Call Rate

No call rate*	Repeatability	Reproducibility – Instrument-to-Instrument	Reproducibility - Lot-to-Lot	Reproducibility - Operator-to-Operator	Reproducibility - Run-to-Run/Day-to-Day
SNVs/Indels	0.05%	0.05%	0.05%	0.05%	0.18%
CNVs	1.78%	1.99%	1.99%	1.95%	1.82%

*For SNVs and Indels, a "no call" is an area that could not be called due to a sample or region-specific limitation like an unfilled coverage gap. The no call (NC) rate for SNVs and Indels is calculated as the proportion of variants with a no call result in the total number of SNV/Indel called variants, assessed across all sample comparisons.

For CNVs, a "no call" is a region that could not be called due to a sample or region-specific limitation like an unfilled coverage gap. The NC rate for CNVs is calculated as the proportion of CNV target regions with a no call result in the total number of target regions, assessed across all sample comparisons.

Table 10. Precision per specimen

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
Study 1									
Specimen 1	SNVs	1204	1204	100%	99.68-100%	776217	776217	100%	>99.99-100%
	Indels	56	56	100%	93.58-100%	197977	197977	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10874	10874	100%	99.96-100%
Specimen 2	SNVs	1106	1106	100%	99.65-100%	776217	776217	100%	>99.99-100%
	Indels	42	42	100%	91.62-100%	198016	198016	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10878	10878	100%	99.96-100%
Specimen 3	SNVs	1512	1512	100%	99.75-100%	776035	776035	100%	>99.99-100%
	Indels	56	56	100%	93.58-100%	198003	198003	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	10892	10892	100%	99.96-100%
Specimen 4	SNVs	1008	1008	100%	99.62-100%	776308	776308	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	198029	198029	100%	>99.99-100%
	CNVs	0	0	N/A***	N/A	10888	10888	100%	99.96-100%
Specimen 5	SNVs	882	882	100%	99.57-100%	776295	776295	100%	>99.99-100%
	Indels	70	70	100%	94.80-100%	198003	198003	100%	>99.99-100%
	CNVs	21	21	100%	84.54-100%	10810	10808	99.98%	99.93-99.99%
Specimen 6	SNVs	997	994	99.70%	99.12-99.90%	776228	776222	>99.99%	>99.99-100%

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	Indels	30	28	93.33%	78.68-98.15%	197972	197969	>99.99%	>99.99-100%
	CNVs	22	22	100%	85.13-100%	10805	10805	100%	99.96-100%
Specimen 7	SNVs	1190	1190	100%	99.68-100%	776152	776152	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198042	198042	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10850	10850	100%	99.96-100%
Specimen 8	SNVs	1036	1036	100%	99.63-100%	776295	776295	100%	>99.99-100%
	Indels	56	56	100%	93.58-100%	198042	198042	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10877	10877	100%	99.96-100%
Specimen 9	SNVs	1120	1120	100%	99.66-100%	776139	776139	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198068	198068	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10888	10888	100%	99.96-100%
Specimen 10	SNVs	1206	1205	99.92%	99.53-99.99%	776128	776125	>99.99%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198055	198055	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10874	10874	100%	99.96-100%
Specimen 11	SNVs	1050	1050	100%	99.64-100%	776334	776330	>99.99%	>99.99-100%
	Indels	63	63	100%	94.25-100%	197994	197990	>99.99%	99.99-100%
	CNVs	14	14	100%	78.47-100%	10864	10864	100%	99.96-100%
Specimen 12	SNVs	1456	1456	100%	99.74-100%	776063	776057	>99.99%	>99.99-100%
	Indels	70	70	100%	94.80-100%	198003	198003	100%	>99.99-100%

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	CNVs	14	14	100%	78.47-100%	10878	10878	100%	99.96-100%
Specimen 13	SNVs	882	882	100%	99.57-100%	776438	776438	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198042	198042	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10869	10869	100%	99.96-100%
Specimen 14	SNVs	1106	1106	100%	99.65-100%	776256	776256	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	198016	198016	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10864	10864	100%	99.96-100%
Specimen 15	SNVs	1080	1074	99.44%	98.79-99.75%	776244	776238	>99.99%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198029	198029	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10892	10892	100%	99.96-100%
Specimen 16	SNVs	952	952	100%	99.60-100%	776373	776373	100%	>99.99-100%
	Indels	56	56	100%	93.58-100%	198003	198003	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10892	10892	100%	99.96-100%
Specimen 17	SNVs	1302	1302	100%	99.71-100%	776113	776113	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198016	198016	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10892	10892	100%	99.96-100%
Specimen 18	SNVs	1694	1694	100%	99.77-100%	775788	775788	100%	>99.99-100%
	Indels	84	84	100%	95.63-100%	197977	197977	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10892	10892	100%	99.96-100%

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
Specimen 19	SNVs	924	924	100%	99.59-100%	776334	776334	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	198016	198016	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10891	10891	100%	99.96-100%
Specimen 20	SNVs	1120	1120	100%	99.66-100%	776308	776308	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	198016	198016	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10878	10878	100%	99.96-100%
Specimen 21	SNVs	1540	1540	100%	99.75-100%	775892	775892	100%	>99.99-100%
	Indels	42	42	100%	91.62-100%	198003	198003	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10878	10878	100%	99.96-100%
Specimen 22	SNVs	1162	1162	100%	99.67-100%	776165	776165	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	198016	198016	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10884	10884	100%	99.96-100%
Specimen 23	SNVs	1032	1028	99.61%	99.01-99.85%	776283	776273	>99.99%	>99.99-100%
	Indels	56	56	100%	93.58-100%	198003	198003	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10878	10878	100%	99.96-100%
Specimen 24	SNVs	924	924	100%	99.59-100%	776399	776399	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	198029	198029	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10864	10864	100%	99.96-100%
	SNVs	1148	1148	100%	99.67-100%	776217	776217	100%	>99.99-100%

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
Specimen 25	Indels	56	56	100%	93.58-100%	197990	197990	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10889	10889	100%	99.96-100%
Study 2									
Specimen 1	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198107	198107	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	10907	10907	100%	99.96-100%
Specimen 2	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198120	198120	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10959	10959	100%	99.96-100%
Specimen 3	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198120	198120	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10988	10988	100%	99.96-100%
Specimen 4	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	14	14	100%	78.47-100%	198146	198146	100%	>99.99-100%
	CNVs	22	22	100%	85.13-100%	11001	11001	100%	99.96-100%
Specimen 5	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198133	198133	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10975	10975	100%	99.96-100%
Specimen 6	SNVs	0	0	N/A	N/A	0	0	N/A	N/A

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	Indels	42	42	100%	91.62-100%	198094	198094	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10792	10792	100%	99.96-100%
Specimen 7	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	14	14	100%	78.47-100%	198133	198133	100%	>99.99-100%
	CNVs	20	20	100%	83.89-100%	10783	10783	100%	99.96-100%
Specimen 8	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	14	14	100%	78.47-100%	198159	198159	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10994	10994	100%	99.96-100%
Specimen 9	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	14	14	100%	78.47-100%	198146	198146	100%	>99.99-100%
	CNVs	17	17	100%	81.57-100%	10991	10991	100%	99.96-100%
Specimen 10	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198120	198120	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	10998	10998	100%	99.96-100%
Specimen 11	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198146	198146	100%	>99.99-100%
	CNVs	22	22	100%	85.13-100%	10990	10990	100%	99.96-100%
Specimen 12	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	0	0	N/A	N/A	198133	198133	100%	>99.99-100%

Specimen Stratified by Variant Type		# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	CNVs	14	14	100%	78.47-100%	10963	10963	100%	99.96-100%
Specimen 13	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198146	198146	100%	>99.99-100%
	CNVs	60	58	96.67%	88.64- 99.08%	10229	10228	99.99%	99.94-100%
Specimen 14	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	14	14	100%	78.47-100%	198159	198159	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10969	10969	100%	99.96-100%
Specimen 15	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	42	42	100%	91.62-100%	198107	198107	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	10938	10938	100%	99.96-100%
Specimen 16	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	10	6	60.00%	31.27-83.18%	167662	167662	100%	>99.99-100%
	CNVs	0	0	0	N/A	11026	11026	100%	99.96-100%
Specimen 17	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	28	28	100%	87.94-100%	198133	198133	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	10958	10958	100%	99.96-100%
Specimen 18	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	42	42	100%	91.62-100%	198068	198068	100%	>99.99-100%
	CNVs	26	26	100%	87.13-100%	10824	10824	100%	99.96-100%

* #Total positive/negative variants: the number of expected positive/negative variants in all replicates of all samples, based on majority call. However, any expected variants in a region that did not pass quality metrics were not counted in this total (these would be accounted for in the no call rate).
** # Detected positive/negative variants: the total number of positive/negative variants that were detected across replicates of the study samples.
*** N/A: values not calculated.

On the gene level, for SNVs, all the genes tested in the study show PPA of 100%, except SDHA (99.15% with 95% CI 98.59-99.50%); for Indels, all the genes tested in the study show PPA of 100%, except SDHA (68.42% with 95% CI 46.01-84.64%); and for CNVs, all the genes tested in the study show PPA of 100%, except NF1 (97.30% with 95% CI 90.67-99.26%) (**Table 11**).

