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: Agilent Resolution ctDx FIRST

Device Procode: PQP

Applicant's Name and Address: Resolution Bioscience, Inc.
Suite 200
550 Kirkland Way
Kirkland, WA 98033

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P210040

Date of FDA Notice of Approval: December 12, 2022

II. INDICATIONS FOR USE

The Agilent Resolution ctDx FIRST assay is a qualitative next generation sequencing-based, *in vitro* diagnostic test that uses targeted hybrid-capture sequencing technology to detect and report single nucleotide variants (SNVs) and deletions in two genes. The Agilent Resolution ctDx FIRST assay utilizes circulating cell-free DNA (cfDNA) isolated from plasma of peripheral whole blood collected in Streck Cell-Free DNA Blood Collection Tubes (BCTs). The test is intended as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) who may benefit from treatment with the targeted therapy listed in Table 1, in accordance with the approved therapeutic labeling.

Table 1. Companion Diagnostic Indication

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*KRAS* G12C	KRAZATI™ (adagrasib)

A negative result from a plasma specimen does not assure that the patient's tumor is negative for genomic findings. Patients with NSCLC who are negative for the biomarker listed in Table 1 should be reflexed to tissue biopsy testing for Table 1 biomarker using an FDA-approved tumor tissue test, if feasible.

Additionally, the test is intended to provide tumor mutation profiling for SNVs and deletions in the *EGFR* gene for use by qualified health care professionals in accordance with professional guidelines in oncology for patients with NSCLC. The test is for use

with patients previously diagnosed with NSCLC and in conjunction with other laboratory and clinical findings.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

The Agilent Resolution ctDx FIRST assay is a single-site assay performed at Resolution Bioscience, Inc.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions are listed below:

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Patients for whom no companion diagnostic alteration is detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.
- Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).
- When collecting the whole blood in the Streck Cell-Free blood collection tube, allow the tube to fill completely until blood stops flowing into the tube. Underfilling of tubes with less than 5 mL of blood (bottom of the label indicates 5 mL fill when tube is held vertically) may lead to incorrect analytical results or poor product performance. The tube has been designed to fill with 10 mL of blood.

V. DEVICE DESCRIPTION

The Agilent Resolution ctDx FIRST assay is a single-site test performed at Resolution Bioscience, Inc. located at 550 Kirkland Way, Suite L100, Kirkland, WA 98033. The test uses next-generation sequencing to analyze small fragments of cfDNA, including tumor-derived cell free DNA fragments (cfDNA), isolated from plasma to detect gene alterations in patients with NSCLC. The test includes reagents, software, and procedures for testing cfDNA from whole blood samples.

Peripheral venous blood samples are collected in Streck cell-free DNA blood collection tubes and shipped to the Resolution Bioscience Clinical Laboratory Improvement Amendments (CLIA) laboratory (Kirkland, Washington) for testing. The Agilent

Resolution ctDx FIRST assay employs a single DNA extraction method (KingFisher Flex™ System) from plasma specimens which undergoes multiple automated and manual steps, including library preparation/amplification (Hamilton Microlab Star Automation), hybridization and capture, sequencing (NovaSeq 6000) and data analysis and reporting (Agilent Resolution ctDx FIRST assay software).

Agilent Resolution ctDx FIRST is designed to sequence 109 genes, but only reports the pre-defined alterations outlined in the indications for use. The assay is not approved for reporting of individual variants outside the variants listed in the indications for use. Agilent Resolution ctDx FIRST detects, but does not differentiate, germline and somatic mutations present in the cfDNA using a targeted panel of gene/region-specific probes, *i.e.,* it is not a whole-genome or whole-exome application.

Test Output
The test report includes variants reported in the following categories: see Table 2.

Table 2. Category Definitions

Category	Agilent Resolution ctDx FIRST			Comments
	Prescriptive Use of Therapeutic Product	Clinical Performance	Analytical Performance	
Category 1: Companion Diagnostic (CDx)	Yes	Yes	Yes	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Agilent Resolution ctDx FIRST has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.
Category 2: ctDNA Biomarkers with Strong Evidence of Clinical Significance	No	No	Yes	ctDNA biomarkers with strong evidence of clinical significance presented by other FDA-approved liquid biopsy companion diagnostics for which Agilent Resolution ctDx FIRST has demonstrated analytical reliability but not clinical performance.

Test Kit Contents
The test includes the Agilent Resolution Sample Collection Kit, which is sent to ordering laboratories. Each Sample Collection Kit contains two Streck Cell-Free DNA blood collection tubes. The Sample Collection Kit also contains supporting packaging materials, instructions for use and a return shipping label.

The Agilent Resolution Sample Collection Kit contains the following components:

1. Kit Instructions-for-Use (IFU): Whole Blood Collection and Shipping
2. Test Requisition Form Sample Collection (TRF)
3. Streck Cell-Free DNA BCT blood collection tubes (2)
4. Adhesive sample identification labels (3)
5. Biohazard bag
6. Refrigerant thermal shipping pack
7. Overnight shipping express envelope
8. Return shipping label

The test also includes commercially available reagents, as well as specific reagents developed by Resolution Bioscience. All reagents included in the Agilent Resolution ctDx FIRST assay process are qualified by Resolution Bioscience and are compliant with the medical device regulations (21CFR820). The kit is assembled into four (4) different boxes (refered to as box 1, 2, 3, and 4) based on the usage of the reagents. The Agilent Resolution ctDx FIRST Reagent Kit includes:

- Resolution ctDx FIRST, IVD (PN 500025)
- Resolution Barcodes 57-80 Kit, IVD (PN 500015) (hereafter referred to as Barcodes)
- Resolution Barcodes 81-104 Kit, IVD (PN 500016) (hereafter referred to as Barcodes)
- Resolution CORE, IVD (PN 500013) (hereafter referred to as CORE)

Instruments
All instruments are qualified under Resolution Bioscience's Quality System and used according to manufacturers' instructions. The Agilent Resolution ctDx FIRST assay is intended to be performed with the following serial number-controlled instruments (Table 3).

Table 3: Serial Number Controlled Instruments

Instrument
Thermo Fisher Scientific Qubit Fluorometer
Thermo Fisher Scientific KingFisher Flex System
Hamilton Star Automated Liquid Handler
Illumina NovaSeq 6000 Sequencing System
Applied Biosystems MiniAmp Thermal Cyclers

Agilent Resolution ctDx FIRST Assay Test Process

Whole Blood Collection and Shipping
Agilent Resolution Sample Collection Kit is used by ordering laboratories/physicians to collect whole blood specimens and ship them Resolution Bioscience. A minimum

of 5 mL whole blood must be received in order to achieve optimal performance for the Agilent Resolution ctDx FIRST assay. Underfilling of tubes with less than 5 mL of blood may lead to incorrect analytical results or poor product performance.

Plasma Isolation and cfDNA Extraction
Whole blood specimens are processed in the Resolution Bioscience Clinical Laboratory within 7 days of blood collection. Plasma is isolated from both tubes of whole blood via centrifugation. One tube of plasma is stored, while the second tube is used for cfDNA extraction using the KingFisher FlexTM System and MagMax cfDNA Isolation kit (Thermo Fisher). The resulting cfDNA is quantified using the Qubit Fluorometer. Input amounts ranging from 15 ng to 50 ng of cfDNA are further processed for each sample.

Sequencing Library Preparation (Hamilton Microlab Star Automation)
Reagents from the Resolution Bioscience are used during library preparation. Unique molecular barcodes, specific to Resolution Bioscience's sequencing chemistry, are ligated to each cfDNA molecule and amplified to create the genomic libraries.

Hybridization
Genomic libraries are pooled, hybridized with biotinylated probes, and further processed to create sequence ready libraries.

Sequencing
Libraries are sequenced on the Illumina NovaSeq 6000 sequencing instrument.

Data Analysis and Reporting
The Agilent Resolution ctDx FIRST Pipeline software uses customized cloud-based analysis software developed by Resolution Bioscience, referred to as the Caller Core. The Caller Core uses the raw data from targeted sequencing, screens for quality controls, and conducts variant detection and analysis by alignment with hg19 human reference genome. All variants must pass variant calling metrics as described in Table 4.

Log likelihood scores are calculated based on mutant allele fraction (MAF) and number of unique variant molecules. This log likelihood score reflects the relative log likelihood of the SNV being a true variant compared to it being noise. The MAF estimate is the calculated allelic fraction of an SNV or indel. The number of DNA molecules describes the minimum unique variant molecules, with both strands represented and with each variant base having a phred quality value greater than or equal to 20 for SNVs. Phred quality scores are directly linked to the error probability of that base call.

Table 4. Variant Calling Threshold/Cut-Off Metrics

SNV Calling Property	Metric
KRAS G12C DNA Molecule Support	≥ 3
KRAS G12C MAF estimate	≥ 0.1%
KRAS G12C Log Likelihood Score	> 6 (if DNA Molecule Support = 3), > 0 (if DNA Molecule Support ≥4)
Other[a] SNVs DNA Molecule Support	≥ 3
Other SNVs MAF estimate	≥ 0.1%
Other SNVs Log Likelihood Score	≥ 10
Indel[b] Calling Property	**Metric**
INDEL 1 nt Molecule Support	≥ 3
INDEL 1nt MAF estimate	≥ 0.1%
INDEL 1nt Log Likelihood Score	≥ 20
INDEL 2-3 nt Molecule Support	≥ 3
INDEL 2-3 nt MAF estimate	≥ 0.1%
INDEL 2-3 nt Log Likelihood Score	≥ 15
INDEL 4-6 nt Molecule Support	≥ 3
INDEL 4-6 nt MAF estimate	≥ 0.1%
INDEL 4-6 nt Log Likelihood Score	≥ 10
INDEL > 6 nt Molecule Support	≥ 4
INDEL > 6 nt MAF estimate	≥ 0.1%
INDEL > 6 nt Log Likelihood Score	> 2

a: *EGFR* L858R and T790M only
b: *EGFR* exon 19 deletions only

Biomarker Status Determination
Biomarker status (*i.e.,* biomarker positivity) is determined based on the custom variant logic applied to the Agilent Resolution ctDx FIRST assay pipeline.

