Agilent **Resolution ctDx FIRST**
Technical Information

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Indications for Use

The Agilent Resolution ctDx FIRST assay is a qualitative next generation sequencing-based, *n 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.

Contraindications

There are no known contraindications.

Warnings and Precautions

- 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.
- Patients for whom no companion diagnostic alteration is detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.
- When collecting the whole blood in the Streck Cell Free BCT® collection tube, allow the tube to fill completely until blood stops flowing into the tube Underfilling of tubes with less than 5 mL of whole 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.
- Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).

Limitations

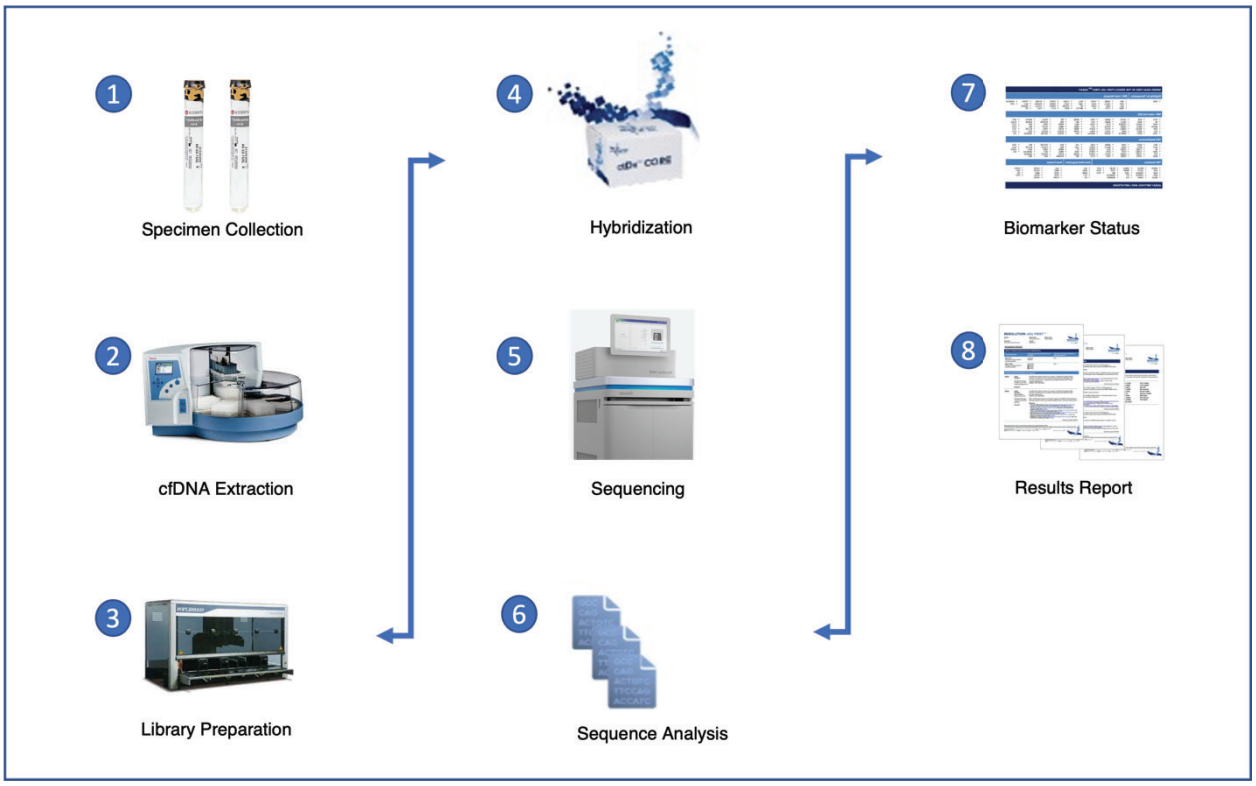
- For in vitro diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The efficacy of KRAZATI™ (adagrasib) has not been established in patients whose KRAS G12C mutations are <0.10% VAF.
- The test is not intended to be used for standalone diagnostic purposes.
- A negative result does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- Genomic findings other than those listed in Table 1 of the indications for use are not prescriptive or conclusive for labeled use of any specific therapeutic product.
- The test has not been reviewed by FDA to report tumor profiling genes or tumor types other than SNVs and deletions in the EGFR gene from NSCLC plasma specimens (please see professional services page).
- The test is intended to be performed on specific serial number-controlled instruments by Resolution Bioscience, Inc.
- The test is not intended to provide information on cancer predisposition.

Test Description

The Agilent Resolution ctDx FIRST assay uses next-generation sequencing to analyze small fragments of cfDNA, including tumor-derived cell free DNA fragments (ctDNA), isolated from plasma to detect gene alterations in patients with NSCLC.

Peripheral venous blood samples are collected in Streck cell-free DNA BCT blood collection tubes and shipped to the Resolution Bioscience 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). A high-level overview of the Agilent Resolution ctDx FIRST assay is presented in Figure 1.

Figure 1: Agilent Resolution ctDx FIRST Test System Overview



The Agilent Resolution ctDx FIRST detects G12C substitutions in KRAS as well as T790M and L858R substitutions and exon 19 deletions in EGFR. It 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.

The following regions are excluded from coverage: KRAS chr12: 25,380,231 to 25,380,233.

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.

Test Kit Contents

The Agilent Resolution ctDx FIRST Sample Collection Kit contains the following components:

Kit Instructions-for-Use (IFU): Whole Blood Collection and Shipping
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

All other reagents, materials and equipment needed to perform the assay are used exclusively in a CLIA-approved laboratory at Resolution Bioscience.

ample Collection and Test Ordering

To order the Agilent Resolution ctDx FIRST assay, the Test Requisition Form (TRF), provided with the Agilent Resolution ctDx FIRST Sample Collection Kit must be fully completed and signed by the ordering physician or other authorized medical professional. Refer to the Sample Collection Kit Instructions-for-Use (IFU) for further details on collection and shipment of blood samples to the Resolution Bioscience clinical laboratory.

To order the Agilent Resolution Sample Collection Kit or obtain an electronic version of the TRF, please contact the Resolution Bioscience Client Services (Tel: 1-800-424-5444, or Email: resolution.support@agilent.com).

Agilent Resolution ctDx FIRST Test System Description

- **Specimen Collection and Preparation:** Whole blood is collected in Streck cell-free DNA BCT blood collection tubes and shipped ambient to Resolution Bioscience.
- **Extraction:** Plasma is isolated through centrifugation and cfDNA is extracted using the KingFisher Flex™ System and MagMax cfDNA Isolation kit (Thermo Fisher).
- **Sequencing Library Preparation (Hamilton Microlab Star Automation):** Unique molecular barcodes, specific to Resolution Bioscience's sequencing chemistry, are ligated to each cfDNA molecule and amplified to create the genomic libraries.

4. **Hybridization:** Genomic libraries are pooled, hybridized with biotinylated probes, and further processed to create sequence ready libraries.
5. **Sequencing:** Libraries are sequenced on the Illumina NovaSeq 6000 sequencing instrument.
6. **Sequencing Data Analysis and Reporting:** Sequencing data is analyzed by the custom, cloud-based informatics pipeline developed by Resolution Bioscience, Agilent Resolution ctDx FIRST Pipeline.

7. **Biomarker Status Determination:** Biomarker status (i.e. biomarker positivity) is determined based on the identification of KRAS G12C via the custom variant logic applied to pipeline outputs.

8. **Reporting:** 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.

9. **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 Resolution Bioscience's HIPAA-compliant Amazon Web Services (AWS) account.

Controls

Quality Controls have been built into 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.

Table 2. Summary of Control Types and Usage

Wet Lab Controls*		Description of Control	Purpose of Control
Control Name	**Control Status**		
Plasma volume	Pass/Fail	Volume measurement	Plasma Sample controlled for the acceptable input volume range
cfDNA concentration	Pass/Fail	Fluorometric Measurement to verify the cfDNA input mass	cfDNA Sample controlled for the acceptable input range
Amplified genomic library concentration	Pass/Fail	Fluorometric Measurement to verify the amplified library concentration of the Patient Sample	Patient Sample controlled for genomic library creation
		Fluorometric Measurement to verify the amplified library concentration of the Positive Control	Positive Control sample controls for FIRST assay performance during genomic library creation
Amplified captured library concentration	Pass/Fail	Fluorometric Measurement to verify that the captured amplified library concentration is within the acceptable range	Captured Amplified Library controlled for sample input range of NovaSeq 6000 sequencing instrument

Software-Based Controls		Description of Control	Purpose of Control
Control Name	**Control Status**		
Inbound Screening QCs	Pass/Fail	Assay Software screens the XML files automatically generated by Illumina sequencing instrument	Run controlled for: Sequencing instrument parameters (sequencing instrument model and run setup on sequencing instrument must match assay-specific parameters) Sequencing consumables (appropriate consumables must be used and consumables used in sequencing run must not have expired) Errors in sequencing run (sequencing run must be completed as planned)
Q30 QC	Pass/Fail	Determines the Phred quality score for each base in the FASTQ file associated with the sequencing run	Run controlled for: Quality of sequencing reads
Pass Filter QC	Pass/Fail	Before demultiplexing, the Assay Software verifies that pass filter criteria are met for a NovaSeq 6000 sequencing run.	Run controlled for: Data quality and total data output

Software-Based Controls		Description of Control	Purpose of Control
Control Name	**Control Status**		
Barcode Assignment QC	Pass/Fail	Determines if barcodes assigned to Run Configuration are detected in the sequencing run	Run controlled for: User errors in barcode assignment or library preparation Sample-swaps Barcode contamination
Whole Genome Read QC	Pass/Fail	Determines if a sample has sufficient whole genome reads to be analyzed by the pipeline software	Sample controlled for: Sufficient whole genome reads per sample Positive Control controlled for: Sufficient whole genome reads per Positive Control
Off-Target Read QC	Pass/Fail	Determines percentage of off-target and unaligned reads in a sample	Sample controlled for: Low percentage of off-target and unaligned reads within sequenced sample Positive Control controlled for: Low percentage of off-target and unaligned reads within sequenced Positive Control
Probe Consistency QC	Pass/Fail	Detection of the number of unique reads for every probe in the assay	Sample controlled for: Uniform probe coverage across targeted by the assay areas Positive Control controlled for: Uniform probe coverage across targeted by the assay areas
Coverage Metrics QC	Pass/Fail	Detection of the number of unique reads for every base targeted by the assay	Sample controlled for: Sufficient read coverage across targeted gene areas Positive Control controlled for: Sufficient read coverage across targeted gene areas
Contamination Detection QC	Pass/Fail	Assesses number of low-allelic frequency (AF) common SNPs in a given sample to determine if the sample is contaminated	Sample controlled for: Between-sample contamination NovaSeq 6000 sequencing instrument carry-over between runs Positive Control controlled for: Between-sample contamination NovaSeq 6000 sequencing instrument carry-over between runs

Software-Based Controls		Description of Control	Purpose of Control
Control Name	**Control Status**		
Positive Control QC	Pass/Fail	Detection of at least 95% of the expected FIRST variants at 1-20% allelic frequency in the FIRST Positive Control by the Positive Control Caller Note: The FIRST Positive Control must also pass the Whole Genome Read QC, Off-Target Read QC, Probe Consistency QC, and Coverage Metrics QC	Run controlled for: Overall Assay Performance Performance of Caller Core bioinformatics pipeline

Reagents and Test Components

Reagents

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 assay utilizes commercially available reagents, as well as specific reagents developed by Resolution Bioscience. Contents of the kit are listed in Table 3.

