SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	ONCO/Reveal™ Dx Lung and Colon Cancer Assay (O/RDx-LCCA)
Device Procode:	PQP
Applicant's Name and Address:	Pillar Biosciences, Inc. 9 Strathmore Road Natick, MA 01760
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P200011
Date of FDA Notice of Approval:	July 30, 2021

II. INDICATIONS FOR USE

The ONCO/Reveal™ Dx Lung and Colon Cancer Assay (O/RDx-LCCA) is a qualitative next generation sequencing based *in vitro* diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs) and deletions in 2 genes from DNA isolated from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients with NSCLC or CRC who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. The O/RDx-LCCA is intended to be used on the Illumina MiSeqDx® instrument.

Table 1. List of somatic variants for therapeutic use

Indication	Gene	Variant	Targeted therapy
Colorectal Cancer (CRC)	*KRAS*	*KRAS* wild-type (absence of mutations in codons 12 and 13)	ERBITUX® (cetuximab), or VECTIBIX® (panitumumab)
Non-Small Cell Lung Cancer	*EGFR*	Exon 19 Deletions and Exon 21 L858R Substitution Mutations	EGFR Tyrosine Kinase Inhibitors approved by FDA*

Indication	Gene	Variant	Targeted therapy
(NSCLC)			

*For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm

III. CONTRAINDICATIONS

The test is not indicated to be used for standalone diagnostic purposes, screening, monitoring, risk assessment, or prognosis.

IV. WARNINGS AND PRECAUTIONS

The warnings/precautions and limitations can be found in the O/RDx-LCCA labeling.

V. DEVICE DESCRIPTION

Test Output
The output of the test includes:

Category 1: Companion Diagnostic (CDx) Claims noted in Table 1 of the Intended Use

Test Kit Contents
The Assay kit is composed of 7 reagents to allow the processing of 48 reactions (46 patient samples and required controls). The reagents are liquids or suspensions, stored in individual vials, and segregated into four labeled sub-containers. The sub-containers allow kit components to be stored at the recommended temperature, which may be room temperature, 4°C, or -20°C, depending on the components. The kits are labelled according to applicable standards and Instructions for Use. Safety Data Sheets are available from Pillar Biosciences. Outer packaging supports international frozen shipment.

Table 2. Assay kit reagents

Kit Box 1: GS-PCR Reagent	Quantity	Storage
Gene Specific PCR Master Mix	1 tube (red cap)	-15°C to -25°C
LC Oligo Pool	1 tube (yellow cap)	-15°C to -25°C
Positive Control (PosCtrl)	1 tube (clear cap)	-15°C to -25°C
Uracil-DNA glycosylase (UDG)	1 tube (blue cap)	-15°C to -25°C
Kit Box 2: Indexing PCR Reagent	**Quantity**	**Storage**
Indexing PCR Master Mix	1 tube (green cap)	-15° to -25°C
Forward indexing primers (A501-A508)	8 tubes (white caps)	-15° to -25°C
Reverse indexing primers (A701-A706)	6 tubes (orange caps)	-15° to -25°C
Kit Box 3: PCR Product Purification Reagent	**Quantity**	**Storage**
Purification Beads	1 bottle	2°C to 8°C
Kit Box 4: Index Tube Caps	**Quantity**	**Storage**
White caps (for A501-A508 primers)	24 caps	Ambient

Kit Box 1: GS-PCR Reagent	Quantity	Storage
Orange caps (for A701-A706 primers)	18 caps	Ambient

Other general lab supplies that are needed to execute the protocol include laboratory gloves, ice, ice buckets, tube racks, etc. For reagents, consumables, and equipment required in both pre- and post-PCR processes, dedicated supplies (including gloves, lab coats, etc.) should be available in both areas.

Materials and equiments and software that are required for the test but are not provided with the assay kit are listed in Tables 3 and 4.

Table 3. Materials required but not provided in the assay kit

Material	Source/Part Number
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific/Q32851 or Q32854
Qubit™ Assay Tubes	Thermo Fisher Scientific/Q32856
PhiX Control v3, 10 nM	Illumina/FC-110-3001
MiSeqDx® Reagent Kit v3 (600 cycles)	Illumina/20012552
Ethanol, 200 proof for molecular biology	General lab supplier
Nuclease-free water	General lab supplier
10 mM Tris-HCl w/ 0.1% Tween-20, pH 8.5	General lab supplier
10 N NaOH or 1 N NaOH	General lab supplier
1.5 mL microcentrifuge tubes	General lab supplier
96-well PCR plates, 0.2 mL	General lab supplier
Microplate sealing film	General lab supplier
Conical tubes, 15 mL	General lab supplier
Conical tubes, 50 mL	General lab supplier
Aerosol filter pipette tips	General lab supplier
Solution basin (trough or reservoir)	General lab supplier

Table 4. Equipment and software required but not provided

Equipment	Source/Part Number
MiSeqDx® Instrument†	Illumina/DX-410-1001
Pillar LC-HS module, v1.1.0.316	Illumina/SW-0001
ONCO/Reveal™ Dx Lung and Colon Cancer Assay™ PiVAT® Software	Pillar Biosciences/SFW-2002
Qubit Fluorometer†	Thermo Fisher Scientific
Vortexer	General lab supplier
Magnetic stand for 96 wells	Life Technologies/12331D or 12027, Beckman Coulter/A32782 or equivalent
Microfuge	General lab supplier
Thermal cycler† with heated lid capability	General lab supplier
Single- and multi-channel pipettes†, 0.5 to 1000 µl	General lab supplier
Centrifuge adapted for PCR plates	General lab supplier

† Equipment should be maintained and/or calibrated according to the manufacturer's instructions

The PiVAT® software is for use with O/RDx-LCCA. PiVAT® performs secondary analysis and report generation from sequencing runs that use the O/RDx-LCCA.

PiVAT® is installed on a stand-alone computer system configured with an Ubuntu operating system and a Chromium browser. The system is configured with no network connectivity.

Test Process
All assay reagents included in the O/RDx-LCCA are qualified by Pillar Biosciences and are compliant with the medical device Quality System Regulation (QSR).

1. **Library Preparation**
   The kit is used to prepare sample libraries from FFPE specimens for NGS analysis that may help to inform treatment regimens through the targeted amplification of regions of the genome often mutated during tumor formation. Specimens must be deparaffinized and digested with protease to liberate the DNA target before purification. After purification, the DNA samples are treated with Uracil-DNA Glycosylase to render formalin damaged DNA non-amplifiable.

   The samples are then amplified using polymerase chain reaction (PCR) and a gene-specific primer pool to enrich the number of assay target sequences.

   The amplification products are purified from remaining primers and each sample is "barcoded" in a second PCR reaction using a unique pair of indexing primers to prepare the samples for pooling and multiplexed analysis.

   After purification of the amplification products from residual indexing primers, the indexed libraries are quantified, normalized, and pooled for sequencing.

2. **Sample Sequencing**
   Sequence information is extracted from the sample library pool using Illumina's MiSeqDx® NGS analyzer and corresponding reagents according to the manufacturer's protocols. The user selects the appropriate analysis panel type (O/RDx-LCCA) from the Pillar Module on the MiSeqDx® to initiate the sequence analysis utilizing the preset parameters for that panel. The Pillar Module is an interface designed for the MiSeqDx® by Illumina to interface with collaborator assays. Its main function is to automatically configure assay-specific sequencing parameters on the instrument, such as read length, when a particular assay is selected from the Module's drop-down menu. As additional assays are developed, they can be added to extend the capabilities of the Module.

   The user must also create a Sample Sheet in Illumina's format that holds information about the samples such as name, whether the sample is a control sample (positive, negative, or no template), and what indices were used to tag that sample. Pillar Biosciences provides a tool that facilitates the aggregation of batches of libraries

prepared across multiple days onto a single MiSeqDx® v3 flow cell, provided that each batch has the required positive and No Template Control (NTC) controls. Up to 48 libraries may be multiplexed onto a single MiSeqDx® v3 flow cell. A successful sequencing run will produce sequence data in Illumina's proprietary Binary Base Call format that is converted to a more universal FASTQ sequence format by the Pillar Module.

The O/RDxLCCA requires the user to run positive control (PosCtrl) and NTC for each "Batch" of up to 46 samples (processed on the same plate). Up to 6 batches may be included in a single sequencing run and analyzed through the PiVAT® software. PosCtrl is a cell line DNA containing the CDx variants with expected variant allele frequencies as shown in Table 5 below. The PosCtrl must generate expected mutations to be valid. If the PosCtrl is invalid, the PiVAT® software will fail the entire batch and no results will be reported for all samples within the batch.

Table 5. Positive Control (PosCtrl)

Gene	Variant	Expected Allelic Frequency, %
EGFR	ΔE746 - A750	2.00%
EGFR	L858R	3.00%
KRAS	G13D	15.00%
KRAS	G12D	6.00%

NTC is a reaction setup using DNA diluent or nuclease-free water with no template/DNA input. The NTC should not detect any mutations. If the NTC is invalid, the PiVAT® software will fail the entire batch and no results will be reported for all samples within the batch.

3. Bioinformatic Analysis

The PiVAT® IVD bioinformatics pipeline is used to convert the raw FASTQ output from the MiSeqDx® into genetic variation observed for each sample. The PiVAT® IVD software is provided on a standalone workstation and raw sequence data are transferred from the MiSeqDx® to the PiVAT® IVD workstation for analysis using a USB drive. Once the required FASTQ files are uploaded to the PiVAT® IVD workstation, the user can select sequence data to be analyzed from the browser-based PiVAT® interface and begin the analysis workflow. The software will deconvolute the mixed sample sequence data using the unique sample index and consolidate matching forward and reverse sequence reads. Filters are used to minimize the result of random variation introduced during sample amplification steps and sequencing. The resulting sequences are aligned to the hg19 human genome sequence framework using the BWA-MEM aligner. Local re-alignments are performed to identify longer insertions and deletions (indels). Filters are applied to isolate likely variation from sequencing noise (Table 6). O/RDx-LCCA is designed to detect and report somatic variants for *EGFR* Exon 19 deletion, *EGFR* L858R mutation and missense mutations in *KRAS* G12 and *KRAS* G13. Non-targeted variants including germline variants are not reported.

After this step, the remaining variants are annotated using HGVS standards, and a PDF format summary report is generated in two parts.

- The Run Summary section of the PiVAT® Customer PDF Report output file contains various statistics that reflect run quality and an overview of all variation to be reported across all samples analyzed within that run. Samples that fail to meet certain NGS quality criteria are reported as not valid and no genetic variants are reported for these samples.

- The Patient Report section of the PiVAT® Patient Report output file aggregates any variation observed at the patient level along with previously established clinical utility. Samples that fail to meet certain NGS quality thresholds are reported as not valid and no genetic variants are reported for these samples. The user can interact with the PiVAT® IVD pipeline utilizing a browser-based visual interface. At the end of analysis, all intermediate data files and reports may be downloaded to a USB drive and transferred to another location for permanent storage.

