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 Manchester Ltd. Skelton House, Lloyd Street North Manchester, UK M15 6SH
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P110027
Date of FDA Notice of Approval:	May 23, 2014
Expedited:	Not applicable

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. The SSED to support the indication is available on the CDRH website and is incorporated by reference here. The current PMA (P110027) was submitted to expand the indication for the *therascreen*® KRAS RGQ PCR Kit.

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. The *therascreen*® KRAS RGQ PCR Kit is intended to aid in the identification of CRC patients for treatment with Erbitux® (cetuximab) and Vectibix® (panitumumab) based on a KRAS no mutation detected test result.

III. CONTRAINDICATIONS

None.

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, Software version 2.1.0, and KRAS Assay Package

Specimen Preparation

Formalin-fixed, paraffin-embedded (FFPE) blocks are sectioned onto glass slides. A stained slide is used to confirm that the tumor content exceeds 20% of the tissue and that a minimum tumor area of $4mm^2$ is available. A single non-stained tissue section is scraped from the slide for DNA extraction. If sections have a tumor content of less than 20%, the section should be macrodissected. DNA is manually extracted and purified from 5 µm glass-mounted sections of FFPE tissue taken from colorectal 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 200 µL of elution buffer. 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. 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 as follows:

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:

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).

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 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 Rotor-Gene Q software 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) 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.

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other alternatives for the correction of colorectal cancer: surgery, radiofrequency ablation, cryosurgery, chemotherapy, radiation therapy, and targeted therapy. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

For the selection of patients who may benefit with Vectibix (panitumumab) targeted therapy, there are no other FDA-cleared or approved alternatives for the testing of colorectal cancer tissue for detecting mutations in the KRAS oncogene.

VII. MARKETING HISTORY

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

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 colorectal cancer treatment. A false positive test result may lead to Vectibix (panitumumab) treatment being withheld from a patient who might have benefitted. A false negative test result may lead to Vectibix (panitumumab) treatment being administered to a patient who is not expected to benefit, and potentially any adverse side effects associated with treatment.

Most common adverse reactions (≥ 20%) in clinical trials of Vectibix (panitumumab) in combination with oxaliplatin-containing chemotherapy are diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, dermatitis acneiform, pruritus, and dry skin.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

The specific performance characteristics of the QIAGEN *therascreen*® KRAS RGQ PCR Kit (henceforth referred to as KRAS Kit) were determined by studies using

formalin-fixed, paraffin-embedded (FFPE) tissue specimens collected from colorectal cancer patients (CRC) and 8 formalin-fixed, paraffin-embedded human cell lines (FFPE cell lines) of which 7 harbor known KRAS mutations, and one KRAS wild-type (i.e., glycine amino acids at codons 12 and 13). Mutation status of specimens was confirmed by bi-directional Sanger sequencing. The similarity between FFPE cell lines and FFPE clinical specimens was demonstrated. FFPE cell lines were sectioned and processed similar to FFPE patient specimens. DNA was extracted and tested according the instructions for use.

1. Comparison to Reference Method

To demonstrate the accuracy of the KRAS Kit relative to Sanger bi-directional sequencing, two accuracy studies with procured specimens were conducted. In the first study, a set of 350 procured tumor specimens from CRC patients was obtained based on characteristics that aligned with patient samples screened in the clinical trial (i.e., intended use population). Variables that impact test performance were described for the procured specimens and compared to the clinical trial specimens. The variables were deemed similar between both groups and consisted of patient demographics (age, genders, race, and country of origin), fixation process, tumor sampling, tumor tissue content, stage, histology, amount of necrotic tissue, and storage conditions. Using a statistical random sampling technique, 150 samples of unknown mutation status were chosen for evaluation. Ten sequential 5µm sections were cut from each sample and mounted onto glass slides. Sections used for testing with the KRAS Kit or for bi-directional sequencing were adjacent to each other. Specimens were processed and tested by the KRAS Kit according to the final product labeling. All sections used for bi-directional sequencing were macrodissected to enrich for tumor content. A Phred score greater than 40 was a pre-specified acceptance criterion for sequencing (seven samples that failed were excluded). Invalid and indeterminate samples were retested according to protocol. The results demonstrated that the KRAS Kit reported two samples as negative. These samples were reported by bi-directional sequencing to be positive for 12ASP or 13ASP. In contrast, three samples were reported as having a KRAS mutation by the KRAS Kit that were not reported as positive by sequencing. In addition, one sample identified as 12ARG by the KRAS Kit was determined to be 12ASP by sequencing. The overall results are shown in the Table below.

KRAS Kit compared to Sanger bi-directional Sequencing

		Mutation Call by Bi-Directional Sequencing									
		Mutation Negative	IND	12ALA	12ARG	12ASP	12CYS	12SER	12VAL	13ASP	Total
KRAS Kit	Indeterminate	3	-	-	-	-	-	-	-	-	3
	Invalid	2	-	-	-	-	-	-	-	-	2
	Mutation-negative	80	-	-	-	1	-	-	-	1	82
	12ALA	-	-	3	-	-	-	-	-	-	3

	Mutation Call by Bi-Directional Sequencing									
	Mutation Negative	**IND**	**12ALA**	**12ARG**	**12ASP**	**12CYS**	**12SER**	**12VAL**	**13ASP**	**Total**
12ARG	-	-	-	-	1	-	-	-	-	1
12ASP	-	-	-	-	20	-	-	-	-	20
12CYS	-	-	-	-	-	3	-	-	-	3
12SER	-	1	-	-	-	-	-	-	-	1
12VAL	2	-	-	-	-	-	-	14	-	16
13ASP	1	-	-	-	-	-	-	-	11	12
Total	88	1	3	0	22	3	0	14	12	143

Concordance between methods for this study was calculated as the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) with the 95% confidence intervals for all samples with valid results. The results demonstrate a PPA of 96.3%, a NPA of 96.3% and an OPA of 96.4%.

Agreement for Samples with both Sanger and KRAS Kit valid Results

Measurement of Agreement	Percentage % (N)	95% CI (%)
Overall percent agreement (OPA)	96.3 (132/137)	92.69 – 98.21
Positive percent agreement (PPA)	96.3 (52/54)	89.41 – 98.77
Negative percent agreement (NPA)	96.4 (80/83)	91.30 – 98.55

A sensitivity analysis was performed to evaluate the agreement between the two methods if all of the KRAS Kit indeterminate and invalid results were treated as mutation-positive or as mutation-negative. Under the condition where all of the invalid/indeterminate calls are assumed to be positive or negative, the OPA is 93.0% and 96.5 %, respectively.

A second unique set of 271 CRC FFPE specimens were procured and compared to Sanger bi-directional sequencing as described above to supplement the data from the first study. The set consisted of 250 specimens of unknown mutations status, and 21 specimens of known mutation status to enrich for rare mutations. A total of 13 (~5%) specimens required macrodissection in accordance with KRAS Kit instructions because the tumor content was less than 20%. Out of the 271 specimens tested, 24 were indeterminate (failed control Ct range). Concordance analysis was carried out on 247 samples with both valid bi-directional and KRAS Kit results. There were 9 discordant samples. One sample from the 247 samples had a mutation positive result with bi-directional sequencing but a mutation negative result with the KRAS Kit. Eight samples were shown to have positive

results with the KRAS Kit but negative results with bi-directional sequencing. The results are shown in the tables below. Overall agreement was 96.4%. The data supports the performance of the *therascreen*® RGQ PCR KRAS Kit.

Comparison of Mutation Calls by KRAS Kit and Bi-Directional Sequencing

		Mutation Call by Bi-Directional Sequencing									
		Mutation Negative	**IND**	**12ALA**	**12ARG**	**12ASP**	**12CYS**	**12SER**	**12VAL**	**13ASP**	**Total**
KRAS Kit call	**Indeterminate**	15	5	1	-	1	1	-	1	-	24
	Mutation-negative	132	-	-	-	-	-	1	-	-	133
	12ALA	-	-	10	-	-	-	-	-	-	10
	12ARG	5	-	-	5	-	-	-	-	-	10
	12ASP	-	-	-	-	31	-	-	-	-	31
	12CYS	1	-	-	-	-	11	-	-	-	13
	12SER	-	-	-	-	-	-	13	-	-	13
	12VAL	2	-	-	-	-	-	-	25	-	27
	13ASP	-	-	-	-	-	-	-	-	11	11
	Total	155	5	11	5	32	12	14	26	11	271

Agreement between Bi-Directional Sequencing and KRAS Kit*

	Percent observed agreement (Lower 95% confidence interval)
Overall percent agreement	96.36% (93.73%)
Positive percent agreement	99.07% (95.64%)
Negative percent agreement	94.29% (89.93%)

* Excluding the 24 KRAS Kit indeterminate results (failed Control Ct range).

2. Analytical Sensitivity

a) Limit of Blank (LoB) – No Template

To assess performance of the *therascreen*® KRAS RGQ PCR Kit in the absence of template and to ensure that a blank sample does not generate an analytical signal that might indicate a low concentration of mutation, samples with no template were evaluated. Ten KRAS Kit runs consisting of specimens with nuclease-free water (no DNA template) were conducted. Each of the runs included positive and negative controls, as well as seven no-template samples. The results demonstrated no detectable control or mutant Ct values in any of the mutation or control reaction wells (Internal control Ct values were all valid). Results are reported as invalid due to failed controls in the absence of DNA.

b) Limit of Detection (LoD)

The *therascreen*® KRAS RGQ PCR Kit does not use a specific concentration of DNA as determined by spectrophotometry. DNA input is based on the Control reaction Ct result which is used to indicate that there is sufficient amplifiable DNA present in the sample. The stated DNA input for the assay is defined by the Control Ct prespecified range of 21.92 to 32.00. For the *therascreen*® KRAS RGQ PCR Kit, the limit of detecting mutant DNA in a background of wild-type (WT) DNA is defined as the lowest dilution factor at which 95% of the test replicates for each mutation positive sample were determined to be positive. Eight FFPE cell lines; seven with known mutant DNA content and one wild-type were used for this evaluation. The proportion of mutant in total amplifiable DNA (percent mutant DNA) was determined previously using a bi-directional Sanger sequencing method from unfixed cells followed by relative peak analysis. In the case of three cell lines the mutant content was 100% (i.e., the cell line DNA was homozygous mutant). The other cell lines were of mixed zygosity. Multiple DNA extractions from each sample were pooled to generate DNA stocks. The DNA stocks were then normalized to achieve target Control reaction Ct values. Normalized mutant DNA extracts were diluted with normalized WT DNA extract to create a dilution series of extracts containing the same level of total amplifiable DNA but different levels of mutant DNA. Serial dilutions were then generated from these samples and 9 replicates for each dilution were run. The first dilution series was created for the mid-range Control reaction Ct value (approximately 26). The percentage of correct calls as a function of the dilution for each mutant reaction is shown below. Shaded boxes indicate the corresponding dilution for each mutant reaction in which greater than 95% of the replicates produced correct calls.

Percentage of Correct Calls

	% Correct Calls						
% Mutation Dilution	**12ALA**	**12ASP**	**12ARG**	**12CYS**	**12SER**	**12VAL**	**13ASP**
0.78	100	0	33.3	55.6	22.2	66.7	0
1.56	100	33.3	100	100	88.9	100	0
3.13	100	77.8	100	100	100	100	66.7
6.25	100	100	100	100	100	100	100
12.5	100	100	100	100	100	100	100
25.0	100	100	100	100	100	100	100
50.0	100*	100	100	100	100	100	100

* Mutation Dilution for this sample was 40.0.

The results of the first dilution series were used to generate dilutions for the confirmation of LoD values using narrower, reaction-specific ranges of percent mutation dilutions at both low and high levels within the input range of the assay. The target value for the High series was approximately Ct 23-

24. Twelve replicates for each dilution were evaluated for the High dilution series. The percentage of correct calls is described for each dilution in the High series and is shown in the tables below. Shaded boxes indicate the percentage in which greater than 95% of the replicates produced correct calls.