Table 3. Agilent Resolution ctDx FIRST Reagents

Box 1 of 4: RESOLUTION ctDx FIRST, IVD (PN 500025)		
Probes, ctDx FIRST (PN 101139-001)	Capture of targeted gene regions	µL
Positive Control, ctDx FIRST (PN 101136-001)	Positive control, genomic DNA mixture of cell line DNA, (NA1851 in NA12878) sheared to similar molecular profile cfDNA representing relevant SNPs and Indels in molecular grade water	6 µL
Box 2 of 4: Resolution BARCODES 57-80 Kit (PN 500015)		
Barcode 57-80 (PN 100490-057 to 90-080)	Unique molecular identifiers for patient samples and external controls of 2 unique kits of 24 interchangeable barcodes	x 140 µL
Box 3 of 4: Resolution Barcodes 81-104 Kit (PN 500016)		
Barcode 81-104 (PN 100490-081 to 90-104)	Unique molecular identifiers for patient samples and external controls of 2 unique kits of 24 interchangeable barcodes	x 140 µL
Box 4 of 4: Resolution CORE, IVD (PN 500013)		
Pre-Amp Components – Resolution CORE Kit		
End Repair Buffer (PN 100422-001)	Optimizes enzyme performance used in reaction	60 µL
End Repair Enzyme (PN 100421-001)	DNA repair enzyme	µL
Ligation Mix (PN 100423-001)	Seals phosphate backbone	550 µL
Library PCR Mix (PN 100424-001)	Library amplification	900 µL
Library Primer (PN 101068-001)	First amplification primers	700 µL
Post-Amp Components – Resolution CORE Kit		
Post AMP PCR Mix (PN 100425-001)	Library amplification	50 µL
Hyb Buffer (PN 101078-001)	Hybridizes Probes to targeted gene regions	580 µL
Post Hyb Primer (PN 100426-001)	Post Hybridization Primer	µL
Wash Buffer (PN 101079-001)	Removes off-target and unbound Probes	520 µL
Genomic Mix (PN 100427-001)	Amplification primers	75 µL
Fwd Seq Primer (PN 101080-001)	Forward sequencing primer	µL

Rev Seq Primer (PN 101081-001)	Reverse sequencing primer	µL

Reagents Required but Not Included

- % SDS Solution (ThermoFisher, Catalog # AM9820, or equivalent)
- Proteinase K, 20 mg/mL (ThermoFisher, Catalog # 25530049, or equivalent)
- MagMAX Cell-Free DNA Isolation Kit containing Lysis/Binding Solution, Magnetic Beads, Wash Solution, and Elution Solution (ThermoFisher, Catalog # A29319)
- NEC Dye Control – 6X Loading Dye (ThermoFisher, Catalog # R0611, or equivalent)
- Molecular Biology Reagent-grade Water (nuclease-free) (Sigma Cat No. W4502, or equivalent)
- Molecular Biology Grade Ethanol (Fisher Scientific Cat No. BP28 81 , or equivalent)
- TEZ (TE Buffer, ThermoFisher Scientific Cat. No. 12090015, or equivalent)
- Agencourt AMPure XP Magnetic Beads (Beckman Coulter Cat. No. A6 882)
- Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Cat. No. Q33 or Q32854)
- Dynabeads MyOne Streptavidin C1 (Thermo Fisher Scientific Cat. No. 65002)
- NaOH, molecular biology-grade (Fisher Scientific Cat No. 352 541L, or equivalent)
- M Tris-HCl, pH 8.0 (Thermo Fisher Scientific Cat No. 15568025, or equivalent)
- NovaSeq 6000 S1 Reagent v1.5 Kit (200 cycles) (Illumina Cat. No. 8318)
- HT1 Buffer (Illumina Cat. No. 20015892)
- Vapor-Lock (Qiagen, Cat No. 981611)

Instruments

The Agilent Resolution ctDx FIRST assay is intended to be performed using serial number-controlled instruments as indicated in Table 4. All instruments are qualified under Resolution Bioscience Quality System and used according to manufacturers' instructions.

Table 4. Instrumentation

Instrument/Device (Manufacturer, Cat. No.)
KingFisher Flex System (Thermo Fisher Scientific, Cat. No. 2407)
Hamilton Star Automated Liquid Handler (Beckman Coulter Life Sciences, customized system)
Qubit Fluorometer for dsDNA quantification (Life Technologies Cat No. Q33216, Q33238, Q33327)
MiniAmp Thermal Cyclers (Thermo Fisher Scientific Cat No. A3783)
NovaSeq 6000 Sequencing System (Illumina, NA) (using Illumina software version 1.7.5)

Test Equipment

- Heat block capable of holding 15 mL conical tubes at 60°C
- MiniAmp Thermal Cyclers
- Qubit Fluorometer for dsDNA quantification
- 4 DW Magnet Head mounted on the KingFisher Flex
- Pipet-Aid
- Plate centrifuge capable of 1000 x g, and holding 96-well PCR plates
- Micro-centrifuge for 1.5 mL tubes
- Micro-centrifuge for 0.2 mL tubes
- Side magnet for 0.2 mL tubes (Life Technologies Cat. No. 12331D or Promega Cat. No. C8351)
- Magnet stand for 1.5 mL tubes (Life Technologies Cat. No. 12321D or Promega Cat. No. Z5342)
- Ring magnet for 0.2 mL tubes (Alpaqua Cat. No. A001322-96S)
- Plate roller and plate sealer
- Single-Channel pipettes: p2, p20, p200, p1000
- Multi-Channel pipettes: p20, p200
- Ice machine
- Laboratory freezer -10°C to -30°C
- Laboratory freezer -15°C to -25°C (Required for Illumina reagents)
- Laboratory Refrigerator 2°C to 8°C

Software

The overall Agilent Resolution ctDx FIRST assay software is a cloud-based software solution delivered by the Resolution Bioscience-managed Amazon Web Service (AWS) cloud account. The same secure HIPAA-compliant AWS configuration that delivers the automated ctDx FIRST Pipeline and automated variant logic also delivers software modules which support automated reporting. The ctDx FIRST Pipeline and biomarker determination are further described below.

The overall Agilent Resolution ctDx FIRST Assay Software was designed to securely support clinical variant detection and analysis and encompasses data and software integrity, whitelisting and security policies, encryption standards, operational monitoring and audit logging, and data backups.

Software Pipeline for Sequence Analysis

The Agilent Resolution ctDx FIRST assay uses a custom bioinformatics pipeline, the ctDx FIRST Pipeline, to call variants associated with the genomic targets of interest. The analysis pipeline operates within a secure HIPAA-compliant cloud account managed by Resolution Bioscience and supports automated analysis of each test sample and positive control within a sequencing run. The automated analysis pipeline includes multiple embedded run-level and sample-level data quality controls. All variants must pass variant calling metrics as described in Table 5.

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 5. Variant Calling Threshold/Cut-Off Metrics

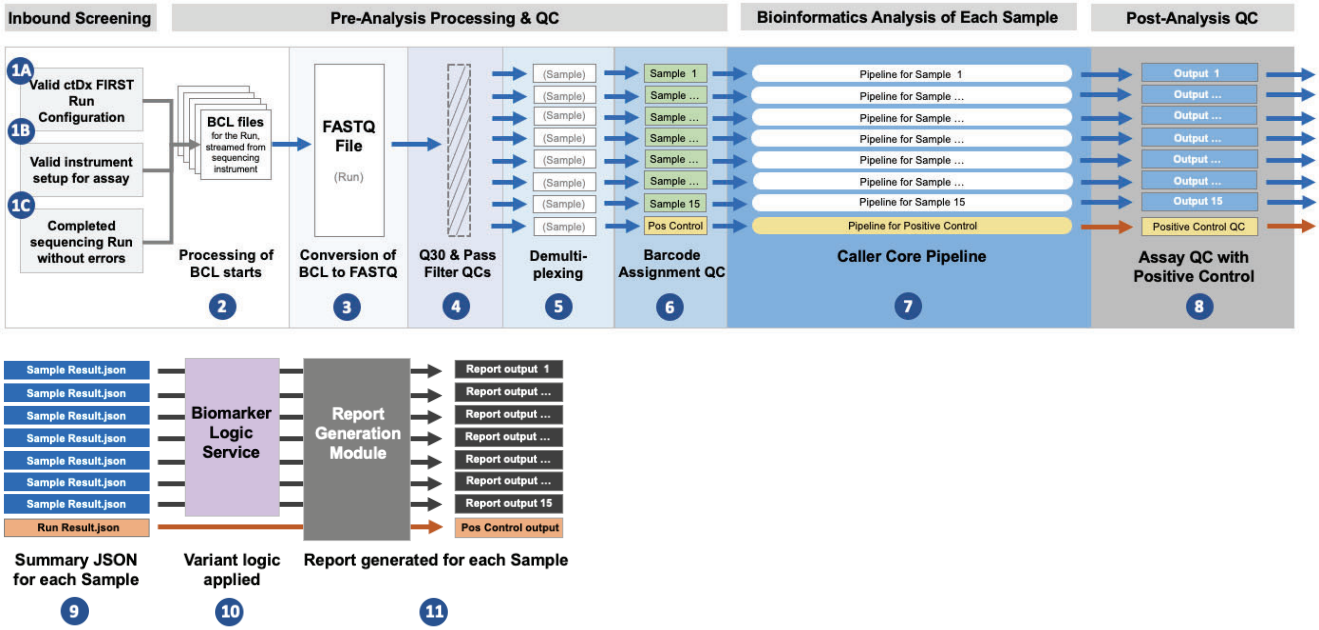
SNV Calling Property	**Metric**
KRAS G12C DNA Molecule Support	≥ 3
KRAS G12C MAF estimate	≥ 0.1%
KRAS G12C Log Likelihood Score	≥ 6 (if DNA Molecule Support = 3), > 0 (if DNA Molecule Support ≥ 4)
Other substitutions[1] DNA Molecule Support	≥ 3
Other substitutions[1] MAF estimate	≥ 0.1%
Other substitutions[1] Log Likelihood Score	≥ 10
Indel[2] Calling Property	**Metric**
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