Table 6. NGS-QC in PiVAT®: Run, Sample and Variant Calling Passing Criteria

Category	QC Metrics	Passing Criteria
Run – FAIL if any QC metric(s) fails	PosCtrl	Expected mutations are detected
	PosCtrl	No unexpected mutation(s) detected
	NTC	No mutation detected
	NTC	Maximum coverage < 50x or < 0.5% of median within-run sample coverage
Sample – NOT VALID if any QC metric(s) fail	Sequencing base quality	Bases (with Q Score ≥ Q30) ≥ 75%
	Amplification specificity	Effective On-Target Rate[2] ≥ 70%
	Coverage[1]	Minimum of the amplicon depths[3] ≥ 1000x
Variant reporting threshold	CDx mutations: non-C>T|G>A	Variant coverage >10x and Total coverage ≥ 1000x and Average variant base Q-score ≥ 30 and VAF ≥ 1%
	CDx mutations: C>T|G>A	Variant coverage >10x and Total coverage ≥1000x and Average variant base Q-score ≥ 30 and VAF ≥ 1.5%

[1] Coverage: the coverage after paired-end assembly by PiVAT®. All CDx markers in the Pillar assay are bidirectional sequenced with 2x150bp sequencing protocol due to the short amplicon sizes (144-162bp including primers). 1x coverage = 1x forward + 1x reverse of sequencing reads. Only uniquely mapped reads are analyzed.

[2] Effective On-Target Rate = Mapping rate * On-target rate

[3] Include the 7-key amplicons: three amplicons covering the CDx markers and other four amplicons covering *EGFR*-exon18, *KRAS*-exon3, *KRAS*-exon4 and *BRAF*-V600E.

Abbreviations: VAF = Variant allele frequency

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several FDA-approved CDx alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in the O/RDx-LCCA intended use statement. The

approved CDx tests are listed in Table 7 below; for additional details see FDA List of Cleared or Approved CDx Devices at https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 7. FDA-approved companion diagnostic (CDx) alternatives to O/RDx-LCCA

Indication	Gene	Device	Company	Technology	Therapy
CRC	*KRAS*	Praxis™ Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX® (panitumumab)
CRC	*KRAS*	**cobas®** KRAS Mutation Test (P140023)	Roche Molecular Systems, Inc.	PCR	ERBITUX® (cetuximab) VECTIBIX (panitumumab)
CRC	*KRAS*	*Therascreen®* KRAS RGQ PCR Kit (P110030; P110027)	QIAGEN Manchester Ltd.	PCR	ERBITUX® (cetuximab) VECTIBIX (panitumumab)
CRC	*KRAS*	FoundationOne® CDx (P170019)	Foundation Medicine, Inc.	NGS	ERBITUX® (cetuximab) VECTIBIX® (panitumumab)
NSCLC	*EGFR*	*Therascreen®* EGFR RGQ PCR Kit (P120022/S018)	QIAGEN Manchester Ltd.	PCR	IRESSA® (gefitinib) GILOTRIF® (afatinib) VIZIMPRO® (dacomitinib)
NSCLC	*EGFR*	**cobas®** EGFR Mutation Test v2 (P120019/S019; P120019/S031)	Roche Molecular Systems, Inc.	PCR	EGFR Tyrosine Kinase Inhibitors approved by FDA
NSCLC	*EGFR*	FoundationOne® CDx (P170019)	Foundation Medicine, Inc.	NGS	GILOTRIF® (afatinib) IRESSA® (gefitinib) TARCEVA® (erlotinib)

					TAGRISSO® (osimertinib)
NSCLC	*EGFR*	Oncomine™ Dx Target Test (P160045; P160045/S019)	Life Technologies Corp.	NGS	IRESSA® (gefitinib)

Abbreviations: NSCLC = Non-Small Cell Lung Cancer, CRC=Colorectal Cancer

VII. MARKETING HISTORY

The O/RDx-LCCA has not been marketed previously in the United States or any foreign country. It has so far been used only in Research Use Only format for research applications.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

For the specific adverse events related to the approved therapeutics, please see approved drug product labels.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Performance characteristics of the O/RDx-LCCA were established using DNA derived from FFPE NSCLC and CRC tumor tissue specimens. Studies included reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. The contrived samples (cell line, reference cell line standards in genomic DNA, FFPE and formalin-compromised DNA formats) were used only in cross-contamination study and reagent kit stability studies.

1. Analytical Accuracy

Analytical accuracy was performed to demonstrate the concordance between the O/RDx-LCCA and an externally validated comparator method for the ability of O/RDx-LCCA to detect reportable SNVs and short and medium deletions for CDx genes *EGFR* and *KRAS*.

A total of 263 samples (177 CRC and 86 NSCLC) were tested. Of these samples, 6 yielded invalid results with the externally validated NGS (evNGS) comparator method and 6 yielded invalid results or did not meet workflow quality control (QC) with O/RDx-LCCA. Among the 251 valid samples, 87 positive and 160

negative samples were concordant between the two assays. There were 4 discordant samples between the O/RDx-LCCA and the comparator assay. The samples included simple SNVs, complex SNV and deletions that are targeted by the O/RDx-LCCA.

The aggregated results at the variant, sample and bin levels are shown in Table 8 below.

Table 8. Overall agreement results by variant, sample, and bin type

Binned by Gene	Test + Comp + TP	Test + Comp - FP	Test - Comp + FN	Test - Comp - TN	Total N	PPA (95%CI)	NPA (95%CI)	PPV (95%CI)	NPV (95%CI)
EGFR variant in NSCLC	18	2	0	7675	7695	100.0% (82.4%, 100.0%)	100.0% (99.9%, 100.0%)	90.0% (69.9%, 97.2%)	100.0% (100%, 100.0%)
KRAS variant in CRC	69	2	0	5199	5270	100.0% (94.7%, 100.0%)	100.0% (99.9%, 100.0%)	97.2% (90.3%, 99.2%)	100.0% (99.9%, 100.0%)
Binned by Sample	**Test + Comp + TP**	**Test + Comp - FP**	**Test - Comp + FN**	**Test - Comp - TN**	**Total n**	**PPA (95%CI)**	**NPA (95%CI)**	**PPV (95%CI)**	**NPV (95%CI)**
Sample (*EGFR*)	18	2	0	61	81	100.0% (82.4%, 100.0%)	96.8% (89.1%, 99.1%)	90.0% (69.9%, 97.2%)	100.0% (94.1%, 100.0%)
Sample (*KRAS*)	69	2	0	99	170	100.0% (94.7%, 100.0%)	98.0% (93.1%, 99.5%)	97.2% (90.3%, 99.2%)	100.0% (96.3%, 100.0%)

Note: O/RDx-LCCA does not have "No Call" in "Valid" samples. Invalid data are excluded from this analysis.
PPA = TP / (TP + FN) x 100%
NPA = TN / (TN + FP) x 100%
PPV = TP / (TP + FP) x 100%
NPV = TN / (TN + FN) x 100%
Comp+ = Sample positive for at least one targeted variant when tested with comparator method
Test+ = Sample positive for at least one target variant when tested with O/RDx-LCCA

Agreement of *EGFR* variants in NSCLC and *KRAS* variants in CRC are summarized Tables 9 and 10 below, respectively.

Table 9. *EGFR* variants (in NSCLC) agreement by variant type and class

Binned by Variant Type	Test + Comp + TP	Test + Comp - FP	Test - Comp + FN	Test - Comp - TN	Total n	PPA (95%CI)	NPA (95%CI)	PPV (95%CI)	NPV (95%CI)
SNV	8	0	0	73	81	100.0% (67.6%, 100.0%)	100.0% (95.0%, 100.0%)	100.0% (67.6%, 100.0%)	100.0% (95.0%, 100.0%)
Complex SNV	0	0	0	243	243	N/A	100.0% (98.4%, 100.0%)	N/A	100.0% (98.4%, 100.0%)
Deletion (15-18bp)	10	2	0	7359	7371	100.0% (72.2%, 100.0%)	100.0% (99.9%, 100.0%)	83.3% (55.2%, 95.3%)	100.0% (99.9%, 100.0%)
Binned by Variant Class	**Test + Comp + TP**	**Test + Comp - FP**	**Test - Comp + FN**	**Test - Comp - TN**	**Total n**	**PPA (95%CI)**	**NPA (95%CI)**	**PPV (95%CI)**	**NPV (95%CI)**
C>T|G>A	0	0	0	0	0	N/A	N/A	N/A	N/A

Non C>T|G>A	18	2	0	7675	7695	100.0% (82.4%, 100.0%)	100.0% (99.9%, 100.0%)	90.0% (69.9%, 97.2%)	100.0% (99.9%, 100.0%)

Table 10. *KRAS* variants (in CRC) agreement by variant type and class

Binned by Variant Type	Test + Comp + TP	Test + Comp - FP	Test - Comp + FN	Test - Comp - TN	Total n	PPA (95%CI)	NPA (95%CI)	PPV (95%CI)	NPV (95%CI)
SNV	69	2	0	1969	2040	100.0% (94.7%, 100.0%)	99.9% (99.6%, 100.0%)	97.2% (90.3%, 99.2%)	100.0% (99.8%, 100.0%)
Complex SNV	0	0	0	3230	3230	N/A	100.0% (99.9%, 100.0%)	N/A	100.0% (99.9%, 100.0%)
Deletion (15-18bp)	0	0	0	0	0	N/A	N/A	N/A	N/A
Binned by Variant Class	**Test + Comp + TP**	**Test + Comp - FP**	**Test - Comp + FN**	**Test - Comp - TN**	**Total n**	**PPA (95%CI)**	**NPA (95%CI)**	**PPV (95%CI)**	**NPV (95%CI)**
C>T|G>A	43	1	0	636	680	100.0% (91.8%, 100.0%)	99.8% (99.1%, 100.0%)	97.7% (88.2%, 99.6%)	100.0% (99.4%, 100.0%)
Non C>T|G>A	26	1	0	4563	4590	100.0% (87.1%, 100.0%)	100.0% (99.9%, 100.0%)	96.3% (81.7%, 99.3%)	100.0% (99.9%, 100.0%)

As the accuracy study samples were enrolled by the O/RDx-LCCA, PPA and NPA values were adjusted using a prevalence of 6.9% for *EGFR* variants and 36.1% for *KRAS* variants in the intended use population. The summary of the agreement statistics is shown in the Table 11 below.

Table 11. Summary of agreement statistics

Binned by Sample	PPV (95%CI)	NPV (95%CI)	Unadjusted		Adjusted	
			PPA (95%CI)	NPA (95%CI)	PPA (95%CI)	NPA (95%CI)
Sample (*EGFR*)	90.0% (69.9%, 97.2%)	100.0% (94.1%, 100.0%)	100.0% (82.4%, 100.0%)	96.8% (89.1%, 99.1%)	100.0% (46.6%, 100.0%)	99.3% (97.7%, 99.8%)
Sample (*KRAS*)	97.2% (90.3%, 99.2%)	100.0% (96.3%, 100.0%)	100.0% (94.7%, 100.0%)	98.0% (93.1%, 99.5%)	100.0% (93.2%, 100.00%)	98.4% (94.6%, 99.6%)

In total, there were 4 samples that were discordant. Among the 4 discordant results, 3 mutations detected by the O/RDx-LCCA (one for *EGFR* and 2 for *KRAS*) had low VAFs, which were below the assay cut-off for the evNGS assay. The remaining discordant variant was a complex *EGFR* Exon 19 deletion, which was detected by O/RDx-LCCA but not the evNGS assay.

To evaluate additional samples with positive calls at the low VAF, DNA from 11 representative positive clinical samples with adequate leftover material from the accuracy study were diluted with normal FFPE DNAs to create samples with low VAFs. In total 16 low VAF samples were generated and tested with O/RDx-LCCA. Of these, 5 NSCLC samples that were positive for 4 different *EGFR* Exon

19 deletions were diluted to 0.6x-2.1x LoD levels, 2 NSCLC clinical samples positive for *EGFR* L858R mutation were diluted to 0.5x-1.7x LoD levels, and 4 CRC samples positive for *KRAS* mutations were diluted to 0.6x-2.2x LoD levels. All these sample runs met the sequencing quality metrics criteria. The results of the diluted samples were positive based on the original calls.