Table 11. Precision per Gene

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
APC	SNVs	2562	2562	100%	99.85-100%	2448957	2448957	100%	>99.99-100%
	Indels	56	56	100%	93.58-100%	838419	838419	100%	>99.99-100%
	CNVs	0	0	N/A***	N/A	10234	10234	100%	99.96-100%
ATM	SNVs	770	770	100%	99.50-100%	2767848	2767848	100%	>99.99-100%
	Indels	168	168	100%	97.76-100%	1086495	1086495	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	37266	37266	100%	99.99-100%
AXIN2	SNVs	1008	1008	100%	99.62-100%	626001	626001	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	79332	79332	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	6018	6018	100%	99.94-100%
BARD1	SNVs	812	812	100%	99.53-100%	708975	708975	100%	>99.99-100%
	Indels	420	420	100%	99.09-100%	182457	182457	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	6621	6621	100%	99.94-100%
BMPR1A	SNVs	210	210	100%	98.20-100%	403335	403335	100%	>99.99-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	Indels	0	0	N/A	N/A	92028	92028	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	8764	8764	100%	99.96-100%
BRCA1	SNVs	1330	1330	100%	99.71-100%	1364151	1364151	100%	>99.99-100%
	Indels	0	0	N/A	N/A	1726671	1726671	100%	>99.99-100%
	CNVs	82	82	100%	95.52-100%	11956	11956	100%	99.97-100%
BRCA2	SNVs	2338	2338	100%	99.84-100%	2694108	2694108	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	2326866	2326866	100%	>99.99-100%
	CNVs	42	42	100%	91.62-100%	16183	16183	100%	99.98-100%
BRIP1	SNVs	952	952	100%	99.60-100%	1005642	1005642	100%	>99.99-100%
	Indels	0	0	N/A	N/A	304578	304578	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	11436	11436	100%	99.97-100%
CDH1	SNVs	882	882	100%	99.57-100%	745317	745317	100%	>99.99-100%
	Indels	0	0	N/A	N/A	173172	173172	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	9632	9632	100%	99.96-100%
CDK4	SNVs	0	0	N/A	N/A	175518	175518	100%	>99.99-100%
	Indels	0	0	N/A	N/A	25350	25350	100%	99.98-100%
	CNVs	0	0	N/A	N/A	4214	4214	100%	99.91-100%
CDKN2A	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	0	0	N/A	N/A	0	0	N/A	N/A

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	CNVs	0	0	N/A	N/A	2310	2310	100%	99.83-100%
CHEK2	SNVs	14	14	100%	78.47-100%	541458	541458	100%	>99.99-100%
	Indels	0	0	N/A	N/A	303225	303225	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	8391	8391	100%	99.95-100%
CTNNA1	SNVs	350	350	100%	98.91-100%	453696	453696	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	64707	64707	100%	99.99-100%
	CNVs	0	0	N/A	N/A	10234	10234	100%	99.96-100%
DICER1	SNVs	420	420	100%	99.09-100%	1110225	1110225	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	276732	276732	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	15652	15652	100%	99.98-100%
EPCAM	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	0	0	N/A	N/A	0	0	N/A	N/A
	CNVs	0	0	N/A	N/A	5418	5418	100%	99.93-100%
GREM1	SNVs	0	0	N/A	N/A	0	0	N/A	N/A
	Indels	0	0	N/A	N/A	0	0	N/A	N/A
	CNVs	21	21	100%	84.54-100%	3530	3528	99.94%	99.79-99.98%
HOXB13	SNVs	294	294	100%	98.71-100%	261696	261696	100%	>99.99-100%
	Indels	0	0	N/A	N/A	181350	181350	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	1204	1204	100%	99.68-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
KIT	SNVs	266	266	100%	98.58-100%	522657	522657	100%	>99.99-100%
	Indels	0	0	N/A	N/A	43278	43278	100%	99.99-100%
	CNVs	0	0	N/A	N/A	12642	12642	100%	99.97-100%
MEN1	SNVs	994	994	100%	99.62-100%	379650	379650	100%	>99.99-100%
	Indels	0	0	N/A	N/A	174147	174147	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	6020	6020	100%	99.94-100%
MLH1	SNVs	224	224	100%	98.31-100%	683862	683862	100%	>99.99-100%
	Indels	0	0	N/A	N/A	457191	457191	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	11984	11984	100%	99.97-100%
MSH2	SNVs	532	532	100%	99.28-100%	904152	904152	100%	>99.99-100%
	Indels	0	0	N/A	N/A	536250	536250	100%	>99.99-100%
	CNVs	83	83	100%	95.58-100%	8096	8096	100%	99.95-100%
MSH3	SNVs	1330	1330	100%	99.71-100%	771639	771639	100%	>99.99-100%
	Indels	518	518	100%	99.26-100%	141462	141462	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	14419	14419	100%	99.97-100%
MSH6	SNVs	602	602	100%	99.37-100%	1285641	1285641	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	628074	628074	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	5978	5978	100%	99.94-100%
MUTYH	SNVs	336	336	100%	98.87-100%	511395	511395	100%	>99.99-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	Indels	0	0	N/A	N/A	104325	104325	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	9632	9632	100%	99.96-100%
NBN	SNVs	1260	1260	100%	99.70-100%	652761	652761	100%	>99.99-100%
	Indels	0	0	N/A	N/A	222897	222897	100%	>99.99-100%
	CNVs	20	20	100%	83.89-100%	9408	9408	100%	99.96-100%
NF1	SNVs	560	560	100%	99.32-100%	1968074	1968074	100%	>99.99-100%
	Indels	0	0	N/A	N/A	1360956	1360956	100%	>99.99-100%
	CNVs	74	72	97.30%	90.67-99.26%	32719	32718	>99.99%	99.98-100%
NTHL1	SNVs	0	0	N/A	N/A	306858	306858	100%	>99.99-100%
	Indels	0	0	N/A	N/A	51675	51675	100%	99.99-100%
	CNVs	0	0	N/A	N/A	3603	3603	100%	99.89-100%
PALB2	SNVs	252	252	100%	98.50-100%	993345	993345	100%	>99.99-100%
	Indels	0	0	N/A	N/A	505269	505269	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	7812	7812	100%	99.95-100%
PDGFRA	SNVs	1330	1330	100%	99.71-100%	585288	585288	100%	>99.99-100%
	Indels	0	0	N/A	N/A	53625	53625	100%	99.99-100%
	CNVs	0	0	N/A	N/A	13242	13242	100%	99.97-100%
PMS2	SNVs	2338	2338	100%	99.84-100%	835626	835626	100%	>99.99-100%
	Indels	0	0	N/A	N/A	244884	244884	100%	>99.99-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	CNVs	112	112	100%	96.68-100%	2279	2279	100%	99.83-100%
POLD1	SNVs	630	630	100%	99.39-100%	834690	834690	100%	>99.99-100%
	Indels	28	28	100%	87.94-100%	144075	144075	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	15652	15652	100%	99.98-100%
POLE	SNVs	1750	1750	100%	99.78-100%	1696266	1696266	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	293832	293832	100%	>99.99-100%
	CNVs	28	28	100%	87.94-100%	29256	29256	100%	99.99-100%
PTEN	SNVs	0	0	N/A	N/A	316410	316410	100%	>99.99-100%
	Indels	0	0	N/A	N/A	268125	268125	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	0	0	N/A	N/A
RAD50	SNVs	70	70	100%	94.80-100%	896586	896586	100%	>99.99-100%
	Indels	0	0	N/A	N/A	211575	211575	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	15050	15050	100%	99.97-100%
RAD51C	SNVs	14	14	100%	78.47-100%	353403	353403	100%	>99.99-100%
	Indels	0	0	N/A	N/A	94953	94953	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	5418	5418	100%	99.93-100%
RAD51D	SNVs	336	336	100%	98.87-100%	314091	314091	100%	>99.99-100%
	Indels	0	0	N/A	N/A	85203	85203	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	6020	6020	100%	99.94-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
SDHA	SNVs	1655	1641	99.15%	98.59-99.50%	523186	523151	99.99%	99.99-100%
	Indels	19	13	68.42%	46.01-84.64%	87287	87280	99.99%	99.98-100%
	CNVs	0	0	N/A	N/A	0	0	N/A	N/A
SDHB	SNVs	378	378	100%	98.99-100%	234600	234600	100%	>99.99-100%
	Indels	0	0	N/A	N/A	73965	73965	100%	99.99-100%
	CNVs	0	0	N/A	N/A	4813	4813	100%	99.92-100%
SDHC	SNVs	0	0	N/A	N/A	121191	121191	100%	>99.99-100%
	Indels	84	84	100%	95.63-100%	23274	23274	100%	99.98-100%
	CNVs	0	0	N/A	N/A	0	0	N/A	N/A
SDHD	SNVs	70	70	100%	94.80-100%	127032	127032	100%	>99.99-100%
	Indels	0	0	N/A	N/A	43875	43875	100%	99.99-100%
	CNVs	0	0	N/A	N/A	2408	2408	100%	99.84-100%
SMAD4	SNVs	14	14	100%	78.47-100%	335493	335493	100%	>99.99-100%
	Indels	0	0	N/A	N/A	91650	91650	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	7224	7224	100%	99.95-100%
SMARCA4	SNVs	378	378	100%	98.99-100%	924498	924498	100%	>99.99-100%
	Indels	0	0	N/A	N/A	107847	107847	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	21070	21070	100%	99.98-100%
STK11	SNVs	154	154	100%	97.57-100%	390417	390417	100%	>99.99-100%

Gene	Type	# Total Positive Variants*	# Detected Positive Variants**	PPA	95% CI of PPA	# Total Negative Variants*	# Detected Negative Variants**	NPA	95% CI of NPA
	Indels	0	0	N/A	N/A	129675	129675	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	5418	5418	100%	99.93-100%
TP53	SNVs	378	378	100%	98.99-100%	346773	346773	100%	>99.99-100%
	Indels	0	0	N/A	N/A	281292	281292	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	7224	7224	100%	99.95-100%
TSC1	SNVs	294	294	100%	98.71-100%	722523	722523	100%	>99.99-100%
	Indels	0	0	N/A	N/A	233940	233940	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	13830	13830	100%	99.97-100%
TSC2	SNVs	490	490	100%	99.22-100%	1499727	1499727	100%	>99.99-100%
	Indels	14	14	100%	78.47-100%	390954	390954	100%	>99.99-100%
	CNVs	0	0	N/A	N/A	25282	25282	100%	99.98-100%
VHL	SNVs	56	56	100%	93.58-100%	300780	300780	100%	>99.99-100%
	Indels	0	0	N/A	N/A	107250	107250	100%	>99.99-100%
	CNVs	14	14	100%	78.47-100%	1764	1764	100%	99.78-100%

* #Total positive/negative variants: the number of expected positive/negative variants in all replicates of all samples, based on majority call. However, any expected variants in a region that did not pass quality metrics were not counted in this total (these would be accounted for in the no call rate).
** # Detected positive/negative variants: the total number of positive/negative variants that were detected across replicates of the study samples.
*** N/A: values not calculated.

2. Linearity:

   Not applicable.

3. DNA input:

During the test procedure, extracted DNA is quantified and normalized to 23ng/uL to create batches for assay processing. To evaluate the impact of different levels of DNA input on the Invitae Common Hereditary Cancers Panel test performance, eight whole blood clinical specimens with representative variants were tested across five input levels spanning 10 fold below and 2 fold above the standard input level of 23ng/uL. These samples include a total of 659 SNVs across 36 genes, 12 Indels across 6 genes, and 13 CNV across 4 genes in different genomic contexts (**Table 12)**. The samples were tested at 1, 5, 10, 23 (standard concentration), and 46 ng/uL, each in triplicate, for a total of 120 samples (8 unique samples x 5 input levels x 3 replicates/ input level = 120 samples). Number of failed samples, number of samples with low coverage, overall concordance, PPA, and NPA compared to the 23ng/uL input were evaluated at each input level.

Results of the study are summarized in **Table 13** to **Table 15.** At all DNA input levels, the overall concordance, PPA and NPA are >99% compared to the standard input. However, five sample failed QC metrics at 1ng/uL, and six samples failed QC metrics at 46ng/uL. Therefore, the minimum DNA input for the Invitae Common Hereditary Cancers Panel was determined to be 5 ng/uL. DNA input higher than 23ng/uL may lead to sample failure and should be normalized to 23ng/uL for assay processing. One variant type – CNV deletions – fell slightly below 95% PPA at 5 ng/uL. This was due to 2 false negative results for the same variant on the NF1 gene, which was in an AT rich region.

