SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: *therascreen*® KRAS RGQ PCR Kit

Device Procode: OWD

Applicant's Name and Address: QIAGEN GmbH
QIAGEN Strasse 1
Hilden, 40724
Germany

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P110027/S012

Date of FDA Notice of Approval: May 28, 2021

The original PMA (P110030) for the *therascreen*® KRAS RGQ PCR Kit was approved on 07/06/2012 and is indicated to aid in the identification of CRC patients for treatment with Erbitux® (cetuximab) based on a *KRAS* no mutation detected test result. PMA P110027 was approved on May 23, 2014 to expand the indication for the *therascreen*® KRAS RGQ PCR Kit as an aid in the identification of CRC patients for treatment with Vectibix® (panitumumab) based on a *KRAS* no mutation detected test result. The current supplement was submitted to expand the intended use of the *therascreen* KRAS RGQ PCR Kit to include a companion diagnostic indication for *KRAS* G12C mutation in patients with Non-small cell lung cancer (NSCLC) using tissue specimens who may benefit from treatment with LUMAKRAS™ (sotorasib).

II. INDICATIONS FOR USE

The *therascreen*® KRAS RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human KRAS oncogene, using DNA extracted from formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue and non-small cell lung cancer (NSCLC) tissue. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic

product labeling.

Table 1. Companion Diagnostic Indications

Indication	Biomarker	Therapy
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
		Vectibix® (panitumumab)
Non-small cell lung cancer (NSCLC)	*KRAS* G12C	LUMAKRAS™ (sotorasib)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the *therascreen*® KRAS RGQ PCR Kit labeling.

V. DEVICE DESCRIPTION

The following components comprise the overall device:

- QIAGEN QIAamp® DSP DNA FFPE Tissue Kit
- QIAGEN *therascreen*® KRAS RGQ PCR Kit
- QIAGEN Rotor-Gene Q MDx, with RGQ Software v2.3, and KRAS Assay Package, version 3.1.1

Specimen Preparation

For NSCLC specimens, formalin-fixed, paraffin-embedded (FFPE) blocks are sectioned onto glass slides. A stained slide is used to confirm the presence of tumor tissue. Two non-stained tissue sections are scraped from the slide for DNA extraction. DNA is manually extracted and purified from 5 µm glass-mounted sections of FFPE tissue taken from non-small cell lung cancer patients using the QIAGEN QIAamp® DSP DNA FFPE Tissue Kit and a modified protocol. The tumor tissue is deparaffinized with xylene and the xylene is extracted with ethanol. The sample is lysed under denaturing conditions with proteinase K for one hour. The sample is heated at 90°C to reverse formalin cross-linking of genomic DNA. The sample is passed through a silica-based membrane so that genomic DNA binds to the membrane and contaminants are removed. Purified genomic DNA is eluted from the membrane into 120 µL of elution buffer in two steps. To elute the bound DNA, 60 µL of elution buffer is added to the membrane and incubated at room temperature for 2.5 minutes after which it is spun in a micro centrifuge at full speed (for 1 minute) into a collection tube. An additional 60 µL of elution buffer is added to the membrane and incubated at room temperature for 2.5 minutes after which it is spun in a micro centrifuge at full speed into the same collection tube to total a volume of 120 µL. Extracted DNA is stored at -

20°C.

PCR Amplification and Detection

The QIAGEN *therascreen*® KRAS RGQ PCR Kit contains reagents for eight separate reactions; seven mutation specific reactions to amplify and detect mutations in codons 12 and 13 in exon 2 of the *KRAS* oncogene, and one Control Reaction that amplifies and detects a region of exon 4 in the *KRAS* oncogene. Each reaction in the KRAS RGQ Kit makes use of an amplification refractory mutation system (ARMS®) allele specific polymerase chain reactions (PCR) to selectively amplify mutated genomic DNA templates (mutation-positive) in a background of non-mutated genomic DNA (mutation- negative; wild-type) combined with a fluorophore-labeled Scorpion® primer to detect any resultant amplification product. The ARMS technology exploits the ability of Taq polymerase to distinguish between a match and a mismatch at the 3' end of a PCR primer. Scorpions are bifunctional molecules containing a PCR primer covalently linked to a probe. The probes incorporate both a fluorophore, [carboxyfluorescein (FAM™)] and a quencher which quenches the fluorescence of the fluorophore. During PCR, when the probe binds to the ARMS amplicon, the fluorophore and quencher become separated leading to a detectable increase in fluorescence.

Before testing with the mutation-specific test reactions, each DNA sample must be tested with the Control Reaction to determine whether the quality and quantity of DNA is sufficient and appropriate for the working range of the assay. The Control Reaction Ct value is used to assess the total amplifiable DNA in a sample and must fall within prespecified ranges for each sample.

The interpretation of the results obtained from the Control reaction is shown in Table 2:

Table 2. Control Reaction Working Range

Control Ct value	Interpretation	Action
> 32.00	Quantity of amplifiable DNA is not sufficient for mutation analysis.	Additional samples should be extracted and tested
< 21.92	Quantity of amplifiable DNA is too high for mutation analysis.	Dilute with the sample diluent water supplied in the kit
21.92 ≤ Control Ct ≥ 32.00	Quantity of amplifiable DNA is suitable for mutation analysis.	---

The run parameters used for assessing the DNA sample with the Control Reaction mix are the same run parameters for mutation analysis using the Mutation Reaction mixes. The run parameters are:

(1) Hold at 95°C for 15 minutes to activate the Taq polymerase;
(2) PCR for 40 cycles of 95°C for 30 seconds, to denature, and 60°C for 1 minute, to anneal/extend.

The PCR cycle at which the fluorescence from a particular reaction crosses the pre-defined threshold value is defined as the Ct value. The seven mutations in codons 12 and 13 of the *KRAS* oncogene detected by the *therascreen*® KRAS RGQ Kit are listed below in Table 3.

Table 3. KRAS Mutations Detected by the *therascreen* KRAS RGQ PCR Kit

Mutation	Base Change
GLY12ALA (G12A)	GGT>GCT
GLY12ASP (G12D)	GGT>GAT
GLY12ARG (G12R)	GGT>CGT
GLY12CYS (G12C)	GGT>TGT
GLY12SER (G12S)	GGT>AGT
GLY12VAL (G12V)	GGT>GTT
GLY13ASP (G13D)	GGC>GAC

Test Controls

Each test run must contain an Internal Control, the Positive Control, and the Negative Control. A test run is considered invalid if the Negative Control indicates that the test run has been contaminated (Ct value above a set value for the FAM channel) or if the Positive Control Ct value lies outside a set range (both FAM and HEX channels). Table 4 shows the Ct ranges for each of the controls to demonstrate test validity.

Table 4. Run Validity Criteria

Reaction Mix	Sample	RGQ Channel	Valid Ct Range*
Control	Positive Control	FAM	23.50 to 29.50
Control	No Template Control	FAM	No Amplification
Control	No Template Control	HEX	31.91 to 35.16
Mutation	Positive Control	FAM	23.50 to 29.50
Mutation	No Template Control	FAM	No Amplification
Mutation	No Template Control	HEX	31.91 to 35.16

*Ranges are inclusive

Internal Control:

All eight reactions contain an additional ARMS primer and a HEX-labeled Scorpion primer for the amplification and detection of a synthetic non *KRAS* related oligonucleotide template that is used as an Internal Control. The Scorpion primer is labeled with HEX to distinguish from the FAM-labeled Scorpions in the control and mutation reactions. In each reaction, the Internal Control reaction is designed to be the weaker of the two reactions. This is achieved through the use of a very low concentration of Internal Control template. The Internal Control reaction is designed to work independently of mutation-specific amplification but can fail in the presence of strong amplification if it is "out-competed" by the FAM reaction. A mutation negative result with a failed Internal Control reaction in any one of the seven mutation reactions will be reported as an invalid result. The Internal Control is used to detect inhibitors or gross reaction failures.

Positive Control:
The positive control is comprised of a mixture of synthetic oligonucleotides representing each of the mutations detected by the *therascreen*® KRAS RGQ Kit. Detection of the positive control confirms the proper functioning of each of the reaction mixes in the Kit.

Negative Control:
The *therascreen*® KRAS RGQ Kit contains nuclease-free water to be used as a no template control (NTC) reaction. The NTC serves as a negative control and assesses potential contamination during assay set up.

