SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic variant detection system
Device Trade Name:	Praxis™ Extended RAS Panel
Device Procode:	PQP
Applicant's Name and Address:	Illumina, Inc. 5200 Illumina Way, San Diego, CA 92122
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P160038

Date of FDA Notice of Approval: June 29, 2017

Priority Review: None

II. INDICATIONS FOR USE

The Praxis™ Extended RAS Panel is a qualitative in vitro diagnostic test using targeted high throughput parallel sequencing for the detection of 56 specific mutations in RAS genes [KRAS (exons 2, 3, and 4) and NRAS (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The Praxis™ Extended RAS Panel is indicated to aid in the identification of patients with colorectal cancer for treatment with Vectibix® (panitumumab) based on a no mutation detected test result. The test is intended to be used on the Illumina MiSeqDx® instrument.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Praxis™ Extended RAS Panel labeling.

V. DEVICE DESCRIPTION

The Praxis® Extended RAS Panel assay is designed specifically for the detection of 56 mutations from exons 2, 3, and 4 of the *KRAS* and *NRAS* genes using the Illumina MiSeqDx instrument. The assay has been configured for 2 runs with a maximum of 10 samples plus 2 controls per run.

The overall diagnostic product consists of the Illumina MiSeqDx sequencing instrument and assay specific software and the Praxis® Extended RAS Panel assay kit which includes reagents, consumables and software required to complete all assay steps: sample qualification, library preparation, sequencing, and report generation. The following components comprise the Praxis™ Extended RAS Panel Kit:

Box Number	Reagent/Consumable
1	Hybridization Buffer
	Extension Ligation Mix
	Index Primers
	PCR Polymerase
	PCR Master Mix
	Library Normalization Diluent
	Library Dilution Buffer
	Universal PhiX Control
2	MiSeqDx Cartridge
3	Stringent Wash Buffer
	Universal Wash Buffer
	PCR Clean-Up Beads
	Library Normalization Wash
	Library Beads
	MiSeqDx Flow cell
4	SBS Solution
5	Filter Plate
	Elution Buffer
	Library Storage Buffer
6	Quality Control Primer
	RAS Panel A
	RAS Panel B
	Extended RAS Panel Control
	QC Template Control
	qPCR Master Mix

Principles of the Procedure

Sample Qualification

The Praxis Extended RAS Panel is intended for use with DNA samples extracted from formalin-fixed paraffin embedded (FFPE) tumor tissue collected from colorectal cancer patients. Due to heterogeneity of tissue and neoplastic cell content, specimens must be

reviewed prior to DNA extraction to ensure adequate tumor is available for testing. Recommended cumulative tissue area using 5µm sections is 240mm^2 or greater with ≥ 50% neoplastic content. For optimal yield of amplifiable DNA, it is recommended to use 8 x 5µm sections or equivalent alternate thickness serial sections.

The Praxis Extended RAS Panel workflow starts with DNA qualification step which is performed to determine if the DNA is of sufficient quality for use with the Praxis Extended RAS Panel. Sample qualification reagents are used to amplify DNA and a control template. The qualification test measures the amount of amplifiable and detectable target DNA by quantitative PCR. The quantification cycle (Cq) of the sample is compared to the Cq of the control template to obtain a ΔCq value for the specimen. Genomic DNA (gDNA) samples giving a ΔCq value less than or equal to 5.00 have sufficient DNA quality for use with the Praxis Extended RAS Panel.

Library Preparation

Library preparation consists of four key steps: hybridization, extension-ligation, PCR amplification and library normalization.

Following DNA extraction and sample qualification, library preparation begins with hybridization of pre-mixed, optimized oligonucleotides specific to the targeted regions of the Praxis Extended RAS Panel upstream and downstream of the regions of interest on sample gDNA. Each probe includes a locus specific sequence and an adapter sequence used in a subsequent amplification reaction. Two oligonucleotide pools (A and B) independently test each strand of DNA and minimize the effect of FFPE artifact on variant detection. At the end of this process, a three-step wash procedure with a filter capable of size selection removes unbound oligonucleotides from the genomic DNA.

The extension-ligation step connects the hybridized upstream and downstream oligonucleotides. A DNA polymerase extends from the upstream oligonucleotides through the targeted region, followed by ligation to the 5' end of the downstream oligonucleotide using a DNA ligase. The result is the formation of products that contain the RAS-specific oligonucleotides flanked by sequences required for amplification.

The extension-ligation products are amplified using universal primers that add index sequences for sample multiplexing as well as the common adapters required for generation of clusters on the MiSeqDx instrument. These primers incorporate sample specific sequence tags that allows for pooling of samples into a single flow cell / sequencing run at the end of the workflow. The primers also incorporate sequences which are complementary to the sequences of the capture oligonucleotides attached to the flow cell. The result is the formation of products that contain the RAS-specific oligonucleotides flanked by the sequences required for amplification.

The presence of PCR product is verified by gel electrophoresis. After PCR product purification, a proprietary bead-based procedure normalizes the quantity of each library to allow for even sample representation in the final pooled library. Pooled amplicon

libraries can be loaded directly onto the included Praxis Extended RAS Panel reagent cartridge without additional processing for sequencing on the MiSeqDx instrument.

Sequencing
After library preparation and loading of the pooled library into the reagent cartridge, the next major process step involves sequencing on the instrument. The Praxis Extended RAS Panel performs a paired 141 base pair sequencing run that is fully bidirectional to ensure that each targeted region for each sample is sequenced in both directions for optimal accuracy. Sequencing-by-synthesis (SBS) chemistry is utilized in which four fluorescently labeled nucleotides are incorporated into the hybridized sample clusters on the flow cell surface in parallel. The nucleotide label serves as a terminator for polymerization, so after each dNTP incorporation, the fluorescent dye is imaged to identify the base and then enzymatically cleaved to allow incorporation of the next nucleotide. This process is repeated for the number of sequencing cycles needed for the run.

Data Analysis
Sequencing data is aligned to the human genome (hg19) and single nucleotide variants and multinucleotide variants are detected using software designed to detect the specific 56 hotspots.

The Praxis Extended RAS Panel module of the Local Run Manager software processes base calls generated during primary analysis and produces information about each sample, called secondary analysis. Secondary analysis includes demultiplexing, FASTQ file generation, alignment, variant calling, and report generation. The primary analysis is performed with Real-Time Analysis (RTA) software and the secondary analysis is performed with MiSeq Reporter (MSR) software.

- Demultiplexing— This is the first step in secondary analysis. Demultiplexing separates data from pooled samples based on the unique sequence indexes that were added during the PCR amplification step.
- FASTQ File Generation—After demultiplexing, the Praxis Extended RAS Panel module generates intermediate files in the FASTQ format, which is a text format used to represent sequences. FASTQ files contain the reads for each sample and the quality scores, excluding reads from any clusters that did not pass filter.
- Alignment—Alignment compares sequences against the reference to identify a relationship between the sequences and assigns a score based on regions of similarity. Aligned reads are written to files in BAM format. Local Run Manager uses a banded Smith-Waterman algorithm that performs local sequence alignments to determine similar regions between two sequences.
- Variant Calling—In order to minimize false positives due to FFPE artifacts, mutations are first called for each strand (oligo pool) separately. Then, mutations from the two pools are compared and combined into a single output file. If found, only mutations listed in Table 1 are reported.
- Report Generation—Results are accessible through Local Run Manager in the Samples and Results tab for the run, and the following simple-text output file is generated:

    - Extended RAS Panel Report—Details whether each sample contains any of the mutations listed in the reference panel.
- Quality Metrics (e.g., read depth, quality scores) are assigned within the Praxis Extended RAS panel and cannot be changed by the user. The assay reports out only the 56 variants. Quality Control Metrics are evaluated for each sample and across samples within the run.

Instrument and Software

Instrument

The MiSeqDx instrument is a bench top integrated sequencing system with a built-in CPU and touch screen monitor, and includes subcomponents such as imagers, motorized stage and a fluidics system.

The imaging subcomponent of the instrument consists of two cameras and two light emitting diodes (LED). Each camera captures fluorescence in two channels, which together allow for the system to recognize the four base pairs. The motorized stage consists of a flow cell stage that is controlled by two motors to move the stage in multiple planes. This movement allows the stage to be moved into view of the two cameras and as well as eject the flow cell for user access. The fluidics system is comprised of tubing, valves, pumps, and one compartment each for the reagent cartridge and buffer bottle. The reagent cartridge compartment contains a chiller to allow for the reagents to be kept cold during the duration of the run. The reagent compartment also contains a motorized sipper with a separate line for each of the positions on the reagent cartridge. The instrument utilizes radio frequency identification tags (RFIDs) for the reagent cartridge, SBS solution bottle, and the flow cell. Each RFID has read/write capability. This allows the instrument to read identifying information as well as record run information indicating that reagents and flow cell have been consumed, which prevents re-use of those consumables.

Software

The MiSeqDx instrument operation is controlled by an assay-agnostic core software architecture, Local Run Manager (LRM), on which assay-specific software modules are run using associated reagents and consumables. The LRM framework provides the ability to run multiple independent assays from a single, cohesive user interface.
The Praxis Extended RAS Panel software module is developed specifically for use with the reagents and consumables included in the assay kit. In addition to common LRM software components, the Praxis Extended RAS Panel software module includes various unique applications developed to setup, operate, scan, quality control and analyze data at different stages during the execution of the assay on the MiSeqDx instrument.

Interpretation of Results

The Praxis Extended RAS Panel report details if a run passed or failed and whether each sample contains any of the mutations listed in the reference panel. Analysis results are summarized in the report, which is available for export if necessary.

Mutations Reported:
The Praxis Extended RAS Panel detects 56 specific mutations in exons 2, 3, 4 of KRAS and NRAS.

Table 1 lists all the mutations in the KRAS and NRAS genes that can be detected by the test. The assay simultaneously determines the presence or absence of each of the 56 mutations in every sequenced sample. Table 1b shows the Sample Results Table.

Table 1a. Praxis Extended RAS Panel Summary of Mutations*

Chromosome	Gene	Exon	Amino Acid	Wild Type > Mutation
chr12	*KRAS*	2	p.Gly13Val	c.38_39GC>TT
chr12	*KRAS*	2	p.Gly13Glu	c.38_39GC>AA
chr12	*KRAS*	2	p.Gly13Asp	c.38_39GC>AT
chr12	*KRAS*	2	p.Gly13Asp	c.38G>A
chr12	*KRAS*	2	p.Gly13Cys	c.37G>T
chr12	*KRAS*	2	p.Gly13Arg	c.37G>C
chr12	*KRAS*	2	p.Gly12Trp	c.34_36GGT>TGG
chr12	*KRAS*	2	p.Gly12Ala	c.35G>C
chr12	*KRAS*	2	p.Gly12Asp	c.35G>A
chr12	*KRAS*	2	p.Gly12Phe	c.34_35GG>TT
chr12	*KRAS*	2	p.Gly12Asn	c.34_35GG>AA
chr12	*KRAS*	2	p.Gly12Val	c.35G>T
chr12	*KRAS*	2	p.Gly12Ser	c.34G>A
chr12	*KRAS*	2	p.Gly12Cys	c.34G>T
chr12	*KRAS*	2	p.Gly12Arg	c.34G>C
chr12	*KRAS*	3	p.Gln61His	c.183A>C
chr12	*KRAS*	3	p.Gln61His	c.183A>T
chr12	*KRAS*	3	p.Gln61Leu	c.182A>T
chr12	*KRAS*	3	p.Gln61Arg	c.182A>G
chr12	*KRAS*	3	p.Gln61Lys	c.181C>A
chr12	*KRAS*	3	p.Gln61Glu	c.181C>G
chr12	*KRAS*	3	p.Ala59Gly	c.176C>G
chr12	*KRAS*	3	p.Ala59Thr	c.175G>A
chr12	*KRAS*	4	p.Ala146Val	c.437C>T
chr12	*KRAS*	4	p.Ala146Thr	c.436G>A
chr12	*KRAS*	4	p.Ala146Pro	c.436G>C
chr12	*KRAS*	4	p.Lys117Asn	c.351A>T
chr12	*KRAS*	4	p.Lys117Asn	c.351A>C
chr1	*NRAS*	2	p.Gly13Glu	c.38_39GT>AG
chr1	*NRAS*	2	p.Gly13Glu	c.38_39GT>AA
chr1	*NRAS*	2	p.Gly13Asp	c.38G>A
chr1	*NRAS*	2	p.Gly13Val	c.38G>T
chr1	*NRAS*	2	p.Gly13Cys	c.37G>T
chr1	*NRAS*	2	p.Gly13Arg	c.37G>C
chr1	*NRAS*	2	p.Gly12Trp	c.34_36GGT>TGG

Chromosome	Gene	Exon	Amino Acid	Wild Type > Mutation
chr1	*NRAS*	2	p.Gly12Ala	c.35G>C
chr1	*NRAS*	2	p.Gly12Val	c.35G>T
chr1	*NRAS*	2	p.Gly12Asp	c.35G>A
chr1	*NRAS*	2	p.Gly12Phe	c.34_35GG>TT
chr1	*NRAS*	2	p.Gly12Asn	c.34_35GG>AA
chr1	*NRAS*	2	p.Gly12Ser	c.34G>A
chr1	*NRAS*	2	p.Gly12Cys	c.34G>T
chr1	*NRAS*	2	p.Gly12Arg	c.34G>C
chr1	*NRAS*	3	p.Gln61His	c.183A>T
chr1	*NRAS*	3	p.Gln61His	c.183A>C
chr1	*NRAS*	3	p.Gln61Arg	c.182A>G
chr1	*NRAS*	3	p.Gln61Leu	c.182A>T
chr1	*NRAS*	3	p.Gln61Lys	c.181C>A
chr1	*NRAS*	3	p.Gln61Glu	c.181C>G
chr1	*NRAS*	3	p.Ala59Gly	c.176C>G
chr1	*NRAS*	3	p.Ala59Thr	c.175G>A
chr1	*NRAS*	4	p.Ala146Val	c.437C>T
chr1	*NRAS*	4	p.Ala146Thr	c.436G>A
chr1	*NRAS*	4	p.Ala146Pro	c.436G>C
chr1	*NRAS*	4	p.Lys117Asn	c.351G>C
chr1	*NRAS*	4	p.Lys117Asn	c.351G>T

* *KRAS* proto-oncogene, GTPase [Homo sapiens (human)] (Gene ID: 3845) - NM_004985.4, NP_004976.2.
* *NRAS* neuroblastoma RAS viral oncogene homolog [Homo sapiens (human)] (Gene ID: 4893) - NM_002524.4, NP_002515.1.