2. **Analytical Sensitivity**

a. Limit of Blank

A Limit of Blank (LoB) of zero was determined across 70 independent sample libraries prepared from four FFPE specimens each of normal (non-tumor) colon and normal (non-tumor) lung tissue. Each sample was tested with 9 replicates spanning low and high DNA input, two reagent lots, and three sequencing analyses. No false positives were reported for the CDx variants.

b. Limit of Detection

The limit of detection (LoD) for each positive variant detected by the O/RDx-LCCA was estimated using the hit rate approach where LoD is defined as the lowest VAF with 100% hit rate. A total of 4 clinical NSCLC and CRC specimens were evaluated, which included CDx SNVs and a deletion/insertion (DelIns) variant which is a complex mutation with a deletion followed by an insertion. Five titration levels and 2 reagent lots were tested. Each level was tested with 10 replicates per sample at the minimum DNA input (30 ng) for each of the two reagent lots (20 replicates per level).

The claimed LoD for each variant summarized in the table below is based on the conservative hit rate approach where the assay produced 100% positive calls. Adequate dilutions were not tested for all samples in the LoD study to determine the lowest VAF at which at least 95% of the test replicates produce correct calls using the probit approach or the lowest level with 100% hit rate (i.e., worst case scenario, if the minimum requirements for the probit approach are not met).

Table 12. Summary of O/RDx-LCCA variant limit of detection

Gene	Variant	Variant Category	Estimated VAF%
KRAS	G13D	SNV	3.3
KRAS	G12D	SNV	3.4
EGFR	L858R	SNV	3.0
EGFR	Exon 19 Del	DelIns (a complex mutation with 19 bp deletion and 1bp insertion)	3.7

Additional Study

Since adequate dilutions were not tested in the original study, a second study was conducted with additional dilutions at lower VAFs with one dilution level

below 100% hit rate for all samples were tested to determine the lowest VAF with 100% hit rate. Samples were tested at a minimum of five titration levels with 20 replicates per dilution level using 2 reagent lots at the minimum DNA input of 30 ng. The newly defined LoD for each variant summarized in the table below is based on the hit rate approach where the assay produced 100% positive calls.

Table 13. Summary of O/RDx-LCCA variant limit of detection (second LoD study)

Gene	Variant	Variant Category	VAF%
KRAS	G13D	SNV	2.6
KRAS	G12D	SNV	1.8
EGFR	L858R	SNV	1.5
EGFR	Exon 19 Del	DelIns (a complex mutation with 19 bp deletion and 1bp insertion)	1.7

c. Tumor Content

The minimum tumor fraction required to support the robustness of the O/RDx-LCCA was evaluated. Four clinical samples with different percentages of initial tumor cell content (30% to 80%) as estimated by an external pathology lab before the study, were diluted with DNA extracted from tissue-matched normal FFPE samples resulting in five levels of final tumor content. These samples were analyzed with 20 replicates per level using the O/RDx-LCCA.

The resulting data show robustness of O/RDx-LCCA in samples with tumor content above 10% at 30 ng DNA input (Table 14). The data supports O/RDx-LCCA requirement of 30% tumor content.

Table 14. Detection rate of diluted tumor content by variant

Gene Exon	Nucleotide Change	Amino Acid Change	Test Level	Detection Rate	VAF Range	VAF Mean	VAF SD	Diluted Tumor Content (%)
KRAS Exon 2	c.35G>A	p.Gly12Asp	L1	20/20	8.7 - 10.7	9.78	0.47	28.8
			L2	20/20	4.3 - 5.8	4.87	0.39	14.3
			L3	20/20	2.7 - 4	3.41	0.37	10.0
			L4	20/20	2.1 - 3	2.52	0.24	7.4
			L5	20/20	1.5 - 2.2	1.81	0.19	5.3
KRAS Exon 2	c.38G>A	p.Gly13Asp	L1	20/20	5.4 - 7.3	6.27	0.53	25.0
			L2	20/20	3.8 - 4.7	4.25	0.26	16.9
			L3	20/20	2.7 - 3.8	3.30	0.32	13.1
			L4	20/20	2 - 3.1	2.60	0.34	10.3
			L5	15/20	1.6 - 2	1.71	0.12	6.8
			L1	20/20	6.2 - 9.7	7.85	1.05	39.9

Gene Exon	Nucleotide Change	Amino Acid Change	Test Level	Detection Rate	VAF Range	VAF Mean	VAF SD	Diluted Tumor Content (%)
EGFR Exon 19	c.2237_2255delinsT	p.Glu746_Ser752delinsVal	L2	20/20	3.1 - 6.4	4.92	0.86	25.0
			L3	20/20	2.4 - 5.1	3.72	0.65	18.9
			L4	20/20	1.6 - 3.4	2.42	0.42	12.3
			L5	20/20	1 - 2.6	1.66	0.41	8.4
EGFR Exon 21	c.2573T>G	p.Leu858Arg	L1	20/20	7.2 - 9.6	8.32	0.70	18.0
			L2	20/20	4.9 - 7.1	6.05	0.62	13.1
			L3	20/20	1.9 - 4.3	3.02	0.56	6.6
			L4	20/20	2 - 3.7	2.59	0.49	5.6
			L5	20/20	1.2 - 1.9	1.53	0.18	3.3

d. DNA Input

The recommended DNA input range of the O/RDx-LCCA is between 30 ng to 80 ng. The DNA input range was evaluated at 5, 10, 20, 40, 80, and 160 ng in duplicate using DNA extracted from 10 FFPE samples containing reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. The expected variants (*KRAS* G12X, *KRAS* G13X *EGFR* Exon 19 deletion and *EGFR* L858R) present in the 10 samples were called correctly at DNA inputs of 5-160 ng. At 5 ng of DNA input, 5 out of 20 samples failed to generate sequencing libraries that met the library yield of ≥ 3.5 nM, which is the minimum library yield requirement recommended for O/RDx-LCCA. At 10 ng of DNA input, 2 out of 20 samples failed the library yield requirement. Seven samples that failed library yield QC requirement were processed to completion to assess results below minimum DNA input of 30 ng/test. The data showed that 10-80 ng of DNA input for the O/RDx-LCCA produced accurate results (at the variant level: PPA=100.0% [95% CI: 95.4%, 100%] (80/80), NPA=100.0% [95% CI: 99.9%, 100%] (9999/10000)); and therefore, supports a DNA input range of 30 ng to 80 ng for the O/RDx-LCCA.

3. Analytical Specificity

a. Interfering Substances

To evaluate the potential impact of interfering substances on the performance of the O/RDx-LCCA, four CRC and four NSCLC FFPE specimens including reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement were evaluated in the presence of exogenous and endogenous substances. Each specimen was assessed with two replicates for each of the eight interferents tested at low and high concentrations for a total of 16 test libraries. Testing was performed at 1-1.5x LoD for the CDx variants for exogenous interfering substances and near the minimum assay requirement of 30 ng DNA input. The following interferents and interferent concentrations were used: paraffin in xylene (0.000002% and 0.000267%), Proteinase K (0.004 and 0.043 ug/mL), lysis buffers Buffer ATL

(0.0002% and 0.0019%), and Buffer AL (0.0002% and 0.0021%), extraction wash buffers AW1 (0.06% and 0.33%), and AW2 (5.7% and 16.7%), ethanol (4.0% and 11.9%) and hemoglobin (1 mg/mL and 2 mg/mL) (Table 15). The concentrations for exogenous interferents is given relative to the eluted DNA sample, and for hemoglobin, relative to the lysis solution post-deparaffinization. No impact on the performance of the O/RDx-LCCA was observed for each substance and at each level tested (Table 16).

Table 15. List of interfering substances tested

Exogenous/ Endogenous	Interfering Substance	Min Value	Max Value	# tested
Endogenous	Control	N/A	N/A	8
	Hemoglobin	1 mg/mL	2 mg/mL	16
Exogenous	Control	N/A	N/A	8
	Buffer AL	0.0002%	0.0021%	16
	Buffer ATL	0.0002%	0.0019%	16
	Buffer AW1	0.06%	0.33%	16
	Buffer AW2	5.7%	16.7%	16
	Ethanol	4.0%	11.9%	16
	Xylene	0.000002%	0.000267%	16
	Proteinase K	0.000004	0.000043	16
	Total			**144**

Table 16. Summary of interference study results

Study	Interfering Substance	Test Value	TP	FP	FN	TN	PPA (95%CI)	NPA (95%CI)
Endogenous	Hemoglobin	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
Endogenous	Buffer AL	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
	Buffer ATL	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
	Buffer AW1	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)

Study	Interfering Substance	Test Value	TP	FP	FN	TN	PPA (95%CI)	NPA (95%CI)
	Buffer AW2	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
	Ethanol	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
	Xylene	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
	Proteinase K	Min	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)
		Max	8	0	0	1000	100% (67.6%, 100%)	100% (99.6%, 100%)

b. Necrotic Fraction

Retrospective analyses of impact of necrotic tissue content in FFPE samples from clinical validation and analytical accuracy studies are shown below. Samples with <20% necrotic tissue content in analytical accuracy study excluded from retrospective analysis. 274 CRC and 276 NSCLC FFPE specimens with varying quantities of necrosis (1% to 70%) were assessed. All samples >20% necrotic content passed library yield QC, PiVAT® results were valid and concordant with comparator assays. Five discordant results were observed in CRC that were not correlated with high necrotic content. Three discordant calls were observed in NSCLC that were not correlated with high necrotic content. For details on the discordant results, see the footnotes in Table 17. No clear trend in decreasing performance with increasing necrotic fraction in the sample was observed supporting the conclusion that the performance of the O/RDx-LCCA is robust within the recommended range of necrotic content less than 50% (Table 17).

Table 17. Summary of necrotic content results

Indication	% Necrotic Bin	# Enrolled/Tetsed	# Library yield QC fail	# Library yield QC pass	# PiVAT® invalid	# PiVAT® valid	# Included in analysis	# of CDx-	# of CDx+	# CDx- Concordant result [1]	# CDx+ Concordant result [1]
CRC	≤10	116	6	110	0	110	96	62	34	61[2]	32[3,4a]
	11-20	11	1	10	0	10	9	6	3	6	3
	21-30	4	0	4	0	4	4	1	3	1	3
	31-40	1	0	1	0	1	1	1	0	1	0
	41-50	1	0	1	0	1	1	1	0	1	0
	>50	1	0	1	0	1	1	0	1	0	1
	Not available	140	9	131	0	131	114	58	56	58	54[3, 4b]
	CRC Total	274	16	258	0	258	226	129	97	128	93
NSCLC	≤10	187	8	179	0	179	176	120	56	120	56
	11-20	15	0	15	0	15	15	12	3	12	3
	21-30	7	0	7	0	7	7	4	3	4	3
	31-40	0	0	0	0	0	0	0	0	0	0
	41-50	1	0	1	0	1	1	1	0	1	0
	>50	1	0	1	0	1	1	0	1	0	1
	Not available	65	6	59	0	59	59	37	22	34[5]	22
	NSCLC Total	276	14	262	0	262	259	174	85	171	85
	CRC + NSCLC	550	550		520		485	485		477	

[1] FDA-approved comparator companion diagnostic (CCD) assay used for concordance analysis of CRC samples in clinical validation studies: *therascreen*® KRAS assay. The CCD assay used for concordant analysis of NSCLC samples in clinical validation studies: **cobas**® EGFR Mutation Test v2. The concordance results above is provided based on the CCD1 and FCD results only. For details on the discordant results, see Table 37 and Table 40.

[2] The replicates of the comparator (CCD1/CCD2 = *KRAS* negative) were discordant with FCD (*KRAS* 13VAL; c.38_39delinsTT). It is inferred that *therascreen*® KRAS is not designed to detect complex SNVs, this result may indicate an error by *therascreen*® KRAS Assay.