Instrument and Software

The Rotor-Gene Q (RGQ) MDx Instrument is a real-time PCR analyzer designed for thermocycling and real-time detection of amplified DNA. The RGQ MDx Instrument controls and monitors PCR reactions and includes the software that determines mutation status based upon PCR results. It incorporates a centrifugal rotor design for thermal cycling during PCR reactions where each tube spins in a chamber of moving air. Samples are heated and cooled in a low-mass-air oven according to a software determined cycle that initiates the different phases of the PCR cycle for a total of 40 cycles for each PCR run. In the RGQ MDx Instrument, samples are excited from the bottom of the chamber by a light emitting diode. Energy is transmitted through the thin walls at the base of the tube. Emitted fluorescence passes through the emission filters on the side of the chamber and is detected by a photomultiplier tube. Detection is performed as each tube aligns with the detection optics; tubes spin past the excitation/detection optics every 150 milliseconds. The fluorescence signals monitor the progress of the PCR reactions. The instrument is capable of supporting up to six optical channels (six excitation sources and six detection filters), however only two of these channels (the FAM and HEX channels) are used with the *therascreen*® KRAS RGQ Kit.

The *therascreen*® *K*RAS Assay Package consists of two templates: the "*therascreen*® KRAS QC Locked Template" (for DNA sample assessment) and the "*therascreen*® KRAS Locked Template" (for detection of *KRAS* mutations). These templates contain the PCR run parameters and calculate the results. The same run parameters are used for both the DNA sample assessment with the Control Reaction Mix and for detection of KRAS mutations using the mutation reaction mixes.

The RGQ MDx Instrument software supports real-time analysis procedures. The software determines Ct values, calculates ΔCt values, and compares these to the mutation-specific cut-off values incorporated into the software as described above. A system of Flags/Warnings is embedded within the software in order to inform the user of potential problems with the assay and to indicate non-valid test runs or non-valid samples within a valid test run (inappropriate level of DNA or Internal Control failure). No results are reported for invalid runs or for non-valid samples. Users of the KRAS RGQ Kit cannot make subjective determinations of mutation status as they do not have access to the Ct or ΔCt values and only see the mutation status calls reported by the software.

Interpretation of Results

The Ct for the control reaction reflects the total amount of amplifiable *KRAS* template in

the sample, while the Ct for the allele specific reactions reflect the amount of KRAS mutation within the sample. The difference in Ct values (ΔCt) between the control reaction and the allele-specific reaction indicates the proportion of mutation within the sample. The ΔCt value approaches 0 as the proportion of mutant DNA in the samples increases. The ΔCt value increases (approaches the threshold for positive vs. negative call) as the proportion of mutant DNA in the sample decreases. When the ΔCt measure exceeds ΔCt cut-off values for the mutant reactions, the assay reports no mutation detected (e.g., negative for the 7 mutations).

For each sample, a calculation is performed by the RGQ MDx Instrument software (v2.3) to determine the ΔCt value (FAM channel) for each of the 7 mutation-specific reactions:

[Mutation reaction Ct value] – [Control Reaction Ct value] = ΔCt

Based on pre-determined analytical Ct and ΔCt values, the RGQ MDx Instrument software (v2.3) qualitatively determines the mutation status of the DNA samples and reports which samples contain which mutation. Each sample will have seven possible ΔCt values (one per mutation). These values are compared to pre-established specifications (cut-off values, shown for each mutation in Table 5) incorporated into the RGQ MDx Instrument software to determine whether a sample is mutation positive or negative and which mutation, if any, is present. When the mutation reaction ΔCt value is less than or equal to the cut-off value for that reaction, the sample is *KRAS* mutation-positive. The assay results will be displayed as "Mutation Positive," "No Mutation Detected," "Invalid" or, if a run control fails, "Run Control Failed." For the mutation-positive samples, specific mutations are reported. The mutation yielding the lowest ΔC_T value will be identified.

Table 5. Cut-off Values for Each Mutation in Each Assay

Mutation Assay	12ALA	12ASP	12ARG	12CYS	12SER	12VAL	13ASP
Cut-Off (ΔCt)	≤ 8.0	≤6.6	≤8.0	≤8.0	≤8.0	≤7.5	≤7.5

Run Validation Criteria

The *therascreen*® KRAS RGQ PCR Kit contains three run controls: a Positive Control, Internal Control and a No Template Control which are used to determine the validity of a test run. The Positive Control contains a synthetic DNA template for each of the eight reactions; it controls for correct set-up of the assays and that the reagents, and process, are working as expected. Each of the eight reaction mixes contains an Internal Control, which employs a non-KRAS related oligonucleotide target sequence, an unlabeled primer, and a Scorpions primer labeled with hexachlorofluorescein (HEX™) in order to distinguish it from the FAM labeled Scorpions in the control and mutation reactions. The No Template Control controls for contamination. Nuclease-free water is provided in the Kit for use as the No Template Control. Ct values for the Positive Control in the seven mutation-specific reactions, and the control reaction, must fall within pre-established specifications for a test run to be considered valid. If values fall outside these specifications, the test run is invalid,

and no patient results will be reported. Ct value for the Internal Control must be below the pre-defined limit of 35.16. The higher Ct value indicates failure of the PCR reaction (e.g., due to the presence of inhibitors). The No Template Control must give negative results in the eight reactions for a test run to be considered valid.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several FDA-approved companion diagnostic (CDx) alternatives for the detection of *KRAS* mutations using CRC tumor specimens for treatment with Erbitux (cetuximab) or Vectibix (panitumumab):

FoundationOne CDx (P170019)

cobas *KRAS* Mutation Test (P140023)

Praxis™ Extended RAS Panel (P160038)

There is one FDA approved CDx alternative for the detection of KRAS G12C mutations in NSCLC patients using plasma specimens for treatment with LUMAKRAS (sotorasib):

Guardant360 CDx (P200010/S002)

VII. MARKETING HISTORY

The QIAGEN *therascreen*® KRAS RGQ PCR Kit has not been marketed in the United States for use with sotorasib; however, it has been marketed in the United States for use with cetuximab since 2012 and for use with panitumumab since 2014.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect KRAS test results, and consequently improper patient management decisions in NSCLC treatment. A false negative test result may lead to LUMAKRAS™ (sotorasib) treatment being withheld from a patient who might have benefitted. A false positive test result may lead to LUMAKRAS™ (sotorasib) treatment being administered to a patient who is not expected to benefit, and potentially subject the patient to any adverse side effects associated with treatment in addition to delaying the start of therapy that may benefit the patient.

For the specific adverse events that occurred in in the clinical study evaluating the efficacy of LUMAKRAS™ (sotorasib) monotherapy in NSCLC subjects with *KRAS* G12C mutation, please see the LUMAKRAS™ (sotorasib) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF PRECLINICAL STUDIES

Laboratory Studies for Non-small cell lung cancer (NSCLC)

The specific performance characteristics of the QIAGEN *therascreen*® KRAS RGQ PCR Kit (henceforth referred to as KRAS Kit) for NSCLC specimens were determined by studies using formalin-fixed, paraffin-embedded (FFPE) tissue specimens collected

from NSCLC patients from the Amgen clinical trial and/or a mixture of procured clinical specimens and cell lines. The studies detailed below included samples for wild type (WT) *KRAS* and seven *KRAS* mutations (i.e., mutations in the glycine amino acids at codons 12 and 13).

1. Analytical Accuracy

An analytical accuracy study was performed with available clinical specimens from NSCLC patients enrolled to Study 20170543 to demonstrate the concordance between KRAS Kit and an externally-validated Droplet Digital PCR (ddPCR) test. From the 126 subjects enrolled to the NSCLC cohort of Study 20170543, 15 of them had multiple samples, giving 142 clinical study samples in total for the accuracy study (1 subject had 3 samples and 14 subjects had two samples). Of these 142 samples, 134 produced evaluable KRAS test results when tested with the KRAS Kit. One sample was not available for ddPCR testing, resulting in 133 NSCLC clinical study samples for the accuracy study. A total of 188 NSCLC samples were obtained from a commercial biobank, with 186 of them yielding valid KRAS test results when tested with the KRAS Kit. The demographics of the procured samples were similar to that of the phase 2 clinical samples. The procured samples were from NSCLC subjects, and the age and gender characteristics were comparable.

Of the 133 clinical study samples tested by ddPCR, 130 yielded valid results after ddPCR testing, and 3 were not evaluable due to insufficient DNA. Of the 186 procured samples, 184 of them yielded valid ddPCR results; one sample was not evaluable due to insufficient DNA and one was lost during the test procedure. A total of 314 samples with both ddPCR and KRAS Kit results were analyzed to assess the positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) of the KRAS Kit with the comparator method. For both methods, samples returning a result other than *KRAS* G12C were considered as mutation negative for this analysis.

The OPA, NPA, and PPA, together with the corresponding two-sided 95% confidence intervals (CI), are summarized in Table 6.