Percentage of Correct Calls for High Dilution Series

		% Mutation Dilution (High)					
12ALA	Dilution Series	0.13	0.27	0.54	**1.08**	2.15	4.30
	% correct calls	0	0	91.7	**100**	100	100
12ASP	Dilution Series	0.56	1.13	2.25	4.50	**9.00**	18.00*
	% correct calls	0	8.3	33.3	83.3	**100**	100
12ARG	Dilution Series	0.16	0.33	0.65	**1.30**	2.60	5.20
	% correct calls	0	0	8.3	**100**	100	100
12CYS	Dilution Series	0.12	0.24	0.49	0.98	**1.95**	3.90
	% correct calls	0	0	8.3	83.3	**100**	100
12SER	Dilution Series	0.31	0.63	1.25	2.50	**5.00**	10.00
	% correct calls	0	0	33.3	66.7	**100**	100
12VAL	Dilution Series	0.17	0.34	0.69	**1.38**	2.75	5.50
	% correct calls	0	0	16.7	**100**	100	100
13ASP	Dilution Series	0.63	1.25	2.50	**5.0**	10.0	20.0
	% correct calls	0	0	0	**100**	100	100

*Eleven valid replicates in this evaluation.

The target Ct value for the Low series was approximately 31. Each dilution was run as 24 replicates unless otherwise indicated. The percentage of correct calls is described for each dilution in the Low series and is shown in the tables below. Shaded boxes indicate the percentage in which greater than 95% of the replicates produced correct calls.

Percentage of Correct Calls for Low Dilution Series

		% Mutation Dilution (Low)						
12ALA	Dilution Series	0.27	0.54	1.08	2.15	**4.30**	8.60	12.90
	% correct calls	12.5	20.8	33.3	83.3	**100**	100	100
12ASP	Dilution Series	0.56	1.13	2.25	4.50	**9.0**	18.0	27.0
	% correct calls	0	16.7	29.2	58.3	**100**	100	100
12ARG*	Dilution Series	0.33	0.65	1.30	2.60	**5.20**	10.4	15.6
	% correct calls	8.3	4.2	29.2	52.2	**95.8**	100	100
12CYS	Dilution Series	0.24	0.49	0.98	1.95	3.90	**7.80**	11.7
	% correct calls	8.3	4.2	20.9	54.2	83.3	**100**	100
12SER	Dilution Series	0.63	1.25	2.50	5.0	10.0	20.0	**30.0**
	% correct calls	0	0	8.3	33.3	70.9	83.3	**100**
12VAL**	Dilution Series	0.34	0.69	1.38	2.75	**5.50**	11.00	16.50

	% correct calls	4.3	16.7	46.7	75.0	**100**	100	100
13ASP	Dilution Series	0.63	1.25	2.5	5.0	10.0	**20.0**	30.0
	% correct calls	0	4.2	8.3	33.3	70.8	**100**	100

*For the 2.60 dilution, the number of valid replicates was 23 for 12ARG.

**Valid replicates for the 12VAL series were 23, 24, 15, 16, 13, 12, and 19.

Logistic Regression models were applied to each assay individually for the low and high input DNA datasets. In these models, the response variable was the binary output of mutation detected (detect = 1) and mutation not detected (detect = 0), the continuous explanatory variable was $\log_2$ % mutation dilution. The LoDs were calculated as the percent mutation dilution which gave a predicted probability of detection of 0.95. The LoDs determined from the dilution series beginning with either the low or high Ct values are shown in the Table below.

Logistics Regression Data for Low and High Ct Dilution Series

	Low	**High**
12ALA	4.25	0.56
12ASP	7.27	0.87
12ARG	10.23	6.43
12CYS	6.90	1.21
12SER	25.75	4.20
12VAL	5.17	0.90
13ASP	18.83	4.16

The data overall supports the manufacturer's Final LoD claims for FFPE cell lines when the input Ct value is between approximately 22 and 27 Ct. At the lower end of the Ct input range, the sensitivity of the assay decreases as the amount of input DNA may not contain sufficient copies to support the same percentage ratios of wild-type to mutant DNA observed within the high and mid points of the working range.

LoD Claims FFPE Cell Lines when Control Reaction Ct Range ~22-27

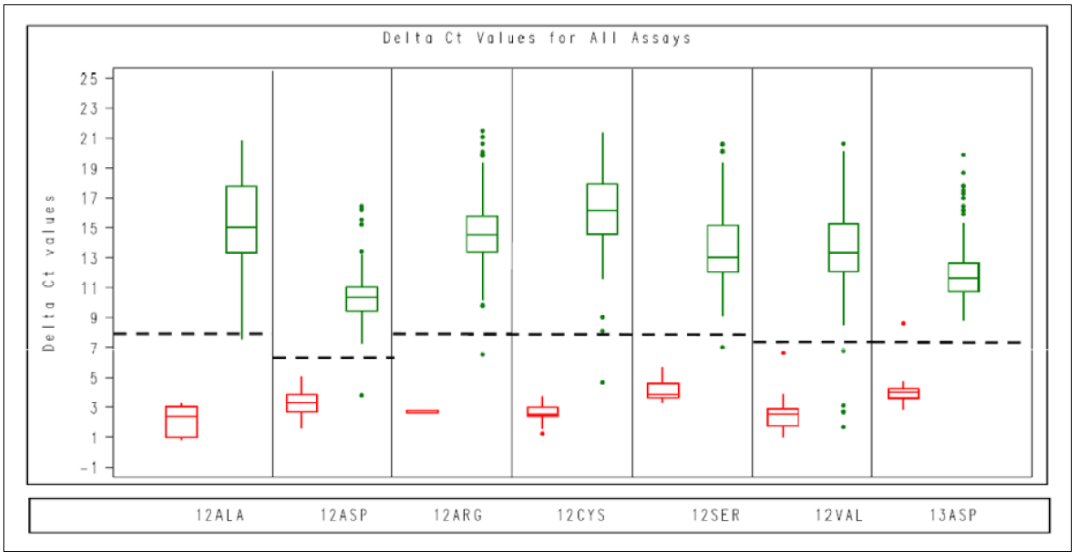
Mutation Reaction	**Final LoD Claim**
12ALA	**0.8**
12ASP	**2.6**
12ARG	**6.4**
12CYS	**1.5**
12SER	**5.6**

12VAL	**1.6**
13ASP	**6.4**

c) Control Ct Range and RFI Validation

For the KRAS Kit, the acceptable Control Ct range that is used to determine the amount of DNA input and the threshold value is set at 0.05 relative fluorescence units (RFI). This value is configured in the KRAS Locked Templates for both the FAM and HEX channels. The threshold value and Control Ct range were defined during development of the KRAS Kit. Briefly, 220 FFPE samples were assayed. Control reaction Ct values were tested for normality and one-side tolerance limits were developed such that 97.5% of the Control Ct values in the mutation reactions fall above the lower limit with a confidence level >90%. Box and Whisker plots of the ΔCt values were based on the assignment of the optimized Control Ct range and are shown in the box below. The line within each box represents the median value in the distribution, while the box represents the 25th and 75th percentile. The hashed line shows the cut-offs, above that are the mutation negative samples, and below that the mutation-positive samples.

Cut-off ΔCt Values by Mutation



3. Effect of DNA Input on ΔCt Values

The DNA input level is defined as the total quantity of amplifiable KRAS DNA in a sample and is determined by the Ct values from the Control reaction. When samples at different total DNA levels contain the same proportion of mutant DNA, it is expected that the measured ΔCt values will remain consistent. The objective of the study was to demonstrate that the performance of the KRAS Kit

is consistent over the total DNA input (Control Ct) range of the assay. DNA extracted from 8 FFPE cell lines was used to prepare pools of DNA with the lowest achievable control reaction Ct. Concentrated DNA stocks were subsequently diluted to generate DNA spanning the working range (total of 5 dilutions including the initial concentrated stock). For mutations 12ALA, 12ASP, 12ARG and 12VAL were diluted 1:10 (range 1 to .0001). For mutations 12CYS, 12SER and 13ASP, the dilutions were 1:5.5 (range 1 to 0.001). For each point within the working range sufficient material was prepared to carry out 6 replicate tests. The dilution range for each mutation reaction, mean Ct value for the Control and Ct reactions in the test runs are shown in the Table below. The results from each mutation pool tested showed Control Reaction Ct values corresponding with the targeted values. For each of the mutations detected by the KRAS Kit, the ΔCt values measured at different total DNA input levels spanning the working range of the assay passed the pre-set acceptance criteria for the study.

Mean Ct Values across the Control Reaction Ct Range – FFPE Cell Lines

	Mean Mutation Ct Value					**Mean Control Ct Value**				
	Dilution 1	2	3	4	5	Dilution 1	2	3	4	5
12ALA	22.75	25.57	28.77	32.31	35.77	21.19	24.32	27.61	31.17	34.51
12ASP	23.45	26.32	29.64	33.28	36.5	20.99	24.14	27.54	31.17	34.66
12ARG	21.37	24.71	28.05	31.49	34.84	20.19	24.08	26.97	30.55	33.78
12CYS	24.32	26.64	28.99	31.45	33.8	23.35	25.94	28.4	30.64	33.13
12SER	25.54	27.19	29.67	32.14	34.61	22.63	24.98	27.52	29.99	32.53
12VAL	21.53	24.72	28.05	31.24	34.41	21.24	24.47	27.9	30.99	34.51
13ASP	26.7	28.54	30.8	33.12	35.65	23.13	25.7	28.26	30.66	33.03

The mean ΔCt values for each mutation reaction and each dilution are displayed as a function of the Control Reaction Ct ranges for the dilutions in the Table below. Although there is a slight increase in ΔCt as DNA input increases, overall, the ΔCt values were consistent across the working range of the KRAS Kit within the prespecified acceptance criteria.

Mean ΔCt Values across the Control Reaction Ct Range - FFPE Cell Lines

	Dilution (1) ~20-21Ct	Dilution (2) ~ 23-24Ct	Dilution (3) ~ 26-27Ct	Dilution (4) ~29-30Ct	Dilution (5) ~32-33Ct
	Mean ΔCt				
12ALA	1.56	1.25	1.16	1.14	1.27
12ASP*	2.46	2.18	2.11	2.11	1.75
12ARG	1.18	0.63	1.08	0.94	1.06
12VAL	0.29	0.25	0.15	0.26	-0.1
	~ 22-23Ct	~ 24-25Ct	~ 27-28Ct	~29-30Ct	~32-33Ct
	Mean ΔCt				
12SER	2.91	2.21	2.15	2.15	2.08
12CYS	0.98	0.71	0.58	0.81	0.67

13ASP	3.57	2.84	2.54	2.46	2.62

*In the case of 12ASP data, total number of replicates was 27.

DNA extracts from FFPE CRC samples were also prepared and diluted to represent three total DNA input levels; nominally High, Medium and Low DNA input levels as defined by the absolute Ct value of the KRAS Kit Control reaction. The High and Medium DNA input levels were within the working range of the assay (i.e., Control Ct range 21.92 – 32.00). The Low DNA input level dilutions were targeted to be within the working range of the assay, however, for the purposes of this study, values that fell outside the working range were also included in the study analysis. The data demonstrates ΔCt values are similarly consistent across DNA input levels.

Effect of DNA Input on ΔCt Values across the Input Ct Range - CRC Samples

	Mean Control Ct			**Mean Mutant Ct**			**Mean ΔCt**		
	Low	**Med**	**High**	**Low**	**Med**	**High**	**Low**	**Med**	**High**
12ALA	31.50	28.36	25.41	33.19	30.14	27.37	**1.69**	**1.78**	**1.95**
12ASP	30.74	27.52	25.44	33.00	29.90	27.16	**2.26**	**2.39**	**2.72**
12ARG	32.12	29.23	27.03	34.18	31.34	29.24	**2.06**	**2.11**	**2.21**
12CYS	30.85	27.36	24.43	33.51	30.10	27.43	**2.66**	**2.74**	**3.00**
12SER	30.35	27.64	25.17	35.04	32.11	30.13	**4.70**	**4.47**	**4.96**
12VAL	30.97	27.48	24.39	32.97	29.60	26.54	**2.00**	**2.12**	**2.16**
13ASP	31.14	27.77	25.13	34.58	31.26	29.05	**3.44**	**3.49**	**3.92**

4. <u>Linearity/Amplification Efficiency as a Function of DNA Input (Part 1)</u>

The linearity and amplification efficiency of PCR for each mutation reaction, relative to the control reaction, across the working range of the KRAS Kit was demonstrated. Amplification efficiency was calculated for each of the mutation reactions and the control reaction as [2(-1/slope)]-1. The largest difference in the amplification efficiencies between the Control reaction and a mutant reaction was observed for the 13ASP (mean difference in efficiencies approximately 14.5%). The amplification efficiency of the control relative to the mutant reactions is consistent across the working range of the assay as shown in the Table below.