Table 1b. Samples Result Output

Column Heading	Description
Sample Name	The sample name provided when the run was created
Sample	Sample Validity Results: **Valid** – Presence or absence of mutations from the panel **Invalid** – Presence or absence of mutations from the panel cannot be determined
Result	Sample results for panel mutation detection **Panel Mutation Detected** – Mutations from the panel are present. Lists the detected panel mutations. **Panel Mutation Not Detected** – Mutations from the panel are not present.
Gene	The gene, *KRAS* or *NRAS* where a mutation is detected, if applicable
Exon	The exon number in which the mutation is presented, if applicable
Amino acid	Human Genome Variation Society (HGVS) amino acid change notation
Nucleotide	HGVS nucleotide change notation
Test Result	**Interpretation**
Panel Mutation Detected	Illumina Praxis Extended RAS Panel mutation is detected in at

	least one locus of interest. The report lists all detected mutations.
Panel Mutation Not Detected	No Praxis Extended RAS Panel mutations are detected in loci of interest
Sample Invalid	The presence or absence of a Illumina Praxis Extended RAS Panel mutation cannot be determined. Repeat library preparation for Invalid samples. If a sample fails 2 times, additional troubleshooting is necessary.

Test Controls

The Praxis Extended RAS Panel requires the user to run positive and negative control samples in parallel.

Positive Control (Panel Control): The Panel Control, required on every run, is a genomic DNA sample containing low mutant allele frequencies (8%) and serves to report both the mutant allele(s) and the wild type allele(s) in both KRAS and NRAS targets. The plasmid has two mutations, KRAS Exon 2 p.Gly12Asp c.35G>A and NRAS Exon 3 p.Gln61Lys c.181C>A. The Panel Control must generate the expected genotype to be valid. If the control is invalid, processing errors may have occurred. The software will fail the entire sequencing run and all samples will appear as Invalid.

Negative Control (No Template Control/ No DNA Control): The use of a negative (no template/no DNA) control (Blank) is required on every run. For a valid run, the Blank sample should appear as Invalid. If an unexpected outcome occurs in the Blank sample, the software will fail the entire sequencing run and all samples will appear as Invalid.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently two FDA-approved PCR-based tests for detection of KRAS mutation status in FFPE CRC tissue in the identification of patients suited for treatment with panitumumab (Vectibix) treatment. The original companion diagnostic used to assess the safety and efficacy of panitumumab is the QIAGEN *therascreen*® KRAS RGQ PCR Kit and was FDA approved in 2014. Subsequently, the Roche **cobas**® KRAS Mutation Test was FDA approved as a follow-on companion diagnostic in 2015. Each test is designed to detect 7 mutations in codons 12 and 13 in exon 2 of the KRAS gene. The Praxis Extended RAS Panel is the first NGS-based test for the detection of 56 mutations in exon 2, 3, 4 of the KRAS and NRAS genes.

VII. MARKETING HISTORY

The Praxis™ Extended RAS Panel assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect Praxis™ Extended RAS Panel results and subsequently improper

patient management decisions in colorectal cancer (CRC) treatment. A false positive test result may lead to Vectibix (panitumumab) treatment being withheld from a patient who might have benefited. A false negative test result may lead to Vectibix (panitumumab) treatment being administered to a patient who may experience adverse side effects associated with treatment without clinical benefit.

Most common adverse reactions (≥ 20%) are skin toxicities (i.e., erythema, dermatitis acneiform, pruritus, exfoliation, rash, and fissures), paronychia, hypomagnesemia, fatigue, nausea, and diarrhea.

No adverse events occurred in connection with the studies conducted in support of the PMA as they were retrospective and used banked specimens.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

CRC FFPE specimens used in the non-clinical studies described below consisted of genomic DNA (gDNA) extracted from FFPE tumor tissue meeting the tissue and tumor requirements described in the product labeling, as well as DNA from cell lines and plasmids to provide panel mutation representation. Samples contained both wild-type (WT) and mutated genotypes, and included rare, multi-nucleotide mutations, and samples with more than one mutation.

1. Analytical Accuracy – Comparison to an Orthogonal Method

Banked FFPE colorectal cancer (CRC) tissue samples collected during the Amgen panitumumab study (protocol no. 20050203 [0203 study]) were tested with the Praxis Extended RAS Panel. Samples were also tested using the reference method, bidirectional Sanger sequencing. The agreements between results from Praxis Extended RAS Panel and Sanger were compared to evaluate the accuracy of the Praxis Extended RAS Panel.

A total of 1183 subjects were included in the 0203 study. Of the 1183 subjects, a total of 685 specimens (57.9%) were available and/or met the specimen tissue requirements for testing. Of those 685 evaluable samples, 169 samples (25%) did not meet the Sample Qualitification (Sample QC) requirement (dCq<5.0) and were excluded from the analysis. An additional 75 specimens failed sequencing quality controls. The reasons for specimen exclusion are summarized listed in Table 2 below, 441 subjects had valid Sanger and Praxis Extended RAS panel results, which were compared.

Table 2. Specimen Accounting

	Cause for Exclusion	Number	% Excluded of Total (x/Total)
Initial Number of Specimens - 1183			
Specimen Quality Failure	No tissue available*	358	
	Insufficient tumor area	127	

	Insufficient neoplastic cells	13	
	Total	498	498/1183 = 42.1%
Total Prior to DNA QC - 685			
DNA Quality Failure	dCq ≤5.0	169	169/685 =24.6%
Total Evaluable For Sequencing - 516			
Sequencing Quality Failure	Gel Check Failure	18	
	Sample sequencing QC invalid	55	
	Error/Processing aborted	2	
	Total	75	75/516 = 14.5%
Total Specimens with Available MiSeq Results		441	441/1183=37.3%

*Some specimens were exhausted during the clinical validation study and were unavailable for Sanger sequencing.

a. Specimen level comparison:

Agreement between the Praxis Extended RAS Panel and Sequencing was assessed first at the specimen level (i.e., mutation detected vs. mutation not detected) in accordance with the intended use, i.e., if any actionable mutation is detected by Praxis Extended RAS Panel, the subject will not be referred for the companion therapeutic treatment. Specimen-level positive percent agreement (PPA) is defined as the proportion of positive specimens as detected by the Praxis Extended RAS Panel among all positive specimens as determined by Sanger Sequencing, where a positive specimen is defined as one or more mutations detected. Specimen-level negative percent agreement (NPA) is defined as the proportion of specimens that were negative (i.e., none of the 56 mutations detected) by the Praxis Extended RAS Panel among all specimens determined to to be negative by Sanger sequencing. At the specimen level, there were 5 specimens that were called positive by the Praxis Extended RAS Panel that were negative by Sanger, and 3 specimens that were negative by the Praxis Extended RAS Panel that were positive by Sanger. The PPA is 98.7% with lower bound of 95% CI at 96.2%; NPA is 97.6% with lower bound of 95% CI at 94.6% (Table 3). Invalid results with the Praxis Extended RAS Panel were obtained for a total of 14.5% of the total specimens that passed the DNA quality check. There were no Sanger Sequencing invalids. The data does not include the Praxis Extended RAS Panel invalids. The results demonstrate that e the Praxis Extended RAS Panel is accurate.

Table 3. Positive and Negative Percent Agreement of Subject-level *RAS* Results (dCq ≤ 5.0) and Excluding Invalids

Praxis Extended RAS Panel	Sanger		Total
	Positive	Negative	
Positive	227*	5	232
Negative	3**	206	209
Total	230	211	441

Performance Summary		
Agreement Statistic	**Point Estimate**	**95% CI Exact**
Subject-level PPA	98.7% (227/230)	(96.2, 99.7)
Subject-level NPA	97.6% (206/211)	(94.6, 99.2)

* There were 224 exact matches for within-subject all mutation level results; for 2 subjects Praxis detected the Sanger detected mutation and one additional mutation; 1 subject had different mutations detected by Praxis and Sanger.

** 1 subject had 2 mutations detected by Sanger; 2 subjects had one mutation detected by Sanger.

b. Mutation level comparison:

Accuracy of the Praxis Extended Panel for each of the variants was analyzed for all 441 specimens as compared to Sanger sequencing. The mutation level comparison is listed in Table 4 for KRAS and Table 5 for NRAS. There were a total of 13 discordant results at the mutation level. Discordant results are shown in the shaded boxes in the tables.

Table 4. Positive and Negative Percent Agreement of KRAS Mutation Results (dCq ≤ 5) and Excluding Invalids

Exon	Mutation	TP	FN	FP	TN	Total	PPA (95% CI*)	NPA (95% CI*)
***KRAS* Exon 2**	c.34G>T	14	0	0	426	440	100.0 (76.8-100.0)	100.0 (99.1-100.0)
	c.34G>A	9	1	0	430	440	90.0 (40.0-100)	100.0 (99.1-100.0)
	c.34G>C	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.34_35GG>TT	1	0	0	439	440	100.0 (2.5-100.0)	100.0 (99.2-100.0)
	c.34_35GG>AA	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.34_36GGT>TGG	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.35G>A	55	0	1	384	440	100.0 (93.5-100.0)	99.7 (98.7-100.0)
	c.35G>T	40	2	2	396	440	95.2 (83.1-100)	99.5 (98.2-100.0)
	c.35G>C	11	0	2	427	440	100.0 (71.5-100.0)	99.5 (98.4-100.0)
	c.37G>T	2	0	0	438	440	100.0 (15.8-100.0)	100.0 (99.2-100.0)
	c.37G>C	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.38G>A	41	0	0	399	440	100.0 (91.4-100.0)	100.0 (99.1-100.0)
	c.38_39GC>TT	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.38_39GC>AA	0	0	0	440	440	NE	100.0 (99.2-100.0)
	c.38_39GC>AT	0	0	0	440	440	NE	100.0 (99.2-100.0)
***KRAS* Exon 3**	c.175G>A	1	0	0	440	441	100.0 (2.5-100.0)	100.0 (99.2-100.0)
	c.176C>G	1	0	0	440	441	100.0 (2.5-100.0)	100.0 (99.2-100.0)
	c.181C>A	0	1	0	440	441	0.0 (0.0-97.5)	100.0 (99.2-100.0)
	c.181C>G	0	0	0	441	441	NE	100.0 (99.2-100.0)

	c.182A>T	1	0	0	440	441	100.0 (2.5-100.0)	100.0 (99.2-100.0)
	c.182A>G	2	0	0	439	441	100.0 (15.8-100.0)	100.0 (99.2-100.0)
	c.183A>C	6	0	0	435	441	100.0 (54.1-100.0)	100.0 (99.2-100.0)
	c.183A>T	1	0	0	440	441	100.0 (2.5-100.0)	100.0 (99.2-100.0)
***KRAS* Exon 4**	c.351A>C	2	1	0	437	440	66.7 (0-100.0)	100.0 (98.4-100.0)
	c.351A>T	2	0	0	438	440	100.0 (15.8-100.0)	100.0 (99.2-100.0)
	c.436G>A	11	0	0	429	440	100.0 (71.5-100.0)	100.0 (99.1-100.0)
	c.436G>C	1	0	0	439	440	100.0 (2.5-100.0)	100.0 (99.2-100.0)
	c.437C>T	3	0	0	437	440	100.0 (29.2-100.0)	100.0 (99.2-100.0)
Total		204	5	5	12114	12328	97.6% (93.5-99.1)	99.96% (99.9-100)

NE = not estimable; NA=not applicable

*For all agreements (PPA, NPA) not equal to 100%, bootstrap method was used to calculate the two-sided 95% confidence intervals (CIs); For all agreements equal to 100%, exact method was used to calculate the 95% CIs. Boot strap confidence intervals are obtained from bootstrap sampling of subjects (maintaining all mutation results nested within subject) with replacement 9,999 times [(1)]

Table 5. Positive and Negative Percent Agreement of NRAS Mutation Results (dCq ≤ 5) and Excluding Invalids