Table 12. Variant Distribution by Genomic Context

Genomic Context	SNVs	Insertions	Deletions	CNVs - Dup	CNVs - Del
Low mappability/complexity	212	2	4	2	7
GC rich regions	11	0	0	0	3
AT rich regions	115	0	3	1	6
All other	370	5	1	0	1

Table 13. DNA input Study Results Summary

DNA Conc	# Samples with Low Coverage*	# Failed Samples	PPA (95% CI)	NPA (95% CI)	Overall Concordance (95% CI)	No call rate**
1 ng/uL	0	5	99.95% (99.72-99.99%)	>99.99% (>99.99%-100%)	>99.99% (>99.99%-100%)	0.32%
5 ng/uL	0	0	99.90% (99.65-99.97%)	>99.99% (>99.99%-100%)	>99.99% (>99.99%-100%)	0.05%
10 ng/uL	0	0	99.95% (99.72-99.99%)	>99.99% (>99.99%-100%)	>99.99% (>99.99%-100%)	0.03%
46 ng/uL	0	6	99.71% (99.36-99.87%)	>99.99% (>99.99%-100%)	>99.99% (>99.99%-100%)	0.02%

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**The no call rate at 23ng/uL is 0.02%.

Table 14. Positive Percent Agreement by Variant Type

DNA Input Level	SNVs				Indels				CNVs			
	TP	FN	PPA	95% CI	TP	FN	PPA	95% CI	TP	FN	PPA	95% CI
1 ng/uL	1976	1	99.9%	99.7-100%	36	0	100%	90.3-100%	37	0	100%	90.6-100%
5 ng/uL	1977	0	100%	99.8-100%	36	0	100%	90.3-100%	39	2	95.1%	83.9-98.7%
10 ng/uL	1976	1	99.9%	99.7-100%	36	0	100%	90.3-100%	39	0	100%	91.6-100%
46 ng/uL	1977	0	100%	99.8-100%	36	0	100%	90.3-100%	29	6	82.9%	67.3-91.9%

TP: true positive; FN: false negative

Table 15. Negative Percent Agreement by Variant Type

DNA Conc	SNVs				Indels				CNVs			
	TN	FP	NPA	95% CI	TN	FP	NPA	95% CI	TN	FP	NPA	95% CI
1 ng/uL	1451671	3	>99.99%	>99.99-100%	364387	2	>99.99%	>99.99-100%	15174	0	100%	>99.99-100%
5 ng/uL	1451671	2	>99.99%	>99.99-100%	364386	3	>99.99%	>99.99-100%	19271	0	100%	>99.99-100%
10 ng/uL	1451673	1	>99.99%	>99.99-100%	364387	2	>99.99%	>99.99-100%	19271	0	100%	>99.99-100%
46 ng/uL	1451669	4	>99.99%	>99.99-100%	364386	3	>99.99%	>99.99-100%	14404	0	100%	>99.99-100%

TP: true positive; FN: false negative

4. Analytical Specificity/Interference:

Two studies were performed to evaluate the impact on assay performance of endogenous and exogenous substances that may be present in test samples due to carry-through from patient samples or due to processing conditions. In each study, seven potential interfering substances, including bilirubin, K2EDTA, wash buffer, TAFT, hemoglobin, and triglycerides, were each spiked at varying levels into specimens sourced from a blood bank. Blood from another donor was also tested in the first study to mimic a stem cell or bone marrow transplant patient. In the first study, samples were tested in three replicates at each level; in the second study, samples, enriched for Indels and CNVs, were tested in two replicates at each level (**Table 16)**. The variants tested included those in challenging genomic contexts such as AT rich, GC rich, and low complexity regions. Results were analyzed for number of failed samples, number of samples with low coverage, overall concordance, PPA, and NPA compared to the un-spiked condition. Results of the interfering substances study are summarized in **Table 17** to **Table 20.** Bilirubin, TAFTs, hemoglobin, and triglycerides were determined not to affect assay performance with the tested levels. Blood from a second donor caused the assay to fail at all levels of contamination as expected. False negative CNVs were reported in the samples with K2EDTA and wash buffer

added. In both cases, the replicates of the control sample were discordant with each other for the same variants. Inappropriate addition of PMS2 amplicon caused all samples to fail QC metrics for CNV detection, as well as decreased PPA for Indel detection. This is because the CNV calling algorithm cannot form a baseline when there is an unexpected read count due to the presence of the inappropriately introduced large amplicons. Therefore, amplicon presence should be stringently controlled in laboratory workflows, through use of filtered tips, automation and other good laboratory practices.

Table 16. Samples and Variants Tested in the Interference Substances Studies

Study	Substance	# Samples	# Replicates	# SNVs	# Indels	# CNVs
1	Bilirubin	5	3	332	15	0
	Wash Buffer	5	3	383	11	0
	TAFTs	5	3	393	18	0
	K2EDTA	5	3	375	12	0
	Amplicon	5	3	395	0	0
	Hemoglobin	5	3	355	9	0
	Triglycerides	5	3	375	12	0
	Donor Blood	10	2	N/A*	N/A	N/A
2	Bilirubin	5	2	0	10	5
	Wash Buffer	5	2	0	11	9
	TAFTs	5	2	0	8	4
	K2EDTA	5	2	0	9	4
	Amplicon	5	2	0	8	0
	Hemoglobin	6	2	0	8	8
	Triglycerides	5	2	0	8	5

*N/A: values not calculated because all samples failed QC metrics

Table 17. Interfering Substances Study Results Summary for SNVs (Study 1)

Substance	Level	# Samples with Low Coverage*	# Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
Bilirubin	0	0	2	N/A**	N/A	N/A
	137 mmol/L	0	1	100% (98.9- 100%)	>99.9% (>99.9- 100%)	>99.9% (>99.9- 100%)
	684 mmol/L	0	1	100% (98.9- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Wash buffer	0	0	0	N/A	N/A	N/A
	15% v/v	0	0	99.7% (98.54- 99.95%)	100% (>99.9- 100%)	>99.9% (>99.9- 100%)
TAFT (molecular barcode)	0	0	0	N/A	N/A	N/A
	5%	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)

Substance	Level	# Samples with Low Coverage*	# Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
	15%	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	30%	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
K2EDTA	0	0	0	N/A	N/A	N/A
	2.8 mg/mL	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	7 mg/mL	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Post-PCR Amplicon	0	0	0	N/A	N/A	N/A
	5%	0	0	96.6% (94.1- 98.0%)	>99.9% (>99.9- 100%)	>99.9% (>99.9- 100%)
	15%	0	0	96.6% (94.1- 98.0%)	>99.9% (>99.9- 100%)	>99.9% (>99.9- 100%)
	30%	0	0	96.6% (94.1- 98.0%)	>99.9% (>99.9- 100%)	>99.9% (>99.9- 100%)
Hemoglobin	0	0	1	N/A	N/A	N/A
	1 mg/mL	0	0	100% (98.9- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	2 mg/mL	0	2	100% (98.9- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Triglycerides	0	0	0	N/A	N/A	N/A
	100 mg/dL	0	0	99.7% (98.5- 100%)	100% (>99.9- 100%)	>99.9% (>99.9- 100%)
	250 mg/dL	0	1	99.7% (98.5- 100%)	100% (>99.9- 100%)	>99.9% (>99.9- 100%)
Bone marrow/ stem cell transplant	0%	N/A	20	N/A	N/A	N/A
	5%	N/A	20	N/A	N/A	N/A
	10%	N/A	20	N/A	N/A	N/A
	25%	N/A	20	N/A	N/A	N/A
	75%	N/A	20	N/A	N/A	N/A
	90%	N/A	20	N/A	N/A	N/A
	95%	N/A	20	N/A	N/A	N/A

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**N/A: values not calculated for reference conditions

Table 18 Interfering Substances Study Results Summary for Indels (Study 1)

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
Bilirubin	0	0	2	N/A**	N/A	N/A
	137 mmol/L	0	1	100%	100%	100%

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
				(79.6- 100%)	(>99.9- 100%)	(>99.9- 100%)
	684 mmol/L	0	1	100% (79.6- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Wash buffer	0	0	0	N/A	N/A	N/A
	15% v/v	0	0	100% (74.1- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
TAFT (molecular barcode)	0	0	0	N/A	N/A	N/A
	5%	0	0	100% (82.4- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	15%	0	0	100% (82.4- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	30%	0	0	100% (82.4- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
K2EDTA	0	0	0	N/A	N/A	N/A
	2.8 mg/mL	0	0	100% (75.8- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	7 mg/mL	0	0	100% (75.8- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Post-PCR Amplicon	0	0	0	N/A	N/A	N/A
	5%	0	0	100% (75.8- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	15%	0	0	100% (75.8- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	30%	0	0	100% (75.8- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Hemoglobin	0	0	1	N/A	N/A	N/A
	1 mg/mL	0	0	100% (81.6- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	2 mg/mL	0	2	100% (81.6- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Triglycerides	0	0	0	N/A	N/A	N/A
	100 mg/dL	0	0	100% (82.4- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
	250 mg/dL	0	1	100% (82.4- 100%)	100% (>99.9- 100%)	100% (>99.9- 100%)
Bone marrow/ stem cell transplant	0%	N/A	20	N/A	N/A	N/A
	5%	N/A	20	N/A	N/A	N/A
	10%	N/A	20	N/A	N/A	N/A
	25%	N/A	20	N/A	N/A	N/A
	75%	N/A	20	N/A	N/A	N/A

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
	90%	N/A	20	N/A	N/A	N/A
	95%	N/A	20	N/A	N/A	N/A

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**N/A: values not calculated for reference conditions

Table 19. Interfering Substances Study Results Summary for Indels (Study 2)

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
Bilirubin	0	0	0	N/A**	N/A	N/A
	137 mmol/L	0	1	100% (80.64- 100%)	100% (99.95-100%)	100% (>99.99- 100%)
	684 mmol/L	0	0	100% (83.89- 100%)	100% (99.95-100%)	100% (>99.9- 100%)
Wash buffer	0	0	0	N/A	N/A	N/A
	15% v/v	0	0	100% (85.13- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
TAFT (molecular barcode)	0	0	0	N/A	N/A	N/A
	5%	0	0	100% (80.64- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
	15%	0	1	100% (78.47- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
	30%	0	0	100% (80.64- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
K2EDTA	0	0	0	N/A	N/A	N/A
	2.8 mg/mL	0	0	100% (82.41- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
	7 mg/mL	0	0	100% (82.41- 100%)	100% (>99.99- 100%)	100% (>99.99- 100%)
Post-PCR Amplicon	0	0	0	N/A	N/A	N/A
	5%	0	0	93.75% (71.67- 98.89%)	100% (>99.99- 100%)	>99.99% (99.99- 100%)
	15%	0	0	87.50% (63.98- 96.50%)	100% (>99.99- 100%)	>99.99% (99.99- 100%)
	30%	0	0	93.75% (71.67- 98.89%)	100% (>99.99- 100%)	>99.99% (99.99- 100%)
Hemoglobin	0	0	0	N/A	N/A	N/A
	1 mg/mL	0	2	100% (80.64- 100%)	100% (>99.99- 100%)	100% (99.9- 100%)
	2 mg/mL	0	1	100% (80.64- 100%)	100% (>99.99- 100%)	100% (99.9- 100%)
Triglycerides	0	0	0	N/A	N/A	N/A
	100 mg/dL	0	0	100% (80.64- 100%)	100% (>99.99- 100%)	100% (99.9- 100%)