PCR Amplification Efficiencies (Part 1)

Sample			Intercept	Intercept Standard Error	Calculated Slope	Standard Error (slope)	Two-Sided 95% CI (slope)	**Amplification Efficiency**	Difference in Amplification Efficiencies
	12ALA	**Control Ct**	21.06	0.060	-1.008	0.007	-1.023, -0.993	0.989	0.03
		12ALA Ct	22.48	0.103	-0.987	0.013	-1.013, -0.961	1.019	
	12ASP	**Control Ct**	20.82	0.083	-1.035	0.01	-1.056, -1.014	0.954	0.056
		12ASP Ct	23.24	0.083	-0.993	0.011	-1.016, -0.97	1.01	
	12ARG	**Control Ct**	20.38	0.13	-1.013	0.016	-1.046, -0.98	0.982	-0.003
		12ARG Ct	21.35	0.065	-1.015	0.008	-1.032, -0.999	0.979	
	12CYS	**Control Ct**	23.43	0.063	-0.981	0.01	-1.003, -0.96	1.026	0.032
		12CYS Ct	24.29	0.039	-0.961	0.006	-0.974, -0.947	1.058	
	12SER	**Control Ct**	22.57	0.050	-1.003	0.008	-1.02, -0.986	0.996	0.105
		12SER Ct	25.21	0.087	-0.934	0.014	-0.963, - 0.904	1.101	
	12VAL	**Control Ct**	21.21	0.047	-0.995	0.006	-1.007, - 0.983	1.007	0.033
		12VAL Ct	21.53	0.043	-0.972	0.005	-0.983, - 0.961	1.04	
	13ASP	**Control Ct**	23.21	0.056	-1.001	0.009	-1.02, -0.982	0.999	0.145
		13ASP Ct	26.47	0.106	-0.909	0.017	-0.945, -0.873	1.144	

5. <u>Linearity/Amplification Efficiency as a Function of %Mutation (Part 2)</u>

The objective of this study was to evaluate the linearity of each mutant reaction across the working range of the assay, when the total amount of DNA is held constant but the percentage of mutant DNA is varied. DNA extracts from FFPE cell lines were initially assessed by OD readings prior to carrying out PCR with the KRAS Kit. DNA stocks were then prepared to a Control Reaction Ct corresponding to ~23Cts. The stocks were diluted serially 2 fold each time using wild-type DNA, in order to maintain the total wild-type DNA constant while varying the percentage mutant DNA in the template. Thus, each of the templates generated had the same absolute quantity and concentration of DNA but differing ratios of wild-type to mutant DNA. The dilutions and Target Ct values are shown below.

Mean Control Reaction Ct Values for Each Dilution Point

Dilution (x100)	Assay	12ALA	12ASP	12ARG	12CYS	12SER	12VAL	13ASP
		Target 22.75	Target 22.75	Target 22.75	Target 23.17	Target 23.00	Target 22.75	Target 23.5
1	**control**	22.33	22.87	22.97	23.68	22.76	22.29	23.51
0.5	**control**	22.42	22.71	22.98	23.45	22.98	22.60	23.75
0.25	**control**	22.51	22.62	22.95	23.40	23.05	22.61	23.75
0.125	**control**	22.54	22.62	22.90	23.36	23.11	22.65	23.65
0.0625	**control**	22.55	22.66	22.90	23.29	23.08	22.67	23.64

Pools of DNA sufficient for 6 replicates per mutation were prepared. The Ct and ΔCt data for each mutation at each dilution point were calculated. The control reaction Cts were consistent over the dilution series of each mutation. For each sample where the control reaction Ct value fell within the specified range (21.92 – 32.00), ΔCt values were calculated. A linear regression model was fitted with mutation reaction Ct versus $\log_2$ DNA input dilution. The slope and 95% confidence intervals were reported. The study showed the dilution of mutations in a background of a constant concentration of wild-type DNA resulted in amplification efficiencies that did not vary significantly outside the values determined in the above linearity study with the amplification efficiencies.

PCR Amplification Efficiencies (Part 2)

	Intercept	Intercept (Std. error)	Slope	95% CI (slope)	Amplification Efficiency
12ALA	23.5	0.025	-0.968	-0.989, -0.947	1.047
12ASP	24.8	0.054	-1.030	-1.075, -0.985	0.960
12ARG	24.2	0.028	-1.008	-1.031, -0.984	0.990
12CYS	24.4	0.027	-0.981	-1.003, -0.959	1.024
12SER	25.4	0.054	-0.892	-0.937, -0.847	1.174
12VAL	22.7	0.035	-1.021	-1.050, -0.992	0.972
13ASP	27.6	0.057	-0.810	-0.857, -0.763	1.353

6. Analytical Specificity

a) Primer and Probe Specificity

The primers and probes have been designed to avoid any known KRAS polymorphisms. A specificity analysis was conducted using the Basic Local Alignment Search Tool (BLAST) to ensure that the primers used in the *therascreen*® KRAS RGQ PCR Kit would amplify only human KRAS sequences and not sequences from other species or to non-KRAS human sequences (e.g., pseudogenes). No non-specific amplification is predicted from non-KRAS genes. In addition, alignments of pairs of oligonucleotides (primers, probes, and templates) used in the KRAS Kit were performed to ensure there is no unexpected binding that could lead to non-specific

amplification. There was no significant homology between the various reagents.

b) Cross-Reactivity/Exclusivity

The *therascreen*® KRAS RGQ PCR Kit is comprised of eight separate reactions; one single control reaction that detects a nonpolymorphic region of the KRAS gene and seven mutation specific reactions. There is no reaction that specifically measures the wild-type KRAS sequence at codon 12 or 13. The KRAS mutation-negative result, (generally wild-type at codon 12 and 13) is determined from the absence of any of the 7 mutations resulting in a positive mutation result. Therefore it is necessary to demonstrate the amount of non-specific amplification, or cross-reactivity that occurs in each reaction with excess amounts of KRAS wild-type DNA, to ensure no false positive results occur. Similarly, non-specific amplification of KRAS mutations for which the reaction is not intended to detect is evaluated to demonstrate that the amount of cross-reactivity between mutant reactions does not result in erroneous mutation calls in the presence of excess amounts of mutant DNA. Since the DNA input for this assay is based on the control Ct range (21.92 to 32.00), the highest concentration of DNA input is based on having a control Ct value of approximately 22. FFPE clinical samples were used for this evaluation, however due to the difficulty of obtaining DNA at the maximum input level, FFPE cell line DNA was also evaluated. Mutation status was confirmed by bi-directional sequencing.

Non-Specific Amplification/Cross-Reactivity: Wild-Type KRAS DNA:

In order to address the amount of non-specific amplification of wild-type (WT) DNA by reaction mixes designed to amplify specific mutations, sixty (60) replicates of WT FFPE cell line DNA, or DNA extracted from CRC tumor tissue at the highest concentration of amplifiable DNA input level, was evaluated using the KRAS Kit. For DNA extracted from FFPE cell line the Control Ct values were approximately 22-23. Control Ct values for three wild-type CRC samples were between 24 and 25. The results demonstrated that the ΔCt values exceeded the established cut-offs. The mean and/or lowest ΔCt values observed for each reaction is shown below.

Lowest Mean ΔCt Observed for Wild-type samples in Mutant Reactions

		WT FFPE cell line	WT Clinical CRC Samples		
Mutant Reaction	**Cut-off**	**Lowest ΔCt Observed**	**Sample 1 ΔCt Mean (Lowest)**	**Sample 2 Mean (Lowest)**	**Sample 3 Mean (Lowest)**
12ALA	**8**	12.76	18.00 (11.40)	18.62 (11.50)	20.03 (19.36)
12ASP	**6.6**	10.35	10.90 (9.62)	10.34 (8.84)	10.68 (9.01)
12ARG	**8**	14.26	20.33 (12.94)	20.02 (13.20)	20.03 (19.36)
12CYS	**8**	13.66	20.62 (17.38)	20.29 (19.62)	20.03 (19.36)
12SER	**8**	11.97	17.26 (11.14)	17.90 (11.42)	18.05 (10.44)
12VAL	**7.5**	11.81	14.87 (11.46)	16.27 (11.50)	18.68 (11.36)
13ASP	**7.5**	10.94	12.35 (9.08)	13.68 (10.69)	14.82 (9.97)

Non-Specific Amplification/Cross-Reactivity/Exclusivity: Mutation-Positive KRAS DNA:

The exclusivity of the KRAS Kit is intended to discriminate between mutation negative and mutation positive status. Mutant samples that have a high concentration of input DNA were tested against all reaction mixes by preparing DNA samples from each of the FFPE cell lines so that the Control Reaction Ct corresponded to approximately 23. Six (6) replicates of each mutation sample were evaluated. The percentage of mutation in the sample was governed by the percentage of mutant in the cell line DNA. The mean ΔCt are presented in the table below and demonstrates that there is cross reactivity between mutant reactions when high concentrations of DNA are evaluated. The 12ALA mutation was amplified and generated ΔCt values below the ΔCt thresholds for the 12CYS, 12SER and 12VAL reactions. The 12VAL mutation was amplified and generated a ΔCt value below the ΔCt threshold for the 12ALA reaction, however in all cases, the results demonstrate that the correct mutation was called with the matched mutation reaction (i.e., the smallest ΔCt value was the correct mutation call). All other test cases were either not detected or outside the ΔCt threshold.

Cross-Reactivity Between Mutation Reactions Using FFPE Cell Line DNA[(1)]

		ΔCt Cut-Off	Mutant Reaction ΔCt Values						
			12ALA	12ASP	12ARG	12CYS	12SER	12VAL	13ASP
Mutant DNA	12ALA	8	**1.42**	12.66	-	5.81	2.78	6.31	13.21
	12ASP	6.6	12.56	**2.42**	-	-	13.44	11.21	13.55
	12ARG	8	13.12	11.56	**1.12**	11.42	-	13.43	12.66
	12CYS	8	14.2	12.48	9.23	**0.98**	-	7.96	12.88
	12SER	8	-	13.39	13.31	-	**3.02**	12.99	13.97
	12VAL	7.5	6.83	-	-	-	13.38	**0.28**	13.74
	13ASP	7.5	-	13.29	13.89	-	-	14.36	**4.5**

(1) ΔCt from matched reactions are shown in bold. Blank cells show no cross-reaction. ΔCt from cross-reactive reactions below the cut-off are shaded.

7. Interference Effects - Necrotic Tissue

To support the performance of the KRAS Kit with tissues with high necrosis, the accuracy of the KRAS Kit for 29 samples with greater than 50% necrosis as determined by a pathologist was evaluated. The KRAS Kit did not falsely detect any mutations in the 21 samples identified as wild-type by bi-directional sequencing. The KRAS Kit correctly called 7 of the 8 KRAS mutant samples. One sample was invalid due to inadequate DNA in the sample. The results support the use of the KRAS Kit with samples with high necrosis.

8. Interference Effects - Exogenous Substances

To evaluate the impact of interfering substance on performance of the KRAS Kit, potentially interfering substances present in the DNA extraction process, were tested at 10x concentration in mutant and wild-type samples with a target Ct value between 27 and 30. The impact of each substance on the ΔCt values and mutation status of the samples was assessed. The substances tested were (1) paraffin wax, (2) xylene, (3) ethanol, and (4) Proteinase K. The difference between the ΔCt of samples with interferent was compared to samples without interferent according to statistical methods outlined in CLSI guidance document EP7-A2. For mutant samples, of the 448 replicates tested (7 mutations x 8 substances x 2 levels X 4 replicates) there were 8 invalid or indeterminate results and 4 false mutation negative results. For wild-type samples, 3 of the 448 replicates tested were invalid or indeterminate and 2 false mutation positive results. The results demonstrated that there was no significant interference.