[1] Other substitutions refer to EGFR T790M and L858R; [2] INDELS refer to Exon 19 deletions

Biomarker Status Determination

After variants are detected by the ctDx FIRST Assay Pipeline, the Agilent Resolution ctDx FIRST assay uses custom biomarker logic to determine biomarker positivity based on the sequence variations. The logic flow for determining biomarker positivity based on detection of KRAS G12C alone or concurrent with EGFR Exon 19 deletions, EGFR L858R, and EGFR T790M mutations (Figure 2). The sequence variants that are required for a positive Agilent Resolution ctDx FIRST status result are listed in the indications for use.

Figure 2: Automated Software Workflow



Category Definitions

The Test Report includes genomic findings reported in the following categories:

Category	Prescriptive Use for Therapeutic Product	Clinical Performance	Analytical Performance	Description
Category 1: Companion Diagnostic (CDx)	YES	YES	YES	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Resolution Bioscience has demonstrated clinical performance shown to support 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 companion diagnostics for which Resolution Bioscience has demonstrated analytical reliability and accuracy but not clinical performance.

Performance Characteristics

Analytical Accuracy: KRAS G12C

KRAS G12C detection capability was compared between the Agilent Resolution ctDx FIRST assay and an externally validated ddPCR assay. A total of 230 cfDNA samples from NSCLC clinical plasma samples were tested across both assays (Institutional Review Board review and informed consent was obtained for clinical samples):

- 76 samples from NSCLC patients enrolled in the Mirati Phase 2 Cohort A 849-001 adagrasib study
- 154 commercially procured plasma samples from NSCLC subjects with non-specified KRAS G12C mutation status representative of the clinical trial population

Seven samples failed to generate valid results in the Agilent Resolution ctDx FIRST assay due to sequencing QC failures; one of these samples also generated invalid results by ddPCR testing. The remaining 223 samples generated valid results by both assays and were evaluated for KRAS G12C concordance (Table 6). Out of the 54 samples that were KRAS G12C positive by ddPCR testing (51 from the 849-001 study), 47 were confirmed positive by Agilent Resolution ctDx FIRST, resulting in a PPA of 87.0% (95% CI: 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% (95% CI: 94.1, 99 4).

Table 6. KRAS G12C Concordance Results Between Agilent Resolution ctDx FIRST and ddPCR

	ddPCR KRAS G12C Positive	**ddPCR KRAS G12C Negative**
ctDx FIRST KRAS G12C Positive	47	4
ctDx FIRST KRAS G12C Negative	7	65
Total	54	69
	PPA: (47/54) 87.0% [75.1, 94.6] **NPA: (165/169) 97.6%** [94.1, 99.4]	

Analytical Accuracy: EGFR Exon 19 Deletions, EGFR L858R, EGFR 90M

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 NSCLC clinical plasma specimens were tested across both assays:

- 91 samples from NSCLC subjects selected from remnant biobanked samples. Due to the low prevalence of EGFR T790M and L858R, all evaluable mutation-positive

samples were tested. Multiple subtypes of EGFR Exon 19 Deletions were included among the tested samples.

- 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 biobanked, 89 all-comers) generated a total of 334 comparative results that were valid across both assays and evaluated for variant concordance (Table 7). 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%). 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 7. Concordance Results Between Agilent Resolution ctDx FIRST and ddPCR for EGFR Variants

	EGFR L858R		EGFR T790M		EGFR Exon 19 Deletions	
	ddPCR Positive	**ddPCR Negative**	**ddPCR Positive**	**ddPCR Negative**	**ddPCR Positive**	**ddPCR Negative**
ctDx FIRST Positive	8		9		9	
ctDx FIRST Negative		88		89		85
Total	9	90		89		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]	

Limit of Detection (LoD) – Analytical Sensitivity

LoD values were established using three pools of NSCLC clinical cfDNA samples with KRAS G12C, EGFR exon 19 deletions, EGFR L858R, and EGFR T790M variants diluted in cfDNA from risk-matched healthy donor plasma. 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.

LoD95 was determined for each variant by calculating the hit rate at each MAF level across samples and performing probit analysis, as described in CLSI EP 7-A2. LoD95 estimates are listed in Table 8.

Table 8. Limit of Detection Estimations for KRAS G12C and EGFR Variants

Variant	LoD95 (%MAF)
KRAS G12C	.581%
EGFR exon 19 deletions	.380%
EGFR L858R	.505%
EGFR T790M	.983%

Limit of Blank (LOB)

LOB was established by profiling plasma from 30 individual risk-matched healthy donors for false positive variant calling. Each of the 30 donors was tested six times for a total of 180 results. Libraries were prepared from 50 ng of cfDNA – the maximum input of the Agilent Resolution ctDx FIRST assay in order to increase the likelihood of detecting false positives – with 3-6 reagent lots, instruments, and operators.

No false positives were observed across the 180 sample replicates, and so the false positive rate was 0% for KRAS G12C, EGFR exon 19 deletions, EGFR L858R, and EGFR T790M variants (Table 9). None of these variants were observed in matched genomic DNA samples.

Table 9. Limit of Blank Study Results – KRAS G12C and EGFR Variants

Variant	No. False Positives/ Sample Replicates	False Positive Rate
KRAS G12C	80	%
EGFR exon 19 deletions	80	%
EGFR L858R	80	%
EGFR T790M	80	%

In Silico Primers (Bait Specificity)

To determine primer and probes specificity for Agilent Resolution ctDx FIRST, panel probes were aligned to the human genome (hg19), modified to include sequences from internal reference genome database for commensal and pathogenic microorganisms, 98.43% of probes uniquely mapped to the human genome. All probes for KRAS G12X and EGFR mapped uniquely to their targets. None of the probes mapped to commensal or pathogenic microbial sequences that are known to colonize or infect humans. The specificity of the primers was evaluated using NCBI's Primer-BLAST tool. No target templates were found for any of the primer pairs.

Precision of Plasma Extraction Procedure

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 singlicate or duplicate.

Forty (40) samples and 169 sample-replicates, 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 10).

There were two cases in which the expected variant (EGFR L858R or EGFR exon19del) was not detected in *one of two* replicates of a precision combination, but there was no pattern by variant or precision combination. The median MAF across the detected replicates for both variants was 5% and 0.50%, respectively.

Table . Precision of Plasma Extraction Variant Detection Results – KRAS G12C, EGFR L858R, and EGFR exon 19 deletions

Agreement Statistic	Number of Detected Pairs			APA (95% CI)	ANA (95% CI)
	Concordant Positive	Concordant Negative	Discordant		
Between-Precision Combination	59	9	4	98.8% (96.8, 100)	99.4% (98.5, 100)
Within-Precision Combination	52	8		98.1% (95.1, 100)	99.1% (97.7, 100)

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 over multiple days across unique combinations of experimental components by analyzing data generated in the LoD establishment study. 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.

Three pools of NSCLC clinical samples with KRAS G12C, KRAS G12V, EGFR exon 19 deletions, EGFR L858R, and EGFR T790M variants were serially diluted in cfDNA from risk-matched healthy donor plasma to a range of MAF levels bracketing the verified LoD95 of the assay. Six or seven MAF levels were tested for each variant, and each MAF level was tested with 35–36 replicates at the minimum allowable assay input of 15 ng cfDNA.

For each variant, PPA and APAs were calculated at each MAF level, and the MAF levels that were closest to 1.5X and 2–3X LoD in each sample were used to evaluate precision. 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 the 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 by Variant at 1.5X and 2-3X LoD

Variant	.5X LoD			–3X LoD		
	%MAF	No. Observed/ Expected	PPA (95% CI [%])	%MAF	No. Observed/ Expected	PPA (95% CI [%])
KRAS G12C	.6–0.7%	4/108	96.3% (90.9, 98.6)	.3–1.5%	8/108	% (96.6, 100)
KRAS G12V	.7%	5/36	97.2% (85.8, 99.9)	.5%	6/36	% (90.4, 100)
EGFR L858R	.5–0.7%	70/72	97.2% (90.4, 99.2)	.2–1.3%	72/72	% (94.9, 100)
EGFR exon 19 deletions	.3–0.5%	70/72	97.2% (90.4, 99.2)	.6–0.8%	72/72	% (94.9, 100)
EGFR T790M	.2%	6/36	% (90.4, 100)	.3%	6/36	% (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 five variants at 1.5X LoD and were 100% for all variants at 2–3X LoD (Table 10). Repeatability was assessed where possible by comparing replicates matched within runs or within operators; it ranged from 96.2% to 100% across the five variants at 1.5X LoD and was 100% across all variants at 2–3X LoD (Table 12).