[3] The replicates of the comparator (CCD1/CCD2 = *KRAS* 12VAL) were discordant with FCD (*KRAS* 12PHE; c.34_35delinsTT). It is inferred that *therascreen*® KRAS is not designed to detect complex SNVs, this result may indicate an error by *therascreen*® KRAS Assay.

[4] The replicates of the comparator were discordant.
[a] CCD1 = *KRAS* 12ALA; CCD2 = *KRAS* 12VAL; FCD = *KRAS* 12VAL

[b] CCD1 = *KRAS* 12ARG; CCD2 = *KRAS* 12CYS; FCD = *KRAS* 12CYS

[5] A total of three unique clinical specimens with *EGFR* L858R mutation showed discordant results. For all three samples, their CCD1/CCD2 results using cobas were both negative and their O/RDx-LCCA results were positive with VAF range 1.9% to 4.9%. These results suggest the discordant cases are likely due to difference in detection sensitivity (Limit of Detection: cobas=5%) and the O/RDx-LCCA results are likely correct.

Abbreviations: Lib=sample library

c. Cross-Contamination

To assess intra-run cross-contamination, 24 replicates of a positive cell line sample containing *EGFR* L858R at ~50% VAF and 24 replicates of NTC were processed on the same plate in a checkerboard format. No false positive calls (0/24, 0%) were detected in all NTC samples. Therefore, no cross-contamination was observed.

To assess inter-run cross-contamination, a retrospective study utilizing sequencing runs generated as part of validation testing were analyzed. Indices that were used in Sequencing Run 1 and theoretically absent from Sequencing Run 2 (unexpected indices) were identified and enumerated in the output of Sequencing Run 2. Reads from index combinations used in Sequencing Run 1 could arise from run-to-run carryover, or they could arise from within run events, such as PCR errors and index hopping. The fraction of reads associated with unexpected indices across all five Run 2 data sets analyzed was less than 1% (≤0.4%) of the minimum number of reads for any sample within that run, well below the level where the unexpected reads could generate false positive results.

d. Cross-Reactivity

An *in-silico* cross-reactivity analysis was performed to evaluate the specificity of the primers used in the OR/Dx-LCCA. The primers were checked for specificity to the human genome (hg19) and the genomes of representative protozoal, viral, fungal, and bacterial human pathogens. A total of 177 human and 259 pathogen non-target sequences with some similarity to the human genome were identified using *in silico* PCR and BLAT analysis. These sequences were converted to FASTQ format and processed through the PiVAT® software. The test samples produced no on-target reads and no variant calls for any of the non-target sequences while producing the expected variant calls for positive controls included in the analysis. These results demonstrated that the primers are specific for the intended targeted sequences.

4. Precision and Reproducibility

The reproducibility of the O/RDx-LCCA was evaluated using 10 clinical samples with target variants adjusted to a variant allele frequency percent (VAF%) in the range of 1-3x of the currently established LoD using DNA extracted from clinically normal tissue. The sample panel included two (2) FFPE CRC specimens with *KRAS* mutations (Gly12Asp and Gly13Asp), two (2) FFPE NSCLC specimens with *EGFR* mutations (Glu746_Ser752delinsVal and Leu858Arg) and

one (1) FFPE CRC specimen negative for CDx variants. Each variant was present at both high and low VAF% levels.

The study was conducted at three sites with 2 operators at each site performing 3 runs on non-consecutive days. One sequencing instrument and 2 reagent lots were used at each site. Each sample was tested in 4 replicates in each run for a total of 36 possible results (3 sites by 3 runs by 4 replicates). The study produced a total of 360 test results.

PPA and NPA values with two-sided 95% confidence intervals were calculated across all tests performed. The observed PPA value for target variants was 100% (98.7%,100%), and NPA was 100% (100%,100%). A variance component analysis was performed for each of the sample/variant level to estimate variability of the assay including site, operator, day (site, operator), replicate and reagent lot. The total standard deviations of VAF% ranged from 0.33% to 0.70%.

Agreement at the Variant and Variant Frequency Level
Observed mean VAF% and positive call rates with 95% confidence intervals across sample variants at both high and low VAF% concentration for the 36 replicates is presented in Table 18 below.

Table 18. Agreement at the variant and variant frequency level

VAF Level	Gene Exon	Nucleotide Change	Amino Acid Change	N	Mean VAF (%)	Fold LoD*	Positive call rate (%)	95% CI (LB, UB)
High	*KRAS* Exon 2	c.35G>A	p.Gly12Asp	36	6.80	2.0	36/36 (100%)	90.4%, 100%
	KRAS Exon 2	c.38G>A	p.Gly13Asp	36	6.91	2.1	36/36 (100%)	90.4%, 100%
	EGFR Exon 19	c.2237_2255delinsT	p.Glu746_Ser752delinsVal	36	5.14	1.4	36/36 (100%)	90.4%, 100%
	EGFR Exon 21	c.2573T>G	p.Leu858Arg	36	8.91	3.0	36/36 (100%)	90.4%, 100%
Low	*KRAS* Exon 2	c.35G>A	p.Gly12Asp	36	4.69	1.4	36/36 (100%)	90.4%, 100%
	KRAS Exon 2	c.38G>A	p.Gly13Asp	36	3.66	1.1	36/36 (100%)	90.4%, 100%
	EGFR Exon 19	c.2237_2255delinsT	p.Glu746_Ser752delinsVal	36	3.02	0.8	36/36 (100%)	90.4%, 100%
	EGFR Exon 21	c.2573T>G	p.Leu858Arg	36	4.96	1.7	36/36 (100%)	90.4%, 100%

* The LoD is based on Table 12.

a. Site-to-Site Reproducibility
Each of the study sites performed a total of 120 tests. An analysis of test performance across study sites, measured as PPA and NPA with 95%

confidence intervals, is provided in Table 19 below. The PPA and NPA observed at each site was 100%. All 4 replicates tested for each sample at both high and low VAF% levels were concordant in each of the 9 runs tested across 3 sites with no false negatives, i.e. 100% concordant (within run precision).

Table 19. Overall agreement by site and 3-sites combined

Bin	N	TP	FP	FN	TN	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
Overall 3 Sites	360	288	0	0	45072	100% (98.7%,100%)	100% (100%,100%)
Site 1	120	96	0	0	15024	100% (96.2%, 100%)	100% (100.0%, 100%)
Site 2	120	96	0	0	15024	100% (96.2%, 100%)	100% 100.0%, 100%)
Site 3	120	96	0	0	15024	100% (96.2%, 100%)	100% (100.0%, 100%)

b. Lot-to-Lot Precision

A total of 3 manufactured reagent lots were used in the study with 2 reagent lots tested at each site. The calculated PPA and NPA values were identical across reagent lots with mean and two-sided 95% confidence intervals of 100% (96.2%,100%) for PPA and 100% (100%,100%) for NPA. The reagent lot component of the total standard deviation of VAF% ranged from 0.08% to 0.33%.

c. Thermocycler Variability

A total of 3 make/model of thermocyclers were used in the study. The calculated PPA and NPA values were identical across thermocyclers with a mean of 100% for PPA and 100% for NPA and with the two-sided 95% confidence intervals as shown in the table below.

Table 20. Precision by Thermocycler

Bin	# tests	TP	FP	FN	TN	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
Eppendorf MasterCycler	120	96	0	0	15024	100% (96.2%, 100%)	100% (100%, 100%)
ABI GeneAmp 9700	40	32	0	0	5008	100% (89.3%, 100%)	100% (99.9%, 100%)
Bio-Rad C1000	200	160	0	0	25040	100% (97.7%, 100%)	100% (100.0%, 100%)

d. Additional Study

An additional single-site precision study was conducted using 11 clinical samples with target CDx variants adjusted to a VAF% in the range of 1-1.5x

of the LoD defined based on the second LoD study (see Section IX.A.2.b, Table 13) using DNA extracted from tissue-matched clinically normal FFPE tissue. The sample panel included three (3) FFPE NSCLC specimens with unique *EGFR* Exon 19 deletion variants, three (3) FFPE NSCLC specimens with *EGFR* Exon 21 L858R mutations, three (3) FFPE CRC specimens with unique *KRAS* G12 variants and two (2) FFPE CRC specimens with *KRAS* G13 variants.

The study was conducted at a single site with 2 operators performing 3 runs on non-consecutive days. Each sample was tested in 4 replicates in each run for a total of 12 possible results (3 runs by 4 replicates).

PPA and NPA values with two-sided 95% confidence intervals were calculated across all tests performed. The observed PPA value for target variants for the site was 99.2% (95.8%, 99.9%) and was 98.6% (92.4%, 99.8%) for *EGFR* and 100% (94.0%, 100%) for *KRAS* on a gene-level. The NPA on a site-level and on a gene-level was 100% for all comparisons.

1. Agreement by Sample at the Variant Level
   Observed mean VAF% and positive call rates with 95% confidence intervals across 11 clinical samples with target variants for the 12 replicates is presented in Table 21 below.

Table 21. Agreement by specimen at the variant level

Gene Exon	Nucleotide Change	Amino Acid Change	Total Calls	Mean VAF (%)	Fold LoD**	Positive call rate (%)	95% CI (LB, UB)
EGFR Exon 19	c.2240_2254del	p.Leu747_Thr751del	12	2.39	1.4	12/12 (100%)	75.8%, 100%
KRAS Exon 2	c.35G>A	p.Gly12Asp	12	2.80	1.6	12/12 (100%)	75.8%, 100%
KRAS Exon 2	c.34G>T	p.Gly12Cys	12	1.94	1.1	12/12 (100%)	75.8%, 100%
EGFR Exon 21	c.2573T>G	p.Leu858Arg	12	2.30	1.5	12/12 (100%)	75.8%, 100%
EGFR Exon 19	c.2236_2250del	p.Glu746_Ala750del	11*	2.23	1.3	11/11 (100%)	74.1%, 100%
KRAS Exon 2	c.38G>A	p.Gly13Asp	12	3.42	1.3	12/12 (100%)	75.8%, 100%
EGFR Exon 21	c.2573T>G	p.Leu858Arg	12	1.73	1.2	12/12 (100%)	75.8%, 100%

Gene Exon	Nucleotide Change	Amino Acid Change	Total Calls	Mean VAF (%)	Fold LoD**	Positive call rate (%)	95% CI (LB, UB)
KRAS Exon 2	c.35G>T	p.Gly12Val	12	1.54	0.9	12/12 (100%)	75.8%, 100%
EGFR Exon 21	c.2573T>G	p.Leu858Arg	12	1.55	1.0	11/12 (91.7%)	64.6%, 98.5%
EGFR Exon 19	c.2235_2249del	p.Glu746_Ala750del	12	1.89	1.1	12/12 (100%)	75.8%, 100%
KRAS Exon 2	c.38G>A	p.Gly13Asp	12	3.16	1.2	12/12 (100%)	75.8%, 100%

* One replicate produced a 'Not Valid" result. Investigation suggests the library was inadvertently excluded during library pooling and was not sequenced.
** The LoD is based on Table 13.

2. Agreement on Site and Gene Level

The study site performed a total of 132 tests. An analysis of test performance on a site and gene level, measured as PPA and NPA with 95% confidence intervals, is provided in Tables 22 and 23 below, respectively. The PPA and NPA observed on a site level was 99.2% (95.8%, 99.9%) and 100% (100%, 100%), respectively. The observed PPA value for target variants was 98.6% (92.4%, 99.8%) for *EGFR* and 100% (94.0%, 100%) for *KRAS* on a gene-level and the NPA on a gene-level was 100% for both genes.