9. Reproducibility

The repeatability and reproducibility of the KRAS Kit was investigated by testing DNA extracted from 8 FFPE CRC tissue blocks representing the 7 KRAS mutations and one wild-type (WT) KRAS specimen, at three sites with 2 operators at each site across 5 non-consecutive days in duplicate. The study

consisted of two-parts: within-laboratory and between-laboratory. Multiple 5-µm sections were cut from each of the blocks and mounted onto glass slides. DNA was extracted using the QIAGEN® QIAamp® DSP DNA FFPE Tissue Kit, pooled and diluted to produce stocks of the same concentration of amplifiable DNA based on target control Ct values. Aliquots of mutant DNA stocks were diluted with WT stock to produce individual DNA samples at the targeted Ct levels representing approximately 3 times the LoD and at the cut-off. (The cut-off sample tested was one that was designed to investigate the variance directly at the cut-off.) The total set consisted of 21 samples; 7 mutation-positive samples at 3xLoD, 7 mutation-positive samples at the cut-off (i.e., at ΔCt cut-off to investigate variance at the cut-off), and 7 wild-type samples.

The within-laboratory precision was determined from a series of 20 runs evaluating both dilution levels and a duplicate on each run resulting in 40 replicates per sample (2 operators and 5 non-consecutive days). Between-laboratory reproducibility was evaluated at two additional sites using 2 operators and 5 non-consecutive days in duplicate resulting in 10 runs and 20 duplicates per sample. Three lots were used in this evaluation. The proportion of correct mutation call of 3xLoD samples testing mutant and WT samples were reported.

The estimated proportion of 3xLOD samples testing mutant and WT samples were reported overall and within each of the sites. For all assays and sample combinations, at least 79 out of 80 replicates gave the correct mutation call. The overall proportion of correct calls was 99.6% (1115/1120); 99.6% (558/560) for mutation-positive (3xLOD) samples and 99.5% (557/560) for wild-type samples.

Proportion of Correct Calls by Assay for Mutation positive and Wild-type samples

Mutant 3X LoD Specimens (Target Control Ct approximately 30)						
12ALA	12ARG	12ASP	12CYS	12SER	12VAL	13ASP
79/80	80/80	80/80	79/80	80/80	80/80	80/80
Wild-Type Specimen						
80/80	79/80	80/80	80/80	79/80	79/80	80/80

Variation (i.e., consistency in calling) was also measured using the GINI index calculated over all laboratories as detailed in the table below. The GINI index measures the variation or consistency in call response. A GINI index of zero (0) means total consistency (i.e., all responses in one single category) while a GINI index of half (0.5) means maximum inconsistency (i.e., the calls are spread equally across the two categories). The results of the GINI analyses when applied to the calls achieved for the 3xLOD and wild-type samples demonstrated very high consistency with the GINI indices over all laboratories less than 0.025 for all assays.

Variance components analyses (random effects models) were used to generate estimates of reproducibility in terms of ΔCt and Ct values. The % CV for the ΔCt and Ct values for each reaction per sample below.

Reproducibility Precision Estimates

Assay	%CV for ΔCt		%CV for Mutant Ct		%CV for Control Ct		
	3xLOD	C50	3xLOD	C50	3xLOD	C50	WT
12ALA	13.14	8.32	1.87	2.02	0.97	1.12	1.12
12ARG	10.79	8.04	1.59	1.96	1.24	1.51	1.15
12ASP	12.86	5.87	1.11	1.00	0.90	0.90	1.04
12CYS	17.61	10.83	1.86	2.02	1.54	1.22	1.15
12SER	13.97	10.43	1.71	2.11	0.94	1.19	1.15
12VAL	9.66	15.47	1.52	1.65	1.11	3.74	1.26
13ASP	13.73	9.35	1.91	2.08	1.11	1.41	1.19

Repeatability Precision Estimates

Assay	%CV for ΔCt		%CV for Mutant Ct		%CV for Control Ct		
	3xLOD	C50	3xLOD	C50	3xLOD	C50	WT
12ALA	10.71	7.51	1.69	1.76	0.77	0.90	0.79
12ARG	9.83	8.04	1.21	1.76	0.84	1.33	0.90
12ASP	10.16	4.08	0.93	0.89	0.80	0.76	0.76
12CYS	13.15	8.80	1.31	1.76	1.40	1.01	0.76
12SER	6.76	6.18	1.10	1.48	0.80	0.90	0.90
12VAL	9.21	15.32	1.40	1.42	0.91	3.49	0.94
13ASP	8.67	7.01	1.30	1.65	0.91	1.19	0.97

10. <u>Sample Handling Variability Across Three Sites (Extraction Study)</u>

To assess sample handling variability as part of the KRAS Kit test system process, 30 sequential 5-µm sections were cut from each of 10 FFPE CRC samples (3 WT and 1 per mutation). Sections were randomized to one of three testing sites so that each site received 10 sections per FFPE sample (100 sections total). Of the 300 DNA extractions tested, 298 samples were valid. There was 99.3% concordance with respect to the KRAS mutation calls between the three sites. The variance of ΔCt values for each assay was estimated, and the contribution of between and within laboratory sources was estimated using an ANOVA variance components model. Variance for within-test site was highest for 12ASP (0.30). Variance between-test site was highest for 12SER (0.05). A comparison by site of mean ΔCt values with corresponding SD for mutant and wild-type samples showed very close agreement for results. The results

demonstrate the agreement of the DNA extraction procedure and sample processing in conjunction with the KRAS Kit.

Comparison by Site of Mean ΔCt (SD) Values for Mutant Type Samples

	12ALA	12ARG	12ASP	12CYS	12SER	12VAL	13ASP
CLA	2.44 (0.1)	2.62 (0.3)	3.03 (0.6)	2.24 (0.1)	2.34 (0.3)	2.51 (0.1)	3.93 (0.4)
HGX	2.44 (0.2)	2.52 (0.4)	3.01 (0.7)	2.29 (0.2)	2.10 (0.4)	2.44 (0.5)	4.15 (0.7)
MAN	2.67 (0.6)	2.52 (0.2)	3.07 (0.5)	2.29 (0.2)	2.74 (0.5)	2.56 (0.2)	3.95 (0.3)

Comparison by Site of Mean ΔCt (SD) Values for Wild-Type Samples

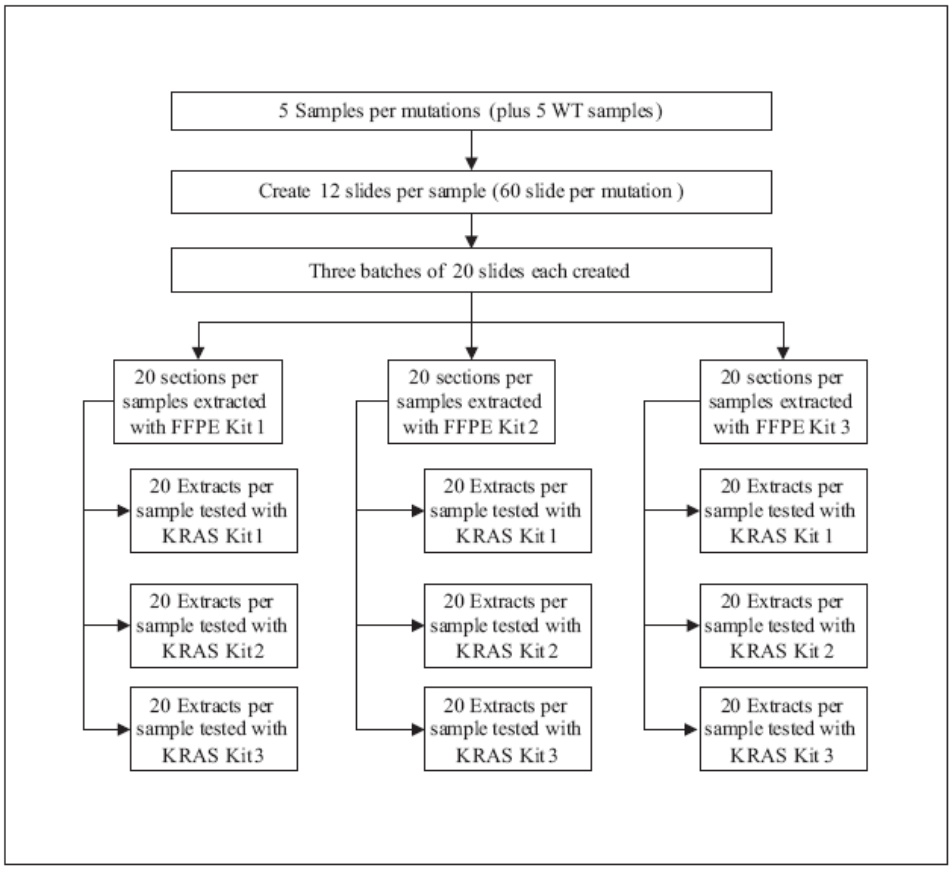
	12ALA	12ARG	12ASP	12CYS	12SER	12VAL	13ASP
CLA	12.46 (0.3)	-	10.37 (0.4)	-	11.84 (0.4)	12.36 (0.5)	11.11 (0.6)
HGX	12.09 (0.6)	13.07 (0.2)	10.17 (0.5)	-	11.71 (0.7)	12.20 (0.6)	11.00 (0.9)
MAN	12.07 (0.2)	-	10.61 (0.4)	-	11.94 (0.3)	12.28 (0.6)	11.82 (0.5)
Note: "-" illustrates a missing value, due to no break through being observed							

11. Specimen Handling – Minimum Tumor content and Macrodissection

To support the consistent mutation calling in macrodissected CRC samples with tumor proportion ≤ 20%, DNA extracted from thirteen samples whose tumor proportion ranged from 3% to 18% were either macrodissected or not. The samples were tested in duplicate. The overall accuracy of the macrodissected samples was compared to the non-macrodissected counterparts as well as ten samples whose tumor proportion was > 20% but close to the cut-off (range 21% to 30%). All results were compared to bi-directional sequencing which were also macrodissected to enrich for tumor. The KRAS Kit did not incorrectly detect a mutation in six (6) samples identified as wild-type by bi-directional sequencing in either the macrodissected and non-macrodissected sections. One (1) wild-type sample produced invalid results with the KRAS Kit across all replicates. Four (4) samples identified as mutant by bi-directional sequencing were also identified as mutant by the KRAS Kit. One (1) mutant positive sample by bi-directional sequencing was correctly detected by the KRAS Kit when the sample was macrodissected but not when the sample was not macrodissected, supporting the use of macrodissection. One sample produced inconclusive results by bi-directional sequencing. The results support the ability of the KRAS Kit to detect mutant in samples with low percentage tumor when the specimen is macrodissected. Additionally, sections from the specimens used in the correlation to bi-directional sequencing studies had tumor proportion (% tumor content) that spanned the range of approximately 10% to 99%. The accuracy results support the use of samples whose tumor proportion is greater than 20% without macrodissection.

12. Lot-to-Lot Reproducibility

The potential for lot-to-lot variability to impact the mutation detection was assessed. In this study, three lots of QIAamp® DSP DNA FFPE Tissue Kit (FFPE Extraction Kit) and *therascreen*® KRAS RGQ PCR Kit with each lot of FFPE Extraction were evaluated. An overview of the workflow for this study is shown below:



For this study, five FFPE CRC specimens (FFPE CRC) for six of the seven mutations, plus five wild-type (WT) samples, were obtained. Only two FFPE CRC specimens were available for the 12ARG mutation, so these studies were supplemented with one 12ARG FFPE cell line. For each mutant sample (except for the 12ARG mutation) and the WT Sample, twelve sequential 5-µm sections were cut and mounted onto glass slides. For the 12ARG mutation, twenty-four sections were cut from each of the two 12ARG FFPE Samples and twelve sections were cut from the FFPE Cell Line for 12ARG. Thus a total of 60 sections per mutation and WT were obtained. These 60 sections were randomly assigned to one of three batches, to give three batches of twenty slides per mutation and WT. DNA was extracted and each extracted DNA sample was tested using the control reaction and its corresponding mutation reaction. Each DNA extract was tested singly on three separate KRAS Kit runs. The acceptance

criterion for this study was that for each of the mutation assays and the three FFPE Kit Lots tested, the proportion of correct mutation calls would be at least 59/60. All samples met the acceptance criteria with the exception of 12SER and 12VAL, the latter of which was found to be due to reasons unrelated to the kits. To ensure quality across lots, a supplemental study was conducted using FFPE cell lines (one wild-type and 7 mutant samples were extracted with 3 lots of FFPE Extraction Kit to yield DNA samples with target control CT values that span the range of total DNA input level for the KRAS Kit. The extracted DNA samples were then tested against 3 lots of KRAS Kit using the strategy outlined above. The mutation status for all samples tested with the different FFPE extractions kit lots and KRAS Kit lots was 100% correct. For the mutant samples, the respective ΔCt value and the corresponding mutation status were calculated. The mutation status for all samples tested with the different FFPE extractions kit lots and KRAS Kit lots was 100% correct. This study criteria was met and the results demonstrated that there is no significant variability between lots of QIAamp® DSP DNA FFPE Tissue kit (FFPE extraction kit) and lots of *therascreen*® KRAS RGQ PCR Kit.