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
	250 mg/dL	0	0	100% (80.64- 100%)	100% (>99.99- 100%)	100% (99.9- 100%)

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**N/A: values not calculated for reference conditions

Table 20. Interfering Substances Study Results Summary for CNVs (Study 2)

Substance	Level	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
Bilirubin	0	0	0	N/A**	N/A	N/A
	137 mmol/L	0	1	100% (70.09- 100%)	100% (99.95-100%)	100% (99.9- 100%)
	684 mmol/L	0	0	100% (72.25- 100%)	100% (99.95-100%)	100% (99.9- 100%)
Wash buffer	0	0	0	N/A	N/A	N/A
	15% v/v	0	0	73.3% (48.05- 89.10%)	99.6% (99.44-99.72%)	99.6% (99.4- 99.7%)
TAFT (molecular barcode)	0	0	0	N/A	N/A	N/A
	5%	0	0	100% (67.56- 100%)	100% (99.95-100%)	100% (99.9- 100%)
	15%	0	1	100% (64.57- 100%)	100% (99.95-100%)	100% (99.9- 100%)
	30%	0	0	100% (67.56- 100%)	100% (99.95-100%)	100% (99.9- 100%)
K2EDTA	0	0	0	N/A	N/A	N/A
	2.8 mg/mL	0	0	87.5% (52.91- 97.6%)	100% (99.95-100%)	>99.99% (99.99-100%)
	7 mg/mL	0	0	87.5% (52.91- 97.6%)	99.99% (99.93-100%)	>99.99% (99.99-100%)
Post-PCR Amplicon	0	0	10	N/A	N/A	N/A
	5%	0	10	N/A	N/A	N/A
	15%	0	10	N/A	N/A	N/A
	30%	0	10	N/A	N/A	N/A
Hemoglobin	0	0	0	N/A	N/A	N/A
	1 mg/mL	0	2	100% (78.47- 100%)	100% (99.95-100%)	100% (99.9- 100%)
	2 mg/mL	0	1	100% (79.61- 100%)	100% (99.95-100%)	100% (99.9- 100%)
Triglycerides	0	0	0	N/A	N/A	N/A
	100 mg/dL	0	0	100% (72.25- 100%)	100% (99.95-100%)	100% (99.9- 100%)
	250 mg/dL	0	0	100% (72.25- 100%)	100% (99.95-100%)	100% (99.9- 100%)

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**N/A: values not calculated for reference conditions

5. Assay Reportable Range:

Not applicable

6. Carry-Over and Cross-Contamination:

Indexing misassignment is minimized through use of dual indexing. To evaluate the rate of contamination, a retrospective analysis was performed of all clinical samples run with the Invitae Common Hereditary Cancers Panel over the course of one year to calculate the percent of clinical samples with contamination above the QC threshold of 2.5% alien DNA. Overall, 0.29% of the samples had evidence of contamination above the QC threshold (**Table 21)**.

Table 21. Summary of Retrospective Analysis for Contamination Rate

Contamination Level	Number of Samples	Percentage of Samples
Below the QC Threshold (<2.5%)	118,123	99.71%
Above the QC Threshold (≥2.5%)	348	0.29%

7. DNA Integrity:

A study was conducted to evaluate the impact of DNA degradation on the performance of the Invitae Common Hereditary Cancers panel test by using an established measurement of "DNA Integrity", which mimics the DNA degradation process in nature. DNA degradation may occur during pre-analytical specimen handling, whether due to processing errors or specimen collection, transport, or storage conditions. In the study, gDNA samples from 5 individual specimens were sheared by sonication to three ranges. These ranges were measured using the DNA Integrity Number as follows: high molecular weight (>7), medium molecular weight (3-7), and low molecular weight (<3). The high molecular weight bin represents DNA that has little or no denaturation; this was therefore considered the control condition. Samples were run in triplicate and analyzed for number of failed samples, number of samples with low coverage, overall concordance, PPA, and NPA compared to the control condition. Results of the study are summarized in **Table 22**. There was 100% concordance, no failed samples or samples with low coverage across all samples and all DNA integrity ranges, indicating that DNA degradation did not impact assay performance. No difference in performance was found between SNVs and Indels; CNVs were not present in the samples therefore could not be evaluated.

Table 22. DNA Integrity Study Results Summary

DIN	Samples with Low Coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
>7	0	0	N/A**	N/A	N/A
3-7	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (100%-100%)

<3	0	0	100% (99.0- 100%)	100% (>99.9- 100%)	100% (100%-100%)

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage
**N/A: values not calculated for reference conditions

8. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Specimen Stability

To evaluate the potential impact of transport and storage on characteristics of blood specimens that may affect assay performance with the Invitae Common Hereditary Cancers Panel, blood specimens were tested for DNA concentration, quality as measured by DIN and variant detection by the Invitae Common Hereditary Cancers Panel at baseline, then stored at 4°C, room temperature, or 40°C. At predetermined time points, samples were tested again for DNA quality, quantity and variant detection by the Invitae Common Hereditary Cancers Panel, and compared to the baseline level. In addition, to simulate the freezing and thawing that may occur in transit, specimens were subjected to up to 3 cycles of freezing at -80°C for 3 hours or overnight, then thawing for 3 hours. Ten specimens were tested for room temperature (including 649 SNVs across 39 genes, 11 Indels across 5 genes and 10 CNVs in 1 gene), 4ºC (including 775 SNVs across 37 genes and 33 Indels across 10 genes), and 40ºC and freeze/thaw (including 886 SNVs across 37 genes and 14 Indels across 8 genes). The samples in the 40ºC and freeze/thaw conditions were tested in 3 replicates.

Results of the study are summarized in **Table 23**. While decrease of the DNA concentrations and quality was observed over time, the concentrations remained higher than the standard input to this test, and the changes in DNA quality did not exceed the threshold required. The PPA and NPA, and overall concordance are >99% at all timepoints for all conditions, supporting the stability of the specimens for the assay performance with the Invitae Common Hereditary Cancers Panel.

Table 23. Specimen Stability Study Results Summary

Condition	Time Point	PPA				NPA				Overall concordance
		TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Concordance (95% CI)
Room Temp	7 days	670	0	100%	99.4-100%	680802	1	>99.9%	>99.9-100%	>99.9% (>99.9-100%)
	30 days	670	0	100%	99.4-100%	679563	0	100%	>99.9-100%	100% (>99.9-100%)
	60 days	670	0	100%	99.4-100%	680815	0	100%	>99.9-100%	>99.9% (>99.9-100%)
	90 days	587*	0	100%	99.4-100%	605178	0	100%	>99.9-100%	>99.9% (>99.9-100%)
4ºC	7 days	807	2	99.8%	99.3-100%	756443	1	>99.9%	>99.9-100%	>99.9% (>99.9-100%)

Condition	Time Point	PPA				NPA				Overall concordance
		TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Concordance (95% CI)
	30 days	412**	0	100%	99.1-100%	452488	0	100%	>99.9-100%	100% (>99.9-100%)
	60 days	807	1	99.9%	99.3-100%	756471	1	>99.9%	>99.9-100%	>99.9% (>99.9-100%)
	90 days	807	1	99.9%	99.3-100%	756469	1	>99.9%	>99.9-100%	>99.9% (>99.9-100%)
40 ºC	3 hr	2612	0	100%	99.9-100%	2190407	0	100%	>99.9-100%	100% (>99.9-100%)
	12 hr	2421***	0	100%	99.9-100%	2039346	2	>99.9%	>99.9-100%	>99.9% (>99.9-100%)
	7 days	2612	0	100%	99.9-100%	2190405	0	100%	>99.9-100%	100% (>99.9-100%)
Freeze/ Thaw	1 cycle	2612	0	100%	99.9-100%	2190407	1	>99.9%	>99.9-100%	>99.9% (>99.9-100%)
	3 cycles	2611	1	>99.9%	99.9-100%	2190402	0	>99.9%	>99.9-100%	>99.9% (>99.9-100%)

* 9 samples were included in the analysis at this time point
**4 samples failed due to an instrument-related failure, unrelated to the study condition.
***2 samples failed due to an instrument-related failure, unrelated to the study condition.

b) Reagent Stability

Studies were performed to establish the stability of the master plates of TAFTs (molecular barcodes), the diluted TAFT plates that are stamped and stored, and later used in production, and the baits. For master stock TAFT plates, five stamped plates from each of 3 stock plate designs from each of three manufactured plate lots, representing 303 ± 94 samples per stock plate were analyzed for the percentage of samples that passed the TAFT QC metrics. For diluted TAFT plates, all available plates older than 1 year at time of use, for each of 3 selected plate designs from each of three manufactured plate lots, representing 450±48 samples run were analyzed for the percentage of samples that passed the TAFT QC metrics. For the baits, control sample data for the genomic regions targeted by the bait components were analyzed for gaps in coverage and concordance with the reference sequence. The results of the study are summarized in **Table 24**. All reagents analyzed met the acceptance criteria, establishing stability as follows:

- Master stock plates stored frozen are stable for at least 36 months
- Diluted plates are stable for at least 12 months
- Baits are stable for at least 33 months

Table 24 Summary of Reagent Stability

Reagent	Passed Number of Reads	Passed Proportion Classified
Master molecular barcode plates	95.3% - 99.2%	99.0% - 100%
Diluted molecular barcode plates	96.4% - 99.0%	96.3% - 100%

Reagent	Gaps in Coverage	Concordance
Baits	0	100%

9. Multiplexing:

The Invitae Common Hereditary Cancers Panel test is multiplexed at two steps:

- At library prep: Libraries are pooled by row to create 8 pools of 12 libraries each.
- At sequencing: Equimolar mixing of hybridization reactions forms sequencing pools that allow each library to be sequenced with a minimum of 4,000,000 clusters on the Illumina flow cell. The number of samples in a pool depends on what other assays are being run at the same time.