Table 6. OPA, PPA, and NPA of KRAS Kit vs. ddPCR (two-sided 95% confidence interval)

Grouping Variable(s)	Proportion		Two-Sided 95% Confidence Limit	
ddPCR G12C Result	Fraction	Percentage	Lower	Upper
PPA	149/150	99.33%	96.34%	99.98%
NPA	157/164	95.73%	91.40%	98.27%
OPA	306/314	97.45%	95.04%	98.89%

The OPA, PPA and NPA (with two-sided 95% confidence intervals) were 97.45% (95.04%, 98.89%), 99.33% (96.34%, 99.98%) and 95.73% (91.40%, 98.27%), respectively. The accuracy study met the prespecified acceptance criteria.

As seen in the following table (Table 7) that compares the results from the two tests on an individual basis, one sample detected as *KRAS* G12C positive by ddPCR was called as negative by the KRAS kit. Six samples returned mutation negative (no mutation detected) results by ddPCR, but positive by the KRAS kit.

Table 7. Direct Comparison of Test Methods (KRAS Kit vs ddPCR)

Table of therascreen *KRAS* Result by ddPCR										
	ddPCR Result									
KRAS-Kit	G12A	G12R	G12C	G12D	G12S	G12V	G13D	Invalid	No Variant Detected	Total
G12A	5	0	0	0	0	0	0	0	0	5
G12R	0	0	0	0	0	0	0	0	0	0
G12C	0	0	149	1	0	0	0	4	6	160
G12D	0	0	1	17	0	0	0	0	0	18
G12S	0	0	0	0	0	0	0	0	0	0
G12V	0	0	0	0	0	20	0	0	1	21
G13D	0	0	0	0	0	0	4	0	0	4
No Variant Detected	0	0	0	0	1	0	0	1	109	111
Total	5	0	150	18	1	20	4	5	116	319

To further investigate the possible impact of sample acquisition method on the KRAS Kit accuracy, the OPA, PPA and NPA were calculated by acquisition method including core needle biopsy (CNB), fine needle aspiration (FNA) and resection. Among the samples used in the accuracy study, for a total of 100 samples the acquisition method was known; 57 samples with CNB, 15 with FNA, and 28 resections. For the remining 214 samples the sample acquisition method was not known. Due to the high concordance between results overall, there was no evidence to suggest a difference in performance across acquisition methods (Table 8 and 9). Likewise, when evaluating the specimens that were invalid by either the KRAS Kit or ddPCR, there were no discernible demographic or other characteristics that could be attributed to their exclusion from the accuracy study.

Table 8. Accuracy Study PPA/NPA by Acquisition Method

Grouping Variable(s)		**Proportion**		**Two-Sided 95% confidence Limits**	
Acquisition Method	ddPCR G12C Result	Fraction	Percentage	Lower	Upper
CNB	G12C Detected	52/53	98.11%	89.93%	99.95%
	G12C Not Detected	1/4	25.00%	0.63%	80.59%
FNA	G12C Detected	15/15	100.00%	78.20%	100.00%
Resection	G12C Detected	25/25	100.00%	86.28%	100.00%

	G12C Not Detected	1/3	33.33%	0.84%	90.57%
Unknown	G12C Detected	57/57	100.00%	93.73%	100.00%
	G12C Not Detected	155/157	98.73%	95.47%	99.85%

Table 9. Accuracy Study OPA by Acquisition Method

Grouping Variable(s)	**Proportion**		**Two-Sided 95% confidence Limits**	
Sample Type	Fraction	Percentage	Lower	Upper
CNB	53/57	92.98%	83.00%	98.05%
FNA	15/15	100.00%	78.20%	100.00%
Resection	26/28	92.86%	76.50%	99.12%
Unklnown	212/214	99.07%	96.66%	99.89%

2. Comparison to the Analytical Reference Method

The purpose of this study was to demonstrate the concordance in overall mutation status of the *therascreen*® KRAS RGQ PCR Kit NSCLC relative to bi-directional Sanger sequencing.

Table 10. Correlation Between Sanger and KRAS RGQ Result

Sanger Status	**KRAS Status**		
Frequency Percent ( )	Mutation Positive	No Mutation Detected	Total
MutationPositive	79 (21.94)	1 (0.28)	80 (22.22)
No Mutation Detected	21 (5.83)	259 (71.94)	280 (77.78)
Total	100 (27.78)	260 (72.22)	360 (100.00)

A set of 360 procured clinical NSCLC FFPE samples representing a mix of samples acquired by resection, FNA and CNB were used to evaluate the concordance between the KRAS Kit and Sanger bi-directional sequencing. DNA was extracted from each sample and tested with the KRAS Kit and bi-directional Sanger sequencing. Of the 360 valid samples, 340 samples produced concordant results between the KRAS Kit and bi-directional sequencing. Two of these concordant samples had one mutation call for the KRAS Kit but a double mutation for the bi-directional Sanger sequencing (one of which was the same as the one call by the KRAS Kit; therefore, a concordant result was given to these samples.

As seen in Table 11, the PPA was 98.75% (94.21, 99.94), the NPA was 92.50% (89.38, 94.92), and the OPA was 93.89% (91.39, 95.83).

Table 11. Agreement for Samples with both Sanger and KRAS Kit valid Results

Measure of Agreement	Frequencies	Percentage Agreement	Exact Binomial Lower Two-sided 90% Confidence Limit	Exact Binomial Upper Two-sided 90% Confidence Limit
Percentage Overall Agreement	338/360	93.89	91.39	95.83
Percentage Positive Agreement	79/80	98.75	94.21	99.94
Percentage Negative Agreement	259/280	92.50	89.38	94.92

There were 20 discordant samples; 19 were WT for the bi-directional Sanger sequencing but gave a mutation result for the KRAS Kit. This, most likely, reflects the increased sensitivity of the KRAS Kit NSCLC compared to bi-directional Sanger sequencing. One sample was WT for the KRAS Kit but gave a mutation positive (13ASP) status for the bi-directional Sanger sequencing.

3. Cell Correlation Study

The objective of this study was to determine the utility of FFPE NSCLC cell line samples as a suitable substitute for clinical FFPE NSCLC samples by comparing hit rates at, below, and above the established Limit of Detection (LoD) for DNA extracted from FFPE cell line samples and DNA extracted from clinical FFPE NSCLC tissue samples. Furthermore, the study was performed to demonstrate that the FFPE cell line samples do not outperform clinical FFPE NSCLC samples when tested using the KRAS Kit.

The study used two KRAS mutation positive FFPE cell line samples (positive as detected by the KRAS Kit) and two *KRAS* clinical FFPE samples of the same mutation status. Six dilution levels that were expected to give C100 (1.5xLoD), C95 (LoD), C75, C50, C25 and C5 percent correct mutation calls were tested for each sample panel member. Two of the most prevalent KRAS codon 12 mutations G12C (12CYS) and G12D (12ASP) were used for this study.

Two Probit models were used for analysis. For the *KRAS* G12D mutation, the data outputs were not statistically significant indicating that there is no difference in performance between the cell line and clinical samples. However, for the KRAS G12C mutation, the positivity rate of the cell line was lower than that of the clinical samples at the different dilution levels, demonstrating that the cell line does not outperform the clinical sample. The study acceptance criteria was met whereby the 12ASP and 12CYS cell lines did not outperform the corresponding clinical samples and this the cell lines were used as substitutes where clinical samples were not

available for validation studies.

4. Equivalency of Sample Acquisition

The purpose of this study was to assess whether the mutation call for NSCLC samples determined by the KRAS Kit was affected by the sample acquisition method. The 3 sample acquisition methods assessed in this study were resection, FNA, and CNB. For this study, "patient-matched" CNB and FNA samples were derived from surgically resected (RES) tumor samples to enable the same tumor to be collected by the 3 acquisition methods. For consistency, each sample was formalin-fixed and paraffin-embedded. DNA was extracted and tested with the KRAS Kit.

The primary analysis was based on the detection of specific mutation across acquisition types. The measures of overall percentage agreement, PPA and NPA were calculated along with the exact two-sided 95% confidence limit for each pair-wise comparison as shown in Table 12.