Reporting
The laboratory and physician receive a qualitative alteration-level result. Test output files, including a PDF test report with patient-specific results, are automatically generated by Resolution Bioscience's Report Generation Module. The Report Generation Module uses an assay-specific Report Template. A sample will receive an overall "Failed" result when any of the QC metrics is failed. Samples failing any QC metric are automatically held and not released.

User Interface
Lab end-users use the cloud-based Resolution Analysis Platform (RAP) Web Application to create Run Configurations for the assay and to download PDF test reports. The RAP Web Application represents the sole user interface for the Agilent Resolution ctDx FIRST assay software and is delivered to Resolution Bioscience's HIPAA-compliant Amazon Web Services (AWS) account.

Quality Control (QC) Measures

Quality Controls have been built into the Agilent Resolution ctDx FIRST assay workflow and functionality to ensure that the assay processes data of sufficient quality and that variants reported by the assay reflect a high level of confidence.

Quality measures have been applied in four QC categories within the Agilent Resolution ctDx FIRST assay:

- Wet Lab Processing & Sequencing QCs
  Wet-lab QCs related to plasma and cfDNA input QCs, and genomic and target-captured library concentrations.

- Positive Control
  Required in each Agilent Resolution ctDx FIRST Assay Run, where this control is processed in parallel with each set of clinical samples. The Agilent Resolution ctDx FIRST Assay Positive Control is comprised of a genomic DNA mixture of cell line DNA, sheared to similar molecular profile cfDNA representing relevant SNVs and Indels in molecular grade water.

- Software QCs Included in Run Configuration Data
  Lab designation of the samples belonging to a specific Agilent Resolution ctDx FIRST Assay Run, including assignment of a unique sample barcode to each Sample in a Run Configuration Template. Software QCs are run against the Run Configuration File to confirm proper sequencing run setup.

- Assay Pipeline Software QCs
  Automated screening, processing, and sample analysis of raw sequence data received from sequencing instruments by the assay pipeline software. The Agilent Resolution ctDx FIRST Assay Pipeline verifies percentage of on-target reads, sequence read quality, overall data quality, generates unique consensus reads, and calls variants, applies the biomarker logic, and generates the biomarker report.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *KRAS* G12C mutations for the identification of patients with NSCLC for treatment with KRAZATI (adagrasib). However, there is one FDA approved CDx alternative for the detection of *KRAS* G12C mutation in patients with NSCLC using tissue specimens for treatment with KRAZATI (adagrasib): QIAGEN *therascreen*® KRAS RGQ PCR Kit. Each alternative has its own advantages and disadvantages. A patient should fully discuss any alternative with their physician to select the most appropriate method that best meets expectations and lifestyle. For additional details see list of FDA Cleared or Approved Companion Diagnostic Devices at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

VII. MARKETING HISTORY

The Agilent Resolution ctDx FIRST has not been marketed in the United States or any foreign country.

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, may lead to inappropriate patient management decisions. Patients with false positive results may undergo treatment with the therapy listed in the intended use statement without clinical benefit and may experience adverse reactions associated with these therapies. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

For the specific adverse events that occurred in the clinical studies, please see the KRAZATI (adagrasib) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of the Agilent Resolution ctDx FIRST for detecting *KRAS* G12C mutations and *EGFR* mutations (*EGFR* L858R, *EGFR* T790M, *EGFR* Exon 19 deletions) in patients with NSCLC was from the data generated using intended use specimens across all validation studies presented below. The clinical samples consisted of pools of cfDNA from patients with NSCLC.

1. Analytical Accuracy/Concordance with an Orthogonal Method

a. Concordance between Agilent Resolution ctDx FIRST and an orthogonal method for *KRAS* G12C CDx variant

The Agilent Resolution ctDx FIRST was compared to the results of an externally validated digital droplet polymerase chain reaction (ddPCR) assay. A total of 230 cfDNA samples from patients with NSCLC were tested across both assays [76 *KRAS* G12C mutation-positive samples from KRAZATI efficacy population (KRYSTAL-1 clinical study) and 154 commercially procured plasma samples representative of the clinical trial population from patients with NSCLC with non-specified *KRAS* G12C mutation status]. Of these, seven samples failed to generate valid results (2 from *KRAS* G12C mutation-positive samples and 5 from *KRAS* G12C mutation-negative samples) in the Agilent Resolution ctDx FIRST assay due to sequencing QC failures. The remaining 223 samples generated valid results by both assays and the concordance between Agilent Resolution ctDx FIRST and ddPCR was calculated (Table 5). A summary of positive percent agreement (PPA) and negative percent agreement (NPA) in reference to ddPCR assay and corresponding 95% two-sided exact confidence intervals (CIs) is provided in

Table 5, below. Out of the 54 samples that were *KRAS* G12C positive by ddPCR testing (51 from the KRYSTAL-1 study), 47 were confirmed positive by Agilent Resolution ctDx FIRST, resulting in a PPA of 87.0% (75.1, 94.6). Out of 169 samples that were *KRAS* G12C negative by the ddPCR assay, 165 were confirmed as negative by the Agilent Resolution ctDx FIRST assay, resulting in a NPA of 97.6% (94.1, 99.4).

Table 5: *KRAS* G12C Concordance Results Between Agilent Resolution ctDx FIRST and ddPCR

		ddPCR *KRAS* G12C Positive	ddPCR *KRAS* G12C Negative	Total
Agilent Resolution ctDx FIRST	***KRAS* G12C-Positive**	47	4	51
	***KRAS* G12C-Negative**	7	165	172
	Total	54	169	223
	PPA: 87.0% (75.1, 94.6) **NPA: 97.6%** (94.1, 99.4)			

In total, there were 11 discordant results between the two assays (Table 5). However, the average coverage depth by the Agilent Resolution ctDx FIRST assay was equivalent between the discordant samples (4579 depth) and all tested samples (4579 depth).

The four discordant samples were *KRAS* G12C positive by the Agilent Resolution ctDx FIRST Assay but negative by ddPCR. These four samples were detected at low percent mutant allele frequency (%MAF) for *KRAS* G12C (0.18%, 0.13%, 0.11% and 0.40%), which is below LoD by the Agilent Resolution ctDx FIRST Assay.

Of the seven samples that were *KRAS* G12C negative by the Agilent Resolution ctDx FIRST Assay but positive by ddPCR, one sample had a *KRAS* AG11AC call, which is a dinucleotide substitution functionally equivalent to *KRAS* G12C. While, in five of the six remaining samples, *KRAS* G12C was detected but filtered out by the variant disclosure threshold of the Contamination QC due to low level contamination. Further, among these five samples, three were from KRYSTAL-1 Cohort A and had stable disease, and two were from commercial sources with no clinical information. Therefore, only one sample out of the seven had a true discordant call.

b. Concordance between the Agilent Resolution ctDx FIRST and a validated orthogonal method for clinically significant *EGFR* variants

The detection capability for *EGFR* exon 19 Deletions, *EGFR* L858R, and *EGFR* T790M variants was compared between the Agilent Resolution ctDx FIRST assay and an externally validated ddPCR assay. A total of 183 cfDNA samples from plasma specimens from patients with NSCLC were tested across

both assays (91 samples from patients with NSCLC were selected from the Resolution Bioscience biobank based on Resolution Bioscience CLIA assays and 92 commercially procured plasma samples from NSCLC subjects with non-specified *EGFR* mutation status). Twenty-nine (29) samples failed to generate valid results in the Agilent Resolution ctDx FIRST assay. The remaining 154 samples (65 remnant bio-banked and 89 commercially procured plasma samples) generated a total of 334 comparative results that were valid across both assays and evaluated for variant concordance (Table 6). The PPA was 96.6% (28/29) for *EGFR* L858R, 90.0% (9/10) for *EGFR* T790M, and 93.5% (29/31) for *EGFR* exon 19 Deletions. Of the 264 total results that were mutation-negative by the ddPCR assay, only two were discordant and classified as positives by the Agilent Resolution ctDx FIRST assay; both variants were very low frequency mutations (0.19%, 0.21%), which are below the limit of detection of the test. Therefore, NPA ranged from 97.8% to 100% across the three variants. The reported PPA and NPA were not adjusted for the distribution of samples selected using Resolution Bioscience LDT results.

Table 6: Concordance Results Between Agilent Resolution ctDx FIRST and ddPCR for clinically significant *EGFR* variants

		EGFR L858R		**EGFR T790M**		**EGFR exon 19 Deletions**	
		ddPCR Positive	**ddPCR Negative**	**ddPCR Positive**	**ddPCR Negative**	**ddPCR Positive**	**ddPCR Negative**
Agilent Resolution ctDx FIRST	**Positive**	28	2	9	0	29	0
	Negative	1	88	1	89	2	85
	Total	29	90	10	89	31	85
		PPA: 96.6% (82.2, 99.9) **NPA: 97.8%** (92.2, 99.7)		**PPA: 90.0%** (55.5, 99.7) **NPA: 100%** (95.9, 100)		**PPA: 93.5%** (78.6, 99.2) **NPA: 100%** (95.8, 100)	

2. Analytical Sensitivity

a. Limit of Blank (LoB)

The LoB was established in cfDNA extracted from healthy plasma from risk-matched healthy donors. Thirty (30) donor samples were processed using 50 ng of cfDNA input with the Agilent Resolution ctDx FIRST (highest DNA input for the assay) across three to six lots of reagents, instruments, and operators. Each of the 30 donor samples were tested with six replicates for a total of 180 results to assess the false positive rate of Agilent Resolution ctDx FIRST assay. This study demonstrated a zero false positive rate for *KRAS* G12C (CDx) and level 2 *EGFR* variants (Table 7).

