SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: **cobas**® KRAS Mutation Test

Device Procode: OWD

Applicant's Name and Address: Roche Molecular Systems, Inc. (RMS)
4300 Hacienda Drive
Pleasanton, CA 94588-2722

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P140023

Date of Notice of Approval: May 7, 2015

Expedited: Not Applicable

II. INDICATIONS FOR USE

The **cobas**® KRAS Mutation Test, for use with the **cobas**® 4800 System, is a real-time PCR test for the detection of seven somatic mutations in codons 12 and 13 of the KRAS gene in DNA derived from formalin-fixed paraffin-embedded human colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab) may be indicated based on a no mutation detected result.

Specimens are processed using the **cobas**® DNA Sample Preparation Kit for manual sample preparation and the **cobas z** 480 analyzer for automated amplification and detection.

III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions can be found in the **cobas**® KRAS Mutation Test product labeling.

V. DEVICE DESCRIPTION

The **cobas**® KRAS Mutation Test consists of two reagent kits and a system platform. Details of the **cobas**® 4800 System platform is described below in "Instrumentation and Software". The reagent kits provide the necessary reagents to perform the two major processes of the test:

1. The **cobas**® DNA Sample Preparation kit provides reagents for manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tissue (FFPET).
2. The **cobas**® KRAS Mutation Test kit provides reagents for automated real-time PCR amplification and detection of the KRAS mutations.

Specimen Preparation

FFPET specimens are processed and genomic DNA isolated using the **cobas**® DNA Sample Preparation Kit, a manual specimen preparation based on nucleic acid binding to glass fibers. A deparaffinized 5 µm section of an FFPET specimen is lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The amount of genomic DNA is spectrophotometrically determined and adjusted to a fixed concentration of 2 ng/µL. Twenty-five (25) µL of the eluate containing 50 ng genomic DNA is combined with 25 µL of each activated master mix reagent, from the **cobas**® KRAS Mutation Test kit, in a 96 well plate. Once the controls and samples have been added to the 96 well plate, the plate is transferred to the **cobas z** 480 analyzer for automated amplification and detection.

PCR Amplification

Target Selection and Amplification

The **cobas**® KRAS Mutation Test kit uses primers that define an 85 base pair sequence for exon 2 containing KRAS codons 12 and 13 in human genomic DNA. Amplification occurs only in the region of the KRAS gene between the primers; the entire KRAS gene is not amplified. The intended target mutations are provided in Table 1 below.

Table 1: List of Intended Target Mutations for the cobas® KRAS Mutation Test

Codon	Mutation ID	Nucleotide Change	AA Change	COSMIC ID
12	c.34G>T	GGT>TGT	G12C	516
	c.34G>A	GGT>AGT	G12S	517
	c.34G>C	GGT>CGT	G12R	518
	c.35G>T	GGT>GTT	G12V	520
	c.35G>A	GGT>GAT	G12D	521
	c.35G>C	GGT>GCT	G12A	522
13	c.38G>A	GGC>GAC	G13D	532

A derivative of *Thermus* species Z05 DNA polymerase is utilized for target amplification. First, the PCR reaction mixture is heated to denature the genomic DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The Z05 DNA polymerase, in the presence of a divalent metal ion and excess dNTPs, extends each annealed primer, thus synthesizing a second DNA strand. This completes the first cycle of PCR, yielding a double-stranded DNA copy which includes the targeted 85 base pair region of the KRAS gene. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of amplicon DNA.

Selective amplification of target nucleic acids from the specimen is achieved in the **cobas**® KRAS Mutation Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme catalyzes the destruction of DNA strands containing deoxyuridine but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA but is always present in amplicons due to the use of dUTP in place of thymidine triphosphate as one of the nucleotide triphosphates in the Reaction Mix reagent; therefore, only the amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicons susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. The AmpErase enzyme is inactive at temperatures above 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy the target amplicon.

Automated Real-time Mutation Detection

The **cobas z** 480 analyzer measures, in real-time, the amount of fluorescence generated by specific PCR products. After amplification, each amplicon generated using the **cobas**® KRAS Mutation Test is subjected to a melting program in which the temperature is ramped from 40°C to 95°C (TaqMelt). The wild-type specific probe is bound to both the wild-type and mutant amplicon at low temperatures. In the bound state, the fluorescein reporter dye on the 5′ end of the probe is sufficiently far away from the 3′ end quencher dye, allowing the fluorescent dye to emit a specific wave length of light. As the temperature rises, the probe dissociates from the amplicon, allowing the quencher dye to come into close proximity to the fluorescent dye, decreasing the amount of measurable fluorescence. An amplicon with a perfect match to the probe (wild-type) melts at a higher temperature than an amplicon with one or more mismatches (mutant). The amount of fluorescence at each temperature increment is measured and the melting

temperature(s) are calculated. The presence of a mutant KRAS sequence in codons 12 and 13 can be detected when the melting temperatures are within specified ranges. To avoid detection of codon 12 and codon 13 silent mutations (no amino acid change), a modified base serves as a universal base and produces a melting temperature within the wild-type range.