Table 12. Percent Agreement (PPA, NPA, OPA) Using Samples Valid With All Three Acquisition Methods

Comparision	Agreement	Frequencies	Percentage (%)	Lower Two-sided 90% Confidence Limit	Upper Two-sided 90% Confidence Limit
CNB vs. FNA with CNB as Reference	Overall Percent Agreement	148/156	94.87	90.15	97.76
	Positive Percent Agreement	29/35	82.86	66.35	93.44
	Negative Percent Agreement	119/121	98.35	94.16	99.80
CNB vs. RES with CNB as Reference	Overall Percent Agreement	148/156	94.87	90.15	97.76
	Positive Percent Agreement	29/35	82.86	66.35	93.44
	Negative Percent Agreement	119/121	98.35	94.16	99.80
FNA vs. CNB with FNA as Reference	Overall Percent Agreement	148/156	94.87	90.15	97.76
	Positive Percent Agreement	29/32	90.63	74.98	98.02

	Negative Percent Agreement	119/124	95.97	90.84	98.68
FNA vs. RES with FNA as Reference	Overall Percent Agreement	152/156	97.44	93.57	99.30
	Positive Percent Agreement	30/32	93.75	79.19	99.23
	Negative Percent Agreement	122/124	98.39	94.30	99.80
RES vs. CNB with RES as Reference	Overall Percent Agreement	148/156	94.87	90.15	97.76
	Positive Percent Agreement	29/32	90.63	74.98	98.02
	Negative Percent Agreement	119/124	95.97	90.84	98.68
RES vs. FNA with RES as Reference	Overall Percent Agreement	152/156	97.44	93.57	99.30
	Positive Percent Agreement	30/32	93.75	79.19	99.23
	Negative Percent Agreement	122/124	98.39	94.30	99.80

The lowest two-sided lower 95% confidence limit values for OPA for all the paired acquisition method comparisons is 90.15%, PPA 66.35%, and NPA 90.84%. The low value of the lower two-sided 95% CI for the PPA is due to the small sample size for positive samples.

At the individual mutation level, a pairwise comparison between acquisition levels was also assessed. In all instances, as shown in Table 13, the percentage agreement between pairs exceeded 95%.

Table 13. Overall Agreement and the Lower Limit of the Two-sided 90% Confidence Interval for KRAS Kit Pair-wise Acquisition Method Comparison by Mutation Reaction Mix

Mutation Reaction Mix	Acquisition Methods	Frequencies	Percentage Agreement	Exact Binomial Lower Two-side 90% Confidence Limit
12ALA	Comparison of CNB with FNA	156/156	100.00	98.10
12ALA	Comparison of	161/161	100.00	98.16

	CNB with RES			
12ALA	Comparison of FNA with RES	156/156	100.00	98.10
12ARG	Comparison of CNB with FNA	155/156	99.38	97.00
12ARG	Comparison of CNB with RES	160/161	99.40	97.09
12ARG	Comparison of FNA with RES	156/156	100.00	98.10
12ASP	Comparison of CNB with FNA	155/156	99.38	97.00
12ASP	Comparison of CNB with RES	160/161	99.40	97.09
12ASP	Comparison of FNA with RES	156/156	100.00	98.10
12CYS	Comparison of CNB with FNA	150/156	97.52	92.55
12CYS	Comparison of CNB with RES	155/161	96.39	92.78
12CYS	Comparison of FNA with RES	152/156	98.76	94.23
12SER	Comparison of CNB with FNA	156/156	100.00	98.10
12SER	Comparison of CNB with RES	161/161	100.00	98.16
12SER	Comparison of FNA with RES	156/156	100.00	98.10
12VAL	Comparison of CNB with FNA	155/156	100.00	97.00
12VAL	Comparison of CNB with RES	160/161	100.00	97.09
12VAL	Comparison of FNA with RES	156/156	100.00	98.10
13ASP	Comparison of CNB with FNA	156/156	100.00	98.10
13ASP	Comparison of CNB with RES	161/161	100.00	98.16
13ASP	Comparison of FNA with RES	156/156	100.00	98.10

The results from the study that evaluated mutation status of bi-directional Sanger sequencing versus KRAS Kit demonstrated an overall percentage agreement of 97.28% across all sample acquisition types. Together, the results from this study demonstrate that the KRAS Kit provides equivalent results across the three collection methods studied.

5. Analytical Sensitivity

a. LOB (Non-Specific Amplification)

The objective of this study was to assess the level of background amplification in

wild type (WT) samples that have a high concentration of input DNA (Control Reaction Ct of approximately 22.00 - 23.00) within the working range of the KRAS Kit (Control Reaction Ct 21.92 - 32.00). The KRAS Kit is designed to determine the mutation status of a patient by analysis of DNA extracted from clinical FFPE NSCLC sections using the FFPE Extraction Kit. The level of breakthrough or the 'Limit of the Blank' (LOB) was used to determine a cut-off value that can discriminate mutation samples from WT samples (determined in the testing of the KRAS Kit for colorectal cancer samples). Breakthrough is non-specific amplification that occurs at a low level and defines the LOB. In terms of the KRAS Kit, this refers to non-specific amplification of WT DNA template in a reaction mix designed to amplify a specific mutation. Sixty valid replicates of pooled clinical surgical resection (Res) FFPE NSCLC WT samples were used to assess the background amplification with regard to high input DNA level (Control Reaction Ct approximately 22.00-23.00). All sixty WT sample replicates were tested with the KRAS Kit and presented ΔCt values that exceeded the assay cut-off values. Thus, all 60 WT sample replicates were called WT by the KRAS Kit. The results demonstrated that the acceptance criterion of "at least 95% (≥57 out of 60) of WT replicates to be called WT" was met.

b. Limit of Detection (LOD)

The LOD values for the KRAS Kit were previously determined using DNA extracted from FFPE cell lines. The objective of this study was to re-verify that the LOD of each of the mutation assays with NSCLC specimens is below or equal to 10% mutation content in the background of wild type DNA, when the concentration of DNA is not a limiting factor (i.e., when the concentration of input DNA was within the working range of the KRAS Kit).

In Part 1 of the study, FFPE cell lines derived from lung (8 FFPE Cell lines and 1 WT) were evaluated and in Part 2 clinical FFPE NSCLC samples along with cell line or Patient-Matched samples were used (twenty one samples representing each of the seven KRAS mutation assays plus three samples representing WT for each of the three different sample acquisition methods were used). All samples were diluted in clinical FFPE NSCLC WT samples. DNA extracted from mutation positive test samples, and wild type samples (to be used as diluents) were normalized to a medium DNA input level (approximately Ct of 26.00). Thereafter, the mutation positive samples were diluted to low levels of mutant DNA content (defined as previously determined LOD). 60 valid replicates of each mutant FFPE NSCLC Cell Line (Part 1) and 96 valid replicates of clinical FFPE NSCLC samples/FFPE Cell-Lines/Patient-Matched samples (Part 2) for each mutant sample diluted to their respective LOD mutant content were used to verify the LOD previously determined values. All valid replicates for FFPE Cell Lines (Part 1) presented positive and correct mutation calls at 100.0% for all samples assessed. The valid replicates for the samples using clinical FFPE NSCLC, patient-matched and FFPE cell line samples (Part 2) presented positive and correct mutation calls at 100.0% for 12ALA, 12ASP, 12ARG, 12VAL and 13ASP samples and presented positive and correct mutation calls at 95.8% for 12SER and 12CYS samples. The final LOD values are shown in Table 14.

Table 14. LOD Claims FFPE Cell Lines when Control Reaction Ct Range ~22-27

Mutation Reaction	Final LOD Claim (% mutant DNA in wild type DNA)
12ALA	0.8
12ASP	6.4
12ARG	2.6
12CYS	1.5
12SER	5.7
12VAL	1.6
13ASP	6.4

c. DNA Input and Linearity

To demonstrate that the performance of the KRAS Kit is consistent across the DNA input range a series of five dilutions with varying DNA input levels with the upper and lower levels being outside of the Control Reaction Ct working range (21.92–32.00 Ct) were evaluated with mutation positive samples.

To address the effect of input DNA levels on ΔCt values, DNA extracted from nine FFPE NSCLC samples (4 Cell Lines and 5 Clinical) were used in the study. Samples were diluted to a Control Reaction Ct value of approximately 20.00-21.00 Ct and from this dilution a pool of DNA for each mutation sufficient for 6 replicates was serially diluted 10-fold over 4 points to correspond to a Ct value of approximately 32.00–33.00 Ct (Table 15).

Table 15. DNA levels in Dilution series for study on input ΔCT values

Dilution Factor	Initial Pool	X10 (dilution 1)	X100 (dilution 2)	X1000 (dilution 3)	X10000 (dilution 4)
Expected Control Ct Values	Approx. 20.00-21.00 Ct	Approx. 23.00-24.00 Ct	Approx. 26.00-27.00 Ct	Approx. 29.00-30.00 Ct	Approx. 32.00-33.00 Ct

Linear regression plots were generated to show the ΔCt values for each mutation type across the dilutions within the Control Ct working range. The linear regression lines and corresponding two-sided 95% confidence limits for the 3 dilutions within the Control Ct working range fell within the mean ± 1.96x Standard Deviation for Reproducibility as calculated in a Repeatability and Reproducibility study. Therefore, the ΔCt values are consistent across the working range of the KRAS Kit for all KRAS mutation assays, demonstrating that the mutation calling for samples is not impacted by the DNA input levels.