13. <u>Guard Band Studies</u>

The potential impact of using different proteinase K digestion times during the DNA extraction process as well as altering the RGQ PCR cycling parameters for the KRAS Kit were investigated. Changes were assessed for any potential effects on mutation reporting. The following studies were conducted to assess the robustness of the Extraction Kit and the KRAS Kit:

Proteinase K Digestion Times: FFPE CRC specimens were used in the evaluation of different proteinase K digestion times on the potential to impact mutation calling using the KRAS Kit. The current proposed labeling for the KRAS Kit states that proteinase K digestion of samples should be carried out for 60 minutes. Therefore, in order to assess the robustness of this limit, FFPE samples representing the wild-type and each of the 7 seven KRAS mutations, detected by the KRAS Kit, were extracted using digestion times that bracket the stated time. Five time points were tested in total; 50, 55, 60, 65, and 70 minutes, respectively. Six replicate extractions were carried out at each time interval. A total of 240 extractions were carried out (6 replicates x 5 time intervals x 8 FFPE CRC Samples). Across all assays, there was 1 replicate of 240 that resulted in a false negative at the 65 minute time point. The KRAS Kit PCR is robust to varying the Proteinase K digestion step by ±10 minutes, from the stated 60 minute digestion.

RGQ PCR Cycling Analysis: Denaturing and annealing temperatures were investigated. Denaturing temperatures are required for the complete separation of target DNA strands and annealing temperature is required for the specific binding of primers prior to extension. The KRAS Kit cycling uses a denaturing temperature of 95°C and an annealing temperature of 60°C. The combined effect of altering each of these parameters was tested in 9 combinations (inclusive of the standard conditions). These combinations are presented in Table below. The KRAS Kit PCR is robust to varying the melting and annealing temperatures of up

to ±1°C (in any combination). All mutation calls for each of the FFPE samples tested at 9 different combinations of cycling conditions returned a 100% correct mutation status. Changes in the cycling temperatures had no impact on the results of mutation status.

Guard Band PCR Cycling Conditions

		Melting Temperature		
		59°C	60°C	61°C
Denaturing Temperature	94°C	94°C /59°C	94°C/60°C	94°C/61°C
	95°C	95°C/59°C	95°C/60°C	95°C/61°C
	96°C	96°C/59°C	96°C/60°C	96°C/61°C

PCR Set-up and Stability Times: The goal of the study was to determine the robustness of the *therascreen*® *K*RAS RGQ PCR Kit to different temperatures for different periods of time before the reactions are loaded on the MDx Instrument. Three parameters were tested in the study:

1. Stability of reaction mixes/Taq polymerase and Positive Control was assessed to determine the effect of time on the stability of reaction mixtures at room temperature in the event that some experiments take longer to set up than the stipulated 1 hour. The following times were tested: (1) 1 hour at room temperature, (2) 4.5 hours at room temperature or (3) 6 hours at room temperature.
2. The temperature of storage of the Rotor-Gene® Q (RGQ) tubes between start of PCR set up and start of the RGQ run was assessed to determine the effect of temperature on the stability of the reaction mixes/Taq with the DNA sample. Room temperature and 2-8°C were tested.
3. The time between start of PCR set up and start of the RGQ run was assessed to determine the stability of the reaction mixes/Taq with the DNA sample added. Tubes were kept for 1 hour, 2 hours, 7 hours or overnight (18 hours).

The following samples were tested:

(1) Positive Control - Sample guaranteed to give a Ct value within the acceptable range.

(2) Wild-type and 3X LOD samples - Samples aimed to challenge the assay (i.e., low positive Control).

(3) No Template control - Negative Samples

The impact of time and temperature on the performance of the KRAS Kit was assessed by comparing the Ct values generated for all samples across 11 experimental conditions (interchangeable times and temperatures described above). Each known mutation sample was tested with its appropriate reaction mix while the WT sample was tested with the Control reaction mix. Each assay

was run in triplicate. The data demonstrates that pre- and post-setup, the KRAS Kit can be stored on the bench-top or at 4°C for sufficient periods of time to allow customers flexibility in the working day. The times within which the KRAS Kit can be stored both for pre- and post-setup are detailed in the table below.

Times within which the KRAS Kit can be stored for pre- and post-setup

Thaw Time		Storage Temp after PCR setup	PCR Setup and Storage time
Minimum	Maximum		
1 hour	4.5 hours	Room Temperature	7 hours
1 hour	4.5 hours	2-8°C	18 hours

Note: PCR setup is to be performed at room temperature. 'Storage' refers to the time between completion of PCR setup and start of the PCR run on the RGQ platform.

14. Cross-Contamination

Studies were performed to demonstrate the absence of cross-contamination between test samples. Two FFPE cell lines were used for this study: one containing WT cells only, and the other containing cells harboring the 12ALA mutation. The 12ALA mutation was selected for this study because the 12ALA reaction is the most sensitive reaction in the KRAS Kit as determined in the analytical sensitivity studies, and therefore most prone to exhibit false positive results from contamination. Multiple serial 5-µm sections were prepared and mounted onto glass slides for testing. DNA was extracted and assayed using one lot of reagents and one RGQ instrument according to protocol. Each extract was tested in 7 replicates using input concentrations based on control Ct in the midrange (approximating Ct 26). The study consisted of ten test runs designed to investigate the potential for contamination both within and between runs. The 10 runs were divided between two sets (5 runs per set) where the sequence in which reagents are added to reaction wells differed; for set "a" the No Template Control preceded the positive control according to instructions for use, and for set "b" the No Template Control preceded the test sample to maximize the opportunity to detect cross-contamination. Results were summarized by Ct, ΔCt and call. The results demonstrated one invalid result from a WT sample replicate. No false positive results were detected. The results of this study indicate no detectable contamination.

15. Stability - Specimen

a) Clinical Specimen, Slide-Mounted

To assess the stability of slides prepared from FFPE CRC tissue samples to determine limits of suitability for the KRAS Kit, ten 5-µm sections were cut

from each of eight FFPE CRC specimens (one for each mutation and one wild-type), mounted onto glass slides, and stored in the dark at room temperature for four weeks. The acceptance criteria were that at each time point, the mutation status must agree with that determined at initial baseline testing, and for each time point the change in ΔCt relative to the corresponding baseline time point must not be statistically different, or the upper 95% CI for the absolute mean change ΔCt must be less than 1. For each time point tested, DNA was extracted from 2 slides, pooled and tested in 5 replicates on Day 7, Day 14 and Day 28. The wild-type slides met the stability criteria across all time points based upon consistent mutation status. Slides from 4 of the 7 mutations (12ALA, 12CYS, 12SER, and 12VAL) were demonstrated to meet stability criteria across all 4 time points. Slides from the 12ASP and 12ARG mutations met all acceptance criteria for all time points where valid results were obtained. No trend in the values was observed for the FFPE samples tested. The results of this study overall support the claim that slides prepared from FFPE samples can be stored for up to 4 weeks at room temperature in the dark prior to testing with the KRAS Kit.

b) Extracted Clinical Specimen DNA

To assess the stability of DNA extracted from FFPE CRC samples (representing 7 mutations and 1 wild-type) using the QIAamp® DSP FFPE Tissue Kit, samples were stores at 2°C to 8°C for 7 days followed by storage at -18°C to -22°C for 5 weeks. During storage at -18°C to -22°C, the samples were subjected to multiple freeze-thaw cycles. DNA extracts were freeze/ thawed over 2 hours at room temperature and then returned to freezer. DNA extracts were tested on days 14, 21, and 35. Five test replicates of all DNA extracts at each time point were evaluated. The acceptance criteria were that the upper limit of the 95% CI cannot exceed the baseline value by more than 1ΔCt). Determination of mutation status was demonstrated to be consistent under the conditions of the study. There was 1 replicate that resulted in false positive call that was attributed to human error. The acceptance criteria were met. DNA extracted from FFPE samples is stable and suitable for use with the KRAS Kit when stored for up to 7 days at 4°C with additional storage at -20°C up to 5 weeks with multiple freeze thaws.

16. Stability - Reagents

The Stability studies conducted for both the *therascreen*® KRAS RGQ PCR Kit and the QIAamp DSP DNA Extraction Kit include (1) Real-time storage conditions for closed bottle conditions, open bottle, and open bottle simulating multiple time use by the user for both the KRAS Kit and the Extraction Kit; (2) Transport conditions (extreme temperatures during storage and shipping, including inversion of reagents), and (3) Stress (freeze/thaw) conditions. FFPE CRC clinical samples and FFPE cell lines were used for this investigation. DNA was extracted according to protocol and each mutation DNA extract was normalized with wild-type DNA to provide test samples corresponding to 3x and 9x the LoD of each of the 7 mutation reactions. Testing was conducted in

triplicate with the exception of the open-bottle (multi-use studies) which was run in singlicate. The acceptance criteria for each time point are that (1) the correct call is made, and (2) the ΔCt values of each sample, when plotted against time, do not indicate a statistically significant trend following regression analysis. Testing was conducted up to 12 months and is planned to continue to 36 months for the KRAS Kit and 24 months for the Extraction Kit. Freeze thaw studies up to 4 months were conducted 12 times. The data supports the following stability claims:

- Storage and shelf life for the KRAS Kit is 12 months at-20°C±5°C
- When used with this assay and the modified protocol, storage and shelf life for the QIAamp kit is 4 months at ambient temperature at 15°C to 25°C except for QIAamp MinElute Columns (5°C ±3°C).

Overview of the Stability Studies and Temperatures

Study*	Storage condition		Cycling condition	
	definition	temperature	definition	temperature
Closed bottle	Real time	-20°C±5°C	n.a.	n.a.
Open bottle	Real time	-20°C±5°C	n.a.	n.a.
Open bottle (light sensitivity)	Ambient temperature	22°C±3°C (Incubator)	n.a.	n.a.
Transport simulation study KRAS kit	Real time	-20°C±5°C	Extreme cold	-90°C to -65°C
			Interim storage	-20°C±5°C
			Thawing	22°C±3°C***
			Freezing	-20°C±5°C
Transport simulation study QIAamp kit	Real time	25°C±3°C (incubator)	Extreme cold	-20°C±5°C
			Extreme warm	45°C±3°C
Stress test	Real time	-20°C±5°C	Thawing	22°C±5°C***
			Freezing	-20°C±5°C

* QIAamp kit stored at (25°C±3°C, incubator), QIAamp MinElute Columns at (5°C±3°C). The cycling for the transport simulation studies performed with all kit components including the QIAamp MinElute columns. *** Tubes are inverted.

Stability- Open bottle study (light sensitivity/ reaction mix + enzyme stability): Master mixes were prepared (Reaction mix + enzyme) in clear tubes and used at predefined times following preparation up to 3 hours. The results indicate that KRAS Kit Master Mix is stable for at least two hours when stored at 32°C.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A clinical performance study was conducted to generate data to support the clinical utility of the *therascreen*® KRAS RGQ PCR Kit (referred to as KRAS Kit) as a companion diagnostic test that aids in the identification of patients for treatment with Vectibix (panitumumab). The objective of the study was to assess whether KRAS status as determined by the *therascreen*® KRAS RGQ PCR Kit can be used to select patients with metastatic colorectal cancer (mCRC) who will benefit from Vectibix (panitumumab) treatment.