Table 12. Precision APA (95% CI [%]) Results Between Experimental Components at .5X and 2-3X LoD

Variant	Closest LoD Level	ctDx FIRST Reagent Lots	Operators	Sequencing Reagent Lots	Sequencers	Within Runs
KRAS G12C	.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)
	-3X	% (96.6,100)	% (96.6,100)	% (96.6,100)	% (96.6,100)	% (96.6,100)
KRAS G12V	.5X	97.1% (90.9,100)	97.1% (90.9,100)	97.1% (90.9,100)	97.1% (90.9,100)	97.1% (90.9,100)
	-3X	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)
EGFR L858R	.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)
	-3X	% (94.9,100)	% (94.9,100)	% (94.9,100)	% (94.9,100)	% (94.9,100)
EGFR exon 19 deletions	.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)
	-3X	% (94.9,100)	% (94.9,100)	% (94.9,100)	% (94.9,100)	% (94.9,100)
EGFR T790M	.5X	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)

Variant	Closest LoD Level	ctDx FIRST Reagent Lots	Operators	Sequencing Reagent Lots	Sequencers	Within Runs
	-3X	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)	% (90.4,100)

For NPA and 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, and 50 ng cfDNA 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, EGFR exon 19, EGFR L858R, or EGFR T790M variant positions, therefore, NPA was 100% for all five variants (Table 13). There was no evidence for differences between operators, Agilent Resolution ctDx FIRST reagent lots, sequencing reagent lots, or sequencers, therefore, the best estimates for ANA matched the NPA estimates.

Table 13. Precision NPA Results

Variant	Number of Replicates	NPA (95% CI)
KRAS G12C	80	% (97.9, 100)
KRAS G12V	80	% (97.9, 100)
EGFR L858R	80	% (97.9, 100)
EGFR exon 19 deletions	80	% (97.9, 100)
EGFR T790M	80	% (97.9, 100)

Interfering Substances

Seven potential endogenous interferents (hemoglobin, albumin, unconjugated bilirubin, conjugated bilirubin, glyceryl trioleate, high molecular weight gDNA, and *Staphylococcus pidermis*) and six potential exogenous interferents (paracetamol, prednisone, dexamethasone, metoclopramide hydrochloride, ethanol, and proteinase K) at medically relevant concentrations, as well as 4 diluent controls (water, phosphate buffered saline, ethanol, and sodium hydroxide) were evaluated.

Plasma sample blends were prepared from cfDNA extracted from NSCLC clinical plasma samples diluted into risk-matched healthy donor plasma to generate two sample blends for KRAS G12C, EGFR L858R, EGFR exon 19 deletion variants, and one sample blend for EGFR T790M, at allelic frequencies close to LoD. Five to six plasma sample replicates were spiked with each interferent and proceeded to testing with the Agilent Resolution ctDx FIRST assay.

Apart from paracetamol, PPA and NPA were 100% for all interferents across all variants (Table 14 and Table 15). In the presence of paracetamol, a single KRAS G12C variant was not detected

as expected, resulting in a PPA of 91.7% (11/12 detected); the median %MAF for KRAS G12C in this sample was below LoD95 (0.93X LoD).

Table 14. Interfering Substances PPA Summary Results

Substance	KRAS G12C		EGFR L858R, Exon 19 Del, T790M	
	Observed/ Expected Positive	PPA (95% CI [%])	Observed/ Expected Positive	PPA (95% CI [%])
Hemoglobin		% (75.8, 100)	8/28	% (87.0, 100)
Albumin		% (75.8, 100)		% (88.6, 100)
Unconjugated Bilirubin		% (75.8, 100)		% (88.6, 100)
Conjugated Bilirubin		% (75.8, 100)		% (88.6, 1)
Glyceryl Trioleate		% (75.8, 100)	8/28	% (87.0, 100)
Paracetamol		91.7% (64.6, 100)		% (88.6, 100)
Prednisone		% (75.8, 100)		% (88.6, 100)
Dexamethasone		% (75.8, 100)		% (88.6, 100)
Metoclopramide		% (75.8, 100)		% (88.6, 100)
Ethanol		% (75.8, 100)		% (88.6, 100)
Proteinase K		% (75.8, 100)		% (88.6, 100)
HMW gDNA		% (75.8, 100)		% (88.6, 100)
S. epidermis		% (75.8, 100)		% (88.6, 100)

Hemoglobin, 10 mg mL; Albumin, 60 mg mL; Unconjugated Bilirubin, 0.4 mg mL; Conjugated Bilirubin, 0.4 mg mL; Glyceryl Trioleate (Triglyceride), 15 mg/mL; Paracetamol, 0.156 mg/mL; Prednisone, 0.099 μg/mL; Dexamethasone, 12 μg/mL; Metoclopramide Hydrochloride, 2.52 μg/mL; Ethanol, 5%; Proteinase K, 0.6 mg mL; High molecular weight gDNA (HMW gDNA), 1:1 (w w); *Staphylococcus epidermis*, 10^6 CFU/mL.

Table 15. Interfering Substances NPA Summary Results

Substance	KRAS G12C		EGFR L858R, Exon 19 Del, T790M	
	Observed/ Expected Negative	NPA (95% CI [%])	Observed/ Expected Negative	NPA (95% CI [%])
Hemoglobin	5 5	% (56.6, 100)		% (85.1, 100)
Albumin	6/6	% (61.0, 100)	4/24	% (86.2, 100)
Unconjugated Bilirubin	6 6	% (61.0, 100)	4/24	% (86.2, 1)
Conjugated Bilirubin	6 6	% (61.0, 100)	4/24	% (86.2, 100)
Glyceryl Trioleate	5 5	% (56.6, 100)		% (85.1, 100)
Paracetamol	6/6	% (61.0, 100)	4/24	% (86.2, 100)
Prednisone	6 6	% (61.0, 100)	4/24	% (86.2, 100)
Dexamethasone	6/6	% (61.0, 100)	4/24	% (86.2, 100)
Metoclopramide	6 6	% (61.0, 100)	4/24	% (86.2, 100)
Ethanol	6/6	% (61.0, 100)	4/24	% (86.2, 100)

Substance	KRAS G12C		EGFR L858R, Exon 19 Del, T790M	
	Observed/ Expected Negative	NPA (95% CI [%])	Observed/ Expected Negative	NPA (95% CI [%])
Proteinase K	6 6	% (61.0, 100)	4/24	% (86.2, 100)
HMW gDNA	6 6	% (61.0, 100)	4/24	% (86.2, 100)
S. epidermis	6/6	% (61.0, 100)	4/24	% (86.2, 100)

See Table 14 footnote for substance details

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 samples obtained from clinical plasma samples 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 plated in a checkerboard pattern to maximize potential 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. No carryover or cross-contamination was observed in this study.

Robustness/Guardbanding

The robustness of the Agilent Resolution ctDx FIRST assay workflow was assessed by challenging the assay outside the specified conditions for probe hybridization and wash conditions (Table 16).

Table 16. Robustness Testing Conditions

Condition	Reference	Low	High
Hybridization Time	– 24 hours	.5 hours	4.5 hours
Wash Buffer Temperature	°C – 25°C	8°C	7°C
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 G1 C, EGFR L858R, and EGFR exon 19 deletion variants that was diluted into a background of 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. Variants were included in analysis if they were identified in all replicates and above 1X LoD in the reference condition.

PPA and NPA were 100% across all conditions and variants, as shown in Table 17. These results indicate that the Agilent Resolution ctDx FIRST assay is robust to changes in hybridization and wash conditions.

Table 17. Robustness Results Summary – KRAS G12C, EGFR L858R, EGFR Exon 19 Deletion

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

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 NSCLC clinical plasma samples 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%.

cfDNA Sample Input Range

The cfDNA sample input range of the Agilent Resolution ctDx FIRST assay (15 to 50 ng) was verified by testing 5 NSCLC clinical sample cfDNA blends with KRAS G12C, EGFR L858R, or EGFR exon 19 deletions. 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 17 and Table 19).

Table 18. cfDNA Input Summary Results – KRAS G12C

cfDNA Input Mass	Observed/ Expected Positive Calls	PPA (95% CI [%])	Observed/ Expected Negative Calls	NPA (95% CI [%])
7.5 ng		% (75.8, 100)		% (72.2, 100)
ng		% (75.8, 100)		% (72.2, 100)
5 ng		% (74.1, 100)		% (72.2, 100)
50 ng	Reference			% (72.2, 100)

75 ng		% (75.8, 100)		% (72.2, 100)

Table 19. cfDNA Input Summary Results – 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		% (72.2, 100)	50/50	% (92.9, 100)
ng		% (72.2, 100)	50/50	% (92.9, 100)
5 ng		% (72.2, 100)	48/48	% (92.6, 100)
50 ng	Reference		50/50	% (92.9, 100)
75 ng		% (72.2, 100)	50/50	% (92.9, 100)

Reagent Lot Interchangeability

The Agilent Resolution ctDx FIRST assay requires 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 conditions were tested comprised of unique combinations from 3 lots of CORE, 3 lots of FIRST, and 3 combinations of reagents from 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 NSCLC clinical plasma samples was diluted into cfDNA extracted from risk-matched healthy donor plasma. Twelve (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 × 12 replicates = 108 expected calls per variant), resulting in a PPA of 100% for each variant (Table 20).

Table 20. PPA Results Across 9 Reagent Interchangeability Conditions

Variant	Observed/Expected Positive Calls	PPA (95% CI [%])
KRAS G12C	8/108	.0% (96.6, 100.0)
EGFR exon 19 deletions	8/108	.0% (96.6, 100.0)
EGFR L858R	8/108	.0% (96.6, 100.0)

Reagent Stability: Real-Time

The Agilent Resolution ctDx FIRST assay requires three reagent kits for sample processing: CORE, FIRST, and BARCODES. The real-time shelf life of these reagents at -20°C ± 10°C will be assessed over four timepoints:

- T0 (reference condition): within one month of kit qualification
- T1: 5 months after T0
- T2: 8 months after T0
- T3: 13 months after T0

cfDNA from NSCLC clinical plasma samples diluted into cfDNA from risk-matched healthy donor plasma to generate a sample blend with KRAS G12C, EGFR L858R, and EGFR exon 19 deletion variants at ~1.5X LoD as well as cfDNA from risk-matched healthy donor plasma were tested.