Location	Mutation	TP	FN	FP	TN	Total	PPA (95% CI*)	NPA (95% CI*)
***NRAS* Exon 2**	c.34G>T	1	0	1	439	441	100.0 (2.5-100.0)	99.8 (98.2-100)
	c.34G>A	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.34G>C	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.34_35GG>TT	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.34_35GG>AA	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.34_36GGT>TG	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.35G>A	4	0	0	437	441	100.0 (39.8-100.0)	100.0 (99.2-100.0)
	c.35G>T	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.35G>C	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.37G>T	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.37G>C	2	0	0	439	441	100.0 (15.8-100.0)	100.0 (99.2-100.0)
	c.38G>A	0	0	1	440	441	NE	99.8 (98.2-100)
	c.38G>T	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.38_39GT>AA	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.38_39GT>AG	0	0	0	441	441	NE	100.0 (99.2-100.0)
***NRAS* Exon 3**	c.175G>A	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.176C>G	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.181C>A	7	0	1	433	441	100.0 (59.0-100.0)	99.8 (98.2-100)
	c.181C>G	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.182A>T	2	0	0	439	441	100.0 (15.8-100.0)	100.0 (99.2-100.0)
	c.182A>G	5	0	0	436	441	100.0 (47.8-100.0)	100.0 (99.2-100.0)

	c.183A>C	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.183A>T	2	0	0	439	441	100.0 (15.8-100.0)	100.0 (99.2-100.0)
***NRAS* Exon 4**	c.351G>C	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.351G>T	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.436G>A	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.436G>C	0	0	0	441	441	NE	100.0 (99.2-100.0)
	c.437C>T	0	0	0	441	441	NE	100.0 (99.2-100.0)
Total		23	0	3	12322	12348	100% (85.2-100.0)	99.98 (99.9-100)

NE = not estimable; NA=not applicable

*For all agreements (PPA, NPA) not equal to 100%, bootstrap method was used to calculate the two-sided 95% confidence intervals (CIs); For all agreements equal to 100%, exact method was used to calculate the 95% CIs. Boot strap confidence intervals are obtained from bootstrap sampling of subjects (maintaining all mutation results nested within subject) with replacement 9,999 times [(1)]

The overall mutation level results and PPA/NPA are summarized in the Table 6. The 95% confidence intervals were obtained using the Clopper-Pearson (Exact binomial) method. The results exclude the Praxis Extended RAS panel invalid results.

Table 6. Total Positive and Negative Percent Agreement of Mutation-Level Results

Location/Mutation	TP	FN	FP	TN	Total	PPA (95% CI)	NPA (95% CI)
KRAS/All	204	5	5	12114	12328	97.6 (94.5-99.2)	99.96 (99.9-100.0)
NRAS/All	23	0	3	12322	12348	100 (85.2-100.0)	99.98 (99.9-100.0)
Total	227	5	8	24436	24676	97.8 (95.0-99.3)	99.97 (99.9-100.0)

Analytical Sensitivity

a. **Limit of Blank (LoB):**
Specimens free of somatic variants ("blank") were analyzed to determine the number of background false positive reads. The threshold for calling a specimen positive was established above this background. Three wild-type (WT) specimens were used in this study. A total of 60 observations were generated per study mutation per lot (20 observations × 3 WT samples). Two lots were used in the study. Specimens free of somatic variants ("blank") were analyzed to determine the number of background positive reads. The threshold for calling a specimen positive was established above this background to minimize false positives. The clinical threshold is set at 2.6%.

b. Limit of Detection (LoD):
The LoD for the Praxis Extended RAS Panel was estimated to determine the lowest allele frequency at which 95% of the tested replicates produced correct calls. Three CRC FFPE blended sample mixes containing 5 common SNVs

and 1 plasmid containing a rare MNV spiked into a mutant CRC FFPE sample were assessed. Each mix was evaluated at 4 mutation frequency levels (10%, 5%, 2.5% , and 1.25%) and were run in duplicate (starting with library preparation) with two reagent lots by 5 operators on 2 non-consecutive days. The sample mixes were prepared at the low level of DNA input (i.e., Specimens blends were created to represent Sample QC near the limit dCq=4-5) (corresponding to approximately 50-25 ng of intact DNA respectively). Two instruments were used per operator.

Using Probit analysis, the LoD for the 6 representative mutations ranged between 4.2%-5.3% (Table 7). The LoD for the MNV sample was shown to be 4.5%. Mutants detected at allele frequencies below the established limit of detection are not detected consistently. Therefore, the LoD claim for the Praxis Extended RAS Panel as defined above is 5.0%

Table 7. Limit of Detection Results

Sample Mix	Gene	Exon	Mutation		LoD
			Amino Acid	Nucleotide	
1	KRAS	2	p.Gly12Asp	c.35G>A	5.0%
2	NRAS	3	p.Gln61Arg	c.182A>G	4.7%
3	KRAS	2	p.Gly13Asp	c.38G>A	4.8%
4	KRAS	4	p.Ala146Thr	c.436G>A	5.3%
5	KRAS	3	p.Gln61His	c.183A>C	4.2%
6	NRAS	2	p.Gly13Glu	c.38_39GT>AA	4.5%

c. DNA Input:

The Praxis Extended RAS Panel workflow involves sample qualification, library preparation and sequencing. Genomic DNA specimens are qualified for use in the assay by measuring amplifiability of the specimen relative to a control DNA template through a real-time quantitative PCR (qPCR) reaction. Measuring amplifiability accounts for both the quantity and quality of the DNA specimen for qualification. A quantification threshold value (Cq) is generated using the Quality Control Template and each genomic DNA specimen. The metric to assess the amplifiability is the change in quantification threshold (Cq) between the specimen and the control template DNA (Delta Cq; dCq). The dCq value is used to determine whether a specimen qualifies for the Praxis Extended RAS Panel assay and is the measure of DNA quality and quantity (DNA Input) for this assay. The acceptable measure for the assay is a Delta Cq $\leq$ 5. DNA input was evaluated over the range of delta Cq values from -0.5 to 5 in order to evaluate assay performance. A Delta Cq level of -0.5 to 5 corresponds to approximately 1200 ng to 25 ng, respectively, of intact DNA used in the assay.

A panel of 9 CRC FFPE specimens each containing 1 unique Extended RAS panel mutation, and 1 CRC FFPE specimen wild type for Extended RAS

panel mutations was evaluated. The mutations represented were in KRAS exons 2, 3, and 4 and NRAS exons 2 and 3. The mutation frequency for this panel ranged from approximately 10% to 20%. The specimen panel was tested in duplicate library preparations at each DNA input level for a total of 20 observations at each DNA input level. Specimens were sequenced at the maximum multiplexing level of 10 samples plus controls per run. Two (2) DNA input levels were tested: 1) dCq -0.5 to 0.5 (nominal high end) 2) dCq 4 to 5 (nominal low end).

All specimens showed a valid result after library preparation and sequencing with a sample first pass rate of 100% (20/20) for each DNA input level. OPA, PPA, and NPA are calculated on a per mutation basis; there are 56 mutations per sample. The overall percent agreement relative to Sanger sequencing was 100% (1120/1120) for each DNA input level. The positive percent agreement was 100% (18/18) and negative percent agreement was 100% (1102/1102) for both DNA input levels relative to Sanger sequencing. Therefore, this study demonstrates the performance of the Praxis Extended RAS Panel assay for the delta Cq (DNA input) range of -0.5 to 5.

To demonstrate the performance with challenging samples that harbor mutations near the limit of detection (LoD) of the mutant allele frequency, an additional study was conducted. Since DNA is qualified based on a dCq cutoff of 5.0, a nominal DNA input concentration range (12.5ng to 50ng) corresponding to approximate values of dCq 6-4 were selected for evaluation. A panel of 5 blended CRC FFPE specimen mixes, each containing 2 unique representative Extended RAS panel mutations, one at a low (approximately 8%) and one at a high (approximately 14%) mutation frequency level, was used. Two (2) of the five (5) sample mixes were spiked with a RAS panel multinucleotide variant (MNV). Two DNA input levels were evaluated for each FFPE blend: a dCq level of 4-5 corresponds to approximately 50-25 ng, respectively, and a dCq level of 5-6 corresponds to approximately 25-12.5 ng, respectively, of intact DNA. Twelve (12) observations of each of the 5 specimen mixes were collected over 3 non-consecutive days per DNA Input/Delta Cq and mutation frequency level. The total number of observations for the study across all specimen mixes was 60 observations; there were 120 observations across all mutation frequency levels per DNA input level
(2 observations × 2 instruments [sequencing runs] × 3 non-consecutive days × 5 mixes). The sample first pass rate was 100% (60/60) for dCq 4 to 5 and 91.7% (55/60) for dCq 5 to 6. For dCq 4 to 5, 100% (120/120) of observations showed the expected Extended RAS Panel mutation result. For dCq 5 to 6, 95% (114/120) of observations showed the expected Extended RAS Panel mutation result. Mean mutation frequencies were similar between Delta Cq levels, whereas specimens with Delta Cq 5 to 6 had more mutation frequency variability in general than the same specimens with Delta Cq 4 to 5. The study confirmed that the Praxis Extended RAS Panel is able to detect mutants

with accuracy at DNA input corresponding to dCq ≤ 5.0 and approximately 25 ng of intact DNA for mutations near the LoD.

2. **Analytical Specificity**

a. Interfering Substances –Exogenous Substances:

To evaluate the potential impact of interfering substances on the performance of the assay, the study evaluated nine exogenous substances: deparaffinization solution, paraffin wax, xylene, ethanol, Proteinase K, and wash solutions from three different commercial extraction kits.

Each substance was added at the specified final concentration to extracted genomic DNA from 15 colorectal cancer FFPE tissue samples. These samples represented wild type (5/15 specimens) and the prevalent RAS mutants across multiple exons for each gene (10/15 specimens). The mutant allele frequency ranged from 0.2-0.7. Results from samples treated with each exogenous substance tested were compared to their respective untreated control condition.

All 15 specimens for all 9 exogenous substances showed agreement at all mutant and non-mutant positions with the untreated control condition. No false positives and no false negatives were observed. Analysis of the DNA quality control dCq identified significant differences (Table 8) but did not invalidate the specimen (all treatments had dCq<5.0). Effects were also analyzed for results. All 15 specimens for all 9 exogenous substances showed agreement with the untreated control condition at all mutant and non-mutant positions. No false positives and no false negatives were observed. Analysis of the interference of exogenous substances on the mutation frequencies observed between control and spike-in showed no significant differences.

Table 8. Effects of Exogenous interfering substances on dCq

Treatment	Treatment	Mean Difference from Untreated	Std Err of Mean Difference	Lower CL of Mean Difference	Upper CL of Mean Difference	*p-Value
Deparaffinization Solution	Untreated Control	0.129	0.045	-0.252	-0.005	0.0377*
Paraffin Wax (in Xylene)	Untreated Control	-0.009	0.045	-0.133	0.115	1.0000
Xylene	Untreated Control	-0.079	0.045	-0.203	0.045	0.4040
Ethanol	Untreated Control	0.177	0.045	0.053	0.301	0.0013*

Proteinase K	Untreated Control	0.051	0.045	-0.073	0.175	0.8388
Wash Solution (Kit 1)	Untreated Control	0.040	0.045	-0.084	0.164	0.9542
Wash Solution (Kit 2)	Untreated Control	0.152	0.045	0.028	0.275	0.0086*
Wash Buffer AW1 (Kit 3)	Untreated Control	0.103	0.045	-0.021	0.226	0.1520
Wash Buffer AW2 (Kit 3)	Untreated Control	0.183	0.045	0.059	0.307	0.0008*

*Dunnett's comparisons from ANOVA model (alpha=0.05, Q=2.72513, p-Value <0.05)

b. Interfering Substances - Endogenous substances:

1. *Necrotic tissue:*

To assess the performance of the assay when specimens contain high amounts of necrotic tissue, 15 colorectal FFPE samples containing 10-80% of necrotic tissue were tested. The dCq ranged from 0.72-4.22 with a mean of 2.94. A total of 4 RAS mutations were represented. The variant frequency ranged from 14%-34% with a mean of 26.9%.

The sample first pass rate was 93.3% (14/15) for endogenous (Necrotic) interfering substance. Of the total possible 840 results (15 specimens X 56 RAS results) the accuracy (overall percent agreement) of the necrotic specimens as compared to sanger sequencing was 93.2% (783/840) when including the specimen that failed to turn results. For the 14 specimens for which results were obtained, the positive percent agreement was 100% (4/4) and negative percent agreement was 99.9% (779/780) for endogenous (Necrotic) interfering substance relative to Sanger sequencing. The one false positive detected was likely due to a sample mutation frequency below limit of detection of Sanger sequencing.

2. *Hemoglobin:*

Hemoglobin was tested at 2mg/mL in the extraction lysis buffer to mimic an endogenous condition in accordance with CLSI EP7. Fifteen (15) colorectal FFPE samples were tested in the presence of this “high” amount of hemoglobin. Each of the samples was also tested in the absence of hemoglobin as an untreated control condition.

The sample qualification (Sample QC) pass rate and first pass rate was 100% (15/15) for samples spiked with endogenous (Hemoglobin) interfering substance. The overall percent agreement was 100% (840/840) for endogenous (Hemoglobin) interfering substance relative to untreated control. The positive percent agreement was 100% (10/10) and negative percent

agreement was 100% (830/830) for endogenous (Hemoglobin) interfering substance relative to untreated control. Analysis of dCq and mutation frequencies identified no significant differences in the presence of endogenous (Hemoglobin) interfering substance.