Instrumentation and Software

The **cobas**® 4800 system is controlled by the **cobas**® 4800 system SR2.1 software which provides the core software engines and user interfaces. This core system software was designed to allow multiple assays to be performed on the system using analyte specific analysis package software (ASAP). The **cobas z** 480 analyzer component of the test system also has its own internal instrument control software which is driven by the core software. All of these software components were developed under design control processes and related software standards.

A dedicated Control Unit computer runs the **cobas**® 4800 SR2.1 System software and provides an interface to the **cobas z** 480 and Laboratory Information System (LIS). The computer also processes the fluorescent signals with the ASAP and stores the test results in a controlled database. The complete system allows a user to create a test work order for each specimen manually. A software wizard guides the user through the necessary steps to perform a run which includes **cobas z** 480 maintenance handling, test selection, specimen ID entry, reagent and microwell plate bar code entry, microwell plate loading and run start.

The **cobas**® 4800 System tracks each specimen during processing and analysis on the **cobas z** 480 analyzer. Once the thermal run is complete the ASAP processes the fluorescence data using data analysis algorithms, assesses the validity of the controls and determines the results using the assay specific result interpretation logic. The software then provides the results to the user in three formats: a printable PDF results report, a Graphical User Interface (GUI) based result viewer and a result export to the LIS. The **cobas**® 4800 System software includes a validated KRAS data analysis algorithm to determine sample results and run validity.

Interpretation of Results

Melting temperature (Tm) and peak height (PH) of the melting curves are calculated by the analysis software, and the values from all Mutant control, Negative control, and Calibrator reactions are used to determine if the run is valid. If the run is valid, the analysis software will determine validity and mutation status of the specimens by evaluating the Tm and PH against acceptable ranges of Tm and PH.

Specimens for which a mutant Tm is observed are reported as “Mutation Detected” and the mutant reaction is reported (Table 2). Due to the number of mutations possible and the nature of melting technology, the specific mutation within the codon is not specified.

Specimens for which no mutant Tm is observed are reported as "No Mutation Detected" (Table 2). Specimens for which the codon 12/13 reaction well is invalid are reported as "Invalid". Specimens for which an out-of-range Tm is observed (neither wild-type nor mutant range) are reported as "Invalid". All valid specimens will have at least one melt peak (mutant, wild-type, or both). Specimens with no melt peaks are invalid.

Table 2: Result Interpretation of the cobas® KRAS Mutation Test

cobas® KRAS Mutation Test Result		**Interpretation**
Test Results	**Mutation Results**	
Mutation Detected	Codon 12/13	Mutation Detected in KRAS Codon 12 or Codon 13
No Mutation Detected	N/A	No Mutation Detected in KRAS Codon 12 or Codon 13
Invalid	N/A	Result is invalid. Repeat the testing of specimens with invalid results following the instructions outlined in the Package Insert
Failed	N/A	Failed run due to hardware or software failure

Test Controls and Calibrator

One KRAS Mutant Control (MC) and one KRAS Negative Control are provided to serve as external run controls. Detection of wild-type codons 12 and 13 by the probe serves as an internal, full process control.

1. *KRAS Mutant Control:* The Mutant Control is a blend of a double mutant linearized DNA plasmid and genomic DNA from a wild-type cell line. The plasmid contains mutant sequence for KRAS codon 12 (Asp, GAT) and KRAS codon 61 (His, CAC). The Mutant Control is included in every run and serves as a process control for every step except sample preparation. The Mutant Control reaction must have KRAS wild-type and KRAS codon 12/13 Mutant Melt Temperatures within the respective acceptable ranges for the run to be considered valid.

2. *KRAS Negative Control:* The Negative Control is a full-process control and serves to identify potential reagent or process contamination for the **cobas®** DNA Sample Preparation kit and the **cobas®** KRAS Mutation kit. The melt peak height values must be below the pre-established cut-off value for the run to be considered valid. No reagent is provided for the Negative Control. DNA Specimen Diluent (DNA SD) is used as Negative Control.