Table 22. Overall agreement by site

Site	Positive /Total Calls	PPA (2-sided 95% CI)	Negative/ Total Calls	NPA (2-sided 95% CI)
Site 1	130/131	99.2% (95.8%,99.9%)	16375/16375	100% (100%,100%)

Table 23. Overall agreement by gene

Gene	Positive /Total Calls	PPA (2-sided 95% CI)	Negative/ Total Calls	NPA (2-sided 95% CI)
EGFR	70/71	98.6% (92.4%, 99.8%)	8875 / 8875	100% (100%,100%)
KRAS	60/60	100% (94.0%, 100%)	7500 / 7500	100% (99.9%, 100%)

Adequate number of samples harboring CDx biomarkers/variants at the LoD levels based on the second LoD study (Table 13) were not evaluated in a 3-site reproducibility study. This was due to the onset of the COVID-19 emergency and associated precautionary measures taken by Pillar, specifically an unanticipated reduction in lab supplies and personnel. Therefore, a post-market 3-site

reproducibility study is planned with samples carrying CDx variants and covering different *EGFR* Exon 19 deletions, *EGFR* Exon 21 L858R mutations and *KRAS* codon 12/13 variants at the newly established LoD levels (Table 13) to supplement the existing studies such that the assay precision is demonstrated to be robust near the true LoD levels of variants that are detected by O/RDx-LCCA.

5. DNA Extraction Method Equivalence

A study evaluating performance of three commercially available FFPE tissue extraction kits was conducted because extraction kits are not included in the O/RDx-LCCA kit. Four FFPE CRC (including one FFPE CRC negative for CDx variants), one normal colon tissue, four FFPE NSCLC (including one FFPE negative for CDx variants), and one normal lung tissue samples were used in the study. The six tumor specimens that were selected to be CDx variant positive included reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. Genomic DNA was extracted using 3 commercially available FFPE extraction kits. Each extracted DNA sample was run in duplicate using the O/RDx-LCCA. PPA was 100% (95% CI: 75.8%,100%) (12/12) and NPA was 100% (95% CI: 99.8%,100%) (2508/2508) at the variant level for each of the two commercially available FFPE DNA extraction kits compared to the validated reference kit. The results demonstrate that the 3 methods yield DNA with comparable quality and quantity to generate reliable results when used with O/RDx-LCCA.

6. Guard Band Studies

The tolerances encompassing the library preparation and sequencing workflow steps were assessed, which correspond to the test's most critical steps that could lead to assay failure. Each workflow step tested included a minimum of 3 test conditions: low; nominal, as defined by the assay instructions for use; and high. The guard-banding range for each study was designed such that the maximum and minimum test points challenged the system, while still being within operational error range.

Ten FFPE-extracted DNA samples were analyzed over 4 sequencing runs to assess library preparation workflow steps such as PCR input and thermal cycling temperature offset. The seven tumor specimens were selected to be CDx variant positive and included reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. The six CDx variant positive tumor specimens were tested for each assay specification. One reference standard DNA (HD799: Quantitative Multiplex Formalin Compromised (Moderate) formalin compromised DNA) containing reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement was prepared and analyzed over 5 sequencing runs to assess library sequencing workflow steps such as library concentration and number of libraries per run. The conditions of testing of the assay's most critical steps are shown in Table 24. All studies resulted in zero failures and 100% agreement across conditions as shown in Table 25.

Table 24. DNA input test levels and sample concentration

Process	Variable	Nominal Value or Range	Test values
Gene-specific PCR	DNA input/test	10 to 80 ng	5 ng 30 ng 160 ng
Indexing PCR	Purified GS-PCR product input volume/test	6 µL	3 µL 6 µL 9 µL
Library pooling	Number of libraries† per sequencing run	12 to 48 libraries per run	6 libraries/ run 12 libraries/run 48 libraries/run 54 libraries/run
Library normalization	Library input per sequencing run	3.5 to 5.0 nM	1 nM 2 nM 3 nM 5 nM 6 nM
Gene-specific and Indexing PCR	Cycling temperatures	Cycling profile in instructions for use	-1°C offset Nominal +1°C offset

† Sample libraries including PosCtrl and NTC

Table 25. Summary of results for guard banding studies

Process	Variable	Nominal value /range	Test values	TP	FP	FN	TN	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
GS-PCR	DNA input/test	30 - 80 ng	5	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
			160	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
I-PCR	Purified GS-PCR product input volume/test	6 µL	3	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
			9	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
GS- & I-PCR	Cycling temperatures	Standard profile in	Standard - 1°C	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)

Process	Variable	Nominal value /range	Test values	TP	FP	FN	TN	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
		User Manual	Standard + 1°C	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
Library pooling	Number of libraries† per sequencing run	12 - 48 libraries/ run	6	4	0	0	500	100% (51.0%, 100%)	100% (99.2%, 100%)
			12	10	0	0	1250	100% (72.2%, 100%)	100% (99.7%, 100%)
			54	52	0	0	6500	100% (93.1%, 100%)	100% (99.9%, 100%)
Library normalization	Library input†† per sequencing run	3.5 – 4.5 nM	1, 2, 3, 4, 5 and 6 nM	46	0	0	5750	100% (92.3%, 100%)	100% (99.9%, 100%)

† Sample libraries including PosCtrl and NTC
†† 1 library each tested at 1, 2, 3, 5 and 6nM; 41 libraries tested at 4 nM (reference)

7. Stability Studies

a. Reagent Kit Shelf-life Stability:

Three separately manufactured kit lots including all components of the O/RDx-LCCA were stored according to the storage conditions specified in product labeling. The stability of the reagents was evaluated by testing at least three (3) reference standard DNA including reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement at specified time points from baseline (Table 26).

- HD701 - Quantitative Multiplex gDNA Multiplex
- HD803 - Quantitative Multiplex Formalin Compromised (Severe) formalin compromised DNA
- HD799 - Quantitative Multiplex Formalin Compromised (Moderate) formalin compromised DNA

Each of the assay QC metrics were evaluated in addition to final calls. Calls and metrics were confirmed against the calls for the kit at the baseline time (i.e., month 0). The shelf-life stability will continue to be evaluated to extend the shelf-life stability claim (see additional studies below [Table 28] for extended stability duration).

Table 26. Summary of reagent kit shelf-life stability studies

Kit Lot	Timepoint (months)	# tests	PPA (2-sided 95% CI)*	NPA (2-sided 95% CI)*
1	2	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	4	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	7	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	9	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	14	6	100% (80.6%, 100%)	100% (99.5%, 100%)
	16	6	100% (80.6%, 100%)	100% (99.5%, 100%)
2	4	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	7	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	10	6	100% (86.2%, 100%)	100% (99.5%, 100%)
3	3.5	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	6.5	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	10	6	100% (86.2%, 100%)	100% (99.5%, 100%)

Additional Studies

Reagent Kit Shelf-Life Stability – Clinical Samples
The stability of the reagents was further evaluated in an additional study by testing three (3) clinical samples with target CDx variants adjusted to a VAF% in the range of 1-1.5x of the LoD (Table 13) using DNA extracted from tissue-matched clinically normal FFPE tissue. The sample panel included one (1) FFPE NSCLC specimens with *EGFR* Exon 19 deletion variant, one (1) FFPE CRC specimen with *KRAS* G12 variant and one (1) FFPE CRC specimen with *KRAS* G13 variant. Three reagent kit lots aged 17, 18 and 24 months were used as representative assay reagent lots to test the samples in replicates of five with each of the 3 reagent lots for a total of 15 replicates per sample at the minimum DNA input of 30 ng.

Each of the assay QC metrics were evaluated in addition to final calls. The detection rate of each sample across all three lots tested was 100% (15/15) as shown in Table 27 below.

Table 27. Performance of each reagent kit lot across clinical samples

Gene Exon	Nucleotide Change	Mean VAF (%)	Fold LoD*	Total Calls	Lot	Detection rate (%)
KRAS Exon 2	c.35G>A	2.49	1.4	5	1	5/5 (100%)
				5	2	5/5 (100%)
				5	3	5/5 (100%)
KRAS Exon 2	c.38G>A	3.53	1.4	5	1	5/5 (100%)
				5	2	5/5 (100%)
				5	3	5/5 (100%)
EGFR Exon 19	c.2235_2249del	1.94	1.1	5	1	5/5 (100%)
				5	2	5/5 (100%)
				5	3	5/5 (100%)

* The LoD is based on Table 13.

Reagent Kit Shelf-Life Stability – Extended Stability Duration

The shelf-life stability of reagent kits was evaluated for additional time points to extend the shelf-life stability claim. The data currently available support at least 13 months of stability for O/RDx-LCCA kit components for all 3 lots evaluated as shown in Table 28 below.

Table 28. Summary of reagent kit shelf-life stability studies

Kit Lot	Timepoint (months)	# tests	PPA (2-sided 95% CI)*	NPA (2-sided 95% CI)*
1	2	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	4	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	7	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	9	3	100% (67.6%, 100%)	100% (99.0%, 100%)
	14	6	100% (80.6%, 100%)	100% (99.5%, 100%)
	16	6	100% (80.6%, 100%)	100% (99.5%, 100%)
2	4	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	7	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	10	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	13	6	100% (86.2%, 100%)	100% (99.5%, 100%)

Kit Lot	Timepoint (months)	# tests	PPA (2-sided 95% CI)*	NPA (2-sided 95% CI)*
	17	6	100% (86.2%, 100%)	100% (99.5%, 100%)
3	3.5	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	6.5	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	10	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	13	6	100% (86.2%, 100%)	100% (99.5%, 100%)
	16	6	100% (86.2%, 100%)	100% (99.5%, 100%)

b. Reagent Kit Transport Stability:

The reagent kit stability studies were performed as one large study that included data points for in-use freeze-thaw stability and transport stability testing under recognized summer and winter profiles for international shipments (Table 29). The transport stability study was performed to demonstrate that the shipping configurations for all kit components provide adequate thermal and physical protection as packages are transported from the manufacturing site to customers. Three (3) separately manufactured kits and component reagent lots were exposed to simulated transport challenges intended to simulate the longest estimated international shipping times of 72 hours and 144 hours. The simulated transport conditions included both physical and temperature challenges, which include 2 packaging configurations (one (1) kit per shipping box and four (4) kits per shipping box) and 4 temperature profiles (72-hour summer, 72-hour winter, 144-hour summer and 144-hour winter). The 144-hour profiles correspond to two runs of the 72-hour profiles. The 72-hour international profile is considered to be a worse case than the 48-hour domestic profile, so a domestic profile was not performed.

Temperature challenge was performed at 3, 4, and 8 months to simulate shipping of aged components. After each temperature challenge, kits produced QC metrics and variant calls equivalent to baseline (month 0) QC metrics and variant calls of the control kit in the lot. No individual kit boxes experienced temperature higher than -15°C. No sign of deterioration or degradation was observed for all labels.

Physical challenge was performed only at 0 months, since the acceptance criteria was visual integrity rather than function (i.e., physical challenge is extremely unlikely to affect the functional integrity of the reagents, and so it was not tested). No packages having undergone simulated transport shipping

displayed signs of physical damage which may impede the function of the assay or workstation and monitor.

Each of the kits and components undergoing temperature challenge was functionally tested using at least three (3) reference standard DNA containing reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement to establish transport stability. The data demonstrate that all kit components show acceptable transport stability at the simulated time points (see Table 30).