The clinical trial 20050203 (ClinicalTrials.gov number NCT00364013) was a multicenter, prospective, open-label, randomized phase 3 study to assess the efficacy of panitumumab in combination with oxaliplatin, 5-fluorouracil (5-FU), and leucovorin (FOLFOX) vs. FOLFOX alone in patients with previously untreated, recurrent metastatic colorectal cancer (mCRC).

Banked tumor samples from patients in study 20050203 were tested with the KRAS Kit to identify two subgroups: KRAS mutation-positive (mutant) and KRAS mutation-negative (wild-type), according to whether at least one or none of seven KRAS mutations in codons 12 and 13 of exon 2 in the KRAS oncogene was detected. In retrospective analyses, a pre-specified efficacy data from study 20050203 were stratified by KRAS subgroup. The primary objective of the KRAS analysis was to assess whether an overall improvement in PFS for panitumumab plus FOLFOX relative to FOLFOX alone was significantly greater among subjects with KRAS wild-type tumors compared to subjects with KRAS mutant tumors.

Note: The KRAS Kit is designed to specifically detect 7 KRAS mutations in codon 12 and 13 of the KRAS gene. It is not designed to specifically detect the wild-type sequence at these codons. The results of the test are reported out as "Mutation-positive" and "No mutation detected." Generally, discussions of patient response to Vectibix (panitumumab) therapy in the context of KRAS status have referred to two groups; KRAS wild-type and KRAS mutant. In the report that follows, the *therascreen*® "no mutation detected" result is referred to as KRAS mutation-negative (wild-type) to be consistent with the pharmaceutical manufacturer's designations in the Vectibix (panitumumab) product label. Patients in the clinical study who were KRAS mutation-positive tested positive for one or more of the 7 mutations detected by the *therascreen*® KRAS RGQ PCR Kit (i.e., G12A, G12D, G12R, G12C, G12S, G12V, or G13D). Patients in the clinical study who were KRAS mutation-negative (wild-type) tested negative for the 7 mutations detected by the *therascreen*® KRAS Kit. However these patients may have harbored mutations in the KRAS gene not identified by the KRAS Kit such as 13CYS, or elsewhere in the gene such as codon 61).

The data presented in this PMA support the clinical utility of the *therascreen*® KRAS RGQ PCR Kit and the corresponding changes to the Vectibix (panitumumab) labeling for the mCRC indication. The results demonstrated that the efficacy of Vectibix (panitumumab) in prolonging overall survival (OS) and progression-free survival (PFS)

was statistically significant in patients with KRAS mutation-negative (wild-type) status. In patients with KRAS mutation-positive tumors, no statistically significant difference was observed between the two treatment groups in OS or PFS.

A. Study Design

1. *Study 20050203*

Study 20050203 was a multicenter, prospective, open-label, randomized phase 3 study to assess the efficacy of panitumumab in combination with oxaliplatin, 5-fluorouracil (5-FU), and leucovorin (FOLFOX) vs. FOLFOX alone in patients with previously untreated, recurrent metastatic colorectal cancer (mCRC) subjects with wild-type KRAS tumors compared to subjects with mutant KRAS tumors. It was initiated on August 23, 2006 and closed to randomization on September 30, 2008. Investigators at 133 centers in Europe, Canada, Australia and South America enrolled patients. Patients with no prior treatment for mCRC were enrolled; however, adjuvant 5 FU-based therapy was allowed if disease progression occurred >6 months after completion. Prior oxaliplatin was not allowed.

The primary objective of the KRAS analysis was to assess whether an overall improvement in PFS for panitumumab plus FOLFOX relative to FOLFOX alone was significantly greater among subjects with KRAS wild-type tumors compared to subjects with KRAS mutant tumors. Secondary objectives were to assess whether an overall improvement in overall survival OS for panitumumab plus FOLFOX relative to FOLFOX alone was greater among subjects with KRAS wild-type tumors compared to subjects with KRAS mutant tumors; to compare PFS and OS by KRAS status overall and within each treatment group; and to assess whether an overall improvement in the objective response rate for panitumumab plus FOLFOX relative to FOLFOX alone was greater among subjects with KRAS wild-type tumors compared to subjects with KRAS mutant tumors. Other key endpoints included OS, objective response rate (ORR), time to progression (TTP), and duration of response.

Assessment of KRAS mutation status was not prospectively planned in the original protocol for the Study 20050203 because evidence for the impact of KRAS mutation on therapeutic response was unavailable at the time. However, based on emerging data demonstrating KRAS mutation status as a potential predictive biomarker for EGFR-targeted monoclonal antibody therapies, available tumor samples from patients in the Study 20050203 were conducted for inclusion in the primary study analysis using an Investigational Use Only (IUO) device, which was the Therascreen® K-RAS Mutation Kit (LC 480 KRAS Kit) (referred to as IUO below). The IUO was used to investigate if KRAS status predicted patient response to panitumumab treatment. The results are the basis for the development of planned, retrospective analyses evaluating the influence of KRAS mutation status on OS and PFS with a specific KRAS companion diagnostic, i.e., the *therascreen*® KRAS RGQ PCR Kit (referred to as the MRT below) (1).

2. *Patients*

Patients were males and females who were at least 18 years of age and had the following key inclusion criteria:

- Metastatic adenocarcinoma of the colon or rectum
- No prior EGFR inhibitor therapy
- Measurable disease
- Adequate hemotologic, renal and hepatic function
- ECOG performance status 0-2
- Paraffin-embedded tumor tissue available for central biomarker testing
- EGFR expression and KRAS status were not required at entry.
- Signed informed consent

Demographic and baseline characteristics were summarized separately for each study in the MRT KRAS Evaluable analysis set and the MRT KRAS Unevaluable analysis set overall and by treatment arm.

3. *Tumor Specimens and Testing*

Tumor KRAS testing was not a requirement for enrollment into these studies; rather, it was used to define specific patient populations for the purposes of the primary analyses. KRAS testing for both the IUO and MRT versions was performed according to the Instructions for Use (IFU) at an independent specialty laboratory in Belgium (HistoGeneX). KRAS testing was performed for the purposes of the study analyses, after completion of subject enrollment. Investigators remained blinded to KRAS status results up until the primary analyses of each study. Determination of KRAS mutation status was made by an independent laboratory. The laboratory was blinded to treatment assignment and clinical outcomes data. DNA samples extracted from FFPE clinical samples met the defined minimum tumor acceptance criteria.

4. *Clinical Inclusion and Exclusion Criteria*

a. <u>Inclusion and Exclusion Criteria for Specimen Testing</u>

KRAS testing was performed on FFPE samples collected from subjects randomized into Studies 20050203. A hematoxylin and eosin stain (H&E) was performed for each FFPE Sample. In Study 20050203, performance of KRAS testing required at least 20% tumor area and a tumor area of 4 mm^2, as evaluated by an experienced pathologist. Samples with less than 20% tumor underwent macro-dissection.

b. <u>Inclusion and Exclusion Criteria for Patient Enrollment into Study 20050203</u>

A summary of the pertinent criteria for enrollment into the trial was limited to patients who met the following inclusion and exclusion criteria.

Inclusion criteria:

- Histologically or cytologically-confirmed adenocarcinoma of the colon or rectum in subjects who are presenting with metastatic disease
- At least 1 uni-dimensionally measurable lesion of at least 20mm per modified RECIST guidelines (all sites of disease must be evaluated ≤ 28 days prior to randomization)
- Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2
- Paraffin-embedded tumor tissue from the primary tumor or metastasis available for central analyses of EGFR and biomarker testing
- Man or woman ≥ 18 years of age
- Hematology and biochemistry tests performed (≤ 7 days prior to randomization7 days) with initial values within specified ranges.
- Negative pregnancy test ≤ 72 hours prior to randomization (females of childbearing potential only)
- Competent to comprehend, sign, and date an IEC/IRB-approved informed consent form
- Life expectancy ≥ 3 months

Exclusion criteria:

- History or known presence of central nervous system (CNS) metastases
- History of another primary cancer, except
    - Curatively treated in situ cervical cancer, or
    - Curatively resected non-melanoma skin cancer, or
    - Other primary solid tumor curatively treated with no known active disease present and no treatment administered for > 5 years prior to randomization
- Prior chemotherapy or systemic therapy for the treatment of metastatic colorectal carcinoma with the following exceptions:
    - Subject may have received adjuvant fluoropyrimidine-based chemotherapy if disease progression is documented at least 6 months after completion of chemotherapy
    - Subjects may have received prior fluoropyrimidine therapy if administered solely for the purpose of radiosensitization
- Prior oxaliplatin therapy
- Prior anti-EGFR antibody therapy (e.g., cetuximab) or treatment with small molecule EGFR inhibitors (e.g., erlotinib)
- Any investigational agent or therapy ≤ 30 days prior to randomization
- Radiotherapy ≤ 14 days prior to randomization (subjects must have recovered from all radiotherapy related toxicities)

- Known allergy or hypersensitivity to platinum-containing medications, 5-FU or leucovorin
- Active infection requiring systemic treatment or any uncontrolled infection ≤ 14 days prior to randomization
- Clinically significant cardiovascular disease (including myocardial infarction, unstable angina, symptomatic congestive heart failure, cardiac arrhythmia) ≤ 1 year prior to randomization
- History of interstitial lung disease (eg, pneumonitis or pulmonary fibrosis) or evidence of interstitial lung disease on baseline chest CT scan
- Active inflammatory bowel disease or other bowel disease causing chronic diarrhea (defined as > CTC grade 2, [CTCAE version 3.0])
- Known positive tests for human immunodeficiency virus (HIV) infection, hepatitis C virus, acute or chronic active hepatitis B infection
- Any co-morbid disease or condition that could increase the risk of toxicity, e.g., dihydropyrimidine deficiency, significant ascites or pleural effusion
- Peripheral sensory neuropathy with functional impairment (> CTC grade 3 [CTCAE version 3.0] neuropathy, regardless of causality)
- Any uncontrolled concurrent illness or history of any medical condition that may interfere with the interpretation of the study results
- Major surgical procedure (requiring general anesthesia) ≤ 28 days or minor surgical procedure (excluding central venous catheter placement) ≤ 14 days prior to randomization. Subjects must have recovered from surgery related toxicities.
- Subject who is pregnant or breast feeding
- Woman or man of child-bearing potential not consenting to use adequate contraceptive precautions i.e., double barrier contraceptive methods (e.g., diaphragm plus condom), or abstinence during the course of the study and for 6 months after the last study drug administration for women, and 1 month for men
- Subject unwilling or unable to comply with study requirements
- Previously randomized into this study protocol

5. ***Follow-up Schedule***

Patients were monitored according to protocol during the prospective trial.

6. ***Clinical Endpoints***

The primary objective was to compare progression-free survival (PFS) in the two treatment arms of the study. The primary statistical goal is to assess whether the addition of panitumumab to chemotherapy significantly prolongs PFS among subjects with wild-type KRAS tumors (the Wild-type KRAS Efficacy Analysis Set) as well as subjects with mutant KRAS tumors (the Mutant KRAS Efficacy Analysis Set) and to characterize and compare overall survival (OS) stratified by

the randomization factors. Significance levels described will be 2-sided unless stated otherwise. PFS in the Wild-type KRAS Efficacy Analysis Set will be compared at a significance level of 5%. PFS in the Mutant KRAS Efficacy Analysis Set and OS in the Wild-type KRAS Efficacy Analysis Set will be compared at a significance level of 5% conditional on first demonstrating a significant treatment effect in PFS in the Wild-type KRAS Efficacy Analysis Set. If the analysis demonstrates a significant treatment effect on PFS in the Mutant KRAS Efficacy Analysis Set, then OS in the mutant KRAS Efficacy Analysis Set will be compared at a significance level of 5%.

B. Accountability of PMA Cohort

The patients used for analysis populations were the randomized patients. In addition, the following subsets were created based on the test result by IUO and MRT.