3. **Precision and Reproducibility**

a. Site-to-site Reproducibility Study:

The reproducibility of the Praxis Extended RAS panel was evaluated using a total of 18 specimens: 4 FFPE CRC specimens without mutations (wild type), 6 FFPE specimens with KRAS mutations (Gly12Ala, Gly12Asp, Gly12Val, Gly13Asp, Gly12Ser, and Gly12Cys) and 8 FFPE cell lines with KRAS mutations (Gly12Arg, Gln61His, Lys117Asn, Ala146Thr, and NRAS Gly12Asp, Gln61Arg, Lys117Asn, Ala146Thr). The study was conducted at three sites with 2 operators at each site across 3 non-consecutive days. One instrument was used at each site and only 1 master reagent lot was used in the study. Each unique specimen/mutation frequency level sample (tested in duplicate in each run) had 36 possible results (3 sites X 2 operators per site X 3 days/one run per day X 2 replicates).Twelve (12) mutations were prepared at both high and low mutation frequency (refer to Table 9) and 2 mutations were prepared at a low mutation frequency only and the corresponding high mutation frequency were not included in the sample panel. The dCq values for each of the reproducibility sample panel members ranged from 3.64-4.08. Sample pass rate and percent correct call (PCC) were calculated.

Combining sites, the sample pass rate was 94.7% for the first run of the sample. Table 9 shows PCCs for each of the 26 unique mutant specimen/mutation frequency level samples (combining replicates across 2 panel members) and corresponding 95% 2-sided score CIs. The two –sided 95% bootstrap confidence intervals for the PCC were also calculated to account for potential correlation between duplicates for unique mutant specimen/mutation frequency (MF) levels within a panel run/day when PCC < 100%. The percent correct call (PCC) across all mutant samples was 99.6% (lower bound 95% CI: 99%) when excluding samples with failed sample QC. The data demonstrate the Praxis Extended RAS Panel is reproducible when dCq<5.0.

Table 9. Mutation-Level Percent Correct Call for Individual Unique Mutant Specimens/Mutation Frequency Level Samples (Evaluable Analysis Set)

VAF Level	Exon	Amino Acid Change	Base Change	N	Percent Correct Call (%)	95% CI[1]
Low	*KRAS* exon 2	p.Gly12Cys	c.34G>T	36	36/36(100%)	(90.4, 100)
		p.Gly12Arg	c.34G>C	36	36/36(100%)	(90.4, 100)
		p.Gly12Ser	c.34G>A	35	35/35(100%)	(90.1, 100)
		p.Gly12Ala	c.35G>C	35	35/35(100%)	(90.1, 100)
		p.Gly12Asp	c.35G>A	36	36/36(100%)	(90.4, 100)

VAF Level	Exon	Amino Acid Change	Base Change	N	Percent Correct Call (%)	95% CI[1]
		p.Gly12Val	c.35G>T	36	36/36(100%)	(90.4, 100)
		p.Gly13Asp	c.38G>A	35	35/35(100%)	(90.1, 100)
	KRAS exon 2(all samples)			249	249/249(100%)	(98.5, 100)
	KRAS exon 3	p.Gln61His	c.183A>C	33	33/33(100%)	(89.6, 100)
	KRAS exon 4	p.Lys117Asn	c.351A>T	35	33/35(94.3%)	(81.4, 98.4)
		p.Ala146Thr	c.436G>A	36	36/36(100%)	(90.4, 100)
	KRAS exon 4(all samples)			71	69/71(97.2%)	(90.3, 99.2)
	KRAS(all exons)			353	351/353(99.4%)	(98.0, 99.8)
	NRAS exon 2	p.Gly12Asp	c.35G>A	31	31/31(100%)	(89.0, 100)
	NRAS exon 3	p.Gln61Arg	c.182A>G	33	33/33(100%)	(89.6, 100)
	NRAS exon 4	p.Lys117Asn	c.351G>T	35	35/35(100%)	(90.1, 100)
		p.Ala146Thr	c.436G>A	35	34/35(97.1%)	(85.5, 99.5)
	NRAS exon 4(all samples)			70	69/70(98.6%)	(92.3, 99.7)
	NRAS(all exons)			134	133/134(99.3%)	(95.9, 99.9)
	RAS(all exons)			487	484/487(99.4%)	(98.2, 99.8)
High	*KRAS* exon 2	p.Gly12Arg	c.34G>C	33	33/33(100%)	(89.6, 100)
		p.Gly12Ala	c.35G>C	36	36/36(100%)	(90.4, 100)
		p.Gly12Asp	c.35G>A	36	36/36(100%)	(90.4, 100)
		p.Gly12Val	c.35G>T	35	35/35(100%)	(90.1, 100)
		p.Gly13Asp	c.38G>A	36	36/36(100%)	(90.4, 100)
	KRAS exon 2(all samples)			176	176/176(100%)	(97.9, 100)
	KRAS exon 3	p.Gln61His	c.183A>C	35	35/35(100%)	(90.1, 100)
	KRAS exon 4	p.Lys117Asn	c.351A>T	36	36/36(100%)	(90.4, 100)
		p.Ala146Thr	c.436G>A	35	35/35(100%)	(90.1, 100)
	KRAS exon 4(all samples)			71	71/71(100%)	(94.9, 100)
	KRAS(all exons)			282	282/282(100%)	(98.7, 100)
	NRAS exon 2	p.Gly12Asp	c.35G>A	34	34/34(100%)	(89.8, 100)
	NRAS exon 3	p.Gln61Arg	c.182A>G	34	34/34(100%)	(89.8, 100)
	NRAS exon 4	p.Lys117Asn	c.351G>T	36	35/36(97.2%)	(85.8, 99.5)
		p.Ala146Thr	c.436G>A	36	36/36(100%)	(90.4, 100)
	NRAS exon 4(all samples)			72	71/72(98.6%)	(92.5, 99.8)
	NRAS(all exons)			140	139/140(99.3%)	(96.1, 99.9)
	RAS(all exons)			422	421/422(99.8%)	(98.7, 100)
Overall	**All panel mutations**			**905/909(99.6%)**		**(98.9, 99.8)**

[1] 95% two-sided confidence interval calculated using the Wilson score method.

The variance component analysis of mutation frequency results for each source of variation and total variation for the evaluable analysis set (for mutation frequency)were conducted and results are shown for each unique

mutant specimen/mutation frequency level sample, combining sites (Table 10).

Table 10. Variance Component Estimates for Individual Mutant Specimen and Mutation Level (Evaluable Analysis Set for Mutation Frequency)

Mutation Level	Exon	Amino Acid Change	Base Change	N	Mean Mutation Frequency	Site SD (%CV)	Operator (Site) SD (%CV)	Day(Site, Operator) SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
Low	*KRAS* exon 2	p.Gly12Cys	c.34G>T	36	0.080	0.003 (4.0)	0.003 (4.0)	0.000 (0.0)	0.009 (10.8)	0.010 (12.2)
		p.Gly12Arg	c.34G>C	36	0.078	0.020 (25.2)	0.004 (5.3)	0.005 (6.6)	0.008 (9.8)	0.022 (28.3)
		p.Gly12Ser	c.34G>A	35	0.080	0.005 (6.1)	0.000 (0.0)	0.000 (0.0)	0.011 (13.9)	0.012 (15.2)
		p.Gly12Ala	c.35G>C	35	0.072	0.000 (0.0)	0.000 (0.0)	0.010 (13.8)	0.013 (17.5)	0.016 (22.2)
		p.Gly12Asp	c.35G>A	36	0.072	0.000 (0.0)	0.000 (0.0)	0.002 (3.4)	0.013 (18.7)	0.014 (19.0)
		p.Gly12Val	c.35G>T	36	0.083	0.002 (3.0)	0.000 (0.0)	0.000 (0.0)	0.009 (11.1)	0.010 (11.5)
		p.Gly13Asp	c.38G>A	35	0.092	0.000 (0.0)	0.000 (0.0)	0.015 (16.7)	0.017 (18.9)	0.023 (25.3)
	KRAS exon 3	p.Gln61His	c.183A>C	33	0.086	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.011 (12.9)	0.011 (12.9)
	KRAS exon 4	p.Lys117Asn	c.351A>T	33	0.090	0.000 (0.0)	0.000 (0.0)	0.003 (3.1)	0.010 (10.7)	0.010 (11.2)
		p.Ala146Thr	c.436G>A	36	0.099	0.005 (4.8)	0.000 (0.0)	0.000 (0.0)	0.009 (9.4)	0.010 (10.6)
	NRAS exon 2	p.Gly12Asp	c.35G>A	31	0.078	0.002 (2.9)	0.000 (0.0)	0.000 (0.0)	0.009 (12.1)	0.010 (12.4)
	NRAS exon 3	p.Gln61Arg	c.182A>G	33	0.097	0.000 (0.0)	0.000 (0.0)	0.002 (1.8)	0.009 (9.5)	0.009 (9.6)
	NRAS exon 4	p.Lys117Asn	c.351G>T	35	0.086	0.001 (1.7)	0.002 (2.4)	0.003 (3.9)	0.007 (8.4)	0.008 (9.7)
		p.Ala146Thr	c.436G>A	35	0.080	0.006 (6.9)	0.000 (0.0)	0.000 (0.0)	0.016 (20.5)	0.017 (21.7)
High	*KRAS* exon 2	p.Gly12Arg	c.34G>C	33	0.230	0.047 (20.4)	0.004 (1.7)	0.000 (0.0)	0.019 (8.1)	0.051 (22.1)
		p.Gly12Ala	c.35G>C	36	0.281	0.009 (3.3)	0.000 (0.0)	0.008 (2.9)	0.013 (4.7)	0.018 (6.5)
		p.Gly12Asp	c.35G>A	36	0.259	0.000 (0.0)	0.000 (0.0)	0.005 (2.0)	0.015 (5.8)	0.016 (6.2)
		p.Gly12Val	c.35G>T	35	0.206	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.020 (9.9)	0.020 (9.9)
	KRAS exon 2	p.Gly13Asp	c.38G>A	36	0.263	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.024 (9.3)	0.024 (9.3)
	KRAS exon 3	p.Gln61His	c.183A>C	35	0.259	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.013 (5.0)	0.013 (5.0)

Mutation Level	Exon	Amino Acid Change	Base Change	N	Mean Mutation Frequency	Site SD (%CV)	Operator (Site) SD (%CV)	Day(Site, Operator) SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
	KRAS exon 4	p.Lys117 Asn	c.351A>T	36	0.267	0.008 (3.0)	0.007 (2.6)	0.003 (1.2)	0.014 (5.3)	0.018 (6.7)
		p.Ala146 Thr	c.436G>A	35	0.277	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.015 (5.6)	0.015 (5.6)
	NRAS exon 2	p.Gly12 Asp	c.35G>A	34	0.274	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.013 (4.8)	0.013 (4.8)
	NRAS exon 3	p.Gln61 Arg	c.182A>G	34	0.281	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.012 (4.3)	0.012 (4.3)
	NRAS exon 4	p.Lys117 Asn	c.351G>T	36	0.258	0.010 (3.9)	0.010 (3.7)	0.012 (4.5)	0.045 (17.3)	0.048 (18.7)
		p.Ala146 Thr	c.436G>A	36	0.258	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)	0.014 (5.6)	0.014 (5.6)

b. Lot-to-lot Precision:

A Lot-to-Lot Precision study was conducted to evaluate the performance of the Praxis Extended RAS Panel across manufactured reagent kit lots. Three (3) reagent kit lots were tested using a panel of 5 blended CRC FFPE specimens. Each CRC FFPE specimen contained 2 unique representative RAS mutations: one at a low (approximately 8%) mutation frequency level and one at a high (approximately 14%) mutation frequency level. Twelve (12) observations of each of the 5 specimen mixes were collected over 3 nonconsecutive days on 2 instruments with 2 replicates (3 days x 2 instruments x 2 replicates=12 observations per mutation frequency level and reagent lot). Across all lots and days, 99.7% (359/360) of observations showed the expected Extended RAS Panel mutation result. The mutation p.Gln61Leu (c.182A>T) in NRAS exon 3 was called wrong at low allele frequency in the specimen mix 1. A variance component analysis was performed for each of the mutations/mutation frequency levels to estimate the variability of the system including kit lot, day, and sequencing run. The total standard deviations of mutation allele frequency ranged from 0.011 to 0.029. The reagent lot component of the total standard deviation of mutation allele frequency ranged from 0 to 0.015.

4. **FFPE Cell Line Equivalency to Clinical Samples (Contrived Sample Functional Characterization Study)**

To support the use of cell lines as an alternative for difficult to acquire clinical samples that are required for some analytical validation studies, contrived sample functional characterization study was conducted by comparing the performance of Praxis Extended RAS Panel for detecting variants in low mutant allele frequency (AF) level clinical FFPE specimens and cell line FFPE specimens.

Two FFPE blended sample mixes each containing two common RAS panel mutations were created. Two cell line blended sample mixes each containing two

common RAS panel mutations matching those of the FFPE sample mixes were created. The mutation frequencies at each level were estimated using an analytically validated comparator method. Each sample mix was prepared to target the following mutation frequency levels: 10%, 5%, 2.5%, and 1.25%. The target delta Cq was 4.5 +/- 0.5 (the lower level of DNA input) and determined prior to the study. Each specimen was tested by 5 operators for 2 days each, with each specimen run in duplicate. Two reagent lots were used and there were 40 results overall for each level of each sample mix.