Table 29. Test configuration for reagent lots 1, 2, and 3

Line ID	Shipping Box	Number of Kits/ Box	Thermal Profile	Purpose of kits used for testing at months…							
				0-1	2-3	4-5	6-8	9-11	12-14	15-17	18
LOT 1											
1	None	Min 1	None	SL	SL	SL	SL	SL	SL	SL	SL
2	1	1	Summer				TC				
3	2	4 *	Summer				TC	IU			
LOT 2											
4	None	Min 1	None	SL	SL	SL	SL	SL	SL	SL	SL
5	3	1	Winter		TC	IU					
6	4	4 *	Winter		TC						
LOT 3											
7	None	Min 1	None	SL	SL	SL	SL	SL	SL	SL	SL
8	5	4 *	Summer	TC; IU							
9	6	1	Summer	TC; IU							
10	7	4 *	Winter	TC; IU							
11	8	1	Winter	TC; IU							
12	9	4	None	0 only, this shipping box and kits was used for simulated transport for physical challenge							
13	10	1	None	0 only, this shipping box and kits wasused for simulated transport for physical challenge							

Abbreviations: SL = Shelf Life; TC = Thermal Challenge; IU = In-Use.

* One (1) representative kit was tested in-use after completion of profile and storage.

c. In-Use Stability:

The in-use stability study evaluated both open vial stability and freeze-thaw stability. For each of Lots 1, 2, and 3, representative kits were subjected to at least five (5) freeze-thaw cycles and four (4) uses. Additional testing was performed for Lot 3 to assess at least five (5) freeze-thaw cycles and two (2)

uses. The reagents were evaluated by testing at least three (3) reference standard DNA containing reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. In-use stability was tested using Lots 1-3 at the baseline (i.e., month 0), 4 and 9 months in a combined study with transport stability (see above).

To perform reagent freeze-thaw cycle, the reagents were removed from freezer storage and placed in a 2°C to 8°C environment overnight (minimum of 12 hours) to simulate use, then returned to freezer storage for a minimum of 12 hours.

Each of the assay QC metrics were evaluated in addition to final calls. The data demonstrate in-use stability for at least 5 freeze-thaw cycles (Table 30).

Table 30. Summary of reagent kit transport stability and in-use stability studies

KIT LOT	Ship Configuration (# kits/shipping box)	Stimulated Thermal Profile*	Freeze-thaw cycles**	PPA (2-sided 95% CI)***	NPA (2-sided 95% CI)***
1	4	Summer	3	100% (75.8%, 100%)	100% (99.0%, 100%)
			4	100% (75.8%, 100%)	100% (99.0%, 100%)
			5	100% (75.8%, 100%)	100% (99.0%, 100%)
			6	100% (75.8%, 100%)	100% (99.0%, 100%)
3	4	Summer	3	100% (75.8%, 100%)	100% (99.0%, 100%)
			4	100% (75.8%, 100%)	100% (99.0%, 100%)
			5	100% (75.8%, 100%)	100% (99.0%, 100%)
			6	92% (64.6%, 99.0%)	100% (99.0%, 100%)
3	1	Summer	5	100% (75.8%, 100%)	100% (99.0%, 100%)
			6	92% (75.8%, 100%)	100% (99.0%, 100%)
3	4	Winter	3	100% (75.8%, 100%)	100% (99.0%, 100%)
			4	100% (75.8%, 100%)	100% (99.0%, 100%)
			5	100% (75.8%, 100%)	100% (99.0%, 100%)

KIT LOT	Ship Configuration (# kits/shipping box)	Stimulated Thermal Profile*	Freeze-thaw cycles**	PPA (2-sided 95% CI)***	NPA (2-sided 95% CI)***
			6	100% (75.8%, 100%)	100% (99.0%, 100%)
2	1	Winter	3	100% (75.8%, 100%)	100% (99.0%, 100%)
			4	100% (75.8%, 100%)	100% (99.0%, 100%)
			5	100% (75.8%, 100%)	100% (99.0%, 100%)
			6	100% (75.8%, 100%)	100% (99.0%, 100%)
3	1	Winter	5	92% (64.6%, 99.0%)	100% (99.0%, 100%)
			6	92% (75.8%, 100%)	100% (99.0%, 100%)

* Summer and winter international profiles per ISTA 7D

** Test kit lots that had undergone simulated thermal challenges were removed from storage and subjected to the indicated freeze-thaw cycles prior to testing.

*** Agreement between test kit lots (temperature challenged and/or freeze-thawed) and control kit lot.

d. FFPE (Section and Block) and DNA Sample Stability

The stability of FFPE clinical samples (section and block) was assessed using both retrospective and a real-time analysis.

Retrospective analysis leveraged data from the clinical validation study and paired observed performance parameters with FFPE block age as determined by the difference between the reported year of specimen collection and the year when specimens were tested. Analysis focused on the QC metrics used throughout the O/RDx-LCCA workflow: sample library concentration, PiVAT® valid/invalid status, and concordance with results determined by the appropriate comparator assay. Results are summarized in Table 31 below. No significant trend in poorer overall performance with increasing FFPE block age was observed, and robust assay performance was observed for samples over 10 years old. A post-market study will be conducted to prospectively test FFPE block stability for CDx variants.

Table 31. Retrospective analysis of the performance of sample libraries prepared from clinical FFPE specimens of various ages and with known comparator test results

Tumor	FFPE Block Age (years)	# Tested	# Lib Yield QC Fail	# Lib Yield QC Pass	# PiVAT® Invalid	# PiVAT® Valid	# Included in Analysis	# of CCD-	# of CCD+	# CCD- Concordant	# CCD+ Concordant	% FCD Discordant
CRC	0-2	29	0	29	0	29	29	16	13	15	13	3.4%
	3-5	38	0	38	0	38	38	28	10	28	10	0.0%
	6-10	20	0	20	0	20	20	14	6	14	6	0.0%
	11-20	1	0	1	0	1	1	1	0	1	0	0.0%
	>20	2	0	2	0	2	2	1	1	1	1	0.0%
	Total	90	0	90	0	90	90	60	30	59	30	1.1%
NSCLC	0-2	0	0	0	0	0	0	0	0	0	0	n/a
	3-5	35	0	35	0	35	35	1	34	1	34	0.0%
	6-10	1	0	1	0	1	1	1	0	1	0	0.0%
	11-20	2	0	2	0	2	2	1	1	1	1	0.0%
	>20	0	0	0	0	0	0	0	0	0	0	n/a
	Total	38	0	38	0	38	38	3	35	3	35	0.0%
All	0-2	29	0	29	0	29	29	16	13	15	13	3.4%
	3-5	73	0	73	0	73	73	29	44	29	44	0.0%
	6-10	21	0	21	0	21	21	15	6	15	6	0.0%
	11-20	3	0	3	0	3	3	2	1	2	1	0.0%
	>20	2	0	2	0	2	2	1	1	1	1	0.0%
	Total	128	128		128		128	128		127		0.5%

Abbreviations: Lib=sample library, CCD=FDA-approved comparator companion diagnostic (+/- indicate whether test was positive or negative), FCD=follow-on companion diagnostic (O/RDx-LCCA)

Stability of FFPE curls was assessed at baseline, 30 days and 60 days to support stability at 30 days. At each timepoint, eight FFPE specimens stored at ambient conditions were extracted using QIAGEN QIAamp FFPE extraction kit and processed for sequencing within 3 days. The samples contained *EGFR* (L858R and Ex19 del) and *KRAS* (G12D and G13D) variants. FFPE curls were stable, as measured by PPA/NPA analysis, at both the 30-day and 60-day time points, supporting a claim of a 30-day stability. PPA was 100% (95% CI: 80.6%,100%) (16/16) and NPA was 100% (95% CI: 99.8%,100%) (2000/2000) compared to baseline testing.

Stability of DNA extracted from FFPE clinical samples using QIAGEN QIAamp FFPE extraction kit was assessed after storage at 4°C or -20°C, and after 5 cycles of freeze-thaw. Stability at 4°C was assessed after 60 days, 8 months, and 8.25 months and stability at -20°C was assessed after 6 months and 6.5 months. FFPE derived DNA was stable, as measured by PPA/NPA analysis, at all the time points tested following storage at 4°C. PPA was 100% (95% CI: 80.6%,100%) (16/16) and NPA was 100% (95% CI: 99.8%,100%)

(2000/2000) for the timepoints 60 days ans 8.25 months compared to baseline testing. For the timepoint 8 month, PPA was 100% (95% CI: 67.6%,100%) (8/8) and NPA was 100% (95% CI: 99.6%,100%) (1000/1000). FFPE derived DNA was stable, as measured by PPA/NPA analysis, at all the time points tested following storage at -20°C. PPA was 100% (95% CI: 80.6%,100%) (16/16) and NPA was 100% (95% CI: 99.8%,100%) (2000/2000) for the timepoints 6 months and 6.5 months compared to baseline testing.

Data presented here supports a claim of DNA storage stability at 8 months at 4°C and 6 months at -20°C. The DNA stability at -20°C will continue to be evaluated to extend the stability claim. FFPE derived DNA that was subjected to 5 cycles of freeze-thaw was stable, as determined by PPA/NPA analysis using DNA that undergone 1 cycle of freeze-thaw as a control. PPA was 100% (95% CI: 80.6%,100%) (16/16) and NPA was 100% (95% CI: 99.8%,100%) (2000/2000). This data supports a DNA freeze-thaw stability claim of 5 cycles.

e. Stability of Assay Intermediates

The workflow for the O/RDx-LCCA incorporates several optional stopping points to hold assay intermediates. The stability of the intermediate products was evaluated by incorporating two optional stopping points specified in the assay instructions for use. Ten FFPE-extracted DNA samples were included in this study which contained reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. Each sample was processed to completion for sequencing at baseline and the resulting intermediates stored. The intermediates were then removed from storage at different time points and processed to completion to assess the impact of storage on assay performance.

The study results support the conclusion that the 60-day hold of Gene-Specific PCR (GS-PCR) products and 90-day hold on indexed libraries at recommended storage condition did not result in a decrease in O/RDx-LCCA performance as shown in Tables 32 and 33.

Table 32. Summary of results for stability of assay intermediates study

Assay intermediate	Timepoint (days)	TP	FP	FN	TN	PPA (2-sided 95% CI)*	NPA (2-sided 95% CI)*
GS-PCR	**0**	N/A	N/A	N/A	N/A	N/A	N/A
GS-PCR	**35**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
GS-PCR	**62**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)

Assay intermediate	Timepoint (days)	TP	FP	FN	TN	PPA (2-sided 95% CI)*	NPA (2-sided 95% CI)*
GS-PCR	**90**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
I-PCR	**35**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
I-PCR	**90**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
I-PCR	**233**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)
Combo*	**GS-PCR 35 + I-PCR 27**	6	0	0	1254	100% (61.0%, 100%)	100% (99.7%, 100%)

* Combination intermediate stability was tested by performing indexing on GS-PCR products that were 35 days old. The resulting indexed libraries was stored for 27 days at -25 to -10°C before being sequenced.

Table 33. Stability of assay intermediates

Assay intermediate(s)	Storage condition	Intermediate stability
GS-PCR products	-15°C to -25°C	60 days
Indexed libraries	-15°C to -25°C	90 days

B. Animal Studies

No animal studies were conducted using the O/RDx-LCCA.

C. Additional Studies

No additional studies were conducted using the O/RDx-LCCA.

Software verification and validation activities, including unit testing, integration testing, and system testing were performed for the PiVAT® Software.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The reasonable assurance of safety and effectiveness for the O/RDx-LCCA CDx claims were established through clinical concordance studies using a non-inferiority statistical testing approach. Two clinical concordance studies were conducted to support the CDx claims indicated in Table 1 of the Indications for Use statement for *EGFR* Exon 19del/L858R in NSCLC and *KRAS* wild-type (absence of mutation in codons 12 and 13) in CRC. A non-inferiority statistical testing approach was used according to Li (2016)[1]. O/RDx-LCCA test, considered the follow-on companion diagnostic (FCD), was compared to an FDA-approved CDx test, considered the comparator companion diagnostic (CCD) test

for each of the clinical concordance study, using samples representative from the intended use population for that specific device.