Unique molecular barcodes are added to individual samples during extraction to ensure that sample identification is maintained throughout the process, even when multiplexed. QC checks built into the test system verify that the molecular barcodes have performed as expected. As demonstrated in the interfering substances study, the molecular barcodes - referred to as TAFTs - spiked at 5%, 15%, and 30% above the standard operational levels did not impact performance of the test. An additional analysis was performed using data from the analytical validation studies. In these studies, each sample has, on average, over 60 variant calls distributed across the 47 loci. Thus, each sample has a nearly unique sequencing footprint. This provides high confidence that the performance of the molecular barcodes for preserving sample identity fidelity can be confirmed using the concordance analysis of the sequencing results. All of the studies performed across all validations were multiplexed with post hybridization multiplexing levels (pool sizes) between 49 to 1485. Pooling sizes and configurations are designed so that read depth can be maintained at a level that meets quality thresholds. Read depth are maintained at a level that meets quality thresholds for all post hybridization multiplexing levels. No trends were observed that suggested that the pool size negatively impacted the read depth.

10. Guardbanding:

A guardbanding study was conducted to establish the robustness of the Invitae Common Hereditary Cancers Panel test by assessing the impact of varying critical input parameters – the DNA input concentration at the library preparation, hybridization, and sequencing steps - related to test run quality metrics. To assess the impact of varying these parameters, eight DNA samples from clinical specimens were run in duplicate with a series of manual manipulations during runs, wherein the input concentrations were varied by +/-5% and +/-10% at each step. The samples contained representative variants across variant types and different genomic context, including 753 SNVs across 39 genes, 14 insertions/deletions across 7 genes and 8 CNVs across 6 genes. Assay results were analyzed for number of failed samples, number of samples with low coverage, overall concordance, PPA, and NPA compared to the standard condition. Results of the study are summarized in **Table 25**. The assay was demonstrated to be robust when input concentrations for the library prep, hybridization, and sequencing steps were varied up to 10%.

Table 25. Guard banding Study Results Summary

DNA Input Concentration		Samples with Low coverage*	Failed Samples	PPA (95% CI)	NPA (95% CI)	Concordance (95% CI)
Library Prep	-10%	1	0	99.9% (99.53-99.96%)	100% (100-100%)	>99.99% (>99.99-100%)
	-5%	0	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+5%	0	0	99.9% (99.43-99.93%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+10%	0	0	99.9% (99.43-99.93%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
Hybridization	-10%	0	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	-5%	0	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+5%	0	0	100% (99.75-100%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+10%	0	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
Sequencing	-10%	1	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	-5%	0	0	99.9% (99.53-99.96%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+5%	0	0	100% (99.75-100%)	>99.99% (>99.99-100%)	>99.99% (>99.99-100%)
	+10%	0	0	100% (99.75-100%)	100% (100-100%)	100% (100-100%)

* Samples with >400 bp (SNV and Indel analysis) or 138 target regions (CNV analysis) at <20x depth of coverage

11. Detection Limit:

    Not applicable

12. Assay Cut-Off:

    Not applicable

13. Accuracy (Instrument):

    Refer to method comparison in Section VI. B

B Comparison Studies:

1. Accuracy – Comparison to Orthogonal Method(s):

a) Comparison Study using Non-Clinical Samples

The primary analysis for this study used five Genome in a Bottle (GIAB) samples with well characterized genome sequence data. Of the 169,458 base positions in the 47 genes, over 155,000 are characterized in each of the GIAB samples, for a total of 778,829 data points generated across five samples. This includes both wild type bases and homozygous and heterozygous SNV and Indel variants across different genomic contexts (total of 389 SNVs across 34 genes and 7 Indels across 4 genes). The performance of the assay was assessed based on concordance with the reference data, in the form of PPA, NPA and Technical Positive Predictive Value (TPPV).

To expand the variant types represented in the analysis, additional 92 supplemental cell line samples were also tested, including a total of 24 SNVs across 14 genes, 101 insertions/deletions across 18 genes and 10 CNVs across 8 genes. While these samples each have at least one variant that has been identified and reported, data is not available for the other positions overlapping the reportable range for this assay. Therefore, results for these samples were included in the calculation of PPA and TPPV, but not NPA.

The results of the study are summarized in **Table 26** to **Table 28**. The percentage of no calls/invalid calls is 0%. The PPA, NPA and TPPV are 100% across all samples for all variant types.

Table 26. Accuracy Study with Cell Lines Results by Variant Type

Analysis	Samples	# Variants	PPA (95% CI)	NPA (95% CI)	TPPV (95% CI)	% No Call
Overall	GIAB	396	100% (99.1-100%)	100% (100-100%)	100% (99.1-100%)	0%
	Supplemental	135	100% (97.2-100%)	N/A*	100% (97.2-100%)	0%
SNVs	GIAB	389	100% (99.0-100%)	N/A	100% (99.0-100%)	0%
	Supplemental	24	100% (86.2-100%)	N/A	100% (86.2-100%)	0%
Indels	GIAB	7	100% (64.6-100%)	N/A	100% (64.6-100%)	0%
	Supplemental	101	100% (96.3-100%	N/A	100% (96.3-100%	0%
CNVs	Supplemental	10	100% (70.1-100%)	N/A	100% (70.1-100%)	0%

**N/A: values not calculated or data not available for calculation

Table 27. Accuracy Study with Cell Lines Results by Genomic Contexts (GIAB samples)

Analysis	# Variants	PPA (95% CI)	NPA (95% CI)	TPPV (95% CI)	No Call (95% CI)
GIAB - low mappability	24	100% (86.2-100%)	100% (100-100%)	100% (86.2-100%)	0%
GIAB - high mappability	372	100% (99.0-100%)	100% (100-100%)	100% (99.0-100%)	0%

Table 28. Accuracy Study with Cell Lines Results by Zygosity (GIAB samples)

Analysis	# Variants	PPA (95% CI)	NPA (95% CI)	TPPV (95% CI)	No Call (95% CI)
GIAB - Homozygous	171	100% (97.8-100%)	N/A	100% (97.8-100%)	0%
GIAB - Heterozygous	218	100% (98.3-100%)	N/A	100% (98.3-100%)	0%

b) Comparison Study using Clinical Specimens

A study was performed to evaluate the accuracy of the Invitae Common Hereditary Cancers Panel using clinical specimens tested at Invitae. Specimens were selected for representation across the panel using consecutive sampling in a pre-specified time period to minimize bias. Specimens were from patients diagnosed with cancer and individuals tested for predisposition assessment. Specimens were first tested with the Invitae Common Hereditary Cancers Panel, and results were compared with a validated orthogonal method. The analyses were adjusted to account for the method of specimen selection (i.e., technical positive predictive value or TPPV)[1]. Positive variants were of clinical significance (Pathogenic, Likely Pathogenic or VUS).

For SNVs and Indels, a total of 6014 samples were tested, including 2181 SNVs distributed across 44 target genes and 3914 Indels across 40 genes. The results of the Invitae Common Hereditary Cancers Panel were compared to a validated high-throughput sequencing platform. In addition to evaluation of false positives, evaluation of false negatives (i.e., technical negative predictive value or TNPV) was performed by interrogating the wild-type flanking sequence spanning 100 to 800 bp for each variant of interest generated by the Invitae Common Hereditary Cancers Panel. Using this approach, over 72% of the entire reportable range was compared between the Invitae Common Hereditary Cancers Panel and the orthogonal method. For CNVs, a total of 3542 samples were tested, including 3601 CNVs distributed across 44 target genes. The results of the Invitae Common Hereditary Cancers Panel were compared to a validated multiplexed PCR based test or a validated microarray. Data from the variant sites were used to calculate TPPV of the test. To evaluate TNPV of the test, additional 106 clinical specimens with prior negative results for CNVs by the Invitae Common Hereditary Cancers Panel were tested across 476 target regions on 28 genes using the validated multiplexed PCR based test. Results were compared between the two assays and TNPV values were calculated.

Summary of the results are shown in **Table 29**. The overall TPPV is 99.9% (95% CI 99.7- >99.9%) for SNVs, 100% (95% CI 99.9- 100%) for Indels and 99.5% (95% CI 99.2- 99.7%) for CNVs. The overall TNPV is 100% for SNVs (95% CI >99.9%- 100%), 100% for Indels (95% CI >99.9- 100%), and 99.7% for CNVs (95% CI 99.6- 99.7%).

[1] For methods of calculation, refer to Section B, Test Performance Characteristics in the Guidance Document Considerations for Design, Development, and Analytical Validation of Next Generation Sequencing (NGS) – Based In Vitro Diagnostics (IVDs) Intended to Aid in the Diagnosis of Suspected Germline Diseases; Guidance for Stakeholders and Food and Drug Administration Staff.

Table 29. Summary of Accuracy Study with Clinical Specimens

Gene	# Samples	# Variants	TP	TN	FP	FN	TPPV (95% CI)	TNPV (95% CI)
SNVs	2151	2181	2180	413004	1	0	99.9% (99.7- >99.9%)	100% (>99.9%- 100%)
Indels	3900	3914	3914	1127320	0	0	100% (99.9- 100%)	100% (>99.9- 100%)
Insertions	1240	1240	1240	781671	0	0	100% (99.7-100%)	100% (>99.9-100%)
Deletions	2671	2674	2674	345649	0	0	100% (99.9-100%)	100% (>99.9-100%)
CNVs	3648	3601	3582	95965	19	317	99.5% (99.2- 99.7%)	99.7% (99.6- 99.7%)
CNV deletions	2983	3110	3103	95965	7	292	99.8% (99.5-99.9%)	99.7% (99.6-99.7%)
CNV duplications	484	491	479	95965	12	25	97.6% (95.8-98.6%)	99.97% (99.96-99.98%)

TP: True positive; TN: True negative; FP: False positive; FN: False negative

Accuracy result on the gene level is shown in **Table 30** and **Table 31**. For SNVs and Indels (**Table 30**), all genes tested show TPPV of 100%, except SDHA (99.0% with 95% CI 94.4-99.8%). For CNVs (**Table 31**), 32 genes show TPPV of 100%, 10 genes show TPPV ranging between 90% to 100%, and 2 genes show TPPV between 80% to 90%, including SMAD4 (84.6% with 95% CI 57.8-95.7%) and TSC2 (88.9% with 95% CI 56.5-98.0%).