For each variant, the average AF associated with each test blend was computed per lot, and the corresponding proportion of correct calls (hit rate) was also determined for clinical sample and cell lines separately. Separate regression analyses were fitted for each mutation for each sample type, with the observed mutation frequencies generated by NGS as the dependent variable (Y-axis) and mutation frequencies obtained by the comparator method as the independent variable (X-axis). The regression coefficients were compared between clinical samples and cell lines, with the differences of intercepts between two samples types ranging from -0.0395 of two-sided 95% CI of (-0.0550, -0.0234) to 0.0098 (-0.0071, 0.0268) and slopes ranging from -0.1140 (-0.1977, -0.0298) to 0.1541 (0.0779, 0.2275).

The predicted estimates of three levels (at clinical cut-off, around C75, between C75 and C95, where C75 and C95 refer to the AF levels corresponding to hit rates of 75% and 95%, respectively) were computed from the regression models fitted above for each sample type (clinical and cell line). In addition, the differences of the predicted estimates of those three levels between the cell lines and the clinical samples as well as the corresponding two-sided 95% confidence intervals using bootstrap method were also calculated. The results show that the differences of the predicted estimates of those three levels between the cell lines and the clinical sample for any of the variants under investigation are small and are clinically acceptable. The results demonstrate that performance with cell lines is comparable to clinical specimens.

5. **FFPE Extraction Method Equivalency Study**
Since extraction kits are not included in the Praxis Extended RAS Panel kit, a study evaluating performance of three commercially available FFPE tissue extraction kits was conducted.

Ten FFPE CRC tissue sample were used in the study, including 8 prevalent mutations and 2 wild types. Genomic DNA was extracted using each of the 3 commercially available FFPE extraction kits. Results were verified by Sanger sequencing. Each specimen was run in duplicate.

The sample first pass rate was 100% (20/20) for each of the three FFPE DNA extraction kits. A total of 1120 results were obtained (10 specimens x 56 possible results x 2) The overall percent agreement was 100% (1120/1120) for each of the

three FFPE DNA extraction kits. The positive percent agreement was 100% (16/16) and negative percent agreement was 100% (1104/1104) for each of the three FFPE DNA extraction kits. The dCq and allele frequencies for each method are shown in the Table 11 below. DNA from 2 extractions for the same sample per kit were combined to get technical replicates for the next step of library preparation. The combined extracted nucleic acid was split up into 2 library preparations to ensure tumor heterogeneity was not a variable that contributed to assessment of extraction method. The Results demonstrate that the 3 methods yield DNA with comparable quality and quantity to generate reliable results.

Table 11. dCq and allele frequency results from different FFPE DNA extraction methods

Sample Number	Gene/ Exon	Amino Acid	Mutation	dCq			Allele Frequency					
				Method1	Method2	Method3	Method1		Method2		Method3	
							Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
DNA 1	KRAS Exon 2	p.Gly12 Asp	c.35G>A	0.01	0.48	0.28	0.327	0.340	0.327	0.330	0.354	0.350
DNA 2	KRAS Exon 2	p.Gly12 Cys	c.34G>T	0.6	0.41	-0.02	0.273	0.288	0.279	0.302	0.310	0.319
DNA 3	Wild Type			1.68	1.9	1.56	N/A	N/A	N/A	N/A	N/A	N/A
DNA 4	Wild Type			2.14	2.18	1.99	N/A	N/A	N/A	N/A	N/A	N/A
DNA 5	KRAS Exon 4	p.Ala146 Thr	c.436G>A	2.54	1.61	1.3	0.579	0.587	0.552	0.564	0.588	0.584
DNA 6	KRAS Exon 3	p.Gln61 His	c.183A>C	-0.12	0.73	0.21	0.253	0.252	0.266	0.268	0.266	0.262
DNA 7	KRAS Exon 2	p.Gly12 Ala	c.35G>C	0.06	-0.04	-0.44	0.300	0.290	0.264	0.271	0.291	0.284
DNA 8	KRAS Exon 2	p.Gly12 Asp	c.35G>A	0.76	0.65	0.82	0.400	0.389	0.366	0.375	0.408	0.406
DNA 9	KRAS Exon 2	p.Gly12 Val	c.35G>T	-0.43	-0.14	-0.59	0.314	0.302	0.282	0.284	0.304	0.306
DNA 10	KRAS Exon 2	p.Gly13 Asp	c.38G>A	0.46	-0.08	0.36	0.409	0.402	0.409	0.405	0.407	0.407

6. **PCR Thermocycler System Comparison**

Since PCR thermocycler instrument is not included as part of the system, this study evaluated performance of three commercially available PCR thermocyclers used during the PCR amplification step.

Three commercially available and commonly used brands of PCR thermocyclers were evaluated. Ten genomic DNA from FFPE tissue with duplicates were used in the study, including 7 prevalent mutations and 3 wild types. Three sequencers were used. OPA, PPA, NPA were calculated. Mutation frequencies were analyzed to demonstrate whether there is significant variation made by PCR thermocycler. The genotypes of ten specimens across the loci of interest in NRAS and KRAS was verified by bidirectional Sanger sequencing.

The sample first pass rate was 100% (20/20) for each of the three thermocyclers. The overall percent agreement was 100% (1120/1120) for each of the three thermocyclers. The positive percent agreement was 100% (14/14) and negative percent agreement was 100% (1106/1106) for each of the three thermocyclers.

7. **qPCR System Comparison (DNA Qualification)**
The Praxis Extended RAS panel kit includes reagents for sample qualification (Quality Control Primers, Quality Control Template, and qPCR Master Mix) but a qPCR system is not specified. Therefore a study demonstrating the concordance of the dCq values obtained with each qPCR method was conducted.

A total of 4 commercially available qPCR instruments were used to test 20 specimens of gDNA from FFPE tissue with pre-characterized dCq (18 with dCq below and 2 above sample qualification cutoff requirement). The number of correct sample qualification judgments for all qPCR system were 60/60; 100% point estimate with 94.0% lower confidence bound (two-tailed). The delta Cq values measured by each qPCR system for each sample are shown in Figure 1. Each instrument is represented by a different color in the Figure.

Figure 1. Evaluation of delta Cq values by Sample and qPCR systems

8. **Sample Carry-over**
The Sample Carryover study was conducted to evaluate whether false positive results due to carryover from well to well contamination during sample library preparation, as well as run to run contamination between consecutive sequencing runs, occur in less than or equal to 1% of test events, and have no negative impact on test results. The sample panel consisted of 4 genomic DNA samples from cell lines each containing different panel mutations in the KRAS and NRAS genes. The specimens were qualified to a high DNA input level (dCq < 1) or a low DNA input level (dCq > 2). Well-to-well (within-run) carryover and run to run (between run) carryover were evaluated in this study.

a. Well-to-well carryover:
Well to well carryover is defined as a failure mode potentially created by manual processing steps (pipetting, sample mix-up etc.). In order to evaluate carryover from one sample well to another, two test runs were performed. 1) A checkerboard layout of a high input genomic DNA (gDNA) sample containing a KRAS mutation (KRAS1 high) alternating with a NRAS mutant sample of low gDNA input (NRAS1 low) referred to as Plate AA was run. 2) A checkerboard

layout of a high input genomic DNA (gDNA) sample containing a NRAS mutation (NRAS2 high) alternating with a KRAS mutant samples of low gDNA input (KRAS2 low) referred to as Plate BB was run. Each test run contained 11 samples plus one blank for a total of 12 test events per run, and 24 total test events for well to well carryover. Any KRAS mutation reported in a well designated as an NRAS mutant sample, and vice versa, would illustrate carryover.

b. Run-to-Run Carryover:

Run to run carryover is defined as a failure mode potentially created by residue from a previous sequencing run. In order to determine if there is carryover between sequencing runs, two plates each containing 11 replicates of a single unique sample of high DNA input plus a blank sample were prepared and sequenced consecutively on one MiSeqDx instrument. The first plate contained 11 replicates of an NRAS mutant sample (NRAS 2 high) plus one blank referred to Plate CC. The second plate contained 11 replicates of a KRAS mutant sample (KRAS2 high) plus one blank referred to as Plate DD. The NRAS mutant sample library (CC) was sequenced first followed by a subsequent sequencing run with the KRAS mutant sample library (DD), followed by another repeat sequencing run of the NRAS mutant sample libraries (CC2). If any NRAS mutations are observed in a KRAS mutant- only run, and vice versa, this would indicate carryover.

Zero false positives due to well to well (0/24, 0%) and run to run (0/24, 0%) carryover were reported. All expected mutations were detected.

9. **Specimen Handling**

The study focused on temperature storage and freeze thaw of DNA extracted from CRC FFPE tissue and the storage conditions' effect on Praxis Extended RAS Panel Assay performance. A 20-specimen panel of gDNA extracted from FFPE samples was tested in a variety of temperature and freeze-thaw (F/T) conditions. The specimens consisted of both wild type samples and those containing prevalent Praxis Extended RAS Panel mutations.

The specimen handling conditions tested (in addition to a time zero condition as a study control) were:

1. Storage at 4°C (+/- 4°C) for 21 days, 28 days, and an additional time point of a range of days (36, 49, 51) tested together
2. Storage at -20°C (+/- 6°C) for 62 days, 76 days, an additional time point of a range of days (161, 173, 175) tested together, and an additional time point of a range of days (166, 178, 180) tested together
3. 2x and 3x freeze/thaw (F/T)

Each condition evaluated in this study used the defined 20 CRC FFPE tissue sample panel. The genotype of the 20 specimens across the NRAS and KRAS loci of interest was verified by bidirectional Sanger sequencing. Agreements (PPA, NPA, OPA) were calculated by comparing testing condition to time zero control.

For each condition and time point, the PPA, NPA and OPA are 100% (16/16, 1104/1104 and 1120/1120) for all storage and handling conditions at 4°C, -20°C, and freeze/thaws. The temperature and storage conditions thus being recommended in product labeling for extracted gDNA prior to testing are as follows: 28 days at 4°C, 161 days at -20°C, and 2x freeze/thaw.

10. **Guard Banding Studies**

The Praxis Extended RAS Panel workflow guard banding studies evaluated selected workflow steps of Sample Qualification, Library Preparation, and Sequencing. Test samples included FFPE genomic DNA and cell line genomic DNA to increase mutation diversity The Library preparation conditions were tested at the maximum multiplexing level of the assay at 10 specim ens per run.

a. Sample Qualification (qPCR step):

Five genomic DNA samples were tested at various storage times of qPCR master mix and reaction mix. Since both the qPCR master mix and reaction mix are used right away, the testing conditions included holding the qPCR master mix at room temperature for 1 hour in ambient light, and the reaction mix for 10 minutes on ice. Average dCq differences of less than 0.25 were observed indicating stability of the qPCR master mix and reaction mixes.

b. Library Preparation:

The robustness of the various library preparation steps were evaluated and ranged from 90-100% accuracy demonstrating the assay robustness.

Condition	Test	Valid Results
Hybridization and Extension-Ligation incubation times and temperatures	+/- 5% of the specified value	100%
Concentration and incubation times for sodium hydroxide removal of the extension-ligation product from the filter plate during PCR amplification.	+/- 5% of the specified value	90% of specimens tested at low sodium hydroxide concentration and 100% of specimen tested at all other conditions
Storage times of PCR working solution, and hold times of the PCR plate (post-amplification)	+/- 5% of the specified value	100%
PCR clean-up and Library Normalization steps (Incubation, shakin times, concentrations of	+/- 5% of the specified value:	90% of specimens tested for the longer PCR clean up elution incubation; 100% of specimens

ethanol and Sodium hydroxide solution)		tested at all other conditions
For the Library Pooling step, times for heat denaturation and cooling	+/- 5% of the specified value	90% of specimens tested at the longer cooling, and 100% of specimens tested at all other heat denaturation and cooling conditions
DAL (Diluted Amplicon Library) storage	tested up to 84 days (stored at -20°C)	100%

c. Sequencing:

Robustness of deep sequencing is achieved with a minimum sequencing depth requirement for the samples to be called "valid". A total of 40 specimens plus controls were tested by 2 operators for meeting the minimum coverage of 1800X across both pools (900x per pool) at the maximum multiplexing level of the assay at 10 specimens per run. All specimens met the minimum coverage requirement with an average coverage of approximately 31000x across both pools.

11. **Stability Studies**

a. Open Tube Stability:

This study was designed to test reagent function and detect reagent failures after being opened and put into use under standard operating conditions (opened tube, freeze/thaw) specified in the Praxis Extended RAS Panel Package Insert. Six kits from one reagent lot were tested. A freeze/thaw condition was considered as passing if the test results for all three kits met the functional test acceptance criteria after open tube/freeze thaw use. The claimed open tube condition was one F/T condition previous to the last passing F/T condition.