A. Study Design

O/RDx-LCCA Concordance Study for *EGFR* Exon 19del/L858R in NSCLC

The concordance of *EGFR* Exon 19 in frame deletions and exon 21 L858R substitution mutation results was determined between the O/RDx-LCCA (FCD) and the approved Roche Molecular Systems' **cobas®** v2 EGFR Mutation Test (CCD). As the *EGFR* mutations are relatively infrequent in the intended use population, a stratified design was used with a target endpoint of 30 to 50% positives in the study population. The acceptance criterion for the clinical study was that the margin of non-inferiority achieved be less than 10%.

O/RDx-LCCA Concordance Study for *KRAS* Wild Type (Absence of Mutation in Codons 12 and 13) in CRC

The concordance of *KRAS* codon 12 and 13 mutation results was determined between the O/RDx-LCCA (FCD) and the approved QIAGEN *therascreen*® KRAS RGQ PCR (CCD). A stratified design was used with a target endpoint of 30 to 50% *KRAS* positive specimens in the study population. The acceptance criterion for the clinical study was that the margin of non-inferiority achieved be less than 10%.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the concordance study was limited to patients who met the following inclusion criteria:

- Patients diagnosed with NSCLC or CRC
- Patients with lung cancer may include any histological subtype that is not small cell lung cancer, including squamous cell carcinoma, adenocarcinoma and large cell carcinoma
- All stages of NSCLC and CRC are eligible
- Male and female patients > 18 years of age
- Tissue samples should be derived from primary cancers, not metastatic sites

Patients were not permitted to enroll in the concordance study if they met any of the following exclusion criteria:

- Any histological subtype that is not CRC or NSCLC
- Patients with only benign tumors such as polyps or adenomas of the colon, or hamartomas, papillomas, schlerosing hemangiomas, alveolar adenomas, etc. of the lung.

2. Follow-up Schedule

None required for this concordance study.

3. Clinical Endpoints

None required for this concordance study.

B. Accountability of PMA Cohort

O/RDx-LCCA Concordance Study for *EGFR* Exon 19del/L858R in NSCLC

A total of 331 DNA samples extracted from NSCLC FFPE specimens were submitted for testing using two successful replicates of CCD. The first replicate of CCD (CCD1) was used to enroll samples into the study. After exclusion of ineligible or failed samples, 257 samples remained for the concordance analysis. Details regarding sample accountability are presented in Table 34 below.

Table 34. Sample accounting for the NSCLC - *EGFR* arm of the clinical study

<table>
<tr><td rowspan="5">331
Enrolled for
CCD1</td><td colspan="3">CCD1 – COBAS EGFR</td><td colspan="3"></td></tr>
<tr><td rowspan="2">CCD DNA QC
(n = 331)</td><td>Invalid</td><td>0</td><td colspan="3"></td></tr>
<tr><td>Valid</td><td>331</td><td colspan="3"></td></tr>
<tr><td rowspan="2">CCD1 test
(n = 331)</td><td>Invalid</td><td>22</td><td colspan="3"></td></tr>
<tr><td>Valid</td><td>309</td><td colspan="3"></td></tr>
<tr><td rowspan="9">309
Enrolled for
CCD2 & FCD</td><td colspan="3">CCD2 – COBAS EGFR</td><td colspan="3">FCD - O/RDx-LCCA</td></tr>
<tr><td rowspan="3">CCD2 test
(n = 309)</td><td rowspan="2">Insufficient DNA</td><td rowspan="2">0</td><td rowspan="3">FCD DNA QC
(n = 309)</td><td><10 ng</td><td>33</td></tr>
<tr><td>10-30 ng</td><td>60</td></tr>
<tr><td>Invalid</td><td>1</td><td>>30 ng</td><td>216</td></tr>
<tr><td rowspan="2">Invalid</td><td rowspan="2">1</td><td rowspan="2">Library yield QC
(n = 276)</td><td>Fail</td><td>19</td></tr>
<tr><td rowspan="4">Valid</td><td rowspan="4">308</td><td>Pass</td><td>257</td></tr>
<tr><td rowspan="3">FCD test
(n = 257)</td><td>CCD2 invalid</td><td>0</td></tr>
<tr><td>PiVAT® invalid*</td><td>0</td></tr>
<tr><td>PiVAT® valid</td><td>257</td></tr>
<tr><td rowspan="2">257
Included in
EGFR analysis</td><td rowspan="2">Matched FCD data</td><td>No</td><td>51</td><td rowspan="2">Matched CCD data</td><td>No</td><td>0</td></tr>
<tr><td>Yes</td><td>257</td><td>Yes</td><td>257</td></tr>
</table>

* PiVAT® invalid rate = 0%

O/RDx-LCCA Concordance Study for *KRAS* Wild Type (Absence of Mutation in Codons 12 and 13) in CRC

A total of 374 DNA samples extracted from CRC FFPE specimens were submitted for testing using two successful replicates of CCD. The first replicate of CCD was used to enroll samples into the study. After exclusion of ineligible or failed samples, 219 samples remained for the concordance analysis. Details regarding sample accountability are presented in Table 35 below.

Table 35. Sample accounting for the CRC - *KRAS* arm of the clinical study

374 Enrolled for CCD1	**CCD1 - THERASCREEN KRAS**					
	CCD DNA QC (n = 374)	Invalid	76			
		Valid	298			
	CCD1 test (n = 298)	Invalid	13			
		Valid	285			
285 Enrolled for CCD2 & FCD	**CCD2 - THERASCREEN KRAS**			**FCD - O/RDx-LCCA**		
	CCD2 test (n = 285)	Insufficient DNA	6	**FCD DNA QC (n = 285)**	<10 ng	16
					10-30 ng	29
					>30 ng	240
		Invalid	11	**Library yield QC (n = 269)**	Fail	36
					Pass	233
		Valid	268	**FCD test (n = 233)**	CCD2 invalid	1
					PiVAT® invalid*	0
					PiVAT® valid	232
219 Included in *KRAS* analysis	**Matched FCD data**	No	49	**Matched CCD data**	No	13
		Yes	219		Yes	219

* PiVAT® invalid rate = 0%

C. <u>Study Population Demographics and Baseline Parameters</u>

Clinical FFPE tumor specimens used for these studies were ethically collected, anonymized, remnant samples obtained from several sources and were provided with detailed clinical and demographic data which is summarized in Table 36 below.

Table 36. Summary data for various attributes associated with the FFPE tumor specimens used for clinical testing

	Indication		**CRC**	**NSCLC**
	# Included in analysis		**219**	**257**
Patient demographics	**Donor Age**	N †	219	246
		Range	23 - 93	18 - 89
		Mean ± SD	63.4 ± 13.5	66.3 ± 9.8
	Gender	Female	101	138
		Male	118	112
		Unknown	0	7
	Race	Caucasian	107	177
		Asian	19	50
		Black	9	4
		Hispanic	3	2
		American Indian	1	1

		Unknown	80	23
FFPE sample information	**Tumor content**	N †	105	196
		Range	10 - 95	15 - 100
		Mean ± SD	58.1 ± 18.2	68.5 ± 19.6
	Necrotic content	N †	105	198
		Range	0 - 20	0 - 70
		Mean ± SD	4.6 ± 4.7	5.3 ± 7.9
	FFPE Sample Age	N †	181	216
		Range	1 - 28	3 - 28
		Mean ± SD	8.5 ± 7.1	11.4 ± 7.5
O/RDx-LCCA	**DNA input**	10 - 30 ng	23	51
		30 - 80 ng	196	206

† The difference between the numbers included in the analysis (219 and 257 for CRC and NSCLC, respectively) and specified N values is related to the number of samples for which the relevant category data was not available.

D. Safety and Effectiveness Results

O/RDx-LCCA Concordance Study for *EGFR* Exon 19del/L858R in NSCLC

Test outcomes from the 257 samples with valid CCD1, CCD2, and FCD results are tabulated below. Details regarding all discordant calls are provided in the legend.

Table 37. 2x2x2 concordance matrix for testing outcomes (NSCLC)

	Enrollment CCD+ (CCD1+)			Enrollment CCD- (CCD1-)		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FCD+	85	0	85	0	3[1]	3
FCD -	0	0	0	0	169	169
Total	85	0	85	0	172	172

[1] A total of three unique clinical specimens with *EGFR* L858R mutation showed discordant results. For all three samples, their CCD1/CCD2 results using cobas were both negative and their O/RDx-LCCA results were positive with VAF range 1.9% to 4.9%. These results suggest the discordant cases are likely due to difference in detection sensitivity (Limit of Detection: cobas=5%) and the O/RDx-LCCA results are likely correct.

The agreements for the non-inferiority test proposed by Li (2016)[1] using the data from the 2x2x2 contingency table above are shown in the table below. Note that, since a mutation enriched population drawn from the intended use population was used for the study, a correction was performed to adjust the observed PPA and NPA based on the prevalence of the *EGFR* mutations in the intended use population (see Li (2016)[1] for details).

All the upper bounds of the 95% confidence intervals were determined to be equal or less than 4%, supporting a conclusion that the agreement between the O/RDx-LCCA

and **cobas®** EGFR Mutation Test v2 is non-inferior to the agreement between two replicates of CCD by a margin of 4%.

Table 38. Calculation of PPA and NPA (NSCLC)

Parameter	Agreement (%)
PPA_{C1F}	100.0
PPA_{C1C2}	100.0
NPA_{C1F}	98.3
NPA_{C1C2}	100.0
PPA_{C2F}	100.0
PPA_{C2F} †	100.0
PPA_{C2C1}	100.0
PPA_{C2C1} †	100.0
NPA_{C2F}	98.3
NPA_{C2F} †	98.3
NPA_{C2C1}	100.0
NPA_{C2C1} †	100.0

See Section 4.2, p.361 in Meijuan Li (2016): Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study, *Statistics in Biopharmaceutical Research*, 8:3, 355-363 for detailed methodology.

The parameter Pc, the "true" minor allele frequency (MAF) for the mutations of interest as analyzed by the O/RDx-LCCA, must be estimated experimentally and was estimated to be 0.07.

† Adjusted for variant enrichment in study design using the parameter Pc.

Confidence intervals for PPA and NPA values were determined using a 1000x bootstrap methodology in SAS. Specifically, 1000x n=257 samples were created using seed-number random selection with replacement from both the CCD1+ (n=257) and CCD1- (n=257) populations. Each of the 1000x CCD1+ (n=257) and CCD1- (n=257) random samples were combined (N=514) and ζppa1, ζppa2, ζnpa1and ζnpa2 were calculated. The lower and upper 95% confidence limits for the x1000 ζppa1, ζppa2, ζnpa1and ζnpa2 values are reported in the following table.

Table 39. Hypothesis testing parameter output for *EGFR* - NSCLC

Parameter	Mean %	95% Upper Confidence Interval
ζppa1	0.0	0.0
ζppa2	0.0	0.0
ζnpa1	1.7	4.0
ζnpa2	1.7	4.0

The following hypotheses were tested:

- H0: ζppa1 = (100.0 - 100.0 = 0%) ≥ 10% vs. Ha: ζppa1 < 10%
- H0: ζppa2 = (100.0 - 100.0 = 0%) ≥ 10% vs. Ha: ζppa2 < 10%
- H0: ζnpa1 = (100.0 - 98.3 = 1.7%) ≥ 10% vs. Ha: ζnpa1 < 10%

- H0: ζnpa2 = (100.0 - 98.3 = 1.7%) ≥ 10% vs. Ha: ζnpa2 < 10%

All upper bounds of the 95% confidence intervals were determined to be equal or less than 4%, supporting the conclusion that the agreement between the O/RDx-LCCA and **cobas®** EGFR Mutation Test v2 is non-inferior to the agreement between two replicates of CCD by a margin of 4%.