- *IUO KRAS Evaluable Analysis Set:* Subset of subjects with valid KRAS result from the IUO kit
- *MRT KRAS Evaluable Analysis Set:* Subset of subjects in the IUO KRAS Evaluable analysis set with a valid KRAS result from the MRT kit
- *MRT KRAS Un-evaluable Analysis Set:* Subset of subjects in the IUO KRAS set without a valid KRAS result from the MRT kit

Overall, 97% (1064/1100) of tumor sample extracts that were evaluable for KRAS testing with the IUO kit were retested with the MRT kit and 92% (1014/1100) of sample extracts were evaluable for MRT KRAS status. The main reasons that sample extracts were un-evaluable for MRT KRAS status included no sample available for testing and MRT sample or assay failure (KRAS test failure).

MRT KRAS Status in IUO KRAS Evaluable Analysis Set

	IUO/LDT Wild-type *KRAS* (N=659)	IUO/LDT Mutant *KRAS* (N=441)	Total (N=1100)
Number of subjects retested with MRT kit -n(%)	636 (97)	428 (97)	1064 (97)
Number of subjects with evaluable MRT *KRAS* status -n(%)	592 (90)	422 (96)	1014 (92)
MRT *KRAS* Wild-type-n(%)[a]	567 (96)	0 (0)	567 (56)
MRT *KRAS* Mutant-n(%)[a]	25 (4)	422 (100)	447 (44)
Number of subjects with unevaluable MRT *KRAS* status -n(%)	67 (10)	19 (4)	86 (8)
No sample available for testing -n(%)[b]	23 (34)	13 (68)	36 (42)
KRAS test failure -n(%)[b]	44 (66)	6 (32)	50 (58)

IUO = investigational use only; *KRAS* = Kirsten rat sarcoma-2 viral oncogene; LDT = laboratory-developed test; MRT = market-ready test.
[a]Percentage based on number of subjects with evaluable MRT *KRAS* status
[b]Percentage based on number of subjects with unevaluable MRT *KRAS* status

C. Study Population Demographics and Baseline Parameters

Demographics

Demographic characteristics of the study population by MRT KRAS mutation status were typical of patients with mCRC being treated with first-line therapy and were similar to those reported by IUO KRAS mutation status. Demographic characteristics in the MRT Mutant KRAS Efficacy Analysis Set were similar to those of the MRT Wild-type KRAS Efficacy Analysis Set. Demographic characteristics of the MRT Un-evaluable KRAS Efficacy Analysis Set were also similar to those of the MRT Wild-type and Mutant KRAS Efficacy Analysis Sets. Demographic characteristics assessed were country and geographic region, sex, race/ethnicity, baseline age, baseline age group, baseline weight and baseline BSA.

Baseline Variables

Baseline disease characteristics were generally well balanced across the 2 treatment arms in the MRT Wild-type, Mutant, and Un-evaluable KRAS Efficacy Analysis Sets. Baseline disease characteristics assessed were primary tumor type, months since primary diagnosis, months since metastatic disease diagnosis, number of metastatic sites, location of metastatic sites, ECOG performance status, carcinogembryonic antigen concentration, baseline LDH concentration.

D. Safety and Effectiveness Results

1. Safety Results

The first FDA approval action for Vectibix (panitumumab) was September 27, 2006 and was without regard to KRAS status. The safety with respect to treatment with panitumumab will not be addressed in details here; refer to the drug label for more information. Most common adverse reactions (≥ 20%) in clinical trials of panitumumab in combination with oxaliplatin-containing chemotherapy are diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, dermatitis acneiform, pruritus, and dry skin.

Adverse Events in Study 20050203

Safety analyses were performed on the MRT KRAS Safety Analysis Sets by treatment received, using the data from the primary analysis snapshot for OS. The overall safety profile of panitumumab was consistent with the class effects of epidermal growth factor receptor (EGFR) inhibitors and safety results were similar to those obtained previously.

Safety findings for the MRT Wild-type and MRT Mutant KRAS Safety Analysis Sets (primary analysis) were similar. Between-treatment differences (panitumumab plus FOLFOX vs. FOLFOX alone) in subject incidence for serious adverse events were higher in the MRT Mutant KRAS Safety Analysis Set (47% vs. 31%) than in the MRT Wild-type KRAS Safety Analysis Set (42% vs. 35%).

Summary of Adverse Events at Primary Analysis Snapshot is provided below for MRT Wild-type, Mutant, and Un-evaluable KRAS Safety Analysis Sets.

Summary of Adverse Events Based on MRT KRAS Status

	MRT Wild-type *KRAS* Safety Analysis Set		MRT Mutant *KRAS* Safety Analysis Set		MRT Unevaluable *KRAS* Safety Analysis Set	
	Panitumumab Plus FOLFOX (N = 278)	FOLFOX Alone (N = 285)	Panitumumab Plus FOLFOX (N = 222)	FOLFOX Alone (N = 222)	Panitumumab Plus FOLFOX (N = 85)	FOLFOX Alone (N = 77)
Subjects with any adverse event - n (%)	278 (100)	281 (99)	220 (99)	221 (100)	85 (100)	77 (100)
Worst grade of 3[a]	159 (57)	142 (50)	131 (59)	117 (53)	35 (41)	37 (48)
Worst grade of 4[a]	81 (29)	53 (19)	47 (21)	48 (22)	21 (25)	19 (25)
Worst grade of 5[a]	12 (4)	17 (6)	16 (7)	8 (4)	11 (13)	3 (4)
Any Serious	117 (42)	100 (35)	105 (47)	69 (31)	40 (47)	29 (38)
Leading to permanent discontinuation of any study drug	69 (25)	40 (14)	49 (22)	30 (14)	18 (21)	14 (18)
Not serious	55 (20)	28 (10)	40 (18)	19 (9)	11 (13)	10 (13)
Serious	20 (7)	15 (5)	16 (7)	11 (5)	9 (11)	5 (6)
Subjects with any treatment-related adverse event[b] - n (%)	277 (100)	273 (96)	219 (99)	216 (97)	85 (100)	76 (99)
Worst grade of 3[a]	165 (59)	138 (48)	136 (61)	123 (55)	43 (51)	31 (40)
Worst grade of 4[a]	67 (24)	38 (13)	40 (18)	36 (16)	16 (19)	16 (21)
Worst grade of 5[a]	2 (1)	3 (1)	2 (1)	1 (0)	4 (5)	1 (1)
Any Serious	74 (27)	42 (15)	64 (29)	28 (13)	24 (28)	19 (25)
Leading to permanent discontinuation of any study drug	62 (22)	27 (9)	40 (18)	24 (11)	15 (18)	12 (16)
Not serious	52 (19)	24 (8)	34 (15)	19 (9)	10 (12)	10 (13)
Serious	13 (5)	6 (2)	9 (4)	5 (2)	6 (7)	3 (4)

FOLFOX = 5-fluorouracil, leucovorin, and oxaliplatin; *KRAS* = Kirsten rat sarcoma-2 viral oncogene; MRT = market-ready test.
Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) v12.0.
[a]Severity graded using the Common Terminology Criteria for Adverse Events (CTCAE) v3.0, with the exception of some dermatology/skin adverse events that were graded using the CTCAE v3.0 with modifications. Fatal adverse events are classified as grade 5.
[b]The investigator considered there to be a reasonable possibility that the event may have been caused by study drug.
The data cutoff date for this analysis is 28 August 2009.

2. Effectiveness Results

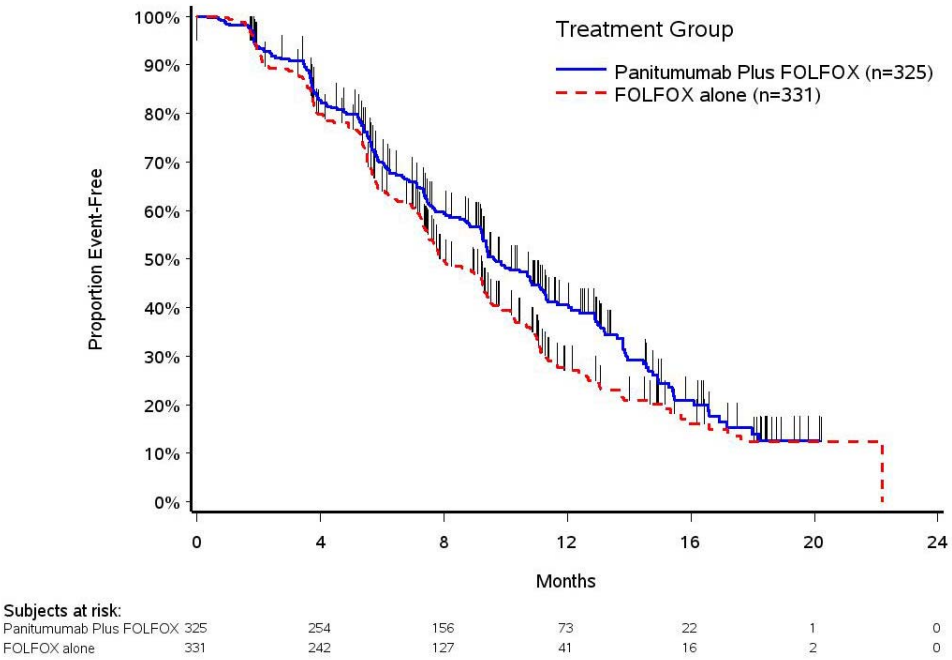
A. Overall Efficacy of Vectibix (panitumumab)

The efficacy of panitumumab in combination with FOLFOX was evaluated in a randomized, controlled trial in mCRC patients with the primary endpoint of PFS. The pre-specified primary efficacy endpoint was PFS in the group of patients (n = 656) with wild-type KRAS mCRC as assessed by a blinded independent central review of imaging. Other key efficacy endpoints included OS and ORR. The efficacy results in mCRC patients with wild-type KRAS (as determined by IUO) are presented in the table below.

Results in Patients with Wild-type KRAS mCRC

	Primary Analysis	
	Panitumumab plus FOLFOX	**FOLFOX Alone**
Wild-type KRAS population	**(n = 325)**	**(n = 331)**
PFS		
Median (months) (95% CI)	9.6 (9.2, 11.1)	8.0 (7.5, 9.3)
Hazard ratio (95% CI) p-value	0.80 (0.66, 0.97) p = 0.02	
ORR		
% (95% CI)	54% (48%, 59%)	47% (41%, 52%)

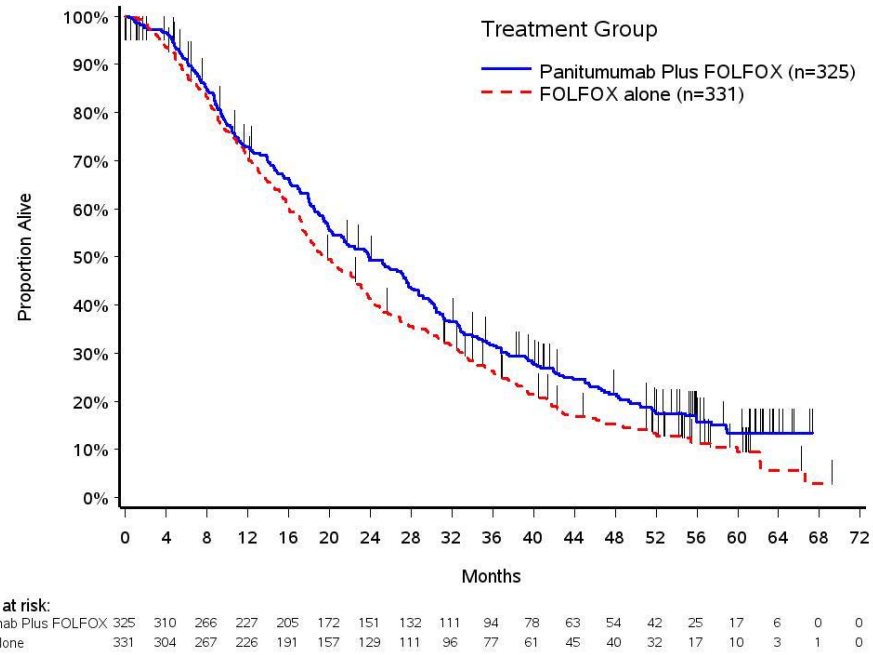
Kaplan-Meier Plot of Progression-Free Survival in Patients with Wild-type KRAS mCRC



Among patients with KRAS mutant tumors, median PFS was 7.3 months (95% CI: 6.3, 8.0) in the 221 patients receiving Vectibix plus FOLFOX versus the median PFS of 8.8 months (95% CI: 7.7, 9.4) in the 219 patients who received FOLFOX alone (HR = 1.29, 95% CI: 1.04, 1.62). Median OS was 15.5 months (95% CI: 13.1, 17.6) in patients receiving Vectibix plus FOLFOX versus the median OS of 19.3 months (95% CI: 16.5, 21.8) in patients who received FOLFOX alone (HR = 1.24, 95% CI: 0.98, 1.57).