Table 30. Accuracy by Stratified by Gene – SNVs and Indels

Gene	# Samples	# Variants		TP	TN	FP	FN	TPPV (95% CI)	TNPV (95% CI)
		SNVs	Indels						
APC	165	40	125	165	51987	0	0	100% (97.7-100.0%)	100% (>99.9-100.0%)
ATM	629	255	386	641	108628	0	0	100% (99.1-100.0%)	100% (>99.9-100.0%)
AXIN2	32	28	4	32	7151	0	0	100% (89.3-100.0%)	100% (>99.9-100.0%)
BARD1	89	17	72	89	14267	0	0	100% (95.9-100.0%)	100% (>99.9-100.0%)
BMPR1A	10	4	6	10	1503	0	0	100% (72.2-100.0%)	100% (99.8-100.0%)
BRCA1	606	154	452	606	177627	0	0	100% (99.4-100.0%)	100% (>99.9-100.0%)
BRCA2	1093	97	996	1093	480847	0	0	100% (99.8-100.0%)	100% (>99.9-100.0%)
BRIP1	260	121	139	260	50700	0	0	100% (98.5-100.0%)	100% (>99.9-100.0%)

Gene	# Samples	# Variants		TP	TN	FP	FN	TPPV (95% CI)	TNPV (95% CI)
		SNVs	Indels						
CDH1	39	7	32	39	8539	0	0	100% (91.0-100.0%)	100% (>99.9-100.0%)
CDK4	0	0	0	0	0	0	0	N/A	N/A
CDKN2A	128	62	66	128	26877	0	0	100% (97.1-100.0%)	100% (>99.9-100.0%)
CHEK2	354	170	185	355	48503	0	0	100% (98.4-100.0%)	100% (>99.9-100.0%)
CTNNA1	7	6	1	7	1379	0	0	100% (64.6-100.0%)	100% (99.7-100.0%)
DICER1	24	6	18	24	8360	0	0	100% (86.2-100.0%)	100% (>99.9-100.0%)
EPCAM	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
GREM1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HOXB13	2	2	0	2	607	0	0	100% (34.2-100.0%)	100% (99.4-100.0%)
KIT	6	5	1	6	1038	0	0	100% (61.0-100.0%)	100% (99.6-100.0%)
MEN1	77	22	55	77	28100	0	0	100% (95.2-100.0%)	100% (>99.9-100.0%)
MLH1	102	14	88	102	16999	0	0	100% (96.4-100.0%)	100% (>99.9-100.0%)
MSH2	154	105	49	154	27897	0	0	100% (97.6-100.0%)	100% (>99.9-100.0%)
MSH3	74	35	39	74	10489	0	0	100% (95.1-100.0%)	100% (>99.9-100.0%)
MSH6	365	30	335	365	104841	0	0	100% (99.0-100.0%)	100% (>99.9-100.0%)
MUTYH	85	52	35	87	14587	0	0	100% (95.8-100.0%)	100% (>99.9-100.0%)
NBN	93	15	78	93	17339	0	0	100% (96.0-100.0%)	100% (>99.9-100.0%)
NF1	396	241	156	397	71058	0	0	100% (99.0-100.0%)	100% (>99.9-100.0%)
NTHL1	43	37	6	43	9795	0	0	100% (91.8-100.0%)	100% (>99.9-100.0%)
PALB2	167	50	117	167	50788	0	0	100% (97.8-100.0%)	100% (>99.9-100.0%)
PDGFRA	6	6	0	6	1191	0	0	100% (61.0-100.0%)	100% (99.7-100.0%)
PMS2	293	123	171	294	65417	0	0	100%	100%

Gene	# Samples	# Variants		TP	TN	FP	FN	TPPV (95% CI)	TNPV (95% CI)
		SNVs	Indels						
								(98.7-99.9%)	(>99.9-100.0%)
POLD1	15	13	2	15	2621	0	0	100% (79.6-100.0%)	100% (>99.9-100.0%)
POLE	22	16	6	22	3899	0	0	100% (85.1-100.0%)	100% (99.9-100.0%)
PTEN	21	12	8	20	3877	0	0	100% (77.3-99.2%)	100% (99.9-100.0%)
RAD50	143	17	127	144	30893	0	0	100% (97.4-100.0%)	100% (>99.9-100.0%)
RAD51C	17	3	14	17	1521	0	0	100% (81.6-100.0%)	100% (99.8-100.0%)
RAD51D	18	9	9	18	2175	0	0	100% (82.4-100.0%)	100% (99.8-100.0%)
SDHA	97	79	18	96	12367	1	0	99.0% (94.4-99.8%)	100% (>99.9-100.0%)
SDHB	47	15	32	47	5752	0	0	100% (92.4-100.0%)	100% (>99.9-100.0%)
SDHC	5	2	3	5	470	0	0	100% (56.6-100.0%)	100% (99.2-100.0%)
SDHD	11	2	9	11	1554	0	0	100% (74.1-100.0%)	100% (99.8-100.0%)
SMAD4	18	4	14	18	2822	0	0	100% (82.4-100.0%)	100% (99.9-100.0%)
SMARCA 4	19	19	0	19	3770	0	0	100% (83.2-100.0%)	100% (99.9-100.0%)
STK11	15	6	9	15	2576	0	0	100% (79.6-100.0%)	100% (99.9-100.0%)
TP53	289	260	32	294	50845	0	0	100% (97.6-99.8%)	100% (>99.9-100.0%)
TSC1	9	5	4	9	2516	0	0	100% (70.1-100.0%)	100% (99.9-100.0%)
TSC2	26	11	15	26	5342	0	0	100% (87.1-100.0%)	100% (99.9-100.0%)
VHL	4	4	0	4	810	0	0	100% (51.0-100.0%)	100% (99.5-100.0%)

TP: True positive; TN: True negative; FP: False positive; FN: False negative

*N/A: values not calculated (for CDK4) or not evaluated as the test does not offer SNV/Indel detection (for EPCAM and GREM1)

Table 31 Accuracy Stratified by Gene – CNVs

Gene	# Samples	# Variants	TP	TN	FP	FN	TPPV*	TNPV
APC	**113**	**117**	**114**	**3149**	**3**	**15**	**97.4% (92.7-99.1%)**	**99.5% (99.2-99.7%)**
ATM	177	183	183	7745	0	37	100% (97.9-100%)	99.5% (99.4-99.7%)
AXIN2	1	1	1	N/A	0	N/A	100% (20.7-100%)	N/A**
BARD1	66	67	67	N/A	0	N/A	100% (94.6-100%)	N/A
BMPR1A	26	26	26	1995	0	4	100% (87.1-100%)	99.8% (99.5-99.9%)
BRCA1	577	580	577	3208	3	3	99.5% (98.5-99.8%)	99.91% (99.7->99.9%)
BRCA2	**181**	**183**	**181**	**6895**	**2**	**25**	**98.9% (96.1-99.7%)**	**99.64% (99.5-99.8%)**
BRIP1	63	69	69	1995	0	7	100% (94.7-100%)	99.65% (99.3-99.8%)
CDH1	59	59	59	2396	0	6	100% (93.9-100%)	99.75% (99.5-99.9%)
CDK4	2	2	2	N/A	0	N/A	100% (34.2-100%)	N/A
CDKN2A	12	12	12	1886	0	8	100% (75.8-100%)	99.6% (99.2-99.8%)
CHEK2	263	266	266	1717	0	8	100% (98.6-100%)	99.5% (99.1-99.8%)
CTNNA1	12	12	12	N/A	0	N/A	100% (75.8-100%)	N/A
DICER1	10	10	10	N/A	0	N/A	100% (72.3-100%)	N/A
EPCAM	113	114	113	523	1	5	99.1% (95.2-99.8%)	99.1% (97.8-99.6%)
GREM1	**23**	**24**	**23**	**N/A**	**1**	**N/A**	**95.8% (79.8-99.3%)**	**N/A**
HOXB13	2	2	2	N/A	0	N/A	100% (34.2-100%)	N/A
KIT	1	1	1	N/A	0	N/A	100% (20.7-100%)	N/A
MEN1	13	13	13	2730	0	5	100% (77.2-100%)	99.8% (99.6-99.9%)
MLH1	134	135	135	4505	0	18	100% (97.2-100%)	99.6% (99.4-99.75%)
MSH2	285	297	297	3147	0	7	100% (98.7-100%)	99.8% (99.5-99.9%)
MSH3	**45**	**45**	**44**	**N/A**	**1**	**N/A**	**97.8% (88.4-99.6)**	**N/A**

Gene	# Samples	# Variants	TP	TN	FP	FN	TPPV*	TNPV
MSH6	60	63	63	2306	0	4	100% (94.3-100%)	99.8% (99.6-99.9%)
MUTYH	20	21	21	5879	0	4	100% (84.5-100%)	99.9% (99.8-99.97%)
NBN	67	68	68	1787	0	9	100% (94.7-100%)	99.5% (99.1-99.7%)
NF1	104	108	107	11296	1	39	99.1% (94.9-99.8%)	99.7% (99.5-99.8%)
NTHL1	7	7	7	1048	0	7	100% (64.6-100%)	99.3% (98.6-99.68%)
PALB2	255	255	255	1674	0	7	100% (98.5-100%)	99.6% (99.1-99.8%)
PDGFRA	1	1	1	N/A	0	N/A	100% (20.7-100%)	N/A
PMS2	414	420	418	3428	2	26	99.5% (98.3-99.9%)	99.3% (98.9-99.5%)
POLD1	3	3	3	N/A	0	N/A	100% (43.9-100%)	N/A
POLE	10	10	10	N/A	0	N/A	100% (72.3-100%)	N/A
PTEN	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RAD50	33	33	33	735	0	2	100% (89.6-100%)	99.7% (99.0-99.9%)
RAD51C	116	116	116	1050	0	6	100% (96.8-100%)	99.4% (98.8-99.7%)
RAD51D	43	44	44	N/A	0	N/A	100% (92.0-100%)	N/A
SDHA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SDHB	35	35	35	N/A	0	N/A	100% (90.1-100%)	N/A
SDHC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SDHD	**17**	**18**	**17**	**N/A**	**1**	**N/A**	**94.4% (74.2-99.0%)**	**N/A**
SMAD4	**12**	**13**	**11**	**1888**	**2**	**3**	**84.6% (57.8-95.7%)**	**99.8% (99.5-99.95%)**
SMARCA4	4	4	4	N/A	0	N/A	100% (51.0-100%)	N/A
STK11	50	50	50	1302	0	12	100% (92.9-100%)	99.1% (98.4-99.5%)
TP53	**33**	**33**	**32**	**3045**	**1**	**10**	**97.0% (84.7-99.5%)**	**99.7% (99.4-99.8%)**
TSC1	17	17	17	3450	0	6	100% (81.6-100%)	99.8% (99.6-99.9%)
TSC2	**9**	**9**	**8**	**14258**	**1**	**32**	**88.9% (56.5-98.0%)**	**99.78% (99.7-99.8%)**
VHL	55	55	55	928	0	2	100%	99.78%

Gene	# Samples	# Variants	TP	TN	FP	FN	TPPV*	TNPV
							(93.5-100%)	(99.2-99.9%)

TP: True positive; TN: True negative; FP: False positive; FN: False negative
*Genes in bold are those that did not meet the 99% performance expectation for CNVs. The data showed that the false positives were due to those positives called based on one exon.
**N/A: not evaluated as the test does not offer CNV detection for the genes or data not available for calculation.