Table 7: LoB Study Summary Results

Variant	False Positives/ Sample Replicates	False Positive Rate
KRAS G12C	0/180	0%
EGFR exon 19 deletions	0/180	0%
EGFR L858R	0/180	0%
EGFR T790M	0/180	0%

b. Limit of Detection (LoD)

The LoD for the Agilent Resolution ctDx FIRST variants with CDx claims, and clinically significant (level 2) claims was established using three pools of clinical cfDNA samples from patients with NSCLC with *KRAS* G12C, *EGFR* exon 19 deletions, *EGFR* L858R, and *EGFR* T790M variants diluted in cfDNA from risk-matched healthy donor plasma samples. Sample pools were serially diluted to a range of MAF levels spanning 0.049–4.0% MAF. For each sample pool, six or seven MAF levels were tested, and 35–36 sample replicates were tested at each MAF level at the minimum allowable cfDNA input of 15 ng.

The LoD was determined for each variant by calculating the hit rate at each MAF level across samples and performing probit analysis, as described in CLSI EP17-A2. LoDs for *KRAS* G12C and *EGFR* variants are summarized in Table 8.

Table 8: Established LoDs for *KRAS* G12C and *EGFR* Variants in NSCLC clinical Samples

Variant	LoD (MAF)
KRAS G12C	0.581%
EGFR exon 19 deletions	0.380%
EGFR L858R	0.505%
EGFR T790M	0.983%

3. Analytical Specificity

a. Interfering Substances

To evaluate the performance of the Agilent Resolution ctDx FIRST in the presence of potential interfering substances, a total of 13 potential interferents were evaluated. These potential interfering substances included six endogenous interferents [hemoglobin, albumin, unconjugated bilirubin, conjugated bilirubin, glyceryl trioleate (Triglycerides), and high molecular weight gDNA], six exogenous interferents (paracetamol, prednisone, dexamethasone, metoclopramide hydrochloride, ethanol, and proteinase K) and one microbial interferent, *Staphylococcus epidermidis* (*S. epidermidis*).

Two samples/variant were tested to evaluate the potential interference of hemoglobin, albumin, unconjugated bilirubin, conjugated bilirubin, glyceryl trioleate, high molecular weight gDNA, *S. epidermidis,* paracetamol,

prednisone, dexamethasone, metoclopramide hydrochloride, ethanol, and proteinase K. Plasma sample blends were prepared from cfDNA extracted from NSCLC clinical plasma samples diluted into risk-matched healthy donor plasma to generate sample blends at allelic frequencies close to LoD with *KRAS* G12C (1.1 - 2.1x LoD), *EGFR* L858R (1.6 - 2.9x LoD), *EGFR* exon 19 deletions (1.3 - 4.7x LoD), and *EGFR* T790M (1.5 - 2.2x LoD) variants. Five to six plasma sample replicates were spiked with each interferent and proceeded to testing with the Agilent Resolution ctDx FIRST assay. For each potential interferent, concordance of alteration calls for Positive and Negative Percent Agreement (PPA and NPA, respectively) was calculated relative to diluent control of the respective sample (without interferent). Apart from paracetamol, PPA and NPA were 100% for all interferents across all variants (Table 9 and Table 10). In the presence of paracetamol, a single *KRAS* G12C variant was not detected, resulting in a PPA of 91.7% (11/12 detected); the median %MAF for *KRAS* G12C in this sample was below LoD (0.93x LoD).

Table 9: Interfering Substances PPA/NPA Summary Results for *KRAS* G12C

Substance	Observed/ Expected Positive	PPA (95% CI [%])	Observed/ Expected Negative	NPA (95% CI [%])
Hemoglobin, 10 mg/mL	12/12	100% (75.8, 100)	5/5	100% (56.6, 100)
Albumin, 60 mg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Unconjugated Bilirubin, 0.4 mg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Conjugated Bilirubin, 0.4 mg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Glyceryl Trioleate (Triglycerides), 15 mg/mL	12/12	100% (75.8, 100)	5/5	100% (56.6, 100)
Paracetamol, 0.156 mg/mL	11/12	91.7% (64.6, 100)	6/6	100% (61.0, 100)
Prednisone, 0.099 µg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Dexamethasone, 12 µg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Metoclopramide, 2.52 µg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)

Substance	Observed/ Expected Positive	PPA (95% CI [%])	Observed/ Expected Negative	NPA (95% CI [%])
Ethanol, 5%	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
Proteinase K, 0.6 mg/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
High Molecular Weight genomic DNA (HMW gDNA), 1:1 (w/w)	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)
S. epidermidis, 10^6 CFU/mL	12/12	100% (75.8, 100)	6/6	100% (61.0, 100)

Table 10: Interfering Substances PPA/NPA Summary Results for *EGFR* L858R, *EGFR* exon 19 deletions, *EGFR* T790M

Substance	Observed/ Expected Positive	PPA (95% CI [%])	Observed/ Expected Negative	NPA (95% CI [%])
Hemoglobin, 10 mg/mL	28/28	100% (87.0, 100)	22/22	100% (85.1, 100)
Albumin, 60 mg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Unconjugated Bilirubin, 0.4 mg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Conjugated Bilirubin, 0.4 mg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Glyceryl Trioleate (Triglycerides), 15 mg/mL	28/28	100% (87.0, 100)	22/22	100% (85.1, 100)
Paracetamol, 0.156 mg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Prednisone, 0.099 µg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Dexamethasone, 12 µg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Metoclopramide, 2.52 µg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Ethanol, 5%	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
Proteinase K, 0.6 mg/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
HMW gDNA, 1:1 (w/w)	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)
S. epidermidis, 10^6 CFU/mL	30/30	100% (88.6, 100)	24/24	100% (86.2, 100)

b. *In Silico* Specifity analysis (Primer Specificity)

An *in silico* specificity study was conducted to evaluate the potential for Agilent Resolution ctDx FIRST probes to amplify non-specific products from human DNA and to assess the potential for incorrect results due to commensal microorganism contamination. For this *in silico* study, the probe sequences were aligned to the human genome (hg19).

In addition, a modified version of the human genome was generated by adding sequences from internal reference genome database for commensal and pathogenic microorganisms. Finally, sequencing primer sequences were checked for specificity using NCBI's primer BLAST tool.

When mapped to the human genome, probes for *KRAS* G12X and *EGFR* were unique to those genes. None of the probes for *KRAS* G12X and *EGFR* mapped to the representative microbial contaminant genomes. No off-target amplicons <4000 bp were predicted for the primers using the web-based NCBI Primer-BLAST tool.

Based on the results, all of the panel coverage results for *KRAS* G12X and *EGFR* level 2 variants satisfy the desired performance. There is minimal risk of Agilent Resolution ctDx FIRST producing a false positive patient result for *KRAS* G12X and *EGFR* level 2 variants due to detection of microbial sequences or mis-alignment of Agilent Resolution ctDx FIRST primers and probes in the human genome.

4. Precision

The purpose of the precision studies was to demonstrate the repeatability and within-site reproducibility of Agilent Resolution ctDx FIRST through closeness of agreement between measured qualitative output obtained in replicate testing using different combinations of reagent lots, instruments, operators, and days. Additionally, to demonstrate precision of analytically blank samples, precision was evaluated in 30 risk-matched healthy donor samples tested in the LoB study.

a. Precision from cfDNA

Precision of variant detection for *KRAS* G12C, *EGFR* exon 19 deletions, *EGFR* L858R, and *EGFR* T790M from cfDNA was evaluated in the Agilent Resolution ctDx FIRST by analyzing the LoD establishment dilution levels (which are performed over multiple days across unique combinations of experimental components) that were closest to ~1.5x and 2-3x LoD based on the estimated LoD. LoB data is used for calculating negative precision.

Data from three pools of NSCLC clinical samples with *KRAS* G12C, *EGFR* exon 19 deletions, *EGFR* L858R, and *EGFR* T790M variants that were diluted in cfDNA from risk-matched healthy donor plasma and tested with 35–36 replicates at the minimum allowable assay input of 15 ng cfDNA were used. Repeatability including intra-run performance and reproducibility including inter-run performance were assessed and compared across different precision combinations. Each combination was compiled from six sequencing reagent lots, six Agilent Resolution ctDx FIRST reagent lots, two Illumina NovaSeq 6000 sequencers with two sides per instrument, and six operators.

For each variant, PPA and Average Percent Agreement (APA) were calculated at the MAF levels that were closest to 1.5x and 2–3x LoD. Across all variants, PPA ranged from 96.3% to 100% at 1.5x LoD and was 100% at 2–3x LoD (Table 11). In addition to demonstrating robust precision for detecting *KRAS* G12C mutation, the Agilent Resolution ctDx FIRST demonstrated adequate precision to distinguish *KRAS* G12C (C>A at position 2539825) and *KRAS* G12V (C>A at position 2539824), as shown in Table 11.

Table 11: Precision PPA Results Summary for *KRAS* G12C, and *EGFR* variants

Variant	1.5x LoD			2–3x LoD		
	%MAF	Positive/Expected	PPA (95% CI)	%MAF	Positive/Expected	PPA (95% CI)
KRAS G12C	0.6–0.7%	104/108	96.3% (90.9, 98.6)	1.3–1.5%	108/108	100% (96.6, 100)
KRAS G12V	0.7%	36/36	100% (85.8, 99.9)	1.5%	36/36	100% (90.4, 100)
EGFR L858R	0.5–0.7%	70/72	97.2% (90.4, 99.2)	1.2–1.3%	72/72	100% (94.9, 100)
EGFR exon 19 deletions	0.3–0.5%	70/72	97.2% (90.4, 99.2)	0.6–0.8%	72/72	100% (94.9, 100)
EGFR T790M	1.2%	36/36	100% (90.4, 100)	2.3%	36/36	100% (90.4, 100)

For each experimental component (Agilent Resolution ctDx FIRST reagent lots, operators, sequencing reagent lots, Illumina NovaSeq 6000 sequencers), APAs between individual components ranged from 96.2% to 100% across the four variants at 1.5x LoD and were 100% for all variants at 2–3x LoD (Table 12). Repeatability was assessed where possible by comparing replicates matched within runs or within operators; it ranged from 96.2% to 100% across the four variants at 1.5x LoD and was 100% across all variants at 2–3x LoD (Table 12). In addition to demonstrating robust precision for detecting *KRAS* G12C mutation, the Agilent Resolution ctDx FIRST demonstrated adequate precision to distinguish *KRAS* G12C (C>A at position 2539825) and *KRAS* G12V (C>A at position 2539824), as shown in Table 12.