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.

At each timepoint T0, T1, and T2, 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 21), indicating Agilent Resolution ctDx FIRST assay reagent kits are stable for at least 7 months at -20°C ± 10°C.

Stability testing is ongoing, and results from T3/13 months testing will be evaluated.

Table 21. Reagent Real-Time Stability Results at 5 and 8 months

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	% (64.6, 100)	% (64.6, 100)
		R2	7 7	7 7	% (64.6, 100)	% (64.6, 100)
		R3	7 7	7 7	% (64.6, 100)	% (64.6, 100)
	T2 (8 months)	R1	7 7	7 7	% (64.6, 100)	% (64.6, 100)
		R2	7 7	7 7	% (64.6, 100)	% (64.6, 100)
		R3	7 7	7 7	% (64.6, 100)	% (64.6, 100)

Variant	Timepoint	Reagent Kit Assembly	Observed Expected Positive	Observed Expected Negative	PPA (95% CI[%])	NPA (95% CI[%])
EGFR L858R, EGFR exon 9 deletions	T1 (5 months)	R1	4/14	4/14	% (78.5, 100)	% (78.5, 100)
		R2	4/14	4/14	% (78.5, 100)	% (78.5, 100)
		R3	4/14	4/14	% (78.5, 100)	% (78.5, 100)
	T2 (8 months)	R1	4/14	4/14	% (78.5, 100)	% (78.5, 100)
		R2	4/14	4/14	% (78.5, 100)	% (78.5, 100)
		R3	4/14	4/14	% (78.5, 100)	% (78.5, 100)

Reagent Stability: In-Use

The Agilent Resolution ctDx FIRST assay requires three reagent kits for sample processing: CORE, FIRST, and BARCODES. The in-use stability of these reagents was assessed over three timepoints/stability conditions using a single assembly comprised of each reagent kit type:

- T0 (reference condition): initial thaw from -20°C ± 10°C
- T1: 2 freeze-thaw cycles and 16–18 days at -20°C ± 10°C
- T2: 3 freeze-thaw cycles and 32–37 days at -20°C ± 10°C

Three sample blends were tested in this study: two were cfDNA extracted from NSCLC clinical plasma samples diluted into cfDNA extracted from risk-matched healthy donor plasma to generate sample blends with KRAS G12C, EGFR L858R, and EGFR exon 19 deletion variants close to LoD, and the third was 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.

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 % across both T1 and T2 stability conditions (Table 22), ind icating Agilent Resolution ctDx FIRST assay reagents are stable for at least 2 freeze-thaw cycles and 30 days at -20°C ± 10°C.

Table 22. Reagent In-Use Stability Results

Variant	Timepoint	Observed/ Expected Positive	Observed/ Expected Negative	PPA (95% CI [%])	NPA (95% CI [%])
KRAS G12C	T1	4/14	7 7	% (78.5, 100)	% (64.6, 100)
	T2	4/14	7 7	% (78.5, 100)	% (64.6, 100)
	T1	8/28	4/14	% (87.9, 100)	% (78.5, 100)

Variant	Timepoint	Observed/ Expected Positive	Observed/ Expected Negative	PPA (95% CI [%])	NPA (95% CI [%])
EGFR L858R, EGFR exon 19 deletions	T2	8/28	4/14	% (87.9, 100)	% (78.5, 100)

Stability of Whole Blood Samples from Healthy Donors

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:

- **Day 0:** Reference Condition. Storage at room temperature (18–25) and processing to plasma within 4 hours of collection.
- **RT Day 1:** Storage at room temperature (18–25°C) until processing to plasma on Day 1 after collection.
- **RT Day 8:** Storage at room temperature (18–25°C) 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 room temperature (18–25°C) 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 room temperature (18–25°C) until processing to plasma on Day 2 after collection.

For each donor, 5 × 10 mL whole blood samples were collected in Streck Cell-Free DNA BCTs, and one tube was tested in each stability condition. 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 Agilent Resolution ctDx FIRST assay. To ensure that performance differences could be detected, a donor and all associated replicates were included in the study if the Day 0 reference condition replicate reached a minimum cfDNA concentration of 0.5 ng/µL following plasma extraction. Stability was determined in 20 donors that met this criterion by evaluating assay QC pass rates, average coverage depth, and NPA (Table 23).

Table 23. Healthy Donor Whole Blood Stability Results

Stability Condition	Sample Assay QC Pass Rate	ΔCoverage Depth	NPA (95% CI) (Detection Rate)
Day 0	% (20/20)	Reference	% (95.4, 100) (80/80)
RT Day 1	% (20/20)	- .4%	% (95.4, 100) (80/80)
RT Day 8	95% (19/20)	-8.3%	% (95.2, 100) (76/76)
Low Humidity	% (20/20)	-5.1%	% (95.4, 100) (80/80)
High Humidity	% (20/20)	-5.4%	% (95.4, 100) (80/80)

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

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.

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 KRAS G12C or EGFR exon 19 deletions diluted into risk-matched healthy donor plasma were tested over four timepoints and two storage conditions.

- T0 (reference condition): cfDNA processed in the Agilent Resolution ctDx FIRST assay within 2 hours of extraction.
- hours: cfDNA stored at 2 – 8°C for 12–24 hours
- 5 days: cfDNA stored at -20°C ± 10°C for 15 days
- 31 days: cfDNA stored at -20°C ± 10°C °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. Since no false positives were observed, NPA was 100% across all conditions.

Short-term Stability of Assay Intermediate Products

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

- T0 (reference condition): Intermediate products tested within 2 hours of creation.
- 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.

All 60 sample replicates (12 replicates at T0 and 12 replicates at the other two timepoints per intermediate product) 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 % across both intermediate products and storage timepoints.

Clinical Validation Bridging Study

This study evaluated baseline plasma samples originally collected from Cohort A of the Phase 2 portion of the Mirati 849-001 study of adagrasib. Patients with NSCLC that were eligible for the clinical bridging study were those with measurable disease at baseline that comprised the sponsor's Full Analysis Set with Blinded Independent Central Review (FAS-BICR). Cohort A patients were enrolled based on having a KRAS G12C mutation detected in tumor tissue.

The primary endpoint for efficacy in the adagrasib clinical study was the objective response rate (ORR) defined as the fraction of the cohort experiencing tumor response (Partial Response [PR] or Complete Response [CR]), where objective disease response was assessed in individual patients in accordance with Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST).

As part of the adagrasib clinical study, subjects were required to submit a screening, pre-treatment whole blood sample in Streck cell-free DNA blood collection tubes to Resolution Bioscience's CLIA laboratory. Samples were plasma separated, frozen, and biobanked at Resolution Bioscience. Samples were then tested with the Agilent Resolution ctDx FIRST assay as part of the bridging study. All available plasma samples were tested for this study.

The FAS-BICR Cohort A comprised NSCLC patients with a KRAS G12C mutation identified in tissue, and so does not represent the entire possible population of patients who might be selected if only the Agilent Resolution ctDx FIRST assay had been used for selection. Commercially procured, paired plasma and tissue samples from an additional random cohort (Cohort R) of NSCLC subjects with non-specified KRAS G12C status were collected to assess the chance that a subject with no KRAS G12C mutation detected in tissue would have a KRAS G12C mutation detected by the Agilent Resolution ctDx FIRST assay. This was used to assess the sensitivity of the efficacy results to the inclusion of patients with a KRAS G C variant identified by the Agilent Resolution ctDx FIRST assay but who had no KRAS G12C mutation identified in tissue. The paired tissue samples from Cohort R were tested with the most commonly used tissue test used for tissue screening of Cohort A.

Cohort A FAS-BICR Study Population

A total of 116 patients were enrolled in Cohort A, however, 4 patients were excluded from the sponsor's primary analysis due to lack of measurable disease at baseline; therefore, there were 112 evaluable enrolled patients (FAS-BICR). Figure 3 summarizes the disposition of samples. Results were considered "Complete" if a valid Agilent Resolution ctDx FIRST result was available (n=71) and "Incomplete" if a Agilent Resolution ctDx FIRST result was missing (Unevaluable Result or Sample Not Tested, n=41).

Figure 3. Sample Disposition of Cohort A FAS-BICR Samples for Bridging Study

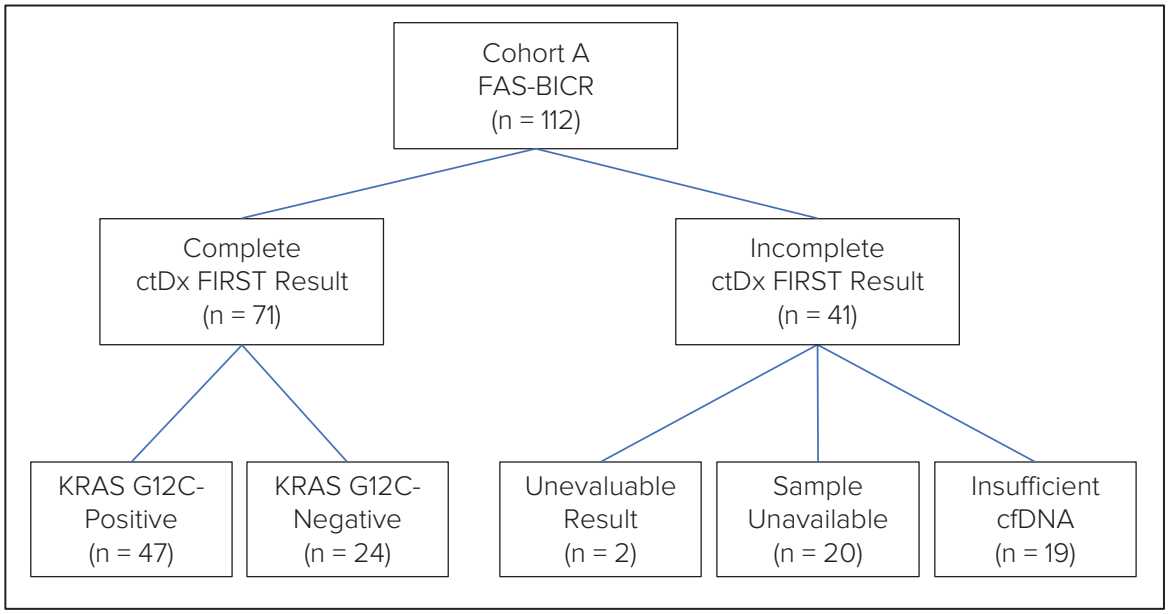


Table 24 summarizes the demographic and baseline characteristics for the study population, including summaries separated by the availability of valid Agilent Resolution ctDx FIRST results (Complete/Incomplete). For the continuous variable "Age", a t-test was used to compare the Complete and Incomplete subsets. For all other (categorical) variables, a Fisher exact test was used to compare the counts in the Complete and Incomplete subsets. There were no significant differences ($\alpha = 0.05$) for any of the demographic and baseline clinical characteristics.