An exploratory analysis of OS with updated information based on events in 82% of patients with wild-type KRAS mCRC estimated the treatment effect of Vectibix plus FOLFOX compared with FOLFOX alone. Median OS among 325 patients with wild type KRAS mCRC who received Vectibix plus FOLFOX was 23.8 months (95% CI: 20.0, 27.7) versus 19.4 months (95% CI: 17.4, 22.6) among 331 patients who received FOLFOX-alone (HR = 0.83, 95% CI: 0.70, 0.98).

Kaplan-Meier Plot of Overall Survival in Patients with Wild-type KRAS mCRC



B. MRT vs. IUO Agreement Analysis

The analytical accuracy of the *therascreen*® KRAS RGQ PCR Kit (MRT) using the IUO as the reference method for the aggregated result of KRAS wild-type and KRAS mutant was conducted and used to estimate drug efficacy in MRT KRAS population.

A total of 1017 out of 1183 (86%) subjects had MRT KRAS results and 1014 subjects had both IUO and MRT KRAS results for comparison. As shown in table below, no samples identified as wild-type by MRT were identified as mutant by IUO; while some samples identified as mutant by MRT were identified as wild-type by IUO. Thus, the MRT identified a smaller KRAS wild-type population than the IUO. The positive percentage agreement (PPA) and negative percentage agreement (NPA) are summarized below.

MRT vs. IUO – Agreement Analysis Results (Sample Numbers)

Number of Samples (n)		IUO^ Valid Results		
		WT*	MT*	Total
MRT# Valid Results	WT	567	0	567
	MT	25	422	447
	WT+MT	592	422	1014

* WT denotes wild-type; MT denotes mutant
# MRT is the market-ready test, i.e., therascreen® KRAS RGQ PCR Kit
^ IUO is the investigational use only assay

MRT vs. IUO – Agreement Analysis Result (NPA and PPA)

	Number of Samples (n)	Percent Agreement (95% CI)
Negative Percent Agreement (NPA)	567/592	95.78% (93.84%, 97.12%)
Positive Percent Agreement (PPA)	422/422	100% (99.1%, 100%)

3. Sensitivity Analyses

Efficacy of Vectibix (panitumumab) in MRT KRAS Population

Results for OS were assessed for subjects in the MRT KRAS Evaluable and Un-evaluable Analysis Sets (i.e., all subjects in the Intent-to-Treat Analysis Set), assuming that MRT Un-evaluable test results were missing at random (MAR). The missing (Un-evaluable) MRT results were imputed by multiple imputation methods together with a bootstrapping method. The estimated hazard ratios from multiple analyses of OS, in which missing (Un-evaluable) MRT results were imputed by modeling of other variables, were generally consistent with results based on the primary MRT analyses and IUO analyses.

The following MAR models were created for imputing missing MRT test results. The 9 imputation models below include different sets of covariates as predictors for imputing the missing MRT results. In the analysis, the uncertainty of an imputation actually being the underlying true value for the missing result was included.

- Model 1 (Full MAR Model Without IUO Result or Other Sample Characteristics): included baseline disease characteristics and clinical outcome (PFS and censoring indicator plus interaction)
- Model 2 (Full MAR Model Without IUO Result, Other Sample Characteristics or Clinical Outcome): included baseline disease characteristics

- Model 3 (Full MAR Model): included IUO results, baseline disease characteristics, sample characteristics, and clinical outcome (PFS and censoring indicator plus interaction)
- Model 4 (Full MAR Model Without Clinical Outcome): included IUO result, baseline disease characteristics and sample characteristics
- Model 5 (Reduced MAR Model 3): included IUO result, selected baseline disease characteristics, selected sample characteristics, and clinical outcome (PFS and censoring indicator plus interaction)
- Model 6 (Reduced MAR Model 3Without Clinical Outcome): included IUO result, selected baseline disease characteristics and selected sample characteristics
- Model 7 (Reduced MAR Model 3): include IUO result, selected sample characteristics and clinical outcome (PFS and censoring indicator plus interaction)
- Model 8 (Reduced MAR Model 3Without Clinical Outcome): included IUO results and selected sample characteristics
- Model 9 (Reduced MAR Model 3): included the IUO results

Quantitative Interaction Test for Treatment and MRT KRAS Status on Survival Time with MRT KRAS Status Modeled by Multiple Imputation Method – Overall Survival

	Subjects with Wild-type MRT KRAS Result		Subjects with Mutant MRT KRAS Result		Quantitative Interaction Test P-Value[b]
	Hazard Ratio (95% CI)[a]	Log rank p-value[b]	Hazard Ratio (95% CI)[a]	Log rank p-value[b]	
Baseline Disease Characteristics and PFS	0.843 (0.709, 1.004)	0.0610	1.096 (0.894, 1.336)	0.2630	0.0440
Baseline Disease Characteristics	0.846 (0.708, 1.009)	0.0630	1.087 (0.896, 1.334)	0.2940	0.0520
Baseline Disease Characteristics, Sample Characteristics, and PFS	0.826 (0.694, 0.987)	0.0470	1.119 (0.901, 1.376)	0.2530	0.0390
Baseline Disease Characteristics and Sample Characteristics	0.824 (0.690, 0.993)	0.0480	1.121 (0.895, 1.363)	0.2600	0.0400
Selected Baseline Disease Characteristics, Selected Sample Characteristics, and PFS	0.818 (0.686, 0.973)	0.0540	1.133 (0.919, 1.389)	0.2390	0.0300
Selected Baseline Disease Characteristics and Selected Sample Characteristics	0.820 (0.692, 0.978)	0.0510	1.123 (0.913, 1.376)	0.2540	0.0310
Selected Sample Characteristics and PFS	0.818 (0.687, 0.974)	0.0510	1.131 (0.922, 1.377)	0.2370	0.0240
Selected Sample Characteristics	0.820 (0.686, 0.976)	0.0460	1.121 (0.912, 1.370)	0.2560	0.0360
IUO/LDT *KRAS* Results	0.819 (0.686, 0.981)	0.0520	1.130 (0.918, 1.389)	0.2610	0.0320

CI = confidence interval; IUO = investigational use only; *KRAS* = Kirsten rat sarcoma-2 viral oncogene; LDT = laboratory-developed test; MRT = market-ready test; PFS = progression-free survival.
The results are based on bootstrapping of 1000 re-samples, and within each re-sample the missing MRT *KRAS* results are estimated by multiple imputation method with 1 imputation. The imputation is based on logistic regression model with the specified covariates for baseline disease characteristics, sample characteristics, or IUO/LDT *KRAS* status.
[a]Hazard Ratio (Panitumumab plus FOLFOX vs FOLFOX alone) is the exponential of the average log(HR) across bootstrap samples. 95% CIs are based on the 2.5% and 97.5% percentiles of log(HR) across bootstrap samples.

[b]The p-value is based on the studentized bootstrap method described in Section 4.4.1 of Davison and Hinkley (Bootstrap Methods and their Application, Cambridge, 1998).

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included retrospective testing at one site. No test was used for patient enrollment. 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. 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 was not referred to the Immunology Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions.

The clinical benefit of the *therascreen*® KRAS RGQ PCR Kit was demonstrated in a retrospective analysis of efficacy and safety in patients without KRAS mutations. Overall, a significant efficacy benefit for Vectibix (panitumumab) + FOLFOX vs. FOLFOX was observed in the subset of patients with KRAS mutation-negative (wild-type) tumors, whereas no such benefit was observed in the subset of patients with KRAS mutation-positive tumors. Results in the KRAS mutation-negative (wild-type) subset were consistent across both efficacy endpoints of OS and PFS. Results from the sensitivity analyses consistently demonstrate an improvement in overall survival in the KRAS mutation-negative (wild-type) group, and no meaningful improvement in the KRAS mutation-positive group.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies to support PMA approval as described above, as well as data collected in a clinical studies conducted to support Vectibix (panitumumab) approval. The safety profile in the subset of subjects without KRAS mutations in codons 12 and 13 is consistent overall with that reported for the Intent-to-Treat population and with the known safety profile of Vectibix (panitumumab). 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 colorectal cancer treatment. A false positive test result may lead to Vectibix (panitumumab) treatment being withheld from a patient who might have benefitted. A false negative test result may lead to Vectibix (panitumumab) treatment being administered to a patient who is not expected to benefit, and potentially any adverse side effects associated with treatment.

C. Benefit-Risk Conclusions

Colorectal cancer is the third most commonly diagnosed cancer and the third-leading cause of cancer-related death in both men and women in the United States (American Cancer Society [ACS] 2011). For 2011, the American Cancer Society predicts approximately 141,210 new cases and 49,380 deaths in the United States. Deaths from CRC account for about 9% of all cancer deaths (ACS 2010). The incidence and death rates for CRC increase with age, with over 90% of new cases and deaths occurring in patients (subjects) 50 years and older (ACS 2011). Overall, 1- and 5-year relative survival rates for patients with CRC are 83% and 67%, respectively (ACS 2010, 2011). When CRC is detected at an early, localized stage, the 5-year survival rate is 90%; however, only 39% of patients with CRC are diagnosed at this stage. After the cancer has spread regionally to involve adjacent organs or lymph nodes, the 5-year survival rate drops to 70%. When the disease has spread to distant organs (metastatic disease), the 5-year survival rate is 12%.

The probable benefits of the device are based on data collected in the clinical study conducted to support PMA approval as described above. The clinical benefit of the *therascreen*® KRAS RGQ PCR Kit was demonstrated in a retrospective analysis of efficacy and safety data obtained from patients with metastatic colorectal cancer from Study 20050203 determined to be KRAS mutation-negative (wild-type) by the KRAS Kit (reported as no mutation detected). A statistically significant benefit in progression free survival was observed in the KRAS mutation-negative (wild-type) subset. In addition, the median overall survival was 23.8 months in the KRAS mutation-negative (wild-type) subset treated with panitumumab plus FOLFOX compared to 19.4 months for KRAS mutation-negative (wild-type) patients who received FOLFOX alone. In KRAS mutation-negative (wild-type) tumors as determined by the therascreen® KRAS RGQ PCR Kit, subjects treated with panitumumab + FOLFOX demonstrated significant improvement in OS compared with subjects treated with FOLFOX alone in the sensitivity analyses.

The risks of the KRAS Kit are associated the potential mismanagement of patients resulting from false results of the test. 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 colorectal cancer treatment. A false positive test result may lead to panitumumab treatment being withheld from a patient who might have benefitted. A false negative test result may lead to panitumumab treatment being administered to a patient who is not expected to benefit, and potentially any adverse side effects associated with treatment. The device is a key part of diagnostic evaluation for colorectal cancer in decisions regarding treatment with panitumumab. There is currently no FDA approved test for the selection of candidate metastatic CRC patients for treatment with panitumumab.

In conclusion, given the available information above, the data support the use of the *therascreen*® KRAS RGQ PCR Kit as an aid in the identification of CRC patients for

panitumumab treatment based on a KRAS Kit no mutation detected test result, and the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from Study 20050203 support the utility of the therascreen® KRAS RGQ PCR Kit as an aid in the identification of patients with metastatic CRCs for treatment with panitumumab.

XIII. CDRH DECISION

CDRH issued an approval order on May 23, 2014. The final conditions of approval can be found in the approval order.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling. Refer to the drug label for Vectibix (panitumumab) for additional information related to the use of the drug.

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

1. Douillard JY, Siena S, Cassidy J, et al: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *Journal of Clinical Oncology*. 28:4697-705, 2010.