To evaluate the effect of a serially diluted mutant positive sample on amplification efficiency a second study was performed to test samples with varying percentage mutation at a high DNA input level (Control Reaction of approximately 23.00Ct). DNA from clinical FFPE NSCLC and FFPE cell line sample pools was diluted to optical densities corresponding to Control Reaction Ct values of approximately 22.00-23.00 Ct. Mutant DNA was serially diluted 2-fold into normalized wild-type DNA to create a series of 5 dilutions per mutant DNA sample that varied in their mutation percentage (targeting 100%, 50%, 25%, 12.5%, and 6.25% percentage mutant).

Table 16 summarizes all the amplification efficiencies for each assay. All the linear regression plots showing the Ct and ΔCt values for each mutation type across the full dilution range tested were linear. Amplification efficiencies of wild-type DNA relative to mutant DNA present in a sample did not impact ΔCT values and thus the mutation calls were not affected.

Table 16. Amplification efficiencies of the mutation assay

Sample	Amplification Efficiency
12ALA	0.921
12ASP	1.034
12ARG	0.916
12CYS	0.988
12SER	1.047
12VAL	1.082
13ASP	1.028*

* Value calculated with outlier excluded from analysis with outlier included amplification efficiency = 1.085

6. Analytical Specificity

a. Primer and Probe Specificity

For this information please see the SSED for P110027.

b. Cross-reactivity/Exclusivity

The objective of this study was to assess whether cross reactivity has been correctly accounted for in the setting of the analytical ΔCt cut-off values. In this study, FFPE cell line samples containing their maximum percentage of mutant DNA (i.e., not diluted in WT DNA) at a DNA input close to the maximum (Control Ct 21.92) was evaluated. The mean ΔCt data from the 6 replicates demonstrated that in some cases, in addition to the desired mutation being detected in the particular sample, there is also cross-reactivity between mutation reactions. However, where cross reactivity was observed, this was seen above the cut-off for the assay, or at a larger ΔCt than observed for the intended target sequence. This issue has been addressed as a limitation in the product labeling. Specifically, the test has been designed such that each mutant reaction is most sensitive for the specific mutation being measured. However, in samples where a mutation is detected, cross-reactivity may occur with other mutation reactions. If more than one mutant reaction is positive, the result is

the one with the lowest ΔCt result. (Additional exclusivity information for the test was reported in the in the SSED for P110027).

7. Interfering Substances

a. Exogenous Substances

This study was designed to demonstrate that the presence of a potentially interfering substance used in the extraction process would not produce any false positive or false negative results when tested with the KRAS Kit. Eight (8) potentially interfering substances from the DNA extraction process were identified: paraffin wax, xylene, ethanol, Buffer ATL, Proteinase K, Buffer AL, Buffer AW1, and Buffer AW2. Each substance was tested against eight FFPE cell lines, representing each of the seven mutations detected by the KRAS Kit, and a wild type sample. The mutation samples were tested at a level corresponding to approximately three times the limit of detection (3x LOD).

Both analytes (mutation and wild-type samples) were spiked with each of the eight potential interfering substances at three different levels: the highest expected carry over (1x), ten-times the highest expected carry over (10x) and one hundred-times the highest expected carry over (100x). Both analytes were also spiked with Buffer ATE, the final elution buffer in the DNA extraction process to serve as negative controls. The study demonstrated that the substances identified from the FFPE DNA extraction kit which might carry over into the KRAS Kit did not have any adverse effect on the performance of the assay at the 1x level of interferent; the correct mutation call was made, and the presence of the interfering substance did not have a statistically significant effect on difference in ΔCT for the majority of sample conditions tested (58 out of 64 conditions, at 1x level). For the six samples that did show a statistically significant difference, the observed difference in the mean for each sample was within the study acceptance criterion of ±2xSD (SD estimate taken from a reproducibility study). The study also demonstrated that the KRAS Kit was tolerant to higher levels of each of the substances being tested, with the correct mutation call being given when the interfering substance was present at 10x the highest expected concentration.

b. Hemoglobin

Another study was performed to assess the impact of Hemoglobin on the KRAS Kit. In this study, procured clinical resection samples for the 12ALA, 12ASP, 12ARG, 12VAL, 12CYS, and 13ASP mutations were used. No 12SER clinical sample was assessed due to the rarity of the mutation (this mutation was previously tested using a cell line sample in a separate study, where it met the acceptance criteria). A clinical KRAS wild type FFPE was incorporated into the sample set to assess any potential interference in samples with a mutation negative status and also used to dilute the mutant samples to 2x LOD. Test samples were spiked with hemoglobin at two different concentrations; half the highest expected carry over (0.5x) and the highest expected carry over (1x). The recommended test concentration (1x) was taken from CLSI EP07-A2 as 2g/L. An interferent-free control or null set (no hemoglobin present) was also produced for comparison. This study demonstrated

that the detection of the 12ASP, 12ARG, 12VAL, 12CYS, and 13ASP mutations by the KRAS Kit is not impacted by the potential interferant Hemoglobin. Detection of the 12ALA mutations by the KRAS kit however is impacted at a concentration of 0.50 µg/µl Hb or higher.

c. **Necrosis**

Samples from the Equivalency of Sample Acquisition Method Study were used along with available data from pathologist's examination of the hematoxylin & eosin (H&E) slides to investigate possible trends due to the extent of necrotic or tumor tissue. For this study, CNB and FNA samples were derived from surgically resected (RES) tumor samples. The samples represented all 7 of the KRAS mutations detected by the test. Linear regression analyses were performed investigating both the percentage of necrotic and tumor tissue on the relevant ΔCt values for resected, CNB and FNA samples. The results indicated that there was no statistically significant difference in ΔCt values with increasing values of the percentage of necrotic or tumor tissue.

8. Repeatability and Reproducibility

The precision of the *therascreen* KRAS RGQ PCR Kit within-laboratory (repeatability) was assessed. Both the accuracy of mutation results and the precision of ΔCT values (the difference in CT values between a Mutation Reaction and the Control Reaction) are reported. In total, 15 panel members were prepared; one for each of the 7 mutations detected by the KRAS kit (at LOD and 2xLOD), and a wild type (WT) panel member. Mutant panel members were represented by either an FFPE cell line or a clinical sample depending on availability. All samples were normalized to a control Ct of 27 and mutant samples were diluted in wild type DNA to generate sufficient material for samples at mutation levels of 1x LOD and 2x LOD. Across all three testing sites, a total of 120 replicates per panel member at LOD, 84 replicates per panel member at 2xLOD and 84 replicates of the wild type were analyzed. The acceptance criteria for reproducibility were the following: The lower limit of the two-sided exact 95% confidence interval for the percentage of correct mutation calls had to exceed 85%, 90%, 90% for LOD, 2x LOD and WT samples, respectively. The proportion of correct calls for each test panel and the quantitative precision values are presented in the table below. The precision of the KRAS Kit between-laboratories (reproducibility) was assessed. Three different test sites were used. The same test panel was used for this study as for the repeatability study. At each site, laboratory conditions were varied by RGQ instrument, operator, KRAS Kit lot, and were performed over 22 non-consecutive days. Data generated by Site 1 (QIAGEN Manchester) was used to assess the within-laboratory repeatability (Table 17). Data generated by all three sites was used to determine the between-laboratory reproducibility (Table 18).

Table 17. Repeatability (Site 1)

Grouping Variable(s)			Proportion		Two-sided 95% Confidence Limit	
Sample Level	**Assay**	**Site**	**Fraction**	**Percentage**	**Lower**	**Upper**
2x LOD	12ALA	1	28/28	100.00%	87.66%	100.00%
	12ARG	1	28/28	100.00%	87.66%	100.00%
	12ASP	1	28/28	100.00%	87.66%	100.00%
	12CYS	1	28/28	100.00%	87.66%	100.00%
	12SER	1	28/28	100.00%	87.66%	100.00%
	12VAL	1	28/28	100.00%	87.66%	100.00%
	13ASP	1	28/28	100.00%	87.66%	100.00%
LOD	12ALA	1	39/40	97.50%	86.84%	99.94%
	12ARG	1	40/40	100.00%	91.19%	100.00%
	12ASP	1	40/40	100.00%	91.19%	100.00%
	12CYS	1	40/40	100.00%	91.19%	100.00%
	12SER	1	40/40	100.00%	91.19%	100.00%
	12VAL	1	40/40	100.00%	91.19%	100.00%
	13ASP	1	38/40	95.00%	91.19%	99.39%
WT	All	1	28/28	100.00%	87.66%	100.00%

As shown in Table 17 (above), repeatability of testing at Site 1 showed a high level of agreement in between-kit, -day, -run, -operator and -instrument testing. As noted above, three different laboratories (test sites) were used to assess test reproducibility. The same test panel was used for this study as for the repeatability study. At each site, laboratory conditions were varied by RGQ instrument, operator, KRAS Kit lot, and runs per day were tested and the proportion of correct calls is shown in Table 18.