Table 4. Cohort A Demographic, Clinical, and Screening Test Characteristics for Bridging Study

Variable (p-value)	**Level**	**Complete (n = 71)**	**Incomplete (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)
	(Minimum, Maximum)	(5, 89)	(9, 84)	(5, 89)
Sex (0.6944)	Female	8 (53.5%)	4 (58.5%)	62 (55.4%)
	Male	(46.5%)	7 (41.5%)	50 (44.6%)
Race (0.9359)	American Indian or Alaska Native	(1.4%)	(0%)	(0.9%)
	Asian	4 (5.6%)	(2.4%)	5 (4.5%)
	Black or African American	6 (8.5%)	(7.3%)	9 (8.0%)
	Other	(4.2%)	(2.4%)	4 (3.6%)
	White	57 (80.3%)	6 (87.8%)	93 (83.0%)
Smoking History (0.1706)	Past Smoker	64 (90.1%)	(78.0%)	96 (85.7%)
	Current Smoker	4 (5.6%)	7 (17.1%)	(9.8%)
	Lifetime Non-Smoker	(4.2%)	(4.9%)	5 (4.5%)
ECOG Status		5 (21.1%)	(7.3%)	8 (16.1%)

Variable (p-value)	Level	Complete (n = 71)	Incomplete (n = 41)	FAS-BICR (n = 112)
(0.0584)		56 (78.9%)	7 (90.2%)	93 (83.0%)
	N/A	(0%)	(2.4%)	(0.9%)
Histology (1.0)	Non-Squamous	69 (97.2%)	40 (97.6%)	9 (97.3%)
	Squamous	(2.8%)	(2.4%)	(2.7%)
Disease Type (0.0529)	Locally Advanced	(15.5%)	(2.4%)	(10.7%)
	Metastatic	60 (84.5%)	40 (97.6%)	(89.3%)
Tissue Sample Location (0.4643)	Lung	44 (62.0%)	9 (46.3%)	63 (56.2%)
	Lymph Node	(18.3%)	9 (22.0%)	(19.6%)
	Adrenal	4 (5.6%)	(4.9%)	6 (5.4%)
	Bone	(4.2%)	(4.9%)	5 (4.5%)
	Liver	(2.8%)	4 (9.8%)	6 (5.4%)
	Other*	5 (7.0%)	5 (12.2%)	(8.9%)
CTA Tissue Test (0.0943)	Tissue Test 1	9 (26.8%)	6 (14.6%)	5 (22.3%)
	Tissue Test 2	(15.5%)	(7.3%)	4 (12.5%)
	Other	41 (57.7%)	(78.0%)	73 (65.2%)

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

Table 25 summarizes the bridging study clinical outcome variables for the study population. There were no statistically significant ($\alpha = 0.05$) differences in the distribution of outcomes between the samples with and without Agilent Resolution ctDx FIRST assay results.

Table 25. Cohort A Outcome Data for Bridging Study

Variable (Fisher exact p-value)	Level	Complete (n = 71)	Incomplete (n = 41)	FAS-BICR (n = 112)
OR Final (0.8455)	Objective Response	(43.7%)	7 (41.5%)	48 (42.9%)
	No Objective Response	40 (56.3%)	4 (58.5%)	64 (57.1%)
Best OR (0.9847)	Complete Response	(1.4%)	(0%)	(0.9%)
	Partial Response	(42.3%)	7 (41.5%)	47 (42.0%)
	Stable Disease	6 (36.6%)	5 (36.6%)	41 (36.6%)
	Progressive Disease	4 (5.6%)	(4.9%)	6 (5.4%)
	Not Evaluable	(14.1%)	7 (17.1%)	7 (15.2%)

Primary Analysis of ORR

For the assessment of the Agilent Resolution ctDx FIRST assay, the calculation of the ORR was restricted to patients who had a KRAS G12C positive result with the Agilent Resolution ctDx FIRST assay.

Of the 112 patients in the FAS-BICR, Agilent Resolution ctDx FIRST assay results were available for 71 of them (63.4%), of which 47 had a KRAS G12C positive result (66.2%). Table 26 shows the ORR for these 47 patients who constitute the primary analysis subset for evaluating

the Agilent Resolution ctDx FIRST assay together with the exact Clopper-Pearson 95% confidence interval. The lower 95% exact confidence limit for the ORR was 36.1%.

Table 26. Objective Response Rate for Primary Analysis Subset

Analysis Set	Objective Response		Total	ORR [95% CI]
	Yes	No		
ctDx FIRST KRAS G12C-Positive	4		47	51.1% [36.1%,65.9%]

For comparison, Table 27 shows the ORR with 95% confidence intervals for patients with a negative KRAS G12C Agilent Resolution ctDx FIRST assay result, for patients without a valid Agilent Resolution ctDx FIRST assay result, and for the full FAS-BICR cohort.

Table 27. Objective Response Rate for Secondary Analysis Sets

Analysis Set	Objective Response		Total	ORR [95% CI]
	Yes	No		
ctDx FIRST KRAS G12C-Negative	7	7	4	9.2% [12.6%,51.1%]
ctDx FIRST KRAS G12C Missing	7	4	41	41.5% [26.3%,57.9%]
FAS-BICR	48	64		42.9% [33.5%,52.6%]

Imputation for 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 KRAS G12C Agilent Resolution ctDx FIRST assay positivity in a logistic regression analysis for inclusion in the imputation analysis. Table 28 shows univariate logistic regression p-values for all the demographic variables and baseline clinical characteristics considered.

Table 28. Univariate p-values for Demographic and Baseline Clinical Variables

Variable	Chi-squared p-value
Smoking History	.37
Tissue Sample Location	.46
Age (Continuous)	.17
Sex	.67
Disease Type	.85
Tissue Test Type	.91
ECOG Performance Status	.97
Race Category	.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 29 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 29. 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]
.4750	.0034	.0033	.0067	47.5% [31.5%,63.5%]

Analysis of Duration of Response Data

Median duration of response (DoR) was estimated using the Kaplan-Meier method for responders in the original FAS-BICR Cohort A, and for the subset of Cohort A who were KRAS G12C positive by the Agilent Resolution ctDx FIRST assay (ctDx FIRST-Positive). The observed percentages of patients with duration of response beyond 6 months is also reported.

Table 30. Duration of Response Analysis for Cohort A

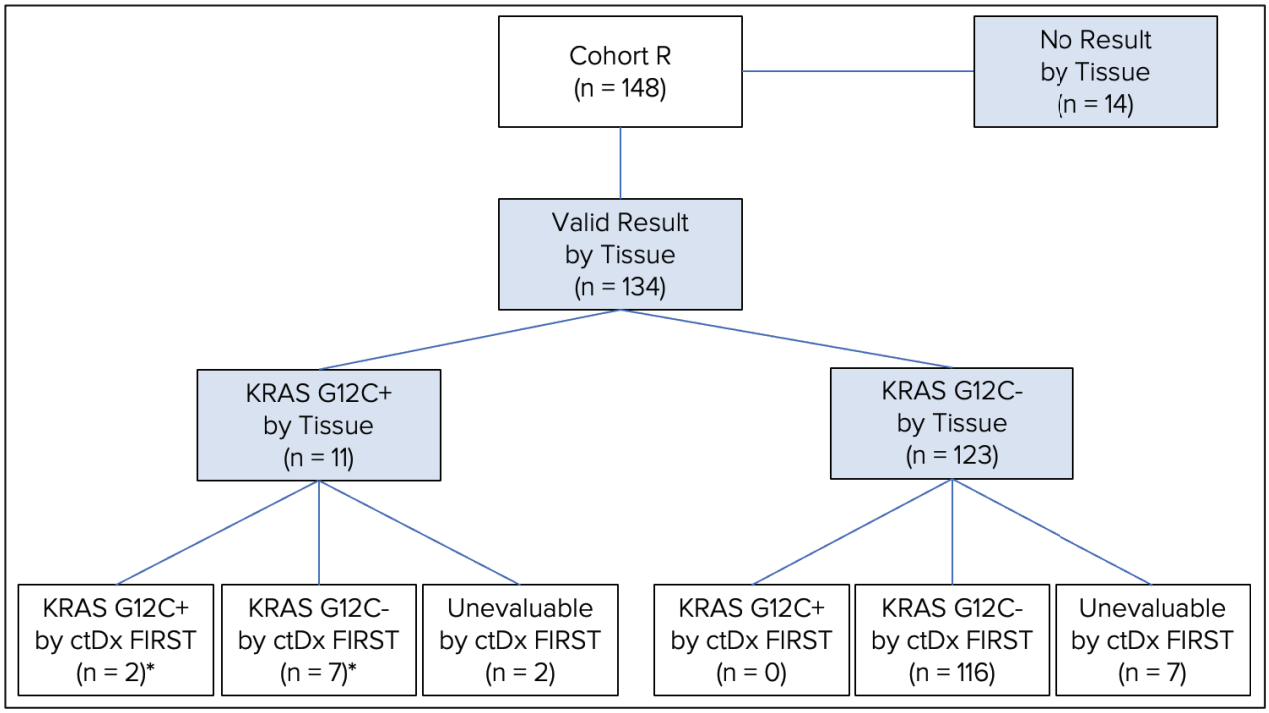
Efficacy Parameter	ctDx FIRST-Positive (n = 47)	FAS-BICR (n = 112)
Median[a] in months (95% CI)	6.9 [3.1,10.6]	8.5 [6.2,13.8]
Patients with duration ≥ 6 months[b], %	46	58

[a] Estimate using Kaplan-Meier method
[b] Observed proportion of patients with duration of response beyond landmark time

Sensitivity Analysis for Primary Efficacy Analysis

As all patients with clinical information had previously been determined to have a KRAS G12C positive result from a tissue assay, there was no clinical information available on patients who might be KRAS G12C negative on a tissue test but KRAS G12C positive on the Agilent Resolution ctDx FIRST assay. If there were such patients that would be found if the Agilent Resolution ctDx FIRST was used as the screening CDx, their presence could potentially diminish the ORR observed in the trial. Providing an estimate of how likely tissue-negative subjects are to be Agilent Resolution ctDx FIRST positive was the reason for testing subjects in a second cohort of commercially procured NSCLC plasma samples (Cohort R) using both the Agilent Resolution ctDx FIRST assay and the most commonly used tissue assay in the original screening for the trial. Figure 4 shows the sample disposition for the 148 subjects in Cohort R tested by at least one of the assays.