All reagent quality control acceptance criteria were met for all kits tested for the execution control, 5X freeze/thaw and 6X freeze/thaw. The claimed open tube condition is one freeze/thaw condition previous to the last passing condition, in this case, up to 5X freeze/thaws for frozen reagents for 2 uses of the kit. Analysis of mutation frequencies showed no significant difference between 5X or 6X freeze thaw and the execution control indicating that mutation frequencies are stable after 2 uses and multiple freeze/thaws.

b. Real-Time Stability:

To establish the shelf life of the Praxis Extended RAS Panel kit, the study evaluated the product throughout the proposed shelf life when stored according to label conditions. Three Praxis Extended RAS Panel kit lots were used utilizing the stability study design described in CLSI EP25-A. The kits were stored in its final kitted configuration for the duration of the study, at

storage conditions defined on the product label. Frozen kit components were stored at -15◦C to -25◦C. Refrigerated kit components were stored at 2◦C to 8◦C. Room-temperature components were stored at 15◦C to 30◦C. Real-time testing occurs at Month 0, 3, 6, 9, 12, 13, 15, 16 and 18. At each test time-point, a sample of product is removed from storage and tested in triplicate.

Three cell line specimens containing highly prevalent RAS mutations (2 in KRAS exon 2, 1 in NRAS exon 2) were run in duplicate. Four replicates of a wild type DNA sample were also run. A time point was considered as passing if the test results for all three kit lots met the functional test acceptance criteria. The shelf life for the product will be one time point previous to the last passing time point where all three lots of reagents meet the acceptance criteria. For shelf life determinations, the age of the kit will be the number of days from the date of manufacture of the youngest tube component to the test date.

All three kit lots met all functional test acceptance criteria for Real Time Stability Study for both the time 0 and the 3 month time point.

c. Shipping Stability:

A summer and winter profile was used to test all reagents. One reagent lot was used in this study. Products which were shipped frozen and refrigerated were shipped in insulated containers. Atmospheric thermal cycling was conducted on packaged products to assess the effects of external temperature on product functionality. Three cell line specimens containing highly prevalent RAS mutations (2 in KRAS exon 2, 1 in NRAS exon 2) were run in duplicate. Four replicates of a wild type DNA sample were also run.

Functional testing was performed after simulated distribution and thermal profiling of product. Specimen are considered valid by presence of PCR product and by software (coverage 2400x and call rate). All test cases passed the shipping stability study for thermal cycling testing and functional testing.

B. Animal Studies

Not Applicable

X. SUMMARY OF CLINICAL STUDY

The safety and effectiveness of the Praxis Extended RAS Panel was evaluated in a retrospective study designed to demonstrate that the Praxis Extended RAS Panel correctly detects the presence of 56 RAS mutants in CRC patients for the purpose of clinically validating the use of the test companion diagnostic test for panitumumab (Vectibix®). Patients without KRAS mutations may benefit from treatment with panitumumab. Banked FFPE colorectal cancer tissue samples collected during the Amgen panitumumab study (protocol no. 20050203 [0203 study]) were tested with the

Praxis Extended RAS Panel. The 0203 study assessed the safety and effectiveness of panitumumab plus FOLFOX in a multicenter, prospective, open-label, randomized phase 3 study that enrolled 1183 subjects with previously untreated mCRC. The first subject was enrolled August 2006. The study was closed to randomization September 2008.

Testing of specimens with Praxis Extended RAS Panel testing occurred over multiple instrument runs spanning approximately 2 months (12 Feb 2016.to 10 Apr 2016). Two testing sites evaluated the samples: Molecular Pathology Laboratory Network, Inc, Geneuity Clinical Research Services (MPLN) for tissue processing, and Expressions Analysis, Q2 Solutions – EA Genomics for Praxis Extended RAS Panel sequencing. Sanger sequencing was conducted by Transgenomics, Inc.

A. Study Design

The study objective was to assess the clinical effectiveness of the Praxis Extended RAS Panel in aiding in the identification of subjects eligible for treatment with panitumumab. Clinical validity was assessed by evaluating the treatment effect of panitumumab plus FOLFOX relative to FOLFOX alone on progression-free survival (PFS) and overall survival (OS) in wild-type RAS subjects (as determined by the Praxis Extended RAS Panel). Primary clinical validation analyses were performed in subjects with specimens with dCq values ≤ 5.0. Additional analyses were also performed.

1. Specimen Inclusion and Exclusion Criteria:

   Inclusion Criteria

   - Sample(s) collected as part of the 0203 study.
   - Samples that met the tissue requirements in the Praxis Extended RAS Panel investigational package insert.
   - Minimum Tissue Area: Minimum total cumulative tissue area of 80 mm^2 with ≥50% neoplastic content by area. Note: 240 mm^2 with ≥50% neoplastic content by area was recommended for optimal results.
   - Sections that had <50% neoplastic content by area could be enriched by macrodissecting the sections to obtain ≥50% neoplastic content by area. To maximize total tissue area captured per slide, macrodissecting to obtain 50% neoplastic content by area was recommended. For optimal yield of amplifiable DNA, using at least 8 µm x 5 µm sections or equivalent alternate thickness serial sections was recommended. Note: If macrodissection was performed, the macrodissected tissue area was used to determine the cumulative tissue area.

   Exclusion Criteria

   Samples that failed to meet the inclusion criteria were excluded from the study.

2. Follow-up Schedule:

   Not Applicable.

3. Clinical Endpoints:

Primary study endpoints for subjects in the efficacy analysis sets were progression-free survival (PFS) and overall survival (OS). The primary analyses evaluated whether there was an improvement in PFS and OS in subjects treated with panitumumab plus FOLFOX versus FOLFOX alone in the wild-type evaluable RAS efficacy analysis set. Analyses were performed using other efficacy analysis sets; these evaluations were descriptive. Time-to-event variables were summarized with stratified log-rank tests, hazard ratios (HRs), Kaplan-Meier (KM) curves, KM estimates of median survival time, the number of subjects censored, and the number of subjects with events (disease progression or death for PFS and death for OS).

B. <u>Accountability of PMA Cohort</u>

Of the 1183 subjects, 983 subjects had FFPE tissue samples available and potentially eligible for testing. There were 824 subjects who had samples that met the tissue requirements, had DNA extracted, and were processed and tested with Praxis Extended RAS Panel reagents. Of the 824 subjects, 567 had sample delta cycle quantification threshold (dCq) values ≤5.0 (i..e, 31.2% of specimens failed the DNA quality criteria to proceed with the assay). Of these, 528 subjects had a valid RAS result by the Praxis Extended RAS Panel and were evaluable for the primary analyses of subjects with dCq values ≤ 5.0 (i.e., 6.9% assay invalid rate). Regardless of dCq value, there were 722 subjects with valid RAS results by the Praxis Extended RAS Panel. Figures 2-4 show accounting of subjects with samples tested with Praxis Extended RAS Panel.

Figure 2. Accounting of subjects with samples potentially eligible for study testing

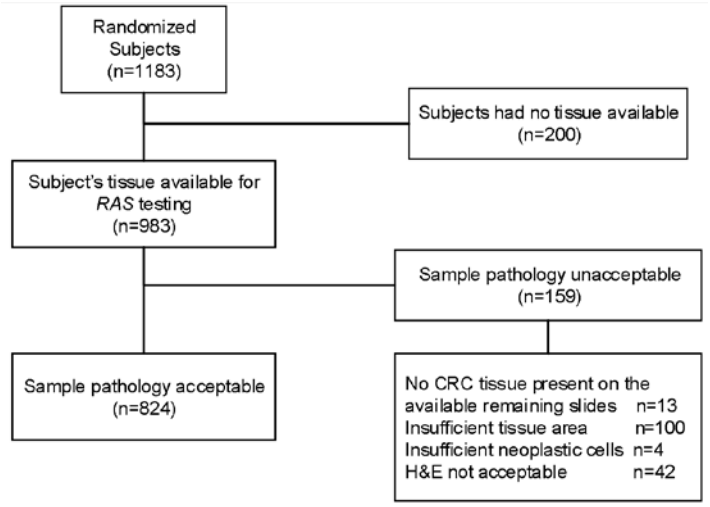
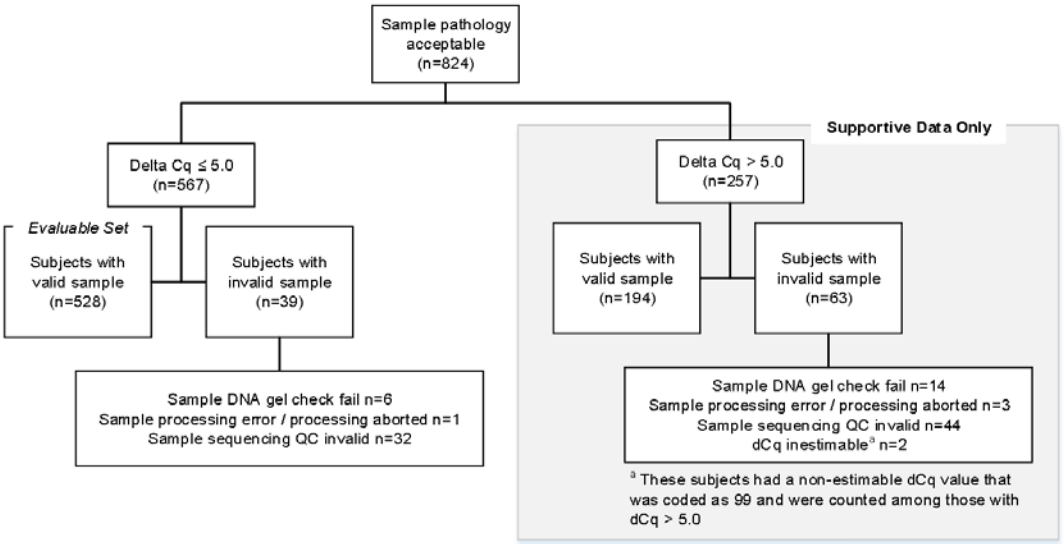


Figure 3 Accounting of Praxis™ Extended RAS Panel results



The evaluable RAS efficacy analysis set was divided into the following subgroups based on the Praxis Extended RAS Panel.

- Wild-type RAS,
- Wild-type KRAS Exon 2 Mutant RAS, and
- Mutant KRAS Exon 2 analysis sets.

The Mutant RAS Efficacy analysis set is the combination of the Wild-type KRAS Exon 2 Mutant RAS Efficacy analysis set and the Mutant KRAS Exon 2 Efficacy analysis set. The Wild-type KRAS Exon 2 Efficacy analysis set is the combination of the Wild-type RAS Efficacy analysis set and the Wild-type KRAS Exon 2 Mutant RAS Efficacy analysis set. The Wild-type KRAS Exon 2 Mutant RAS Efficacy analysis set represents subjects with a mutation detected with the Praxis Extended RAS Panel but not by a KRAS Exon 2 panel. Figure 4 shows an accounting of subjects and RAS results in the Evaluable RAS Efficacy analysis set with dCq values ≤5.0.

Figure 4. Subject Accountability and RAS Results by the Praxis™ Extended RAS Panel for the Evaluable RAS Efficacy Analysis Set, dCq ≤ 5.0

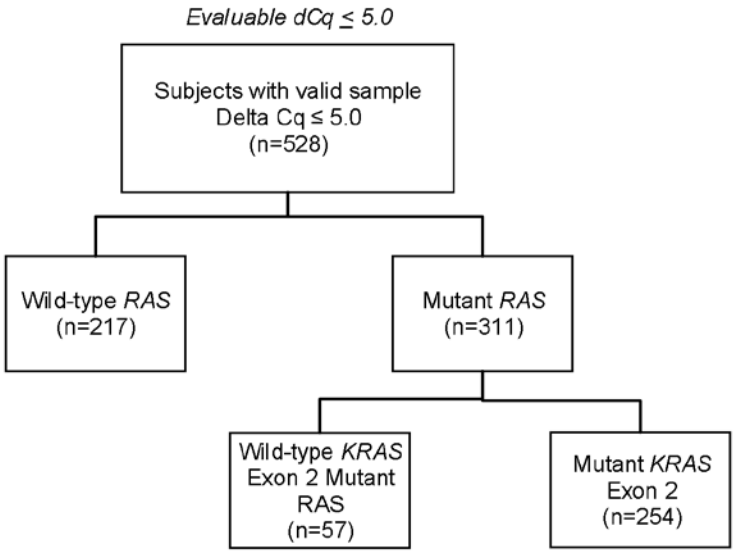


Table 12 shows an accounting of the samples from the 1183 randomized subjects by treatment group. There were 593 subjects (50%) that were randomized to panitumumab plus FOLFOX group and 590 subjects (50%) that were randomized to FOLFOX alone group. The percent of subjects with evaluable results was similar for the panitumumab plus FOLFOX group (269/593, 45%) and the FOLFOX alone group (259/590, 44%). The most common reason (approximately 40%) for an unevaluable outcome was dCq > 5.0.

Table 12. Number of Subjects With Evaluable or Unevaluable *RAS* Results and Reasons for Unevaluability Stratified by Treatment Group

Intent-to-treat Analysis Set	Panitumumab plus FOLFOX	FOLFOX alone (n=590	Total (n=1183)
Total Evaluable *RAS* Efficacy Analysis Set	269	259	528
Total Unevaluable *RAS* Efficacy Analysis Set	324	331	655
Subjects had no tissue available [a] – n (%)	84 (26)[b]	116 (35)	200 (31)
Unacceptable specimen - no CRC tissue on	7 (2)	6 (2)	13 (2)
Insufficient tissue area – n (%)	57 (18)	43 (13)	100 (15)
Insufficient % neoplastic cell content – n	2 (1)	2 (1)	4 (1)
H&E not evaluable – n (%)	28 (9)	14 (4)	42 (6)
Delta Cq > 5.0 – n (%)	129 (40)	128 (39)	257 (39)
Sample DNA gel check failure – n (%)	4 (1)	2 (1)	6 (1)
Sample processing error / processing aborted	1 (<1)	0 (<1)	1 (<1)
Sample sequencing QC invalid – n (%)	12 (4)	20 (6)	32 (5)

[a] Source table describes subjects with no tissue available as "Subjects had no plan for testing of RAS".
[b] Percent based on total unevaluable.