O/RDx-LCCA Concordance Study for *KRAS* Wild Type (Absence of Mutation in Codons 12 and 13) in CRC

Test outcomes from the 219 samples with full CCD1, CCD2, and FCD results are tabulated below. Details regarding all discordant calls are provided in the legend.

Table 40. 2x2x2 concordance matrix for testing outcomes (CRC)

	Enrollment CCD+ (CCD1+)			Enrollment CCD- (CCD1-)		
	CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
FCD+	87	2[1a,1b]	89	0	1[4]	1
FCD -	2[2]	2[3a, 3b]	4	0	125	125
Total	89	4	93	0	126	126

[1] The replicates of the comparator were discordant.
- [a] CCD1 = *KRAS* 12VAL; CCD2 = *KRAS* 12ALA; FCD = *KRAS* 12VAL
- [b] CCD1 = *KRAS* 12ASP; CCD2 = *KRAS* negative; FCD = *KRAS* 12ASP

[2] The replicates of the comparator (CCD1/CCD2 = *KRAS* 12VAL) were discordant with FCD (*KRAS* 12PHE; c.34_35delinsTT). It is inferred that *therascreen®* KRAS is not designed to detect complex SNVs, this result may indicate an error by *therascreen®* KRAS Assay.

[3] The replicates of the comparator were discordant.
- [a] CCD1 = *KRAS* 12ARG; CCD2 = *KRAS* 12CYS; FCD = *KRAS* 12CYS
- [b] CCD1 = *KRAS* 12ALA; CCD2 = *KRAS* 12VAL; FCD = *KRAS* 12VAL

[4] The replicates of the comparator (CCD1/CCD2 = *KRAS* negative) were discordant with FCD (*KRAS* 13VAL; c.38_39delinsTT). It is inferred that *therascreen®* KRAS is not designed to detect complex SNVs, this result may indicate an error by *therascreen®* KRAS Assay.

The agreements for the non-inferiority test proposed by Li (2016)[1] using the data from the 2x2x2 contingency table above are shown in the table below. Note that, since a mutation-enriched population drawn from the intended use population was used for the study, a correction was performed to adjust the observed PPA and NPA based on the natural frequency of the *KRAS* mutations in the intended use population (see Li (2016)[1] for details).

All the upper bounds of the 95% confidence intervals were determined to be less than 5%, supporting a conclusion that the agreement between the O/RDx-LCCA and Qiagen therascreen® KRAS RGQ PCR is non-inferior to the agreement between two replicates of CCD by a margin of 5%.

Table 41. Calculation of PPA and NPA (CRC)

Parameter	Agreement (%)
PPA_{C1F}	95.7
PPA_{C1C2}	95.7

Parameter	Agreement (%)
NPA_{C1F}	99.2
NPA_{C1C2}	100.0
PPA_{C2F}	97.8
PPA_{C2F} †	97.8
PPA_{C2C1}	100.0
PPA_{C2C1} †	100.0
NPA_{C2F}	97.7
NPA_{C2F} †	98.0
NPA_{C2C1}	96.9
NPA_{C2C1} †	97.6

Section 4.2, p.361 in Meijuan Li (2016): Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study, *Statistics in Biopharmaceutical Research*, 8:3, 355-363.

*The parameter Pc, the "true" MAF for the mutation of interest as analyzed by the O/RDx-LCCA, must be estimated experimentally, for the case of *KRAS* codon 12 and 13 mutations, 0.36.

† Adjusted for enrichment

Confidence intervals for PPA and NPA values were determined using a 1000x bootstrap methodology in SAS. Specifically, 1000x n=219 samples were created using seed-number random selection with replacement from both the CCD1+ (n=257) and CCD1- (n=257) populations. Each of the 1000x CCD1+ (n=219) and CCD1- (n=219) random samples were combined (N=438) and ζppa1, ζppa2, ζnpa1and ζnpa2 were calculated. The lower and upper 95% confidence limits for the x1000 ζppa1, ζppa2, ζnpa1and ζnpa2 values are reported in the following table.

The following hypotheses were tested:

- H0: ζppa1 = (95.7 - 95.7 = 0.0%) ≥ 10% vs. Ha: ζppa1 < 10%
- H0: ζppa2 = (100.0 - 97.8 = 2.2%) ≥ 10% vs. Ha: ζppa2 < 10%
- H0: ζnpa1 = (100.0 - 99.2 = 0.8%) ≥ 10% vs. Ha: ζnpa1 < 10%
- H0: ζnpa2 = (97.6 – 98.0 = -0.4%) ≥ 10% vs. Ha: ζnpa2 < 10%

Table 42. Hypothesis testing parameter output for *KRAS* - CRC

Parameter	Mean %	95% Upper Confidence Interval
ζppa1	0.0	3.3
ζppa2	2.2	4.9
ζnpa1	0.8	2.0
ζnpa2	-0.4	0.8

All upper bounds of the 95% confidence intervals were determined to be less than 5%, supporting the conclusion that the agreement between the O/RDx-LCCA and QIAGEN *therascreen*® KRAS RGQ PCR is non-inferior to the agreement between two replicates of CCD by a margin of 5%.

1. **Subgroup Analyses**
   Preoperative characteristics were not evaluated for potential association with outcomes due to this not being a prospective study.

2. **Pediatric Extrapolation**
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The clinical concordance study included 2 investigators of which 1 was full-time or part-time employees of the sponsor and 1 had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0
- Significant payment of other sorts: 0
- Proprietary interest in the product tested held by the investigator: 1
- Significant equity interest held by investigator in sponsor of covered study: 1

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Analytical performance studies were conducted with the O/RDx-LCCA test using FFPE tissue or DNA extracted from FFPE tissue from NSCLC and CRC indications. When the test is used in accordance with the directions provided, the sensitivity for

detecting the tested variants is shown in the studies above. The clinical benefit of the O/RDx-LCCA test in the detection of alterations listed in Table 1 of the intended use statement was demonstrated in two clinical concordance studies using previously approved CDx tests as the comparator methods. All studies based on non-inferiority (NI) statistical testing approach passed the acceptance criteria specified in each study protocol. The concordance observed between the O/RDx-LCCA test and the approved CDx tests supports the effectiveness of the O/RDx-LCCA test to identify patients whose tumors are positive for the alterations listed in Table 1 of the intended use and may respond to the associated therapeutics listed in the same table.

B. Safety Conclusions

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

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

Adverse effects that occurred in the PMA clinical study: None.

This clinical investigation did not involve human subjects but did involve human tissue samples. In all cases, the tissues used were remnant, left-over tissues.

- There were no adverse reactions or complications to patients (due to use of remnant samples) or operators
- There were no instances of patient discontinuation or patient complaints
- There were no instances of clinical investigator complaints
- There were no device failures requiring replacement (assay kits and software were shipped in production-equivalent state)
- Patient death metrics are not available due to this being a retrospective investigation where patient information has been de-identified

C. Benefit-Risk Determination

The probable benefit of the O/RDx-LCCA to select patients with *EGFR* Exon 19del/L858R in NSCLC for treatment with EGFR tyrosine kinase inhibitors approved by the FDA and *KRAS* wild-type (absence of mutation in codons 12 and 13) patients in CRC for treatment with cetuximab or panitumumab was established through clinical concordance studies using a non-inferiority statistical testing approach. For

the *EGFR* Exon 19del/L858R in NSCLC indication, the assay O/RDx-LCCA was compared to two replicates of an approved test (Roche **cobas**® v2 EGFR Mutation Test); and the clinical concordance studies showed non-inferiority to the approved method, indicating probable benefit of this device in selecting patients with these alterations for treatment with EGFR tyrosine kinase inhibitors approved by the FDA. For the *KRAS* wild-type (absence of mutation in codons 12 and 13) indication in CRC, O/RDx-LCCA was compared to two replicates of an approved test (QIAGEN *therascreen*® KRAS RGQ PCR); and the clinical concordance studies show non-inferiority to the approved test, indicating probable benefit of this device in selecting patient without codon 12 and codon 13 *KRAS* mutations for treatment with cetuximab or panitumumab.

There is probable risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user.

The risks of the O/RDx-LCCA for the selection of NSCLC patients with *EGFR* Exon 19del/L858R, for treatment with EGFR tyrosine kinase inhibitors approved by the FDA or CRC patients with the absence of *KRAS* codon 12 or 13 mutations, for treatment with cetuximab or panitumumab, are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The risks of false results are partially mitigated by the analytical and clinical validation results summarized above.

Additional factors to be considered in determining probable risks and benefits for the O/RDx-LCCA included: analytical performance of the device and representation of variants in the major effectiveness studies. The O/RDx-LCCA has been analytically validated as summarized above; however, multiple post-market studies are also planned to supplement and confirm the test performance described above. To supplement the premarket data, some post-market studies are planned as summarized in Section XIV, below. The data support that for the O/RDx-LCCA, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

1. Patient Perspective

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

In conclusion, given the available information above, the data support that for the O/RDx-LCCA, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indication for use. Data from the analytical validation and clinical concordance studies support the performance of O/RDx-LCCA as an aid for the identification of NSCLC and CRC patients for whom the therapies listed Table 1 of the Intended Use statement may be indicated.

XIV. CDRH DECISION

CDRH issued an approval order on July 30, 2021. The final conditions of approval cited in the approval order are described below.

1. In order to provide a more robust assessment of precision near the LoD levels for each CDx variant, Pillar Biosciences must provide robust and high-confidence data from a well-designed 3-site reproducibility study using additional clinical samples carrying CDx biomarkers. The data from this study must be adequate to support precision near LoD for CDx variants in the intended use population.

2. Pillar Biosciences will provide results from the following stability studies using intended use specimen type as follows:

    i. Pillar Biosciences will provide robust and high confidence data from a stability study which uses FFPE blocks from the CDx intended use patients at low tumor content and close to the newly defined LoD levels, to the extent possible, starting from baseline measurement with stability data collected in a controlled prospective manner to support FFPE block stability claim.

    ii. Pillar Biosciences will provide robust and high confidence data from a stability study which uses FFPE-extracted DNA samples from the CDx intended use patients with allelic frequencies near the newly defined LoD (i.e., 1-1.5x LoD) and at low DNA inputs (30 ng) to supplement the DNA stability claim at -20°C for the CDx variants.

    The data from these studies must be adequate to support stability claims for the CDx variants in the intended use population.

3. Pillar Biosciences must provide robust and high confidence data from a study using clinical specimens to demonstrate that different lots of the O/RDx-LCCA reagent kit components may be used interchangeably. The data from these studies must be adequate to support that different lots of the O/RDx-LCCA reagent kit components do not impact the results of the assay.

Pillar Biosciences must provide detailed protocols, including acceptance criteria where appropriate, for the studies that are noted above as conditions of approval. These studies

must be adequate to confirm the safety and effectiveness of the O/RDx-LCCA and must include a detailed description of the numbers of sample to be tested, the type of samples to be tested, the tumor types for each sample, the complete testing protocol, and a robust statistical analysis plan. These protocols must be submitted to FDA no later than 60 days after approval.

The final study data, study conclusions, and labeling revisions should be submitted within 1 year of the PMA approval date, unless stated otherwise.

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

1. Meijuan Li. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. Statistics in Biopharmaceutical Research, 8:3, 355-363 (2016).