Table 12: Precision Results Summary for *KRAS* G12C and *EGFR* Variants

Variant	LoD Level	ctDx FIRST Reagent Lots	Operators	Sequencing Reagent Lots	Sequencers	Within Runs (Repeatability)
KRAS G12C	1.5X	96.2% (92.0, 99.1)	96.2% (92.3, 99.1)	96.2% (92.2, 99.1)	96.2% (92.2, 99.1)	96.2% (92.0, 99.1)
	2-3X	100% (96.6, 100)	100% (96.6, 100)	100% (96.6, 100)	100% (96.6, 100)	100% (96.6, 100)
KRAS G12V	1.5X	97.1% (90.0, 100)	97.1% (90.9, 100)	97.1% (90.9, 100)	97.1% (90.9, 100)	97.1% (90.9, 100)
	2-3X	100.0% (90.4, 100)	100.0% (90.4, 100)	100.0% (90.4, 100)	100.0% (90.4, 100)	100.0% (90.4, 100)
EGFR L858R	1.5X	97.3% (93.0, 100)	97.3% (93.0, 100)	97.3% (93.3, 100)	97.2% (93.2, 100)	97.1% (92.5, 100)
	2-3X	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)
EGFR exon 19 deletions	1.5X	97.3% (93.3, 100)	97.3% (93.3, 100)	97.3% (93.3, 100)	97.2% (94.3, 100)	97.1% (92.5, 100)
	2-3X	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)

Variant	LoD Level	ctDx FIRST Reagent Lots	Operators	Sequencing Reagent Lots	Sequencers	Within Runs (Repeatability)
EGFR T790M	1.5X	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)
	2-3X	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)	100% (90.4, 100)

For NPA and Average Negative Agreement (ANA) analysis, precision was evaluated in 30 risk-matched healthy donor samples tested in the LoB study. Each of the 30 donors was tested 6 times for a total of 180 results using 50 ng of cfDNA input (highest DNA input for the assay). Libraries were prepared with three Agilent Resolution ctDx FIRST reagent lots, three sequencing reagent lots, two Illumina NovaSeq 6000 instruments with two sides per instrument, and six operators over multiple days.

There were no false positives in *KRAS* G12C (CDx) and *EGFR* level 2 variants. There was no evidence for differences between operators, Agilent Resolution ctDx FIRST reagent lots, sequencing reagent lots, or sequencers.

b. Precision of Extraction from Plasma

The purpose of this study was to establish the level of variability in the plasma extraction process of the Agilent Resolution ctDx FIRST assay. Precision of the plasma sample extraction process of the Agilent Resolution ctDx FIRST assay was evaluated across three unique precision combinations of KingFisher instruments, KingFisher reagent lots, and operators over multiple days. NSCLC clinical plasma samples were blended with risk-matched healthy donor plasma to create unique samples with *KRAS* G12C, *EGFR* L858R, or *EGFR* exon 19 deletions variants at allelic frequencies close to LoD. Each 4 mL plasma sample was extracted in either singlicate or duplicate.

Forty (40) samples comprised of 23 *KRAS* G12C, 15 *EGFR* L858R, and 19 *EGFR* exon 19 deletion replicates per precision combination, were evaluated for variant agreement. In each sample, variant concordance was compared between pairs of results within the same precision combination or between precision combinations. Across pooled sample and variant results, between- and within-precision combination APA and ANA were both >98%, and the lower 95% confidence intervals for each statistic were >95% (Table 13). The discordants were from two different individual replicates in which the variant was not seen in two of the three precision combinations. However, there is no pattern to these discordances by variants or precision combinations PC. There were no *KRAS* G12C discordant pairs.

Table 13: Precision of Plasma Extraction for *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 19 deletions

	Number of Detected Pairs			APA (95% CI)	ANA (95% CI)
	Concordant Positive	Concordant Negative	Discordant		
Between-Precision Combination	159	329	4	98.8% (96.8, 100)	99.4% (98.5, 100)
Within-Precision Combination	52	108	2	98.1% (95.1, 100)	99.1% (97.7, 100)

5. Contamination and Carryover

The carryover and contamination study evaluated barcode contamination, sample-to sample cross-contamination, and sample carryover between sequencing runs. This study tested a pool of cfDNA sample obtained from clinical plasma sample harboring high allelic frequency (~50%) *KRAS* G12C variants and *KRAS* G12C-negative risk-matched healthy donor samples at the assay's maximum cfDNA input level of 50 ng to maximize the potential for sample contamination. Twelve replicates of each sample type were run in a checkerboard pattern to maximize the potential to detect sample-to-sample cross-contamination in three separate library preparations.

All 36 healthy donor samples passed the assay's Contamination QC caller, and no *KRAS* G12C variants (0/36) were detected in the negative samples. In conclusion, no carryover or cross-contamination was observed in this study.

6. Robustness/Guardbanding

The study established the tolerance of Agilent Resolution ctDx FIRST assay to variation in critical workflow parameters; namely, hybridization time, wash buffer temperature, and wash time (Table 14).

Table 14: Guardbanding Study Overview for Agilent Resolution ctDx FIRST

Guardbanding Condition	Reference Condition	Low Condition	High Condition
Hybridization Time	12 – 24 hours	11.5 hours	24.5 hours
Wash Buffer Temperature	20 ºC – 25 ºC	18 ºC	27 ºC
Wash Time	5 minutes	4 minutes	6 minutes

Two different sample blends were used to measure robustness. The first blend contained cfDNA extracted from NSCLC clinical plasma samples containing *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 19 deletion variants that was diluted into cfDNA extracted from risk-matched healthy donor plasma. The second sample blend was comprised entirely of cfDNA from risk-matched healthy donors. Seven replicates per sample per condition were tested with the Agilent Resolution ctDx FIRST assay at the minimum cfDNA input of 15 ng.

PPA and NPA were 100% across all conditions and variants tested (Table 15). These results indicate the robustness of Agilent Resolution ctDx FIRST assay to variations in the device's workflow.

Table 15: Guardbanding Study Results Summary for *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 19 Deletion

Guardbanding Condition	Level	PPA Observed/Expected	NPA Observed/Expected
Hybridization Time	Low	100% (21/21)	100% (21/21)
	High	100% (21/21)	100% (21/21)
Wash Buffer Temperature	Low	100% (21/21)	100% (21/21)
	High	100% (21/21)	100% (21/21)
Wash Time	Low	100% (21/21)	100% (21/21)
	High	100% (21/21)	100% (21/21)

7. **Plasma Sample Input Range**
   The Agilent Resolution ctDx FIRST assay requires a minimum plasma volume of 2.0 mL and a nominal/maximum volume of 4.0 mL. The plasma input range was verified by analyzing cfDNA extraction results from plasma samples from patients with NSCLC within this range as well as samples 2x below the minimum (1.0 mL). Across the 2.0–4.0 mL plasma sample input range, the cfDNA concentration QC average sample pass rate was 91.1%.

8. **cfDNA Sample Input Range**
   The purpose of this validation study was to verify the required cfDNA sample input range of the Agilent Resolution ctDx FIRST. The cfDNA sample input range of the Agilent Resolution ctDx FIRST assay (15 to 50 ng) was verified by testing five NSCLC clinical sample cfDNA blends with *KRAS* G12C (1-3.8x LoD), *EGFR* L858R (1.1x LoD), or *EGFR* exon 19 deletions (1.9x LoD). Each sample was tested at 7.5 ng, 12 ng, 15 ng, 50 ng, and 75 ng cfDNA input levels. The nominal cfDNA input of the Agilent Resolution ctDx FIRST assay (50 ng) was used as the reference condition to calculate PPA at each input level. For all variants tested, PPA and NPA was 100% at every cfDNA input level, including 2x below and 1.5x above the assay's allowable input range (Table 16 and Table 17).

Table 16: cfDNA Sample Input Summary Results for *KRAS* G12C

cfDNA Input Mass	Observed/ Expected Positive Calls	PPA (95% CI)	Observed/ Expected Negative Calls	NPA (95% CI)
7.5 ng	12/12	100% (75.8, 100)	10/10	100% (72.2, 100)
12 ng	12/12	100% (75.8, 100)	10/10	100% (72.2, 100)
15 ng	11/11	100% (74.1, 100)	10/10	100% (72.2, 100)
50 ng	Reference		10/10	100% (72.2, 100)
75 ng	12/12	100% (75.8, 100)	10/10	100% (72.2, 100)

Table 17: cfDNA Sample Input Summary Results for *EGFR* L858R and *EGFR* exon 19 Deletions

cfDNA Input Mass	Observed/ Expected Positive Calls	PPA (95% CI)	Observed/ Expected Negative Calls	NPA (95% CI)
7.5 ng	10/10	100% (72.2, 100)	50/50	100% (92.9, 100)
12 ng	10/10	100% (72.2, 100)	50/50	100% (92.9, 100)
15 ng	10/10	100% (72.2, 100)	48/48	100% (92.6, 100)
50 ng	Reference		50/50	100% (92.9, 100)
75 ng	10/10	100% (72.2, 100)	50/50	100% (92.9, 100)

9. Reagent Lot Interchangeability

The Agilent Resolution ctDx FIRST assay uses three reagent kits for sample processing: CORE, FIRST, and Barcodes. This study was designed to demonstrate interchangeability of these reagents by evaluating the reproducibility of results using different combinations of Agilent Resolution ctDx FIRST Assay kits. Nine interchangeability run conditions were tested comprised of unique combinations from 3 lots of CORE, 3 lots of FIRST, and 3 combinations of reagents from 2 lots of Barcodes kits.