Figure 4. Sample Disposition of Cohort R samples for Bridging Study



*Agilent Resolution ctDx FIRST results confirmed by orthogonal cfDNA-based ddPCR assay

Note that the 9 Cohort R samples that were positive by tissue and had valid Agilent Resolution ctDx FIRST results were orthogonally tested by an externally validated ddPCR assay. The ddPCR results for 8 out of the 9 samples were concordant with Agilent Resolution ctDx FIRST results.

Table 31 summarizes the agreement results between tissue and Agilent Resolution ctDx FIRST results for both Cohort A FAS-BICR and Cohort R. Estimates are provided by cohort and for the combined cohort. Based on the PPA of 61% (95% CI between Agilent Resolution ctDx FIRST 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 31. Comparison of KRAS G12C Detection Between Tissue and Agilent Resolution ctDx FIRST Assays (Combined Cohorts A and R)

	Tissue Positive	**Tissue egative**
ctDx FIRST Positive	49	
ctDx FIRST Negative		6
ctDx FIRST Unevaluable	43	7
	PPA: 61.3% [49.7, 71.9] (49/80) **NPA: 100%** [96.9, 100] (116/116)	

The primary objective analysis described above demonstrated adagrasib efficacy in the Agilent Resolution ctDx FIRST(+) CTA(+) subset of the Agilent Resolution ctDx FIRST intended use population. As subjects in the KRYSTAL-1 clinical study were enrolled based on positive tissue testing for KRAS G12C mutations, a sensitivity analysis was assessed using matched tissue and plasma samples (procured from commercial vendors according to the selection criteria similar to the KRYSTAL-1 clinical study). 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 the Agilent Resolution ctDx FIRST(+) population is estimated as the same as the ORR in the Agilent Resolution ctDx FIRST(+)/CTA(+) population.

The Agilent Resolution ctDx FIRST clinical bridging study included 71 (63%) of the 112 subjects enrolled into Cohort A of the KRYSTAL-1 clinical study (efficacy population, n = 112). 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 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 the additional patients supported the efficacy conclusions from the adagrasib primary efficacy population.

Diagnostic Study Conclusions

Among the Cohort A patients recruited into the study based on KRAS G12C detection by a tissue-based test, the ORR among patients with a KRAS G12C variant detected by the Agilent Resolution ctDx FIRST assay was 51.1% [36.1%,65.9%]. Results were confirmed after imputation of the missing data: Cohort A ORR after imputation was 47.5% [31.5%,63.5%]

Additional Information

Glossary of Symbols

Symbol	Meaning
	Sterilized Using Irradiation
	Batch Code
	Catalog Number
	In Vitro Diagnostic Medical Device
	Authorized Representative in the European Community
	Consult Instructions for Use

Symbol	Meaning
CE	CE Mark, European Conformity
Rx ONLY	By Prescription Only
	Biological Risk
	Temperature Limitation
	Do Not Re-use
	Use By
	Date of Manufacture
	Manufacturer

Agilent **Resolution**

ctDx FIRST

Technical Specifications

Intended 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 Detected	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 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.

Summary of Analytical Sensitivity and Specificity

The Agilent Resolution ctDx FIRST assay demonstrated the below analytical performance for sensitivity (limit of detection) and specificity (limit of blank) studies. The limit of detection lists the 95% probability (LOD95) of detection. LOD was determined in NSCLC clinical samples. The specificity was determined by evaluating healthy donors.

Biomarker	Limit of Detection (LOD95)	Limit of Blank (LOB)
KRAS G12C	0.581% VAF	0%
EGFR exon 19 deletions	0.380% VAF	0%
EGFR L858R	0.505% VAF	0%
EGFR T790M	0.983% VAF	0%

VAF: Variant Allele Frequency

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, California 95051
United States of America

IVD

Agilent Resolution ctDx FIRST

Patient:
N/A

Report Date:
31 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

Patient	Physician	Specimen
NAME: N/A **DATE OF BIRTH:** N/A **SEX:** N/A **MEDICAL RECORD #:** N/A	**ORDERING PHYSICIAN:** N/A **NPI:** N/A	**SAMPLE TYPE:** Plasma **COLLECTION DATE:** 10 September 2020 **ACCESSION DATE:** 10 December 2021 **SPECIMEN ID:** BXXXXXX-X **TUMOR TYPE:** Non-Small Cell Lung Cancer

COMPANION DIAGNOSTIC ASSOCIATED FINDINGS

Biomarker	Status	FDA-Approved Therapy
KRAS G12C	**Detected**	KRAZATI™ (adagrasib)

ABOUT THIS TEST: The Agilent Resolution ctDx FIRST assay is a qualitative *in vitro* diagnostic test using targeted next-generation sequencing (NGS) to detect clinically-relevant sequence variations in genes using cell-free DNA (cfDNA) from a patient's plasma sample. For *In Vitro* Diagnostic Use.

Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
31 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

INTENDED USE

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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.

WARNINGS AND PRECAUTIONS

- 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.
- Patients for whom no companion diagnostic alteration is detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.
- When collecting the whole blood in the Streck Cell Free BCT® collection tube, allow the tube to fill completely until blood stops flowing into the tube. Underfilling of tubes with less than 10 mL of whole 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.

LIMITATIONS

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The efficacy of KRAZATI™ (adagrasib) has not been established in patients whose *KRAS* G12C mutations are <0.10% VAF.
- The test is not intended to be used for standalone diagnostic purposes.
- A negative result does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment should be based on the independent medical judgment of the treating physician, taking into consideration all applicable information about the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- Genomic findings other than those listed in Table 1 of the Intended Use are not prescriptive or conclusive for labeled use of any specific therapeutic product.
- The test has not been reviewed by FDA to report tumor profiling genes or tumor types other than SNVs and deletions in the EGFR gene from NSCLC plasma specimens (please see *Professional Services* section).
- This test is intended to be performed on specific serial number-controlled instruments by Resolution Bioscience, Inc.
- Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).
- The test is not intended to provide information on cancer predisposition.

Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
31 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

CATEGORY DEFINITIONS

The test report includes genomic findings reported in the following categories:

Category	Prescriptive Use for Therapeutic Product	Clinical Performance	Analytical Performance	Description
Category 1: Companion Diagnostic (CDx)	YES	YES	YES	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Resolution Bioscience has demonstrated clinical performance shown to support 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 companion diagnostics for which Resolution Bioscience has demonstrated analytical reliability and accuracy but not clinical performance.

PERFORMANCE CHARACTERISTICS

For performance characteristics, refer to the Agilent Resolution ctDx FIRST Technical Information. Clinical Performance has not been established for biomarkers in Category 2.

QUALITY CONTROLS

Multiple quality measures are applied by the Agilent Resolution ctDx FIRST assay during processing and analysis of a patient sample, including:

- **Sample Processing QCs:** Within the Resolution Bioscience CLIA lab, quality metrics related to plasma and cell-free DNA input parameters are monitored on a per-sample basis, as well as amplification concentrations of genomic libraries and target-captured sequencing libraries created in preparation for sequencing a patient sample. A patient sample must pass these quality metrics to be processed and sequenced as part of the Agilent Resolution ctDx FIRST assay.
- **Sequence Data Analysis QCs:** The assay software performs an automated inspection of sequence data quality to ensure high quality analysis of a patient's sample for potential genetic variants. Prior to variant identification, the assay software verifies data suitability for analysis, sequence read quality, and overall data quality. During automated variant identification and variant analysis, the assay software verifies that patient's sequence data has sufficient probe coverage and sufficient sequence coverage uniformity and depth, in addition to meeting acceptance criteria for no or very low contamination. A patient sample must pass these sequence data analysis QCs before its variant, if detected, can be reported.

REFERENCES

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- Awad, et al., (2021) Acquired Resistance to KRAS^{G12C} Inhibition in Cancer. N Engl J Med. 2021 Jun 24;384(25):2382-2393. (PMID: 34161704)
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ANALYSIS VERSION

Assay Software: Agilent Resolution ctDx FIRST v1.3.0 | **Pipeline:** ctDx FIRST Pipeline v1.0.0 | **Report Template:** ctDx FIRST Report v1.2.0

Agilent Resolution ctDx FIRST

Patient:
N/A

Report Date:
31 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

Patient	Physician	Specimen
NAME: N/A **DATE OF BIRTH:** N/A **SEX:** N/A **MEDICAL RECORD #:** N/A	**ORDERING PHYSICIAN:** N/A **NPI:** N/A	**SAMPLE TYPE:** Plasma **COLLECTION DATE:** 10 September 2020 **ACCESSION DATE:** 10 December 2021 **SPECIMEN ID:** BXXXXXX-X **TUMOR TYPE:** Non-Small Cell Lung Cancer

COMPANION DIAGNOSTIC ASSOCIATED FINDINGS

Biomarker	Status	FDA-Approved Therapy
KRAS G12C *	**Detected**	KRAZATI™ (adagrasib)

** The VAF for KRAS G12C detection in this patient is <0.10%. Please refer to the Limitations section.*

ABOUT THIS TEST: The Agilent Resolution ctDx FIRST assay is a qualitative *in vitro* diagnostic test using targeted next-generation sequencing (NGS) to detect clinically-relevant sequence variations in genes using cell-free DNA (cfDNA) from a patient's plasma sample. For *In Vitro* Diagnostic Use.