Accuracy stratified by indel size is shown in **Table 32**. TPPV is 100% across all insertion and deletion sizes.

Table 32. Accuracy Stratified by Indel Size

Variant Type	Size	TP	FP	TPPV (95% CI)
Insertions	1-5 bp	974	0	100% (99.6-100%)
	6-10 bp	92	0	100% (96.0-100%)
	11-20 bp	87	0	100% (95.8-100%)
	21+ bp*	87	0	100% (95.8-100%)
Deletions	1-5 bp	2351	0	100% (99.8-100%)
	6-10 bp	157	0	100% (97.6-100%)
	11-20 bp	114	0	100% (96.7-100%)
	21+ bp*	52	0	100% (93.1-100%)

TP: True positive; FP: False positive
*The largest size tested are 338bp for insertions and 313bp for deletions

Accuracy stratified by CNV size is shown in **Table 33**. TPPV is >99% across all CNV deletion and duplication sizes except that lower TPPV was observed for CNV duplications with single exon or less (95.5% with 95% CI 92.4-97.4%).

Table 33 Accuracy Stratified by CNV Size

CNV type	CNV Type	TP	FP	TPPV (95% CI)
CNV Deletions	≤ Single Exon	1093	6	99.5% (98.8-99.8%)
	2-5 Exons	1075	0	100% (99.6-100%)
	6-9 Exons	345	0	100% (98.9-100%)
	10+ Exons	226	1	99.6% (97.6-99.9%)
	Entire Coding Sequence	308	0	100% (98.8-100%)
	Other (intronic, non-coding, combination)	56	0	100% (93.6-100%)

CNV type	CNV Type	TP	FP	TPPV (95% CI)
CNV Duplications	**≤ Single Exon**	**257**	**12**	**95.5% (92.4-97.4%)**
	2-5 Exons	108	0	100% (96.6-100%)
	6-9 Exons	28	0	100% (87.9-100%)
	10+ Exons	41	0	100% (91.4-100%)
	Entire Coding Sequence	40	0	100% (91.2-100%)
	Other (intronic, non-coding, combination)	5	0	100% (56.6-100%)

TP: True positive; FP: False positive

Accuracy stratified by GC content is shown in **Table 34**. TPPV is >99% across all GC content ranges except for CNV deletions with GC content between 25% to 30% (98.6% with 95% CI 92.3-99.8%), CNV duplications with GC content between 30% to 55% (98.5% with 95% CI 96.6-99.4%), and GC content >55% (95.5% with 95% CI 91.0-97.8%).

Table 34. Accuracy Stratified by Variant Type and GC content

Variant Type	Stratification	# of Variants	TP	FP	TPPV (95% CI)
SNVs	GC content 0-15	0	0	0	-
	GC content 15- 20	1	1	0	100% (20.7-100%)
	GC content 20- 25	236	236	0	100% (98.4-100%)
	GC content 25- 30	216	216	0	100% (98.3-100%)
	GC content 30- 55	1123	1123	0	100% (99.7-100%)
	GC content >55	605	604	1	99.8% (99.1->99.9%)
Insertions	GC content 0-15	0	0	0	-
	GC content 15- 20	0	0	0	-
	GC content 20- 25	14	14	0	100% (78.5-100%)
	GC content 25- 30	183	183	0	100% (97.9-100%)
	GC content 30- 55	837	837	0	100% (99.5-100%)
	GC content >55	125	125	0	100% (97.0-100%)
Deletions	GC content 0-15	0	0	0	-
	GC content 15- 20	1	1	0	100%

Variant Type	Stratification	# of Variants	TP	FP	TPPV (95% CI)
					(20.7-100%)
	GC content 20- 25	74	74	0	100% (95.1-100%)
	GC content 25- 30	408	408	0	100% (99.1-100%)
	GC content 30- 55	2049	2049	0	100% (99.8-100)
	GC content >55	142	142	0	100% (97.4-100%)
Delins*	GC content 0-15	0	0	0	-
	GC content 15- 20	0	0	0	-
	GC content 20- 25	1	1	0	100% (20.7-100%)
	GC content 25- 30	2	2	0	100% (34.24-100%)
	GC content 30- 55	66	66	0	100% (94.5-100%)
	GC content >55	12	12	0	100% (75.8-100%)
CNV Deletions	GC content 0-15	0	0	0	-
	GC content 15- 20	0	0	0	-
	GC content 20- 25	18	18	0	100% (82.4-100%)
	GC content 25- 30	**70**	**69**	**1**	**98.6% (92.3-99.8%)**
	GC content 30- 55	2596	2591	5	99.8% (99.6-99.9%)
	GC content >55	426	425	1	99.8% (98.7-100%)
CNV Duplications	GC content 0-15	0	0	0	-
	GC content 15- 20	0	0	0	-
	GC content 20- 25	0	0	0	-
	GC content 25- 30	0	0	0	-
	GC content 30- 55	**335**	**330**	**5**	**98.5% (96.6-99.4%)**
	GC content >55	**156**	**149**	**7**	**95.5% (91.0-97.8%)**

TP: True positive; FP: False positive

*A delins refers to a sequence change where, compared to a reference sequence, one or more nucleotides are replaced by one or more other nucleotides and which is not a substitution, inversion or conversion.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Refer to Section VI.B, Comparison Study using Clinical Specimens

2. Clinical Specificity:

Refer to Section VI.B, Comparison Study using Clinical Specimens

3. Other Clinical Supportive Data: Evaluation of Variant Classification, Interpretation, and Reporting

a) Genotype-phenotype associations

Genotype-phenotype associations for the 47 panel genes are summarized in **Table 35.**

Table 35. Gene/Disease Associations

Gene*	Syndrome / Cancer
APC	AD familial adenomatous polyposis (FAP), attenuated FAP (AFAP), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), colorectal, small bowel, gastric, liver, brain, pancreatic, and thyroid cancers.
ATM	AR ataxia-telangiectasia, AD predisposition to breast, ovarian, pancreatic and prostate cancer.
AXIN2	AD oligodontia-colorectal cancer syndrome
BARD1	AD breast cancer
BMPR1A	AD juvenile polyposis syndrome (JPS), colorectal, small bowel, gastric and pancreatic cancers
BRCA1	AR Fanconi anemia, AD hereditary breast and ovarian cancer (HBOC) syndrome, breast, ovarian, fallopian tube, peritoneal, pancreatic, and prostate cancers, and affected individuals may be at increased risk for melanoma
BRCA2	AR Fanconi anemia, AD hereditary breast and ovarian cancer (HBOC) syndrome, breast, ovarian, fallopian tube, peritoneal, pancreatic, and prostate cancers.
BRIP1	AD predisposition to ovarian cancer and AR Fanconi anemia.
CDH1	AD hereditary diffuse gastric cancer (HDGC) syndrome, stomach and breast cancers.
CDK4	AD melanoma
CDKN2A	AD hereditary melanoma-pancreatic cancer syndrome, melanoma, pancreatic cancer and neural system tumors
CHEK2	AD breast, colon, thyroid and prostate cancers.

Gene*	Syndrome / Cancer
CTNNA1	AD butterfly-shaped pigmentary macular dystrophy, hereditary diffuse gastric cancer
DICER1	AD pleuropulmonary blastoma familial tumor predisposition syndrome, ovarian sex cord-stromal tumors, rhabdomyosarcoma, renal sarcoma, cystic nephroma, Wilms tumor, thyroid cancer
EPCAM	AR congenital tufting enteropathy, AR constitutional mismatch repair deficiency syndrome (CMMR-D), AD Lynch syndrome, colorectal, uterine, endometrial, ovarian, gastric, bladder, biliary tract, urinary tract, prostate, brain, and small bowel cancers.
GREM1	AD hereditary mixed polyposis syndrome (HMPS), colorectal polyps.
HOXB13	AD prostate cancer
KIT	AD piebaldism, AD gastrointestinal stromal tumors, and AD familial mastocytosis.
MEN1	AD familial isolated hyperparathyroidism, multiple endocrine neoplasia type 1 (MEN1) syndrome, parathyroid, pituitary, thymus, gastric, small bowel (duodenal endocrine cells), pancreatic and adrenal gland tumors
MLH1	AR constitutional mismatch repair deficiency syndrome (CMMR-D), AD Lynch syndrome, colorectal, uterine, endometrial, ovarian, gastric, bladder, biliary tract, urinary tract, prostate, brain, and small bowel cancers.
MSH2	AR constitutional mismatch repair deficiency syndrome (CMMR-D), AD Lynch syndrome, colorectal, uterine, endometrial, ovarian, gastric, bladder, biliary tract, urinary tract, prostate, brain, and small bowel cancers.
MSH3	AR MSH3-associated polyposis, colorectal cancer.
MSH6	AR constitutional mismatch repair deficiency syndrome (CMMR-D), AD Lynch syndrome, colorectal, uterine, endometrial, ovarian, gastric, bladder, biliary tract, urinary tract, prostate, brain, and small bowel cancers.
MUTYH	AR MUTYH-associated polyposis (MAP), colorectal cancer, duodenal adenomas, ovarian, bladder, breast, endometrial, skin and thyroid cancers
NBN	AR Nijmegen breakage syndrome (NBS), lymphoma, medulloblastoma, glioma, and rhabdomyosarcoma.
NF1	AD Neurofibromatosis type 1 (NF1), AD neurofibromatosis-Noonan syndrome, AD Watson syndrome, central nervous system neoplasms, breast cancer, gastrointestinal stromal tumors, pheochromocytoma, and sarcomas.
NTHL1	AR NTHL1-associated polyposis, colorectal cancer.
PALB2	AR Fanconi anemia, AD predisposition to breast, pancreatic, ovarian and male breast cancer
PDGFRA	AD GIST-plus syndrome, gastrointestinal stromal tumors.
PMS2	AR constitutional mismatch repair deficiency syndrome (CMMR-D), AD Lynch syndrome, colorectal, uterine, endometrial, ovarian, gastric, bladder, biliary tract, urinary tract, prostate, brain, and small bowel cancers.
POLD1	AD MDPL syndrome (mandibular hypoplasia, deafness, progeroid features, and lipodystrophy), AD colorectal cancer.