To generate sample blends with *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 19 deletion variants close to LoD, cfDNA extracted from plasma samples from patients with NSCLC was diluted into cfDNA extracted from risk-matched healthy donor plasma. Two sample blends with 12 replicates (6 replicates per sample blend) were tested with each interchangeability condition at the assay's minimum cfDNA input of 15 ng.

All variants were detected in all interchangeability conditions (9 conditions x 12 replicates = 108 expected calls per variant), resulting in a PPA of 100% for each variant (Table 18).

Table 18: Reagent Lot Interchangeability Study Summary Results for *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 20 Deletion

Variant	Observed/Expected Positive Calls	PPA (95% CI)
KRAS G12C	108/108	100.0% (96.6, 100.0)
EGFR exon 19 deletions	108/108	100.0% (96.6, 100.0)
EGFR L858R	108/108	100.0% (96.6, 100.0)

10. Stability

a. Reagent Stability

Evaluation of the stability of critical reagents in the Agilent Resolution ctDx FIRST assay [baseline (T0), 5 months (T1), 8 months (T2), and 13 months (T3)] is ongoing. The reagent stability of Agilent Resolution ctDx FIRST is assessed by analyzing cfDNA from NSCLC clinical plasma samples diluted

into cfDNA from risk-matched healthy donor plasma for *KRAS* G12C (1.5 - 1.9x LoD), *EGFR* L858R, and *EGFR* exon 19 deletion variants (2.1 - 4.5x LoD)) as well as cfDNA from risk-matched healthy donor plasma were tested (NPA).

Three unique lots of each of the reagent kit types (CORE, FIRST, or Barcodes) were randomly assigned to a reagent kit assembly (R1, R2 or R3) comprised of each kit type prior to study initiation. At each timepoint, seven replicates of each sample blend were processed with each reagent kit assembly at the assay's minimum cfDNA input of 15 ng.

To date, baseline, and timepoints T1 and T2 has been analyzed. At these timepoints, all sample replicates passed all in-process assay QCs, including genomic library yield QCs and sample-level sequencing QCs.

For PPA calculations, T0 results served as the reference condition for variant inclusion, and healthy donor samples were used to assess NPA. PPA and NPA for all included variants were 100% at T1 and T2 (Table 19), indicating Agilent Resolution ctDx FIRST assay reagent kits are stable for at least 7 months at -20°C ± 10°C.

Table 19: Reagent Stability Study Summary Results for *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 20 Deletion

Variant	Timepoint	Reagent Kit Assembly	Observed/ Expected Positive	Observed/ Expected Negative	PPA (95% CI)	NPA (95% CI)
KRAS G12C	T1 (5 months)	R1	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
		R2	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
		R3	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
	T2 (8 months)	R1	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
		R2	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
		R3	7/7	7/7	100% (64.6, 100)	100% (64.6, 100)
EGFR L858R, *EGFR* exon 19 deletions	T1 (5 months)	R1	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)
		R2	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)
		R3	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)
	T2 (8 months)	R1	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)
		R2	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)
		R3	14/14	14/14	100% (78.5, 100)	100% (78.5, 100)

b. <u>In-Use Reagent Stability</u>

Evaluation of the in-use stability of critical reagents in the Agilent Resolution ctDx FIRST assay [baseline (T0), 2 freeze-thaw cycles and 16–18 days at -20 °C ± 10 °C (T1), 3 freeze-thaw cycles and 32–37 days at -20 °C ± 10 °C (T2)]. The in-use reagent stability of Agilent Resolution ctDx FIRST is assessed by analyzing cfDNA from three sets of samples: two clinical sample blends (*KRAS* G12C, *EGFR* L858R, and *EGFR* exon 19 deletion variants) and cfDNA extracted from risk-matched healthy donor plasma. Seven replicates of

each sample were tested in each stability condition at the assay's minimum cfDNA input of 15 ng.

All sample replicates passed all in-process assay QCs, including genomic library yield QCs and sample-level sequencing QCs.

For PPA calculations, T0 results served as the reference condition for variant inclusion, and healthy donor samples were used to assess NPA. PPA and NPA for all included variants were 100% across both T1 and T2 stability conditions (Table 20), indicating Agilent Resolution ctDx FIRST assay reagents are stable for at least 2 freeze-thaw cycles and 30 days at -20°C ± 10 °C.

Table 20: Reagent In-Use Stability Study Summary Results for *KRAS* G12C, *EGFR* L858R, and *EGFR* exon 20 Deletion

Variant	Timepoint	Observed/ Expected Positive	Observed/ Expected Negative	PPA (95% CI)	NPA (95% CI)
KRAS G12C	T1	14/14	7/7	100% (78.5, 100)	100% (64.6, 100)
	T2	14/14	7/7	100% (78.5, 100)	100% (64.6, 100)
EGFR L858R, *EGFR* exon 19 deletions	T1	28/28	14/14	100% (87.9, 100)	100% (78.5, 100)
	T2	28/28	14/14	100% (87.9, 100)	100% (78.5, 100)

c. Whole Blood Stability

The objective of this study was to demonstrate the stability of whole blood specimens used for Agilent Resolution ctDx FIRST Assay collected in the RESOLUTION Sample Collection Kit, that is in Streck Cell-Free DNA Blood Collection Tubes (BCT), across the expected range of sample transport and storage conditions for up to 7 days after blood collection prior to plasma isolation. The stability of Streck Cell-Free DNA BCT whole blood samples prior to plasma isolation was evaluated by testing samples from 20 risk-matched healthy donors across five stability conditions (Table 21).

Table 21: Description of Whole Blood Stability Study Storage Conditions

Condition	Storage Condition / Processing
Day 0	Reference Condition. Storage at RT and processing to plasma within 4 hours of collection.
RT Day 1	Storage at RT until processing to plasma on Day 1 after collection.
RT Day 8	Storage at RT until processing to plasma on Day 8 after collection.
Low Humidity	Storage at low humidity (25% relative humidity at 23°C) for 24 hours followed by storage at RT until processing to plasma on Day 2 after collection.
High Humidity	Storage at high humidity (90% relative humidity at 23°C) for 24 hours followed by storage at RT until processing to plasma on Day 2 after collection.

*RT: Room Temperature (18–25°C)

For each donor, five 10 mL whole blood samples were collected in Streck Cell-Free DNA BCTs, and one tube was tested in each stability condition

(Table 21). Following each storage period, whole blood was processed to plasma and frozen until shipment to Resolution Bioscience's CLIA laboratory where it was tested with the Agilent Resolution ctDx FIRST assay. To ensure that performance differences could be detected, a donor and all associated replicates were included in the study only if the Day 0 reference condition replicate reached a minimum cfDNA concentration of 0.5 ng/µL following plasma extraction. Stability was determined using 20 donor samples subjected to the condition described in Table 22.

All 20 samples passed all QC and were included in analysis. Except for a single RT Day 8 replicate that failed to meet the minimum cfDNA QC of the assay (resulting in a 95% sample pass rate for the RT Day 8 condition), all other replicates passed all in-process assay and sequencing QCs, resulting in a 100% sample pass rate for all other stability conditions. Combined NPA was evaluated for *KRAS* G12C, *EGFR* exon 19 Del, *EGFR* L858R, and *EGFR* T790M and was 100% for all stability conditions (Table 22).

Table 22: Whole Blood Stability Study Summary Results

Stability Condition	Sample Assay QC Pass Rate	ΔCoverage Depth	NPA [95% CI] (Detection Rate)
Day 0	100% (20/20)	Reference	100% [95.4, 100] (80/80)
RT Day 1	100% (20/20)	-0.4%	100% [95.4, 100] (80/80)
RT Day 8	95% (19/20)	-8.3%	100% [95.2, 100] (76/76)
Low Humidity	100% (20/20)	-5.1%	100% [95.4, 100] (80/80)
High Humidity	100% (20/20)	-5.4%	100% [95.4, 100] (80/80)

ΔCoverage Depth: median of within-donor differences in average coverage depth between stability and reference condition

For PPA calculations, a post-market study is planned with additional samples from patients with NSCLC harboring *KRAS* G12C and *EGFR* level 2 variants (see section XIII).

d. Short-Term Stability of cfDNA

To evaluate the storage conditions and stability of cfDNA extracted from plasma, six sample blends comprised of NSCLC clinical plasma samples with three *KRAS* G12C and three *EGFR* exon 19 deletions diluted into risk-matched healthy donor plasma were tested over four timepoints and two storage conditions (Table 23) with either singlicate or duplicates at each condition.

Table 23: Description of cfDNA Stability Study Storage Conditions

Condition	Storage Condition
T0	cfDNA processed in the Agilent Resolution ctDx FIRST assay within 2 hours of extraction (Reference Condition).
12 hours	cfDNA stored at 2 – 8 °C for 12–24 hours
15 days	cfDNA stored at -20 °C ± 10 °C for 15 days

Condition	Storage Condition
31 days	cfDNA stored at -20 °C ± 10 °C for 31 days

Across all timepoints and storage conditions, all sample replicates (24 replicates with *KRAS* G12C and 21 with *EGFR* exon 19 deletions) passed all Agilent Resolution ctDx FIRST assay QCs and generated valid data. All expected variants were observed at each stability condition, resulting in PPAs of 100% across all samples, storage conditions, and timepoints for each variant.

e. Short-Term Stability of Assay Intermediate Products

To evaluate the stability of Agilent Resolution ctDx FIRST assay intermediate products, six sample blends comprised of NSCLC clinical plasma samples with three *KRAS* G12C and three *EGFR* exon 19 deletions diluted into risk-matched healthy donor plasma were tested over three timepoints (Table 24).