Of the 528 evaluable subjects with dCq values ≤5.0 and valid RAS results with the Praxis Extended RAS Panel, 5 subjects did not receive at least 1 dose of panitumumab or chemotherapy. The remaining 523 of 528 subjects were included in the safety analysis and analyzed according to the treatment received (eg, subjects who received at least 1 dose of panitumumab were included in the panitumumab plus FOLFOX group for safety analyses, regardless of randomization).

Table 13 shows dataset by RAS gene/exon status and treatment group.

Table 13. Number of Subjects by Evaluable *RAS* Gene/Exon Status and Treatment Group for Efficacy Analysis Sets

***KRAS* Exon 2**	***KRAS* Exon 3**	***KRAS* Exon 4**	***NRAS* Exon 2**	***NRAS* Exon 3**	***NRAS* Exon 4** [a]	**Panitumumab plus FOLFOX (n=269)**	**FOLFOX alone (n=259)**	**Total (n=528)**	**Total by *RAS* Status (n=528)**	**Total by *KRAS* Exon 2 Status and *RAS* Status (n=528)**	**Total by *KRAS* Exon 2 Status (n=528)**
						n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
						106 (39)	111 (43)	217 (41)	Wild-type *RAS* 217 (41)	Wild-type *RAS* 217 (41)	Wild-type *KRAS* Exon 2 274 (52)
				Mut		9 (3)	8 (3)	17 (3)	Mutant *RAS* 311 (59)	Wild-Type *KRAS* Exon 2 Mutant *RAS* 57 (11)	
			Mut			5 (2)	3 (1)	8 (2)			
		Mut				11 (4)	8 (3)	19 (4)			
	Mut					5 (2)	8 (3)	13 (2)			
Mut						133 (49)	119 (46)	252 (48)		Mutant *KRAS* Exon 2 254 (48)	Mutant KRAS Exon 2 254 (48)
Mut			Mut			0 (0)	1 (<1)	1 (<1)			
Mut		Mut				0 (0)	1 (<1)	1 (<1)			

Mut=mutant

[a] No mutations of *NRAS* Exon 4 observed

C. Study Population Demographics and Baseline Parameters

Subject demographics for the Wild-type and Mutant RAS Efficacy analysis sets are provided in Table 14 along with the Wild-type KRAS Exon 2 Mutant RAS and Unevaluable RAS Efficacy analysis sets. Gender, race/ethnicity, and BSA characteristics were nominally similar between groups.

Table 14. Subject Demographics for Efficacy Analysis Sets

	Wild-type *RAS*		**Mutant *RAS***		**Wild-type *KRAS* Exon 2 Mutant *RAS***		**Unevaluable *RAS***	
	Panitumumab + FOLFOX (n=106)	**FOLFOX alone (n=111)**	**Panitumumab + FOLFOX (n=163)**	**FOLFOX alone (n=148)**	**Panitumumab + FOLFOX (n=30)**	**FOLFOX alone (n=27)**	**Panitumumab + FOLFOX (n=324)**	**FOLFOX alone (n=331)**
Demographics								
Country and Geographic Region (Western Europe, Canada, Australia)[a] – n (%)	63 (59)	70 (63)	101 (62)	84 (57)	21 (70)	13 (48)	165 (51)	172 (52)
Female – n (%)	29 (27)	51 (46)	53 (33)	62 (42)	7 (23)	13 (48)	125 (39)	119 (36)
Race/ethnicity –								

White or Caucasian	95 (90)	102 (92)	148 (91)	135 (91)	28 (93)	26 (96)	287 (89)	303 (92)
Black or African American	2 (2)	5 (5)	3 (2)	5 (3)	0 (0)	1 (4)	3 (1)	5 (2)
Hispanic or Latino	7 (7)	3 (3)	9 (6)	4 (3)	1 (3)	0 (0)	26 (8)	19 (6)
Asian	2 (2)	1 (1)	1 (1)	3 (2)	0 (0)	0 (0)	5 (2)	0 (0)
Other	0 (0)	0 (0)	2 (1)	1 (1)	1 (3)	0 (0)	3 (1)	4 (1)
Age – mean (SD)	61.3 (10.5)	59.6 (11.6)	63.1 (10.1)	60.3 (10.7)	63.6 (11.4)	61.4 (10.8)	60.7 (9.8)	60.6 (11.0)
Baseline weight (kg)–mean (SD)	76.01 (17.13)	72.25 (16.77)	73.56 (15.12)	70.32 (14.73)	73.96 (12.44)	66.94 (12.87)	72.31 (15.15)	73.63 (16.72)
Baseline height (cm)–mean (SD)	168.9 (9.2)	167.7 (9.1)	167.8 (8.5)	167.1 (9.9)	168.3 (10.3)	166.2 (10.6)	167.0 (9.9)	167.9 (9.6)
Baseline BSA (m^2)–mean (SD)	1.88 (0.24)	1.82 (0.24)	1.84 (0.22)	1.80 (0.22)	1.85 (0.20)	1.75 (0.20)	1.82 (0.23)	1.84 (0.24)

BSA = Body surface area
a Versus rest of the world.
Percentages are based upon the number of subjects randomized.
The data cut-off date for this analysis was 28 August 2009.

Subject baseline disease characteristics for the Wild-type and Mutant RAS Efficacy analysis sets are provided in Table 15 along with the Wild-type KRAS Exon 2 Mutant RAS and Unevaluable RAS Efficacy analysis sets.

Table 15. Baseline Disease Characteristics for Efficacy Analysis Sets

	Wild-type *RAS*		**Mutant *RAS***		**Wild-type *KRAS* Exon 2 Mutant *RAS***		**Unevaluable *RAS***	
	Panitumumab+ FOLFOX (n=106)	**FOLFOX alone (n=111)**	**Panitumumab+ FOLFOX (n=163)**	**FOLFOX alone (n=148)**	**Panitumumab+ FOLFOX (n=30)**	**FOLFOX alone (n=27)**	**Panitumumab+ FOLFOX (n=324)**	**FOLFOX alone (n=331)**
Baseline Disease Characteristics								
Primary tumor type								
Colon cancer – n (%)	78 (74)	72 (65)	117 (72)	115 (78)	20 (67)	17 (63)	199 (61)	211 (64)
Rectal cancer – n (%)	28 (26)	39 (35)	46 (28)	33 (22)	10 (33)	10 (37)	125 (39)	120 (36)
Adenocarcinoma differential of primary tumor well or moderately differentiated – n (%)	71 (67)	70 (63)	123 (76)	109 (74)	28 (93)	18 (67)	219 (68)	223 (67)
Months since primary diagnosis[a] – mean (SD)	10.0 (18.1)	7.7 (14.3)	8.7 (15.4)	7.4 (15.0)	6.5 (10.3)	7.2 (11.9)	9.4 (20.3)	10.4 (18.4)
Months since metastatic disease diagnosis[a] – mean (SD)	2.8 (4.8)	2.1 (1.9)	2.1 (2.1)	2.6 (6.5)	1.7 (0.9)	1.6 (1.1)	2.1 (4.7)	2.2 (3.8)
Number of sites of metastatic disease ≥ 3 – n (%)	39 (37)	47 (42)	74 (45)	66 (45)	14 (47)	14 (52)	166 (51)	150 (45)

SD = standard deviation

[a] Date of randomization minus date of primary diagnosis or metastatic disease diagnosis. Percentages are based upon the number of subjects randomized.
The data cut-off date for this analysis was 28 August 2009.

D. Safety and Effectiveness Results

1. Safety Results

   No clinically relevant changes in the safety profile of panitumumab were observed in the clinical validation study. The most relevant sub population for analysis is the wild-type RAS subjects who will be identified as candidates for treatment with panitumumab plus FOLFOX based on Praxis Extended RAS Panel results.

   To determine if the 2 different approaches (RAS status vs KRAS exon 2 status) for selecting candidates for panitumumab therapy had any effect on safety outcomes, AEs in the 2 subpopulations were compared. In subjects treated with panitumumab plus FOLFOX, the incidence of adverse events (AEs) was similar in wild-type RAS subjects and wild-type KRAS exon 2 subjects. Serious AEs were observed in 44% of wild-type RAS subjects and in 43% of wild-type KRAS exon 2 subjects. AEs leading to permanent discontinuation of study drug were observed in 24% of wild-type RAS and 25% in wild-type KRAS exon 2 subjects.

2. Effectiveness Results

   Efficacy analysis sets are summarized in Tables 16-17 including only samples with acceptable, amplifiable DNA (dCq value ≤ 5.0 per the package insert). The Praxis Extended RAS panel reports results as "Panel Mutation Detected", "Panel Mutation Not Detected", and "Invalid". The latest cutoff dates (PFS: August 2, 2010 and OS: January 24, 2013) were applied to analyze the data. Hazard ratio (HR) (panitumumab plus FOLFOX:FOLFOX alone) were calculated. The Kaplan-Meier plots of PFS and OS based on the latest cutoff date for Wild-type *RAS* Efficacy Analysis set were shown in Figures 6-7 below.

   PFS:

   For subjects with wild-type RAS, the hazard ratio (HR) for PFS from a stratified Cox proportional hazards model was 0.700 (95% CI: 0.516 to 0.948). The stratified log-rank test revealed a statistically significant difference in PFS between the panitumumab plus FOLFOX group versus the FOLFOX alone group (P =0.0206).

   For subjects with mutant RAS, the HR for PFS was 1.242 (95% CI: 0.976 to 1.582). HR results for mutant KRAS exon 2 and wild-type KRAS exon 2 mutant RAS subjects also showed HRs > 1.0.

Table 16. Primary Efficacy Parameters – PFS for Wild Type RAS and Mutant RAS

	Wild Type RAS		Mutant RAS	
Parameter	**Pmab + FOLFOX (n=106)**	**FOLFOX Alone (n=111)**	**Pmab + FOLFOX (n=163)**	**FOLFOX Alone (n=148)**
Events n (%)	86 (81)	93 (84)	147 (90)	133 (90)
Median[a] (95% CI)	10.0 (9.2, 12.8)	9.2 (7.7, 10.3)	7.4 (6.3, 9.0)	9.2 (7.7, 10.1)
HR[b] (95% CI)	0.700 (0.516, 0.948)		1.242 (0.976, 1.582)	
P-value[c]	0.0206		0.0757	

Pmab = panitumumab

[a] Kaplan-Meier estimate of median time (months) to event; 95% CI is based on a sign test (Brookmeyer and Crowley, 1982).

[b] HR presented as panitumumab plus FOLFOX:FOLFOX alone is estimated from Cox proportional hazard model stratified by region (Western Europe, Canada and Australia vs. Rest of World) and ECOG score (0 or 1 vs. 2).

[c] P-value is based on a 2-sided log-rank test stratified by region and ECOG score.

OS:

For subjects with wild-type RAS, the HR for OS from a stratified Cox proportional hazards model was 0.754 (95% CI: 0.555 to 1.024). However, in additional analysis including relevant covariates in a Cox Proportional Hazard Model, the HR for overall survival was 0.655 (95% CI: 0.464 to 0.924). The stratified log-rank test did not reveal a statistically significant difference in OS between the panitumumab plus FOLFOX group versus the FOLFOX alone group (P =0.0694).

For subjects with mutant RAS, the HR was 1.156 (95% CI: 0.906, 1.476). HR results for mutant KRAS exon 2 and wild-type KRAS exon 2 mutant RAS subjects also showed HRs > 1.0.

Table 17. Primary Efficacy Parameters – OS for Wild Type RAS and Mutant RAS

	Wild Type RAS		Mutant RAS	
Parameter	**Pmab + FOLFOX (n=106)**	**FOLFOX Alone (n=111)**	**Pmab + FOLFOX (n=163)**	**FOLFOX Alone (n=148)**
Events n (%)	81 (76)	91 (82)	140 (86)	132 (89)
Median[a] (95% CI)	26.0 (19.9, 32.5)	23.1 (17.2, 26.9)	17.4 (13.8, 20.2)	19.6 (15.9, 22.6)
HR[b] (95% CI)	0.754 (0.555, 1.024)		1.156 (0.906, 1.476)	
P-value[c]	0.0694		0.2441	

Pmab = panitumumab

a Kaplan-Meier estimate of median time (months) to event; 95% CI is based on a sign test (Brookmeyer and Crowley, 1982).

b HR presented as panitumumab plus FOLFOX:FOLFOX alone is estimated from Cox proportional hazard model stratified by region (Western Europe, Canada and Australia vs. Rest of World) and ECOG score (0 or 1 vs. 2).
c P-value is based on a 2-sided log-rank test stratified by region and ECOG score.