Table 18. Reproducibility (All 3 sites). Proportion of Correct Mutation Calls Across all Sites

Grouping Variables		Proportion		Two-Sided 95% Confidence Interval	
Sample level	**Assay**	**Fraction**	**Percentage**	**Lower**	**Upper**
2xLOD	12ALA	84/84	100.00%	95.70%	100.00%
	12ARG*	84/84	100.00%	95.70%	100.00%
	12ASP	84/84	100.00%	95.70%	100.00%

	12CYS	84/84	100.00%	95.70%	100.00%
	12SER*	84/84	100.00%	95.70%	100.00%
	12VAL	84/84	100.00%	95.70%	100.00%
	13ASP*	84/84	100.00%	95.70%	100.00%
LOD	12ALA	118/120	98.33%	94.11%	99.80%
	12ARG	120/120	100.00%	96.97%	100.00%
	12ASP	120/120	100.00%	96.97%	100.00%
	12CYS	120/120	99.17%	95.44%	99.98%
	12SER*	120/120	100.00%	96.97%	100.00%
	12VAL	120/120	100.00%	96.97%	100.00%
	13ASP*	118/120	98.33%	94.11%	99.80%
WT	All	82/84	97.62%	91.66%	99.71%

* Represented by FFPE cell line

A variance component analysis was used to estimate the standard deviation and %CV for between-run, between-day, between-lot, between-operator, between-instrument, and between-day variability for the reproducibility study. Across all mutant panel members, at 1x LoD, the SD for ΔCt was ≤0.24 for within site, between-lots, between instrument, between days, and between operator variance components, and % CV was in the range of 0.0- 4.55%. At 2x LoD, the SD for ΔCt were ≤0.27 and % CV was in the range of 0.0- 5.2%.

For WT samples, in 2 of the 3 study sites all replicate samples were called WT (56/56). However, in the remaining site, 2 of 28 samples were called 12ARG rather than the expected WT. The overall proportion of all WT was 97.62%, exceeding the study acceptance criteria.

9. <u>Lot Interchangeability</u>

The objective of this study was to establish that the use of different lots of the QIAamp DSP DNA FFPE Tissue Kit for extraction and KRAS Kit would not impact the mutation call determination of a tested sample. Three lots of each kit were used to test FFPE NSCLC samples to establish that the use of different lots of the QIAamp DSP DNA FFPE Tissue Kit and the KRAS Kit did not impact the sample mutation status of tested samples. Twenty-four (24) NSCLC samples, three each for the seven mutations detected by the KRAS Kit plus three wild-type samples were used in the study. Of the 24 NSCLC samples, six clinical FFPE NSCLC samples, seven FFPE patient-matched samples, and eleven FFPE cell lines were used. Each sample was extracted using three lots of the QIAamp DSP DNA FFPE Tissue Kit. All DNA extracts were tested with each of the three lots of KRAS Kit within the same PCR run on the RGQ instrument (Table 19).

Table 19. Frequency of Correct Call for Each Reaction Mix by Kit

Kit	12ALA	12ASP	12ARG	12CYS	12SER	12VAL	13ASP	Total Correct Mutation Call
FFPE Extraction Kit 1	36/36	36/36	36/36	36/36	36/36	36/36	36/36	256/256
FFPE Extraction Kit 2	35/35	35/35	36/36	34/34	35/35	35/35	35/35	245/245
FFPE Extraction Kit 3	36/36	36/36	36/36	36/36	36/36	36/36	36/36	252/252
KRAS Kit NSCLC 1	36/36	36/36	37/37	35/35	36/36	36/36	38/38	254/254
KRAS Kit NSCLC 2	35/35	35/35	35/35	35/35	35/35	35/35	36/36	246/246
KRAS Kit NSCLC 3	36/36	36/36	36/36	36/36	36/36	36/36	37/37	253/253

As seen in the table above, all seven mutation and the WT samples were 100% correct mutation calls, regardless of the KRAS or FFPE kit used in the analysis.

An Analysis of Variance was conducted on the mutation samples (for each sample mutation status) with ΔCt as the response variable and sample as a fixed effect explanatory variable. The ΔCT values (the difference in CT values between a Mutation Reaction and the Control Reaction) and mutation calls were collected for all test samples extracted with different QIAamp DSP DNA FFPE Tissue Kits and tested with different KRAS Kits. The acceptance criterion of "at least 95% of mutation calls must be correct for the overall proportion" was met with each mutation and wild-type being called 100% correctly.

To evaluate FFPE cell line samples containing their maximum percentage of mutant DNA (i.e., not diluted in WT DNA) at a DNA input close to the maximum (Control Ct 21.92) allowed. From the mean ΔCt data from the 6 replicates, it was clear that in a number of instances, in addition to the desired mutation being detected in the particular sample, there is also cross-reactivity between mutation reactions. In this context, cross-reactivity is defined as signal that appears in one mutant assay due to amplification of another mutation. However, where cross reactivity was observed, in most cases it was seen above the cut-off for the assay, or at a larger ΔCt than observed for the target sequence. This has been partially mitigated by the RGQ software since it is programmed to only report the mutation with the lowest Ct resulting in the correct result was reported even if a signal was observed with other mutations.

10. Sample Handling (Variability)

A study was performed to demonstrate that different laboratories produced the same results (specifically DNA extraction) starting from the same clinical samples. This study used a combination of FFPE Cell Line and Clinical FFPE NSCLC samples. Thirteen Clinical FFPE NSCLC samples were used; three for 12ASP and 12CYS, and four for 12VAL mutations detected by the KRAS Kit (a total of 10 mutation samples) plus three wild-type samples. Four FFPE Cell Line samples positive for the mutations 12ALA, 12ARG, 12SER, and 13ASP were used.

There are three types of KRAS NSCLC clinical samples which differ in their method of acquisition: surgical resection (Res), fine needle aspirate (FNA) and core needle biopsy (CNB). As a target, each mutation and WT sample were represented by a Res, FNA and CNB. As insufficient clinical samples were available, patient-matched clinical FFPE NSCLC were used for all CNB and FNA samples. After surgical resection of each tumor, a procedure was performed to acquire a Res, FNA and CNB specimen from that same tumor, with the result being that "patient-matched" samples were obtained. Subsequent to acquisition, all tumor specimens were formalin-fixed and paraffin-embedded (FFPE).

Samples were blinded so that the mutation status was unknown to the operators at each of three test sites. At each of the three sites, DNA extraction was performed on a batch of 20 FFPE sections (that is, 10 pairs) per Mutation and wild-type. A total of 314 extractions (104 extractions at test Site 1, 106 extractions at test Site 2 and 104 extractions at test Site 3) were carried out across the three sites. All sample preparations at 3 individual test sites, when tested with the *therascreen* KRAS RGQ PCR Kit provided a correct mutation call for each of the seven mutations and wild-type samples. The overall call for each of the seven mutations and wild-type samples was 100% correct demonstrating inter-site consistency for the DNA extraction procedure and mutation detection using the KRAS Kit.

An additional Sample Handling study was conducted using clinical FFPE clinical NSCLC samples representative of 12ALA, 12ARG, 12SER, and 13ASP mutations, as the previous study used cell-line samples representing these mutations. This study followed the same study design as the previous study. All sample preparations for 12ALA, 12ARG, and 13ASP mutant samples extracted across all three individual test sites, provided a correct mutation call when tested with the KRAS Kit. The overall correct call for these samples was 100%. Sample preparations for 12SER mutation provided a frequency of correct mutation call of 28/30 (percentage of correct call equal to 93.33%) across all three individual test sites. The results demonstrate the agreement of the DNA extraction procedure and sample processing in conjunction with the *therascreen* KRAS RGQ PCR Kit.

11. Guardband Studies

The objective of the guardbanding studies was to establish the robustness of the KRAS Kit. Changes were assessed for any potential effects on mutation reporting. The following studies were conducted to assess the robustness of the KRAS Kit:

a. Varying Proteinase K Digestion Times

The objective of this study was to provide guardband study data demonstrating the robustness of the *therascreen* KRAS RGQ PCR System to varying proteinase K digestion times (50, 55, 60, 65 and 70 minutes). Eight non-small cell lung cancer (NSCLC) samples, representing each of the seven mutations (four FFPE cell lines and four clinical FFPE NSCLC samples) detected by the KRAS Kit along with one wild type (WT), were extracted using the FFPE Extraction Kit and tested (6 replicates) using one lot of the KRAS Kit NSCLC. The DNA input level of the samples was determined using the KRAS Kit Control Reaction mix. Mutant samples were tested using the Control Reaction mix and relevant Mutation Reaction mix; WT samples were tested using all eight reactions of the KRAS Kit. The ΔCt values (the difference in FAM Ct values between a Mutation Reaction and the Control Reaction) and mutation calls for all samples were obtained at each digestion time point.