Table 24: Description of Agilent Resolution ctDx FIRST Assay Intermediate Products Stability Study Storage Conditions

Condition	Storage Condition
T0	Intermediate products tested within 2 hours of creation (Reference Condition).
15 days	Intermediate products stored at -20 °C ± 10 °C for 15 days
31 days	Intermediate products stored at -20 °C ± 10 °C for 31 days

Two Agilent Resolution ctDx FIRST assay intermediate products were tested: amplified library (following barcoding and amplification, prior to probe hybridization) and sequence-ready library (complete assay product prior to sequencing). All 60 sample replicates that were assessed passed relevant QCs and generated valid data. *KRAS* G12C and *EGFR* exon 19 deletions were observed in all expected samples. PPA and NPA were 100% across both intermediate products and storage timepoints.

B. Animal Studies

No animal studies were conducted using Agilent Resolution ctDx FIRST.

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The safety and effectiveness of the Agilent Resolution ctDx FIRST for selecting patients with NSCLC who have *KRAS* G12C mutation and who may benefit from treatment with KRAZATI (adagrasib) was demonstrated through testing of cfDNA in pre-treatment plasma specimens from patients enrolled into KRYSTAL-1 study used to support the efficacy of adagrasib. A clinical bridging study was conducted to assess clinical agreement between samples with *KRAS* G12C mutation status tested with the clinical trial assays (CTA) and the Agilent Resolution ctDx FIRST in the intent-to-test population. A summary of the Agilent Resolution ctDx FIRST clinical validation study is presented below.

A. Study Design

The KRYSTAL-1 (NCT03785249) clinical study is a multicenter, single-arm, open-label expansion Cohort clinical study of KRAZATI in adult patients with locally advanced or metastatic NSCLC. The KRYSTAL-1 clinical study population comprises of *KRAS* G12C mutation-positive subjects from Cohort A of KRYSTAL-1 study who have received at least one prior systemic therapy. A total of 116 subjects were enrolled based on the presence of *KRAS* G12C mutations by local tissue testing or clinical trial assays (CTA); and of these 116 subjects there were 112 subjects with at least one site of measurable disease at baseline in the efficacy population.

Agilent Resolution ctDx FIRST Bridging Study Design for *KRAS* G12C mutations

Pre-treatment plasma samples from Cohort A of KRYSTAL-1 clinical study were tested with Agilent Resolution ctDx FIRST. The KRYSTAL-1 clinical study did not include patients negative for *KRAS* G12C mutations and therefore did not represent the Agilent Resolution ctDx FIRST positive (+)/ tissue-based CTA negative (-) [Agilent Resolution ctDx FIRST (+) / CTA(-)] subgroup of the Agilent Resolution ctDx FIRST intended use population. As such, supplemental matched tissue and plasma samples were obtained from subjects available through commercial vendors using subject selection criteria similar to those of the KRYSTAL-1 clinical study, and a sensitivity analysis was performed to evaluate the potential impact of the Agilent Resolution ctDx FIRST (+)/CTA(-) population on the efficacy in the Agilent Resolution ctDx FIRST intended use population.

1. Clinical Bridging Study Inclusion and Exclusion Criteria

The inclusion/exclusion criteria for the clinical bridging study are summarized below:

a. Inclusion Criteria for plasma samples from the KRYSTAL-1 clinical study
- Sample must have been collected in Streck tubes
- Sample must contain sufficient plasma for ctDx Lung IUO assay testing
- Sample must be accompanied by requisition form indicating consent from the patient has been obtained
- Sample must have 2 unique identified on the tubes that match the requisition

b. Exclusion Criteria for plasma samples from the KRYSTAL-1 clinical study
- Sample collected in anything other than Streck tubes
- Sample with insufficient plasma for testing
- Sample integrity has been compromised (sample degraded, unable to confirm specimen identify, etc.)
- Sample lacking appropriate documentation regarding consent for testing

2. Follow-up Schedule

The Agilent Resolution ctDx FIRST clinical bridging study involved retrospective testing of plasma samples; as such, no additional patient follow-up was conducted in regard to the clinical bridging study.

3. Clinical Endpoints

The clinical endpoint used to assess adgrasib efficacy in the KRYSTAL-1 clinical study was confirmed objective response rate (ORR) and duration of response by RECIST version 1.1 as assessed by blinded independent central review (BICR).

4. Diagnostic Objective and Endpoints

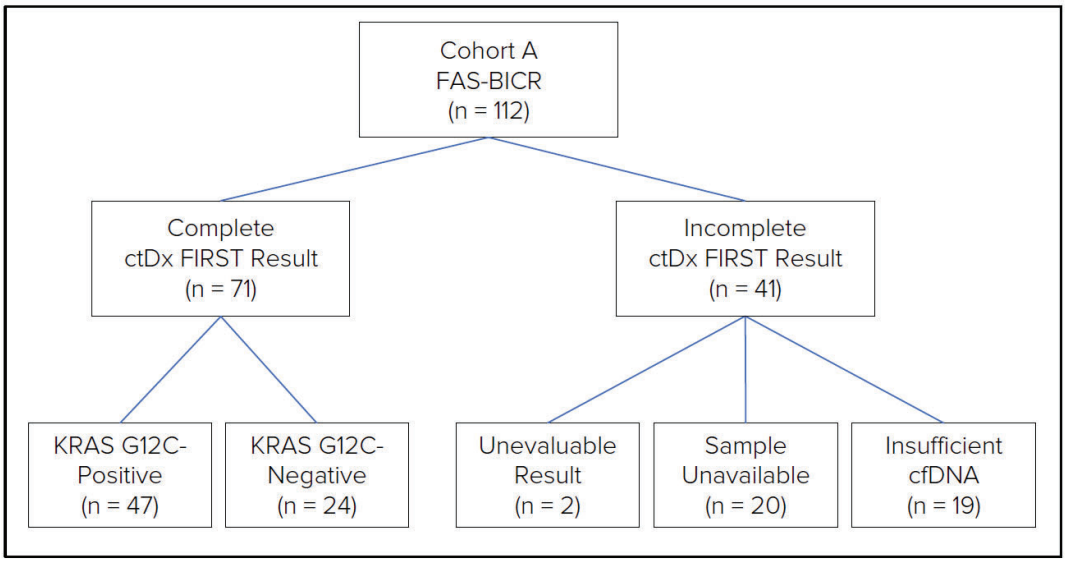
The primary objective of the clinical bridging study is to demonstrate the safety and effectiveness of Agilent Resolution ctDx FIRST for the selection of patients with *KRAS* G12C-mutated NSCLC for treatment with KRAZATI. The primary endpoint is ORR by RECIST version 1.1 as assessed by BICR and compared to the benchmark ORR of the KRYSTAL-1 clinical study.

A sensitivity analysis was conducted to model the impact of the Agilent Resolution ctDx FIRST (+)/CTA(-) population on the efficacy in the intended use population.

B. Accountability of PMA Cohort

The Agilent Resolution ctDx FIRST clinical bridging study included 71 (63%) of the 112 subjects in the efficacy population in Cohort A of the KRYSTAL-1 clinical study (Figure 1). A total of 41 (37%) subjects were missing and were excluded from the study (20 subjects did not have pre-treatment plasma samples available for testing, 19 subjects did not have sufficient cfDNA for testing, and 2 samples resulted in unevaluable results). Of these 71 subjects, 47 subjects (66%) tested *KRAS* G12C mutation positive by Agilent Resolution ctDx FIRST, while 24 (34%) tested negative.

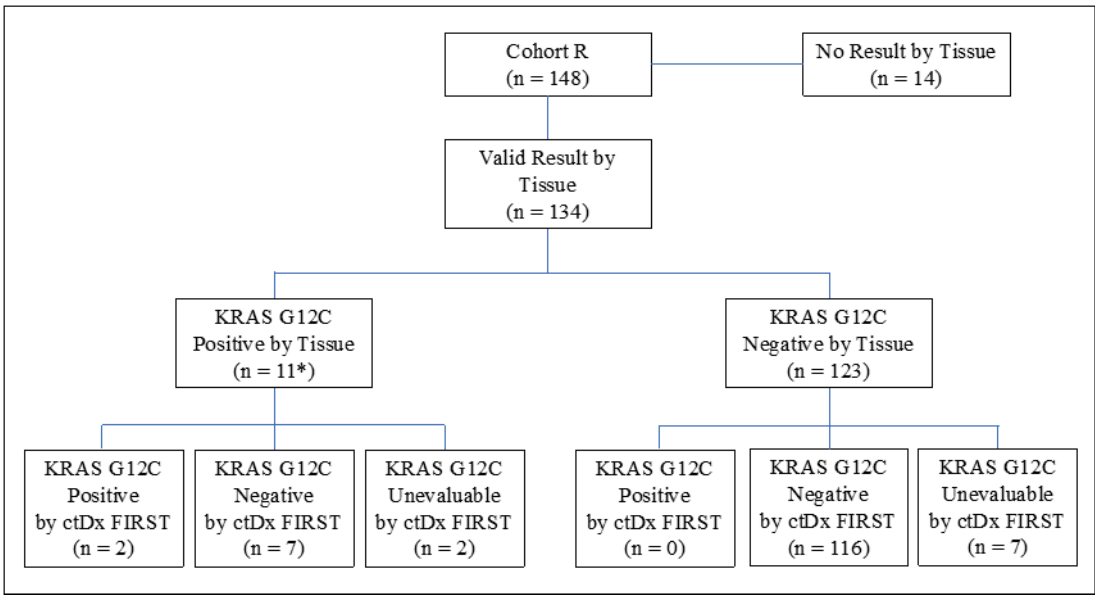
Figure 1: Agilent Resolution ctDx FIRST *KRAS* G12C Mutation Positive Bridging Study Patient Accountability



Plasma samples from biomarker-negative (negative for *KRAS* G12C mutation) patients in KRYSTAL-1 clinical study was not available to represent Agilent Resolution ctDx

FIRST(+)/CTA(-) subgroup of the Agilent Resolution ctDx FIRST-positive intended use population. Therefore, for sensitivity analysis, matched tissue and plasma samples were procured from commercial vendors (Cohort R) to estimate the prevalence of *KRAS* G12C mutation Agilent Resolution ctDx FIRST(+)/CTA(-) population and to assess the potential impact of this population on clinical efficacy. The samples procured from commercial vendors were tested using Foundation One CDx (F1CDx), an NGS based test and a representative CTA used to enroll subjects into the KRYSTAL-1 clinical study. The sensitivity analysis prevalence sub study set (Cohort R) included 148 subjects with matched plasma and tissue samples (Figure 2). Of the 148 subjects, 14 subjects failed screening and were excluded from the analysis, 11 were *KRAS* G12C positive, leaving 123 *KRAS* G12C negative commercially sourced NSCLC samples available for testing by the Agilent Resolution ctDx FIRST. Of the 123 *KRAS* G12C negative samples, 116 samples were called negative by the Agilent Resolution ctDx FIRST and 7 samples were unevaluable. Of the 11 *KRAS* G12C tissue test positive samples, 7 samples were called negative by the Agilent Resolution ctDx FIRST, 2 samples were called positive by the Agilent Resolution ctDx FIRST and 2 samples were unevaluable.