Figure 5 Kaplan-Meier Plot of Progression-free Survival Time by RAS Wild Type vs Mutant and by Treatment, 02 August 2010 (Union of Wild Type RAS Efficacy Analysis Set and Mutant RAS Efficacy Analysis Set)

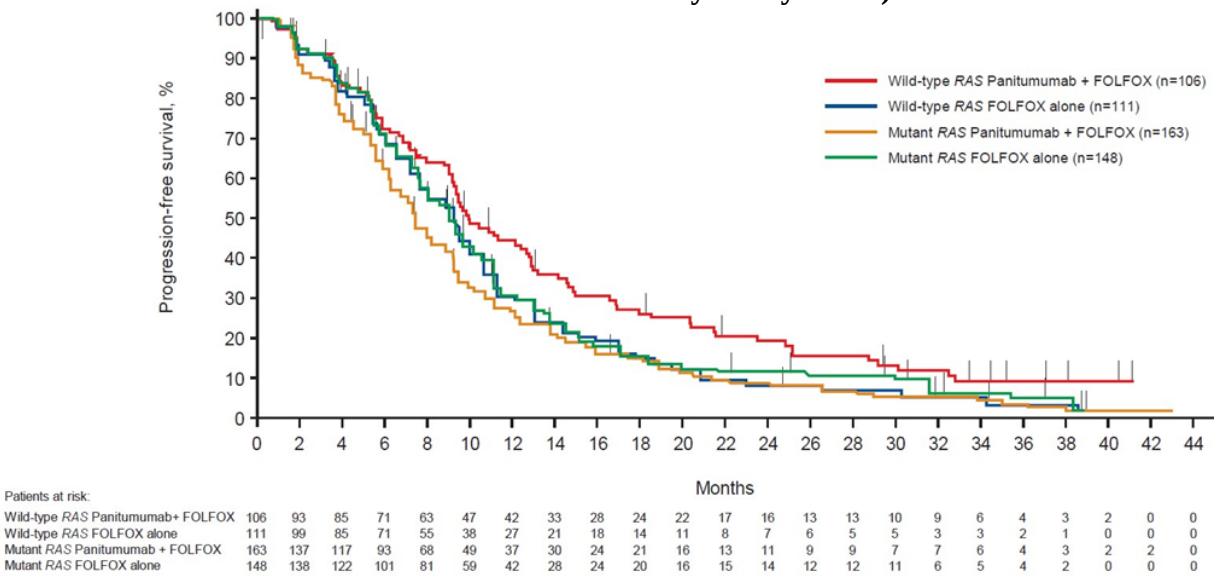
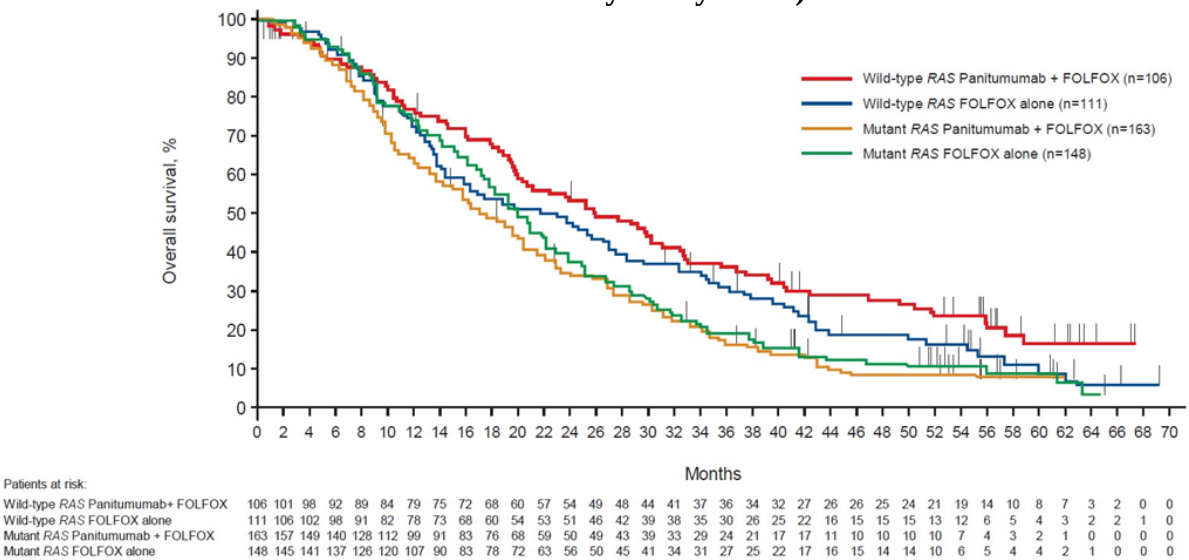


Figure 6 Kaplan-Meier Plot of Survival Time by RAS Wild Type vs. Mutant and by Treatment, 24 January 2013 (Union of Wild Type RAS Efficacy Analysis Set and Mutant RAS Efficacy Analysis Set)



Taken together, PFS and OS results demonstrate a favorable trend among subjects with wildtype RAS, panitumumab plus FOLFOX improved PFS compared to FOLFOX alone. In addition, treatment effect is more favorable for wild-type RAS

subjects in the panitumumab plus FOLFOX group over the FOLFOX alone group when compared to the treatment effect for mutant RAS subjects.

Treatment Effect--Interaction between treatment group and RAS status
Quantitative interaction testing indicated the treatment effect, as measured by panitumumab plus FOLFOX:FOLFOX alone, differed when comparing subjects with wild type RAS to subjects with mutant RAS (PFS: P =0.0038; OS: P = 0.0323), Table 18.

Table 18. Interaction Between Treatment Group (Panitumumab Plus FOLFOX Versus FOLFOX Alone) and *RAS* status (Wild-type Versus Mutant) for PFS Time and OS Time

Endpoint	HR[a] (95% CI) Wild-type *RAS*	HR[a] (95% CI) Mutant *RAS*	Interaction[b]	(95% CI) for Interaction[b]	*P*-value[b]
PFS	0.700 (0.516, 0.948)	1.242 (0.976, 1.582)	0.563	(0.382, 0.831)	0.0038
OS	0.754 (0.555, 1.024)	1.156 (0.906, 1.476)	0.652	(0.441, 0.964)	0.0323

[a] HR presented as panitumumab plus FOLFOX:FOLFOX alone was estimated from Cox proportional hazard model stratified by Region (Western Europe, Canada and Australia vs. Rest of World) and ECOG score (0 or 1 vs. 2).
[b] Calculated based on methods from Gail & Simon 1985.

3. Sensitivity Analyses:
Multivariate Cox PH models were fitted adjusting for all clinical relevant covariates identified as either a predictor for RAS mutation status (wild-type vs. mutant RAS), or predictors for clinical outcome (PFS, OS). For subjects with wild-type RAS, the HR for OS was 0.68 (95% CI: 0.49 to 0.95) and for PFS was 0.76 (0.56, 1.05).

Multiple imputation models were conducted to assess the impact of missing data on the efficacy results. All clinical relevant covariates as described above as well as RAS Sanger Results, and clinical outcome (PFS or OS) were used to impute the missing RAS mutation status. Stratified Cox PH models as described in Section D. 2. were conducted for subjects with wild-type RAS after imputation. For subjects with wild-type RAS after imputation, the HR for OS was 0.78 (95% CI: 0.64 to 0.97) and for PFS was 0.76 (0.61, 0.95).

4. Subgroup Analyses:
To understand the effect of the dCq requirement, including the reduction in number of events, additional analyses mirroring the primary analyses were performed including all samples regardless of the dCq value.

A summary of the primary analyses for PFS and OS for all analysis sets using samples regardless of dCq value are presented in Table 19. For subjects with

wild-type RAS, the log-rank test revealed a difference in PFS between the panitumumab plus FOLFOX group versus FOLFOX alone group (P =0.0279) for the cutoff date (2010). This was also true for mutant RAS but in the opposite direction (P =0.0452) which is consistent with the interaction effect noted in subjects with tissue samples with dCq≤5.0. The log-rank test was not notable for OS. HRs were all nominally less than 1, indicating improved PFS or OS for panitumumab plus FOLFOX group over the FOLFOX alone group.

Table 19. Primary Efficacy Parameters for PFS and OS for *RAS* Efficacy Analysis Sets, Regardless of dCq

		Wild-type *RAS*		**Mutant *RAS***		**Wild-type *KRAS* Exon 2 Mutant *RAS***	
Endpoint/ Data Cutoff Date[a]	**Parameter**	**Pmab + FOLFOX (n=161)**	**FOLFOX alone (n=148)**	**Pmab + FOLFOX (n=209)**	**FOLFOX alone (n=204)**	**Pmab + FOLFOX (n=37)**	**FOLFOX alone (n=41)**
PFS 2010	Events n (%)	136 (84)	125 (84)	190 (91)	182 (89)	32 (86)	34 (83)
	Median[a] (95% CI)	10.0 (9.2, 12.8)	9.3 (7.8, 10.3)	7.5 (7.1, 9.0)	9.0 (8.0, 9.6)	9.2 (6.8, 10.8)	9.2 (7.3, 11.3)
	HR[b] (95% CI)	0.756 (0.588, 0.970)		1.234 (1.003, 1.518)		1.134 (0.687, 1.872)	
	P-value[c]	0.0279		0.0452		0.6283	
OS 2013	Events n (%)	127 (79)	124 (84)	182 (87)	183 (90)	32 (86)	35 (85)
	Median[b] (95% CI)	27.8 (22.5, 31.1)	23.6 (18.1, 26.9)	17.0 (14.0, 20.1)	18.9 (15.7, 21.7)	20.2 (16.3, 30.4)	18.3 (13.0, 31.7)
	HR[c] (95% CI)	0.806 (0.626, 1.036)		1.086 (0.883, 1.336)		0.971 (0.592, 1.591)	
	P-value[d]	0.0920		0.4357		0.9204	

NE= not estimable, Pmab=panitumumab
[a]Data cutoff dates were 30 September 2008 and 02 August 2010 for PFS; and, 28 August 2009 and 24 January 2013 for OS.
[b]KM estimate of median time (months) to event; 95% CI is based on a sign test (Brookmeyer and Crowley, 1982).
[c]HR presented as panitumumab plus FOLFOX:FOLFOX alone is estimated from Cox proportional hazard model stratified by Region (Western Europe, Canada and Australia vs. Rest of World) and ECOG score (0 or 1 vs. 2).
[d]*P*-value is based on a 2-sided log-rank test stratified by Region and ECOG score. Source: Ta

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

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The

pivotal clinical studies included 4 investigators. 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)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the Praxis Extended RAS Panel in the detection of mutations in KRAS and NRAS genes in DNA isolated from FFPE tumor tissue was demonstrated in a retrospective analysis of banked specimens from subjects without RAS mutations. Overall, PFS and OS results demonstrated a favorable trend among subjects with wild-type RAS, panitumumab plus FOLFOX improved PFS compared to FOLFOX compared to FOLFOX alone. In mutant RAS subjects, trend was in the opposite (unfavorable) direction: PFS and OS are longer in the FOLFOX alone group compared to the panitumumab plus FPLSOX group.

Analytical performance studies demonstrated that the accuracy of the Praxis Extended RAS panel in specimens that pass the DNA qualification step is acceptable (PPA = 98.7% , NPA = 97.6%). After exclusion of specimens that fail the DNA qualification step, the assay has an estimated invalid rate of 6.9% to 14.5% as assessed in the retrospective clinical and accuracy studies, respectively.

B. Safety Conclusions

As a diagnostic test, the Praxis Extended RAS Panel involves testing of DNA extracted from CRC FFPE tumor tissue. The risks of the Praxis Extended RAS Panel are associated with the potential mismanagement of patients resulting from false results of the test. A false positive test result may lead to Vectibix (panitumumab) treatment being withheld from a patient 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. Analytical performance of the assay demonstrates the test to be safe for use in mCRC subjects.

C. Benefit-Risk Determination

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. 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 a retrospective analysis of efficacy and safety data obtained from patients with metastatic colorectal cancer from Study 20050203 determined to be RAS mutation-negative (wild-type) by the on data collected with the Illumina PraxisTM Extended RAS Panel (reported as Panel Mutation Not Detected). A statistically significant benefit in progression free survival was observed in the RAS mutation-negative (wild-type) subset (HR: 0.700 95% CI: 0.516 to 0.948) . The median overall survival was 26.0 months in the RAS mutation-negative (wild-type) subset treated with panitumumab plus FOLFOX compared to 23.1 months for RAS mutation-negative (wild-type) patients who received FOLFOX alone. For subjects with wild-type RAS, the HR for OS from a stratified Cox proportional hazards model was 0.754 (95% CI: 0.555 to 1.024). However, in additional analysis including co-variates in a Cox Proportional Hazard Model, the HR for overall survival was 0.655 (95% CI: 0.464 to 0.924). Taken together, the totality of the results, demonstrate a favorable trend among subjects with wildtype RAS for greater probable benefit with panitumumab plus FOLFOX compared to FOLFOX alone.

The risks of the Illumina PraxisTM Extended RAS Panel 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 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 probable risks associated with false results are mitigated by the analytical performance of the device. When compared to Sanger sequencing, the subject level PPA was 98.7% (227/230) and subject level NPA was 97.6% (206/211).

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

In conclusion, given the available information above, the data support the use of the Illumina PraxisTM Extended RAS Panel as an aid in the identification of patients eligible for treatment with panitumumab, 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 Praxis Extended RAS Panel test as an aid in the identification of patients with metastatic CRCs for treatment with panitumumab.

XIII. CDRH DECISION

CDRH issued an approval order on June 29, 2017.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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