At the different Proteinase K digestion time points of 55, 60, and 65 minutes, all samples were called with 100% correct mutation calls, demonstrating the robustness of the KRAS System. Overall, varying Proteinase K digestion times (50 min – 70min) had no significant effect upon the performance of the KRAS NSCLC system. For the entire study, 238/239 valid samples gave the correct mutation call with a single valid WT replicate giving a 12ASP mutation result at 70 minutes. Since the five other replicates for this sample all gave the expected WT call, no investigation was initiated.

An additional study was also performed assessing the 12ARG mutation (using a human lung cell line FFPE sample) in order to address the deficiencies in the initial study where a pancreatic cell line was used to represent the 12ARG mutation. An FFPE cell line sample which represented the 12ARG mutation detected by the KRAS Kit, was tested in this study. Similar to the study described above, the samples processed for 55, 60, 65 and 70 minutes were assessed. All reaction replicates (144) yielded the correct call.

b. Guardband Study on Volumetric Tolerance

The objective of this study was to determine the tolerance of the *therascreen* KRAS Kit to variations in pipetting volumes. Cell lines were used for these studies. This study used eight FFPE cell line samples (12ALA, 12ASP, 12ARG, 12CYS, 12SER, 12VAL, 13ASP and WT), Positive Control (PC) and No template Control (NTC). The volume of each PCR component (i.e., each reaction mix, sample or *Taq* polymerase) of the KRAS Kit was varied individually and incrementally up to approximately ±20% while all other components were used at the standard volumes for the kit. Each variable was considered to be a test condition (TC). Each run was set up in the standard manner, with the exception of the pipetting volumes. The volumes of reagents used for each run were varied so that in total 23 test conditions were analyzed. This guardband study demonstrated that the *therascree*n KRAS RGQ PCR System is robust up to approximately ±20% in varying pipette volumes for all assays other than 12ASP. When the volume of each PCR component of the

KRAS Kit was varied individually and incrementally while all other components were used at the standard volumes, there was no change in mutation call and all differences in mean ΔCt were within ±2 x the intermediate precision observed in Repeatability and Reproducibility for all assays, other than 12ASP. The result obtained with the 12ASP assay with the volumetric tolerance level for -21% *Taq* did not pass the acceptance criteria. The 12ASP sample met the acceptance criteria for ±8% volumetric tolerance; therefore, this level was set for this reaction.

An additional guardband study was also performed assessing the 12ARG mutation (using a human lung cell line FFPE sample) in order to address the deficiencies in the initial study where a pancreatic cell line was used to represent the 12ARG mutation. An FFPE Cell Line sample which represented the 12ARG mutation detected by the KRAS Kit, was tested in this study. The sample was tested with all 23 test conditions, in triplicate. In order to recreate conditions most likely to result in a false positive or false negative result, the volumes of multiple components (Reaction Mix, *Taq* and Sample) were altered simultaneously. The acceptance criterion for the study was that the differences in the mean delta Ct values between the different PCR component levels should either be not significantly different from zero or the estimate should be less than or equal to twice the intermediate precision value calculated during the Repeatability and Reproducibility study. All results fulfilled these requirements. This Guardband study demonstrated that the KRAS Kit is robust enough to tolerate volumetric reagent changes up to ±20% (when all other factors are constant) due to pipetting error.

c. Guardband Study- RGQ PCR Cycling Analysis

This information was included in the SSED for P110027.

d. Guardband Study _PCR Set-up and Stability Times

This information was included in the SSED for P110027.

12. Cross-Contamination

This information was included in the SSED for P110027.

13. Stability-

a. Reagents

Results from studies demonstrating the stability of the reagent components of the *therascreen*® KRAS RGQ PCR Kit and the QIAamp DSP DNA Extraction Kit were included in the SSED for P110027. Expiration dating for this device has been established and approved at 12 months when stored at -15 to -25°C.

b. Stability-FFPE Sections

The main objective of this study was to demonstrate that sections prepared from resected FFPE NSCLC samples were stable when stored in the dark at ambient temperature. Sample sections from five KRAS mutation positive clinical FFPE NSCLC samples (one of each of the mutations 12ALA, 12ASP, 12CYS, 12VAL,

13ASP) and one KRAS wild-type sample were sectioned and stored until required. The sections were tested with the KRAS Kit with five time points from 0 to 75 weeks. All samples provided the correct mutation status call at all the time points, demonstrating that FFPE sections are stable at ambient temperature for 70 weeks (i.e., a timepoint prior to the last tested timepoint).

A second study was performed to demonstrate that sections prepared from FFPE NSCLC FNA samples are stable when stored in the dark at ambient temperature. This study used FNA samples that are wild type for the seven KRAS mutations that are detected by the *therascreen* KRAS RGQ PCR Kit. Sections from ten KRAS wild-type samples were used. These samples were extracted using one lot of the QIAamp DSP DNA FFPE Tissue Kit. Extracted DNA was tested at T0, then at T1 (T0+52 weeks), T2 (~56 weeks) and T3 (~60 week). All of the valid results gave the correct call. The results obtained during this study provide evidence that FFPE NSCLC FNA samples are stable for 56 weeks when stored in the correct conditions.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of the *therascreen* KRAS RGQ PCR Kit for the detection of *KRAS* G12C mutations in NSCLC patients was demonstrated in a multi-center, non-randomized, open-label study from subjects enrolled in Amgen clinical Study 20170543 for LUMAKRAS™ (sotorasib). Data generated from Study 20170543 supported the clinical validation of the KRAS Kit for identification of G12C mutation positive subjects with NSCLC who may benefit from LUMAKRAS™ (sotorasib) treatment.

A. Study Design

The Amgen 20170543 clinical study was a phase 1/2 multicenter, non-randomized, open-label study of orally administered LUMAKRAS™ (sotorasib) in subjects with NSCLC. The primary sotorasib registration population comprises *KRAS* G12C mutation-positive subjects from the Amgen 20170543 study. Eligible patients were required to have locally advanced or metastatic *KRAS* G12C mutated NSCLC with disease progression after receiving an immune checkpoint inhibitor and/or platinum-based chemotherapy, an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 or 1, and at least one measurable lesion as defined by Response Evaluation Criteria in Solid Tumors (RECIST v1.1). The outcomes of the phase 2 portion of the study was used to support the approval of the LUMAKRAS™ (sotorasib) under NDA 214665. The primary objective of the phase 2 portion of clinical study 20170543 was to evaluate tumor objective response rate (ORR) assessed by response evaluation criteria in solid tumors (RECIST) 1.1 of sotorasib as monotherapy in subjects with KRAS G12C mutations in NSCLC. The primary endpoint was ORR (complete response [CR] + partial response [PR]), measured by computed tomography [CT] or magnetic resonance imaging [MRI] and assessed by blinded independent central review (BICR) per RECIST 1.1. The benchmark ORR to exclude for clinical study 20170543 was selected as 23% for NSCLC based on a large phase 3 clinical trial (REVEL) for second-line treatment after disease progression on platinum-based therapy showed that an ORR of 23% (95% CI: 20, 26) was observed with ramucirumab plus docetaxel.

As of the clinical cut-off date of September 1, 2020, a total of 126 NSCLC patients with *KRAS* G12C mutations were enrolled in the phase 2 study. For NSCLC cohort, the mutation was confirmed in adult subjects (≥ 18 years of age) prospectively by central testing using the *therascreen* KRAS RGQ PCR Kit prior to enrollment.

Accountability of sPMA Cohort

The *therascreen* KRAS RGQ PCR Kit was used prospectively to select NSCLC patients with *KRAS* G12C mutations to the clinical study. A total of 126 subjects with NSCLC were enrolled in phase 2, all of whom received ≥1 dose of LUMAKRAS™ (sotorasib). 142 specimens were obtained from 126 patients. Eight (8) were not evaluable (2 no tumor present, 5 failed sample assessment, 1 failed mutation assessment). Replicate samples procured from 8 patients and, in each instance, one of the specimens yielded a valid result. The full analysis set, which included treated subjects with measurable lesions at baseline as assessed by Blinded Independent Central Review (BICR), consisted of 124 subjects with NSCLC; two subjects were excluded as they did not have ≥ 1 measurable lesion at baseline according to BICR. Since the *therascreen* KRAS RGQ PCR Kit was used to test all specimens enrolled to the clinical trial, no sensitive analysis was needed to impute for the missing samples.