Figure 2: Agilent Resolution ctDx FIRST *KRAS* G12C Mutation Negative Bridging Study Patient Accountability



Concordance Between Agilent Resolution ctDx FIRST and Tissue Testing

Concordance between Agilent Resolution ctDx FIRST and tissue-based CTA testing using matched plasma and tissue samples from Cohort A of the KRYSTAL-1 clinical study, along with the sensitivity analysis prevalence sub-study group, is shown in Table 25 below. While all samples from the KRYSTAL-1 clinical study population were positive for *KRAS* G12C mutation by tissue testing as a requirement for enrollment in the clinical study, the sensitivity analysis prevalence sub-study subject samples were identified and commercially procured in an effort to represent the *KRAS* G12C mutation-negative population. For concordance analyses, *KRAS* G12C Positive from both the efficacy population and biomarker-negative population (Cohort R) were

included (please refer to the Table 25 footnotes for distribution of samples between the two Cohorts). Based on the PPA of 61% (95% CI between Res Bio test and tissue CTA), reflex testing using tissue specimens to an FDA approved tissue test is recommended, if feasible, if the plasma test is negative

Table 25. Concordance Between Agilent Resolution ctDx FIRST and Tissue-based CTA

		Tissue-based CTA Testing		
		***KRAS* G12C Positive**	***KRAS* G12C Negative**	**Total**
Agilent Resolution ctDx FIRST				
	KRAS G12C Positive	49[a]	0	49
	KRAS G12C Negative	31[b]	116	147
	Unevaluable	43[c]	7	50
	Total	123[d]	123	246
	PPA [95% CI]	61.3% (49/80) [49.7, 71.9]		
	NPA [95% CI]	100.0% (116/116) [96.9, 100.0]		

a: 47 from KRYSTAL-1 study and 2 from Cohort R (See Figures 2 and 3)
b: 24 from KRYSTAL-1 study and 7 from Cohort R (See Figures 2 and 3)
c: 41 from KRYSTAL-1 study and 2 from Cohort R (See Figures 2 and 3)
d: 112 from KRYSTAL-1 study and 11 from Cohort R (See Figures 2 and 3)

C. Study Population Demographics and Baseline Parameters

The demographics, disease characteristics, and specimen characteristics for the Agilent Resolution ctDx FIRST evaluable and unevaluable patients were assessed and these characteristics were not statistically significant between the Agilent Resolution ctDx FIRST evaluable and unevaluable subgroups (Table 26).

Table 26: Demographics and Baseline Characteristics of the Clinical Effectiveness Analysis Subgroups

Variable (*p*-value)	**Level**	**Agilent Resolution ctDx FIRST**		
		Evaluable (n = 71)	**Unevaluable (n = 41)**	**FAS-BICR (n = 112)**
Age (0.7251)	Mean (SD)	63.9 (10.5)	64.5 (8.0)	64.1 (9.7)
	Median (Q1, Q3)	64 (59, 70)	64 (60, 69)	64 (59.8, 70)
	(Min, Max)	(25, 89)	(39, 84)	(25, 89)
Sex (0.6944)	Female	38 (53.5%)	24 (58.5%)	62 (55.4%)
	Male	33 (46.5%)	17 (41.5%)	50 (44.6%)
Race (0.9359)	American Indian or Alaska Native	1 (1.4%)	0 (0%)	1 (0.9%)
	Asian	4 (5.6%)	1 (2.4%)	5 (4.5%)
	Black or African American	6 (8.5%)	3 (7.3%)	9 (8.0%)
	Other	3 (4.2%)	1 (2.4%)	4 (3.6%)
	White	57 (80.3%)	36 (87.8%)	93 (83.0%)
Smoking History (0.1706)	Past Smoker	64 (90.1%)	32 (78.0%)	96 (85.7%)
	Current Smoker	4 (5.6%)	7 (17.1%)	11 (9.8%)
	Lifetime Non-Smoker	3 (4.2%)	2 (4.9%)	5 (4.5%)

Variable (*p*-value)	Level	Agilent Resolution ctDx FIRST		
		Evaluable (n = 71)	Unevaluable (n = 41)	FAS-BICR (n = 112)
ECOG Status (0.0584)	0	15 (21.1%)	3 (7.3%)	18 (16.1%)
	1	56 (78.9%)	37 (90.2%)	93 (83.0%)
	N/A	0 (0%)	1 (2.4%)	1 (0.9%)
Histology (1.0)	Non-Squamous	69 (97.2%)	40 (97.6%)	109 (97.3%)
	Squamous	2 (2.8%)	1 (2.4%)	3 (2.7%)
Disease Type (0.0529)	Locally Advanced	11 (15.5%)	1 (2.4%)	12 (10.7%)
	Metastatic	60 (84.5%)	40 (97.6%)	100 (89.3%)
Tissue Sample Location* (0.4643)	Lung	44 (62.0%)	19 (46.3%)	63 (56.2%)
	Lymph Node	13 (18.3%)	9 (22.0%)	22 (19.6%)
	Adrenal	4 (5.6%)	2 (4.9%)	6 (5.4%)
	Bone	3 (4.2%)	2 (4.9%)	5 (4.5%)
	Liver	2 (2.8%)	4 (9.8%)	6 (5.4%)
	Other*	5 (7.0%)	5 (12.2%)	10 (8.9%)
CTA Tissue Test (0.0943)	Tissue Test 1	19 (26.8%)	6 (14.6%)	25 (22.3%)
	Tissue Test 2	11 (15.5%)	3 (7.3%)	14 (12.5%)
	Other	41 (57.7%)	32 (78.0%)	73 (65.2%)

*Tissue sample locations with two or fewer occurrences (Brain, Breast, Forearm, Pericardium, Pleura, Pleural Fluid, Unknown)

D. Safety and Effectiveness Results

1. Safety Results

   Data regarding the safety of adagrasib therapy were presented in the drug approval and are summarized in the drug label. Please refer to Drugs@FDA for complete safety information on KRAZATI (adagrasib). No adverse events were reported in the conduct of the diagnostic study to support this PMA as these involved retrospective testing of banked plasma specimens only.

2. Effectiveness Results

   a. ORR in Patients Positive by Agilent Resolution ctDx FIRST for *KRAS* G12C mutations

      The efficacy of adagrasib was evaluated in Mirati's KRYSTAL-1 clinical study. The efficacy of adagrasib in KRYSTAL-1 study population and in those subjects positive for *KRAS* G12C mutations by Agilent Resolution ctDx FIRST is shown in Table 27. The observed diagnostic clinical bridging study ORR [51% (36.1, 65.9)] is similar to the ORR [42.9%, (33.5, 52.6) from the adagrasib efficacy population. As shown in Table 28, the duration of response (DOR) for Agilent Resolution ctDx FIRST clinical efficacy population was 6.9 months (3.1, 10.6).

Table 27. ORR in the Agilent Resolution ctDx FIRST and Adagrasib Study Populations Assessed by Blinded Independent Central Review

	Analysis Set	Objective Response		Total	ORR [95% CI]
		Yes	No		
Agilent Resolution ctDx FIRST *KRAS* G12C	Positive (CTA+/CDx+)	24	23	47	51.1% (36.1%,65.9%)
	Negative (CTA+/CDx-)	7	17	24	29.2% (12.6%,51.1%)
	CTA+/CDx Unevaluable	17	24	41	41.5% (26.3%,57.9%)
Adagrasib efficacy population (CTA+)		48	64	112	42.9% (33.5%,52.6%)

[a]Assessed by BICR. CI= confidence interval

Table 28. DOR in the Agilent Resolution ctDx FIRST and adagrasib Study Populations Assessed by Blinded Independent Central Review

Efficacy Parameter	Agilent Resolution ctDx FIRST KRAS G12C - Positive	Adagrasib Efficacy Population
Median[a] in months (95% CI)	6.9 (3.1, 10.6)	8.5 (6.2, 13.8)
Patients with duration ≥ 6 months[c], %	46%	58%

[a] Estimated using Kaplan-Meier method. [b] Observed proportion of patients with duration of response beyond landmark times

The Agilent Resolution ctDx FIRST clinical bridging study included 71 (63%) of the 112 subjects in the efficacy population in Cohort A of the KRYSTAL-1 clinical study. Since 37% of the efficacy population could not be evaluated in the clinical bridging study, additional clinical data were provided from KRYSTAL-1 clinical study (non-efficacy population) in an effort to mitigate the missingness and to demonstrate the clinical effectiveness of the Agilent Resolution ctDx FIRST assay. The study included a separate Cohort of 47 patients enrolled using blood-based tests. Of the 47 patients, 39 patients were Agilent Resolution ctDx FIRST positive and eight (8) were Agilent Resolution ctDx FIRST negative for *KRAS* G12C. The observed ORR for these additional patients supported the efficacy conclusions from the adagrasib primary efficacy population.

b. Sensitivity Analysis

Sensitivity analyses were conducted to model the impact of the hypothetical Agilent Resolution ctDx FIRST(+) / CTA(-) population and patients without Agilent Resolution ctDx FIRST results.

i. Sensitivity analysis for the unrepresented Agilent Resolution ctDx FIRST(+) CTA(-) subject population

Because all CTA(-) patients are tested as negative by CDx (i.e. NPA=100%) and thus PPV is estimated as 100%, the results do not vary with Pr(CTA+) values and the ORR in Agilent Resolution ctDx FIRST(+) population is estimated as the same as the ORR in Agilent Resolution

ctDx FIRST [CTA(+)/CDx(+)] population.

ii. Sensitivity analysis for the missing Agilent Resolution ctDx FIRST *KRAS* G12C Results

As Agilent Resolution ctDx FIRST assay results were missing for 41 of the 112 patients, multiple imputation was used to predict the missing Agilent Resolution ctDx FIRST assay results. Only one of the demographic or baseline clinical characteristic variables (age) was a sufficiently strong ($p < 0.20$) predictor of Agilent Resolution ctDx FIRST assay positivity for *KRAS* G12C in a logistic regression analysis for inclusion in the imputation analysis (Table 29). Univariate logistic regression *p*-values for all the demographic variables and baseline clinical characteristics considered are shown in Table 29.