Agilent **Resolution** ctDx FIRST

Patient:
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Report Date:
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Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

INTENDED USE

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Table 1. Companion Diagnostic Indication

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- The test is not intended to be used for standalone diagnostic purposes.
- A negative result does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment should be based on the independent medical judgment of the treating physician, taking into consideration all applicable information about the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
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Agilent **Resolution** ctDx FIRST

Patient:
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Report Date:
31 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

CATEGORY DEFINITIONS

The test report includes genomic findings reported in the following categories:

Category	Prescriptive Use for Therapeutic Product	Clinical Performance	Analytical Performance	Description
Category 1: Companion Diagnostic (CDx)	YES	YES	YES	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Resolution Bioscience has demonstrated clinical performance shown to support 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 companion diagnostics for which Resolution Bioscience has demonstrated analytical reliability and accuracy but not clinical performance.

PERFORMANCE CHARACTERISTICS

For performance characteristics, refer to the Agilent Resolution ctDx FIRST Technical Information. Clinical Performance has not been established for biomarkers in Category 2.

QUALITY CONTROLS

Multiple quality measures are applied by the Agilent Resolution ctDx FIRST assay during processing and analysis of a patient sample, including:

- **Sample Processing QCs:** Within the Resolution Bioscience CLIA lab, quality metrics related to plasma and cell-free DNA input parameters are monitored on a per-sample basis, as well as amplification concentrations of genomic libraries and target-captured sequencing libraries created in preparation for sequencing a patient sample. A patient sample must pass these quality metrics to be processed and sequenced as part of the Agilent Resolution ctDx FIRST assay.
- **Sequence Data Analysis QCs:** The assay software performs an automated inspection of sequence data quality to ensure high quality analysis of a patient's sample for potential genetic variants. Prior to variant identification, the assay software verifies data suitability for analysis, sequence read quality, and overall data quality. During automated variant identification and variant analysis, the assay software verifies that patient's sequence data has sufficient probe coverage and sufficient sequence coverage uniformity and depth, in addition to meeting acceptance criteria for no or very low contamination. A patient sample must pass these sequence data analysis QCs before its variant, if detected, can be reported.

REFERENCES

- Jänne, et al., (2022) Adagrasib in Non-Small-Cell Lung Cancer Harboring a KRAS^{G12C} Mutation. N Engl J Med. 2022; 387:120-131 (PMID: 35658005)
- Sabari, et al., (2022) Activity of Adagrasib (MRTX849) in Brain Metastases: Preclinical Models and Clinical Data from Patients with KRASG12C-Mutant Non-Small Cell Lung Cancer. Clin Cancer Res. 2022 Aug 2;28(15):3318-3328. (PMID: 35404402)
- Ou, et al., (2022) First-in-Human Phase I/IB Dose-Finding Study of Adagrasib (MRTX849) in Patients With Advanced KRASG12C Solid Tumors (KRYSTAL-1). J Clin Oncol. 2022 Aug 10;40(23):2530-2538. (PMID: 35167329)
- Awad, et al., (2021) Acquired Resistance to KRAS^{G12C} Inhibition in Cancer. N Engl J Med. 2021 Jun 24;384(25):2382-2393. (PMID: 34161704)
- Chakravarty et al., (2017) OncoKB: A Precision Oncology Knowledge Base. JCO Precision Oncology. 2017:1, 1-16 (PMID: 28890946)

ANALYSIS VERSION

Assay Software: Agilent Resolution ctDx FIRST v1.3.0 | **Pipeline:** ctDx FIRST Pipeline v1.0.0 | **Report Template:** ctDx FIRST Report v1.2.0

Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
19 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

Patient	Physician	Specimen
NAME: N/A **DATE OF BIRTH:** N/A **SEX:** N/A **MEDICAL RECORD #:** N/A	**ORDERING PHYSICIAN:** N/A **NPI:** N/A	**SAMPLE TYPE:** Plasma **COLLECTION DATE:** N/A **ACCESSION DATE:** 27 March 2022 **SPECIMEN ID:** BXXXXXX-X **TUMOR TYPE:** Non-Small Cell Lung Cancer

COMPANION DIAGNOSTIC ASSOCIATED FINDINGS

Biomarker	Status	FDA-Approved Therapy
KRAS G12C	**Not detected**	None

ABOUT THIS TEST: The Agilent Resolution ctDx FIRST assay is a qualitative *in vitro* diagnostic test using targeted next-generation sequencing (NGS) to detect clinically-relevant sequence variations in genes using cell-free DNA (cfDNA) from a patient's plasma sample. For *In Vitro* Diagnostic Use.

Agilent Resolution ctDx FIRST

Patient:
N/A

Report Date:
19 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

INTENDED 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 test, if feasible.

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

WARNINGS AND PRECAUTIONS

- 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.
- Patients for whom no companion diagnostic alteration is detected should be considered for confirmation with an FDA-approved tumor tissue test, if available.
- When collecting the whole blood in the Streck Cell Free BCT® collection tube, allow the tube to fill completely until blood stops flowing into the tube. Underfilling of tubes with less than 10 mL of whole 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.

LIMITATIONS

- For *in vitro* diagnostic use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The efficacy of KRAZATI™ (adagrasib) has not been established in patients whose *KRAS* G12C mutations are <0.10% VAF.
- The test is not intended to be used for standalone diagnostic purposes.
- A negative result does not preclude the presence of this variant in tumor tissue.
- Decisions on patient care and treatment should be based on the independent medical judgment of the treating physician, taking into consideration all applicable information about the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- Genomic findings other than those listed in Table 1 of the Intended Use are not prescriptive or conclusive for labeled use of any specific therapeutic product.
- The test has not been reviewed by FDA to report tumor profiling genes or tumor types other than SNVs and deletions in the EGFR gene from NSCLC plasma specimens (please see *Professional Services* section).
- This test is intended to be performed on specific serial number-controlled instruments by Resolution Bioscience, Inc.
- Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP).
- The test is not intended to provide information on cancer predisposition.

Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
19 October 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

CATEGORY DEFINITIONS

The test report includes genomic findings reported in the following categories:

Category	Prescriptive Use for Therapeutic Product	Clinical Performance	Analytical Performance	Description
Category 1: Companion Diagnostic (CDx)	YES	YES	YES	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which Resolution Bioscience has demonstrated clinical performance shown to support 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 companion diagnostics for which Resolution Bioscience has demonstrated analytical reliability and accuracy but not clinical performance.

PERFORMANCE CHARACTERISTICS

For performance characteristics, refer to the Agilent Resolution ctDx FIRST Technical Information. Clinical Performance has not been established for biomarkers in Category 2.

QUALITY CONTROLS

Multiple quality measures are applied by the Agilent Resolution ctDx FIRST assay during processing and analysis of a patient sample, including:

- **Sample Processing QCs:** Within the Resolution Bioscience CLIA lab, quality metrics related to plasma and cell-free DNA input parameters are monitored on a per-sample basis, as well as amplification concentrations of genomic libraries and target-captured sequencing libraries created in preparation for sequencing a patient sample. A patient sample must pass these quality metrics to be processed and sequenced as part of the Agilent Resolution ctDx FIRST assay.
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REFERENCES

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ANALYSIS VERSION

Assay Software: Agilent Resolution ctDx FIRST v1.3.0 | **Pipeline:** ctDx FIRST Pipeline v1.0.0 | **Report Template:** ctDx FIRST Report v1.2.0

Patient:
N/A

Report Date:

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

Patient	Physician	Specimen
NAME: N/A **DATE OF BIRTH:** N/A **SEX:** N/A **MEDICAL RECORD #:** N/A	**ORDERING PHYSICIAN:** N/A **NPI:** N/A	**SAMPLE TYPE:** na **COLLECTION DATE:** 10 September 2020 **ACCESSION DATE:** 10 December 2021 **SPECIMEN ID:** **TUMOR TYPE:** Non-Small Cell Lung Cancer

COMPANION DIAGNOSTIC ASSOCIATED FINDINGS

Biomarker	Status	FDA-Approved Therapy
KRAS G12C	**Not detected**	None

OTHER BIOMARKERS IDENTIFIED IN PATIENT

Results reported in this section are not prescriptive or conclusive for labeled use of a specific therapeutic product.
See Professional Services Section for additional information.

ctDNA Biomarkers with Strong Evidence of Clinical Significance *

Variant	Status	Additional Information
EGFR L858R	**Detected**	*See Professional Services section for additional information.*
EGFR T790M	**Detected**	*See Professional Services section for additional information.*

This section is limited to specific detected *EGFR* gene variants (*EGFR* L858R, *EGFR* T790M, and *EGFR* exon 19 deletions.)
** Please refer below to Performance Characteristics and Category Definitions section for descriptions of categories.*

ABOUT THIS TEST: The Agilent Resolution ctDx FIRST assay is a qualitative *in vitro* diagnostic test using targeted next-generation sequencing (NGS) to detect clinically-relevant sequence variations in genes using cell-free DNA (cfDNA) from a patient's plasma sample. For *In Vitro* Diagnostic Use.

Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
09 November 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

INTENDED USE

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Agilent **Resolution** ctDx FIRST

Patient:
N/A

Report Date:
09 November 2022

Report Type:
Patient Report

Indication:
Non-Small Cell Lung Cancer

Subject ID:
N/A

CATEGORY DEFINITIONS

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- Chakravarty et al., (2017) OncoKB: A Precision Oncology Knowledge Base. JCO Precision Oncology. 2017:1, 1-16 (PMID: 28890946)

ANALYSIS VERSION

Assay Software: Agilent Resolution ctDx FIRST v1.3.0 | **Pipeline:** ctDx FIRST Pipeline v1.0.0 | **Report Template:** ctDx FIRST Report v1.2.0