B. Demographics

The baseline demographics for NSCLC patients in the phase 2 portion of the study were:

- Sex: 63 men (50.0%); 63 women (50.0%)
- Age: mean (SD; range): 62.9 (9.3; 37, 80) years
- Race: 103 white (81.7%); 2 other (1.6%); 2 black (1.6%), 19 Asian (15.1%)
- Ethnicity: 2 Hispanic or Latino (1.6%), 116 not Hispanic or Latino (92.1%), 8 not Reported (6.3%)

The baseline demographic and disease characteristics of the study population were: median age 64 years (range: 37 to 80) with 48% ≥ 65 years and 8% ≥ 75 years; 50% Female; 82% White, 15% Asian, 2% Black; 70% ECOG PS 1; 96% had stage IV disease; 99% with non-squamous histology; 81% former smokers, 12% current smokers, 5% never smokers. All patients received at least 1 prior line of systemic therapy for metastatic NSCLC; 43% received only 1 prior line of therapy, 35% received 2 prior lines of therapy, 22% received 3 prior lines of therapy; 91% received prior anti-PD-1/PD-L1 immunotherapy, 90% received prior platinum-based chemotherapy, 81% received both platinum-based chemotherapy and anti-PD-1/PD-L1. The sites of known extra-thoracic metastasis included 48% bone, 21% brain, and 21% liver.

An abbreviated list of the study inclusion/exclusion criteria is provided below:

Inclusion Criteria

1. Subject has provided informed consent prior to initiation of any study.
2. Men or women ≥ 18 years old.
3. The mutation will be confirmed by central testing prior to enrollment.

4. Must have progressed after receiving anti-PD1 or anti-PD-L1 immunotherapy (unless contraindicated) AND/OR platinum-based combination chemotherapy **AND targeted therapy** if actionable oncogenic driver mutations were identified (ie, EGFR, ALK, and ROS1)**. S**ubjects must have received no more than 3 prior lines of therapy.
5. Subjects willing to provide archived tumor tissue samples (formalin fixed, paraffin embedded [FFPE] sample collected within 5 years) or willing to undergo pretreatment tumor biopsy.
6. Subjects who have lesions that can be feasibly biopsied will be asked to undergo an optional biopsy at the time of tumor progression.
7. Measurable disease per RECIST 1.1 criteria.
8. Eastern Cooperative Oncology Group (ECOG) Performance Status of ≤ 2 (phase 1) or ≤ 1 (phase 2).
9. Life expectancy of > 3 months.
10. QTc ≤ 470 msec.
11. Adequate hematological, renal, hepatic and coagulation laboratory results.

Exclusion Criteria

1. Active brain metastases from non-brain tumors.
2. History or presence of hematological malignancies unless curativelytreated with no evidence of disease ≥ 2 years.
3. Myocardial infarction within 6 months of study day 1, symptomatic congestive heart failure (New York Heart Association > class II), unstable angina, or cardiac arrhythmia requiring medication.
4. Gastrointestinal (GI) tract disease causing the inability to take oral medication, malabsorption syndrome, requirement for intravenous alimentation, uncontrolled inflammatory GI disease (eg, Crohn's disease, ulcerative colitis).
5. Active infection requiring IV antibiotics within 1 weeks of study enrollment (day 1).
6. Hepatitis infection.
7. Known positive test for HIV.
8. Unresolved toxicities from prior anti-tumor therapy.
9. Anti-tumor therapy (chemotherapy, antibody therapy, molecular targeted therapy, retinoid therapy, hormonal therapy [except for subjects with breast cancer], or investigational agent) within 28 days of study day 1.

Patients will be assessed after treatment as follows: end of Treatment and Safety Follow-up will be performed 30 (+7) days after discontinuation of treatment. Long-term follow-up will be performed every 12 weeks (±2 weeks) from the last dose of investigational product.

C. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with LUMAKRAS™ (sotorasib) is addressed during review of the NDA and is not addressed in detail in this Summary of Safety and

Effectiveness Data. Please refer to Drugs@FDA for complete safety information on LUMAKRAS™ (sotorasib).

2. Effectiveness Results

The effectiveness of the *therascreen* KRAS RGQ PCR Kit to identify *KRAS* G12C mutation positive patients who may benefit from LUMAKRAS™ (sotorasib) is supported by the efficacy results from the primary analysis of Study 20170543. The efficacy results show that LUMAKRAS™ (sotorasib) monotherapy provides a clinically meaningful benefit to NSCLC patients with *KRAS* G12C mutations. The ORR for NSCLC subjects as assessed by BICR per RECIST 1.1 for subjects with NSCLC was 36% (46 of 124 subjects; 95% CI: 28-45%) with 2 subjects (1.6%) achieved complete response and 44 subjects (35.8%) achieved partial response. The lower limit of the 95% CI of 28% showed statistically significant sotorasib efficacy relative to the benchmark ORR of 23% for NSCLC based on the trial REVEL trial (ramucirumab plus docetaxel in second line setting) (Table 20).

Table 20. Summary of effectiveness results in the primary efficacy population by BICR

Efficacy Parameter	LUMAKRAS N=124
Objective Response Rate, N (%) (95% CI)[a]	45 (36) (28,45)
Complete response rate, N (%)	2 (2)
Partail response rate, N (%)	43 (35)
Duration of Response[a]	
Median[b], months (range)	10.0 (1.3, 11.1)
Patients with duration ≥ 6 months[c], %	58%

CI=confidence interval

[a]Assessed by Blinded Independent Central Review (BICR)

[b]Estimate using Kaplan-Meier method

[c]Observed proportion of patients with duration of response beyond landmark time

D. Pediatric Extrapolation

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

E. Financial Disclosures

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA supplement was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates the information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the *therascreen* KRAS RGQ PCR Kit (which was used to enroll patients into the Phase 2 Portion of clinical study 20170543) was demonstrated in analysis of efficacy and safety in NSCLC patients with the *KRAS* G12C mutation. Overall, a significant efficacy benefit for LUMAKRAS™ (sotorasib) was observed. The primary endpoint of ORR (complete response + partial response) measured by CT or MRI and assessed per RECIST 1.1 by BICR laboratory for subjects with *KRAS* G12C-mutated NSCLC was 36 (45 of 124 subjects; 95% CI: 28-45%); 2 subjects (1.6%) achieved complete response and 44 subjects (35.5%) achieved partial response.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies to support PMA Supplement approval as described above, as well as data collected in the clinical study conducted to support LUMAKRAS™ (sotorasib) approval.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect *KRAS* G12C test results, and consequently improper patient management decisions in NSCLC treatment. A false negative test result may lead to LUMAKRAS™ (sotorasib) treatment being withheld from a patient who might have benefitted. A false positive test result may lead to LUMAKRAS™ (sotorasib) treatment being administered to a patient who is not expected to benefit, and potentially subject the patient to any adverse side effects associated with treatment in addition to delaying the start of a therapy that may benefit the patient.

C. Benefit-Risk Conclusions

The probable clinical benefit of the *therascreen* KRAS RGQ PCR Kit for selecting *KRAS* G12C NSCLC patients for treatment with LUMAKRAS™ (sotorasib) was demonstrated in the analysis of efficacy and safety data obtained from the Phase 2 portion of the Phase 1/2 non-randomized, open-label Amgen 20170543 clinical study. For patients who were positive for *KRAS* G12C by the *therascreen* KRAS RGQ PCR Kit, an ORR of 36% (95% CI: 28-45%) was observed which indicates a favorable and clinically meaningful response, demonstrating a probable benefit of the device for selecting *KRAS* G12C positive advanced NSCLC patients for treatment with LUMAKRAS™ (sotorasib).

There is probable risk associated with the use of this device, mainly due to 1) false

positive, false negatives, or failure to provide a result and 2) incorrect interpretation of test results by the user. The risks of the KRAS Kit are associated with the potential mismanagement of patients resulting from false results of the test. A false negative test result may lead to LUMAKRAS™ (sotorasib) treatment being withheld from a patient who might have benefitted. A false positive test result may lead to LUMAKRAS™ (sotorasib) treatment being administered to a patient who is not expected to benefit, and also subjected to potentially adverse side effects associated with treatment with this drug. The probable risks of this device are partially mitigated by the analytical studies for this device, which demonstrated acceptable test performance for the indicated use.

1. Patient Perspectives

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

In conclusion, given the available information above, the data support the use of the *therascreen* KRAS RGQ PCR Kit to identify NSCLC patients for LUMAKRAS™ (sotorasib) treatment based on a *KRAS* Kit G12C mutation detected test result, and the probable benefits outweigh the probable risks, for this indication.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical validation study support the use of *therascreen* KRAS RGQ PCR Kit for the qualitative detection of somatic G12C mutations in the human KRAS oncogene using DNA extracted from formalin fixed paraffin embedded (FFPE) non-small cell lung cancer (NSCLC) tissue as an aid in the identification of NSCLC patients for treatment with LUMAKRAS™ (sotorasib).

XIV. CDRH DECISION

CDRH issued an approval order on May 28, 2021. The final conditions of approval can be found in the approval order.

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling

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