Table 29: Univariate *p*-values for Demographic and Baseline Clinical Variables

Variable	Chi-squared *p*-value
Smoking History	0.37
Tissue Sample Location	0.46
Age (Continuous)	0.17
Sex	0.67
Disease Type	0.85
Tissue Test Type	0.91
ECOG Performance Status	0.97
Race Category	0.97

Age was the only variable that had a *p*-value of less than 0.20 and, thus, was used in subsequent imputation. Imputation was performed 1000 separate times, and the ORR and its variance were calculated within each of the 1000 completed datasets. Table 30 summarizes the components of the total variance and shows the final estimate of the ORR together with the 95% confidence interval. The lower 95% confidence limit for the ORR calculated from datasets completed using multiple imputation was 31.5%.

Table 30: Imputation Analysis Summary with No Demographic or Baseline Clinical Variables as Predictors of Agilent Resolution ctDx FIRST Assay *KRAS* G12C-positivity

ORR Mean	Mean of ORR Variances	Variance of ORR Means	Total Variance	ORR (95% CI)
0.4750	0.0034	0.0033	0.0067	47.5% (31.5, 63.5)

E. Pediatric Extrapolation

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

F. 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 pivotal clinical study included one investigator who was a full-time employee of the sponsor and 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: [0]
- Significant equity interest held by investigator in sponsor of covered study: [1]

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. Statistical analyses were conducted by FDA to determine whether the financial interests/arrangements had any impact on the clinical study outcome. The information provided does not raise any questions about the reliability of the data.

XI. 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, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use of identifying *KRAS* G12C mutations in patients with NSCLC for treatment with KRAZATI (adagrasib), the effectiveness of Agilent Resolution ctDx FIRST was demonstrated through analytical studies using patient samples from the KRYSTAL-1 clinical study. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of Agilent Resolution ctDx FIRST when used in accordance with the indications for use. Data from the KRYSTAL-1 clinical study show that patients with *KRAS* G12C mutations received benefit from treatment with adagrasib and support the CDx indication to Agilent Resolution ctDx FIRST.

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. Agilent Resolution ctDx FIRST is an *in*

vitro diagnostic test, which involves testing of cfDNA extracted from the plasma of whole blood routinely collected as part of the diagnosis and patient care.

Failure of Agilent Resolution ctDx FIRST to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decision in cancer treatment. Patients with false positive results may undergo treatment with KRAZATI (adagrasib) therapy 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.

C. Benefit-Risk Determination

The probable clinical benefit of Agilent Resolution ctDx FIRST for the identification of *KRAS* G12C mutations in patients with NSCLC for treatment with KRAZATI (adagrasib) was demonstrated through retrospective analysis of efficacy data obtained from KRYSTAL-1 (NCT03785249), a multicenter, single-arm, open-label multiple expansion Cohort study. The supporting clinical validation analyses demonstrate that the observed ORR based on BICR assessment for the 47 evaluable subjects that were positive by Agilent Resolution ctDx FIRST for *KRAS* G12C was 51.1% (36.1, 65.9%), which is similar to the ORR observed in the primary efficacy population for adagrasib of [42.9% (33., 52.6)]. This Agilent Resolution ctDx FIRST selected patient population also exhibited an ORR that was greater than the size-adjusted benchmark ORR of 23%. The effectiveness of Agilent Resolution ctDx FIRST for *KRAS* G12C mutations was also evaluated in a separate Cohort of 47 patients enrolled using blood-based tests (non-efficacy population). The observed ORR for these additional patients supported the efficacy conclusions from the adagrasib primary efficacy population. Thus, this device identifies *KRAS* G12C mutations in patients with NSCLC, who have a meaningful clinical response to adagrasib.

There is potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, or failure to provide a result and 2) incorrect interpretation of test results by the user. The key risks of Agilent Resolution ctDx FIRST are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug. However, this risk is partially mitigated by reflex testing recommendation for negative results with an FDA-approved tissue test for the *KRAS* G12C mutation. There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *KRAS* G12C mutations for the identification of patients with NSCLC for treatment with KRAZATI (adagrasib), though there is one FDA approved tissue-based CDx for this indication. This device may fulfill an unmet need in patients who cannot otherwise provide tissue for ascertainment of *KRAS* G12C status.

These risks of this assay are partially mitigated by the clinical and analytical studies for Agilent Resolution ctDx FIRST detection of *KRAS* G12C mutations. An accuracy study of Agilent Resolution ctDx FIRST for the detection of *KRAS* G12C mutations with an externally validated comparator method demonstrating a PPA of 96.2% and NPA of 98% and a LoB study demonstrating a false-positive rate of 0% also indicates a low likelihood of patient misassignment. Together, these results support the use of Agilent Resolution ctDx FIRST as an aid in selecting patients with NSCLC with *KRAS* G12C mutations for KRAZATI (adagrasib) treatment.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with accuracy, mitigating the potential for false results.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, the available data support the probable benefits of Agilent Resolution ctDx FIRST use with the indication above 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 indications for use. Data from the analytical and clinical validation studies support the use of Agilent Resolution ctDx FIRST as an aid for the identification of *KRAS* G12C mutation-positive patients with NSCLC for whom KRAZATI (adagrasib) may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on December 12, 2022. The final conditions of approval cited in the approval order are described below.

1. Resolution Bioscience must provide data from a well-designed and well-controlled short-term frozen plasma stability using intended use clinical samples for *KRAS* G12C and *EGFR* level 2 variants. The data from this study must be adequate to support short-term frozen plasma stability claims for *KRAS* G12C and *EGFR* level 2 variants.

2. Blood Collection Tubes
   a. Resolution Bioscience must demonstrate clinically insignificant variability when different lots of the Agilent Resolution ctDx FIRST Blood Collection tube are used with the Agilent Resolution ctDx FIRST assay. Resolution Bioscience must provide data from a robust and high confidence precision study. This study must confirm the Agilent Resolution ctDx FIRST assay's precision when the Agilent Resolution ctDx FIRST cfDNA Blood Collection tubes are used, and must use replicate samples from each of multiple different patients. Each patient who

donates specimens for this study must have plasma collected in a total of four tubes, each from two tube lots; three lots are required to be represented in the study. This is important to assess variability between tube lots and across patient specimens. Each replicate must be run at or near the minimum standardized cfDNA input (*i.e.,* at a target concentration of 15 ng). The samples must be collected from intended use patients with *KRAS* G12C and *EGFR* level 2 variants that are identified by the Agilent Resolution ctDx FIRST assay. The data from this study must be adequate to minimize clinically significant inaccurate results when used on specimens collected in the Agilent Resolution ctDx FIRST Blood Collection tubes in the intended use population.

b. Resolution Bioscience must provide robust and high confidence data from a well-designed and well-controlled study to evaluate potential interference caused by underfilling the Agilent Resolution ctDx FIRST Blood Collection tubes. Replicates must be run at or near the minimum standardized cfDNA input (i.e., at a target concentration of 15 ng). The samples must be collected from intended use patients with *KRAS* G12C and *EGFR* variants that are identified by the Agilent Resolution ctDx FIRST assay. The data from this study must be adequate to evlaute the potential interference caused by underfilling the Agilent Resolution ctDx FIRST Blood Collection tubes in the intended use population.

c. Resolution Bioscience must evaluate the impact of variations in mixing after blood collection (incomplete mixing). Replicates must be run at or near the minimum standardized cfDNA input (i.e., at a target concentration of 15 ng). The samples must be collected from intended use patients with *KRAS* G12C and *EGFR* variants that are identified by the Agilent Resolution ctDx FIRST assay. The data from this study must be adequate to demonstrate the impact of inadequate or overmixing on the performance of Agilent Resolution ctDx FIRST Blood Collection tubes in the intended use population.

d. Resolution Bioscience must provide robust and high confidence data from a stability study which demonstrates acceptable stability of whole blood collected from the intended use patients (NSCLC), stored, and shipped in the Agilent Resolution ctDx FIRST cfDNA Blood Collection tubes. The study must confirm the claimed whole blood shipping and storage stability for the intended use population.

e. Resolution Bioscience must demonstrate clinically insignificant variability on the performance of the Agilent Resolution ctDx FIRST assay when specimens collected in Agilent Resolution ctDx FIRST cfDNA Blood Collection tubes at modified plasma spinning protocol from that of the manufacturer's recommendations. The study must confirm that the Resolution Bioscience's modified centrifugation protocol has not impacted sample QC metrics and variant calling.

The final study data, study conclusions, and labeling revisions should be submitted within one (1) year of the PMA approval date.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. 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.

XV. REFERENCES

None.