Gene*	Syndrome / Cancer
POLE	AR facial dysmorphism, immunodeficiency, livedo, and short stature (FILS) syndrome, AD predisposition colon cancer.
PTEN	AD PTEN hamartoma tumor syndrome (PHTS), melanoma, breast, thyroid, renal, endometrial, and colorectal cancer.
RAD50	AR Nijmegen breakage syndrome-like disorder
RAD51C	AD breast and ovarian cancer
RAD51D	AD ovarian and breast cancer
SDHA	AR mitochondrial complex II deficiency, AD hereditary paraganglioma-pheochromocytoma (PGL-PCC) syndrome, gastrointestinal stromal tumors (GIST), renal cancer.
SDHB	AR mitochondrial complex II deficiency, AD hereditary paraganglioma-pheochromocytoma (PGL-PCC) syndrome, gastrointestinal stromal tumors (GIST), renal cancer.
SDHC	AD hereditary paraganglioma-pheochromocytoma (PGL-PCC) syndrome, gastrointestinal stromal tumors (GIST), renal cancer.
SDHD	AD hereditary paraganglioma-pheochromocytoma (PGL-PCC) syndrome, gastrointestinal stromal tumors (GIST), renal cancer. AR mitochondrial complex II deficiency
SMAD4	AD juvenile polyposis syndrome (JPS), colorectal, gastric and pancreatic cancer
SMARCA4	AD Coffin-Siris syndrome, AD rhabdoid tumor predisposition syndrome, ovarian cancer.
STK11	AD Peutz-Jeghers syndrome (PJS), breast, ovarian, non-epithelial ovarian cancer (sex cord tumors with annular tubules), testicular, uterine, cervical, colorectal, small bowel, pancreatic, gastric and lung cancers
TP53	AD Li-Fraumeni syndrome (LFS), osteosarcoma, brain, breast, adrenocortical, leukemia, lymphoma, head and neck, renal, lung, laryngeal, skin, ovarian, pancreatic, prostate, testicular and thyroid cancers.
TSC1	AD tuberous sclerosis complex (TSC), hamartomas, facial angiofibromas, ungual fibromas, cortical tubers, subependymal giant cell astrocytomas, cardiac rhabdomyomas, renal angiomyolipomas, retinal nodular hamartomas, lymphangioleiomyomas and pancreatic neuroendocrine tumors.
TSC2	AD tuberous sclerosis complex (TSC), hamartomas, facial angiofibromas, ungual fibromas, cortical tubers, subependymal giant cell astrocytomas, cardiac rhabdomyomas, renal angiomyolipomas, retinal nodular hamartomas, lymphangioleiomyomas and pancreatic neuroendocrine tumors.
VHL	AR familial erythrocytosis, AD von Hippel-Lindau (VHL), hemangioblastomas, paragangliomas, pheochromocytomas, endolymphatic sac tumors, epididymal cystadenomas, pancreatic neuroendocrine tumors, and clear cell renal cell carcinoma

AD = autosomal dominant; AR = autosomal recessive

*****Genes of high clinical significance are defined as those for which the test result(s) may lead to prophylactic screening, confirmatory procedures or treatment that may incur morbidity or mortality to the patient and are shown in bold text.

b) Database

The Invitae Common Hereditary Cancers Panel test system employs multiple databases that store variant information, including one within the VDB software and another within the CROP software.

VDB contains normalized representations of variants, as well as both left- and right-aligned representations of variants. These variants are annotated. As new variants are encountered in patient samples, they are added to the database and go through the annotation process using various annotation sources. Variants that have been encountered before are verified to ensure that annotations are present and up to date, with any missing annotations flagged for manual review.

CROP contains variant classifications (i.e., pathogenic, likely pathogenic, uncertain significance, benign, likely benign) and the supporting evidence supporting the classifications, which has been curated by qualified Invitae staff. Gene-disease relationship curation and variant interpretation is performed according to controlled SOPs by trained individuals who have passed a competency assessment. A variety of external databases are consulted. When a novel variant is encountered, CROP pulls in relevant clinical evidence for evaluation. Variant interpreters review the PMIDs and website resources associated with the variant's HGVS nomenclature and assign an evidence type to each individual piece of evidence. Based on this evidence, a classification is calculated, assigned, reviewed, and finalized. The final interpretation is then stored in CROP. When the variant is encountered again, CROP automatically checks how recently the variant was last seen and triggers a new search for and evaluation of any new evidence.

c) Interpretation Agreement

To evaluate the performance of variant classification, interpretation and reporting, a study was performed to compare result interpretations made by the Invitae Common Hereditary Cancers Panel and independently generated prior clinical laboratory testing results. In this study, results from a total of 975 patients, representing 1874 unique BRCA1/BRCA2 variants, were examined. These patients were referred for hereditary breast/ovarian cancer (HBOC) counseling, or have known familial mutations, or have personal or familial HBOC high risk factors. Patients had prior BRCA1/2 results using an orthogonal method, as well as results obtained from a 29-gene panel, which is a subset of the Invitae Common Hereditary Cancers Panel that included BRCA1 and BRCA2, and utilizes consistent variant annotation framework as the Invitae Common Hereditary Cancers Panel. Variant interpretation was compared between the Invitae results and the prior clinical laboratory testing. Across the total of 975 patients, there was 98.9% concordance (964 patients, 1765 variants) between the Invitae interpretation and the prior results from an orthogonal laboratory.

A second analysis was performed to evaluate the concordance between Invitae variant classifications and ClinVar classifications. At the time of the analysis, 9 of the 47 target genes (BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PMS2, PTEN and TP53) have Expert Panel submissions in ClinVar. All available variants (total of 3190, including 3102 SNVs, 17 Indels and 71 CNVs) were evaluated across these 9 genes, and 97% of Invitae classifications were concordant with ClinVar. Results of the analysis are summarized in **Table 36**. The concordance between Invitae variant classifications and ClinVar classifications is evaluated annually to ensure that the rule sets and the professional application of those rule sets continues to agree with the clinical interpretations issued by other clinical and medical groups.

Table 36 Invitae-ClinVar Variant Classification Concordance

Interpretation		ClinVar		
		Positive (P, LP)	Uncertain	Negative (B, LB)
Invitae	Positive (P, LP)	1783	7	0
	Uncertain	26	52	63
	Negative (B, LB)	0	6	1253

In addition, to evaluate the performance of the Invitae bioinformatics pipeline in appropriately linking variant classification to report level clinical results, a total of 95 cases were evaluated, for which clinical results were determined and reviewed manually by professionals, representing every possible combination, comparison was made between the software output and manual evaluation. 100% concordance of clinical results were observed between manual evaluation and software output, based on variant classification inputs.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

F Other Supportive Performance Characteristics Data:

Not applicable

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
False positive, false negative, or failure to provide a result.	Certain design verification and validation including certain analytical and clinical studies, and mutation annotation and clinical interpretation rules identified in special control (1). Certain labeling information including limitations, device descriptions, methodology and protocols, and performance information identified in special controls (2).
Incorrect interpretation of variants/alterations by the lab.	Certain labeling information including

Risks to Health	Mitigation Measures
	limitations, device descriptions, methodology and protocols, and performance information identified in special controls (2). Certain design verification and validation including certain analytical and clinical studies, and mutation annotation and clinical interpretation rules identified in special control (1).
Incorrect interpretation of test results by healthcare provider.	Certain labeling information including limitations, device descriptions, methodology and protocols, and performance information identified in special controls (2). Certain design verification and validation including certain analytical and clinical studies, and mutation annotation and clinical interpretation rules identified in special control (1).

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The Invitae Common Hereditary Cancers Panel is a qualitative high-throughput sequencing based in vitro diagnostic test system intended for analysis of germline human genomic DNA extracted from whole blood for detection of substitutions, small insertion and deletion alterations and copy number variants (CNV) in a panel of targeted genes. This test system is intended to provide information for use by qualified health care professionals, in accordance with professional guidelines, for hereditary cancer predisposition assessment and to aid in identifying hereditary genetic variants potentially associated with a diagnosed cancer. The test is not intended for cancer screening or prenatal testing. Results are intended to be interpreted within the context of additional laboratory results, family history, and clinical findings.The test is a single-site assay performed at Invitae Corporation.

There are probable benefits to the population(s) for whom the Invitae Common Hereditary Cancers Panel test system is intended, including individuals with a diagnosed cancer, and individuals with a family history of developing a certain type or types of cancer. The benefit includes detection of cancer predisposition variants and is supported by the extensive analytical validation and clinical validation of the device, which indicates that use of this device, may aid in appropriate medical management for patients with identified variants. Overall, the Invitae Common Hereditary Cancers Panel test would inform qualified health care professionals, to act in accordance with professional guidelines, for hereditary cancer predisposition assessment and to aid in identifying hereditary genetic variants potentially associated with a diagnosed cancer. This would provide benefit to patients and other family members, in receiving the appropriate medical management for the mutation/alteration identified. The performance of the analytical accuracy study in particular, supported the probable benefit of this device. A total of 6014 samples with SNVs or Indels and 3648 samples with CNVs were included in the analytical accuracy study. The overall TPPV is 99.9% for SNVs, 100% for Indels and 99.5% for CNVs.

The overall TNPV is 100% for SNVs, 100% for Indels, and 99.7% for CNVs. The totality of the analytical and clinical data provided support a probable benefit of this device, for the uses indicated.

B Summary of the Assessment of Risk:

The probable risk associated with the use of this device are mainly due to 1) analytical false positive, false negatives, or failure to provide a result and 2) incorrect interpretation of variants/alterations by the lab and 3) incorrect interpretation and use of test results by the end-user.

False negatives may lead to patients not being able to avail of appropriate surveillance or management that could benefit the patients. False positives may lead to patients being offered surveillance or management options that are inappropriate for the patients, and that can be associated with other clinical sequelae. Erroneous device results could adversely influence clinical interpretation and consultation for patients. However, this test is not conclusive or prescriptive for the use of any specific therapeutic product or therapeutic pathway and the interpretive statements regarding the clinical implications of a given mutation should not be viewed as a formal treatment or management recommendation.

There is a degree of probable risk of mismanagement of patient care, in accordance with professional guidelines, based on false test results from this test, or incorrect interpretation of test results. These risks are mitigated by the analytical performance of this device, clinical validation and labeling of this device.

C Patient Perspectives:

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

D Summary of the Assessment of Benefit-Risk:

In conclusion, given the available information above, for the following indications for use statement:

The Invitae Common Hereditary Cancers Panel is a qualitative high-throughput sequencing based in vitro diagnostic test system intended for analysis of germline human genomic DNA extracted from whole blood for detection of substitutions, small insertion and deletion alterations and copy number variants (CNV) in a panel of targeted genes.

This test system is intended to provide information for use by qualified health care professionals, in accordance with professional guidelines, for hereditary cancer predisposition assessment and to aid in identifying hereditary genetic variants potentially associated with a diagnosed cancer.

The test is not intended for cancer screening or prenatal testing. Results are intended to be interpreted within the context of additional laboratory results, family history, and clinical findings.

The test is a single-site assay performed at Invitae Corporation.

The probable benefits outweigh the probable risks for the Invitae Common Hereditary Cancers Panel, considering the mitigations of the risks provided in the special controls as well as general controls.

X Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): QVU
Device Type: High throughput DNA sequencing for hereditary cancer predisposition assessment test system
Class: II
Regulation: 21 CFR 866.6095