3. *KRAS Calibrator:* The KRAS Calibrator consists of wild-type genomic DNA and serves as a melting temperature (Tm) calibrator to compensate for run-to-run and instrument-to-instrument temperature variability. The wild-type peak Tm is used by

the analysis software to adjust Tm values of the Mutant Control and all specimens prior to analysis for mutation status.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently one FDA-approved alternative for testing formalin-fixed, paraffin-embedded CRC tissue for KRAS mutation status in the selection of patients who are eligible for cetuximab (Erbitux) or panitumumab (Vectibix) treatment. Namely, the QIAGEN *therascreen* KRAS RGQ PCR kit was approved under PMAs P110030 and P110027.

VII. MARKETING HISTORY

The **cobas**® KRAS Mutation Test has not been marketed in the United States.

The **cobas**® KRAS Mutation Test is currently marketed in Europe, Australia, Brazil, China, Colombia, Ecuador, Indonesia, Korea, Peru, Singapore, Thailand and Venezuela. The test is approved in Canada. The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect KRAS test results, and consequently improper patient management decisions in colorectal cancer (CRC) treatment.

A false positive test result may lead to Erbitux (cetuximab) or Vectibix (panitumumab) treatment being withheld from a patient who might have benefited. A false negative test result may lead to Erbitux (cetuximab) or Vectibix (panitumumab) treatment being administered to a patient who may experience adverse side effects associated with treatment without clinical benefit.

Most common adverse reactions (≥ 25%) in clinical trials of Erbitux (cetuximab) are: cutaneous adverse reactions (including rash, pruritus, and nail changes), headache, diarrhea, and infection. Most common adverse reactions (≥ 20%) in clinical trials of Vectibix (panitumumab) in combination with oxaliplatin-containing chemotherapy are diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, dermatitis acneiform, pruritus, and dry skin.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

CRC FFPET specimens used in the non-clinical studies described below were anonymized leftover specimens with basic characteristics derived from the surgical

pathology reports. Sections of each specimen were stained with hematoxylin and eosin (H&E) to confirm that the specimens were CRC specimens and to determine the tumor content by pathologists. Bi-directional Sanger sequencing and massively parallel sequencing (MPS) were used to select the specimens for testing. Percentage of mutation for each CRC FFPET specimen was determined using a MPS method. In studies described below, bi-directional Sanger sequencing was always conducted twice, i.e., a total of 4 reads including 2 forward and 2 reverse reads.

1. **Correlation to Reference Method**

   Studies were conducted to compare the results of the **cobas**® KRAS Mutation Test to those of Sanger sequencing (reference method) using 94 procured CRC FFPET specimens. A 5-micron section was processed to isolate DNA from each tumor specimen using one **cobas**® DNA Sample Preparation kit lot and the extracted DNA was tested with each of two reagent lots of the **cobas**® KRAS Mutation Test. Tumor stage information for the specimens tested (Table 3), and results of bi-directional Sanger sequencing compared to the **cobas**® KRAS Mutation Test for the 94 specimens (Table 4) are summarized below.

Table 3: Tumor Stage vs. Sanger Sequencing

		Bi-Directional Sanger Sequencing Results			Total	% of Total
		Codon 12	Codon 13	Wild-type		
Tumor Stage	Stage I	3	1	4	8	8.5%
	Stage II	20	2	8	30	31.9%
	Stage III	23	5	6	34	36.2%
	Stage IV	10	7	5	22	23.4%
Total		56	15	23	94	100.0%

Table 4: cobas® KRAS Mutation Test vs. Bi-Directional Sanger Sequencing

cobas® KRAS Mutation Test	Bi-directional Sanger Sequencing		
	Mutation Detected	No Mutation Detected	Total
Mutation Detected	37	3	40
No Mutation Detected	1	53	54
Total	38	56	94
PPA (95% CI)	97.4% (86.5%, 99.5%)		
NPA (95% CI)	94.6% (85.4%, 98.2%)		
OPA (95% CI)	95.7% (89.6%, 98.3%)		

PPA: positive percent agreement; NPA: negative percent agreement
OPA: overall percent agreement; CI: confidence intervals

Another study tested 188 colorectal cancer FFPET specimens with both the **cobas**® KRAS Mutation Test and Sanger sequencing using two lots of **cobas**® KRAS Mutation Test kits stored at 2-8°C. Comparable results were obtained with this procured sample set, with a PPA of 97.5 % (CI: 91.4-99.3%), a NPA of 94.4% (CI: 88.3-97.4%), and an OPA of 95.7% (CI: 91.8-97.8%).

2. Analytical Sensitivity

a. **Analytical Sensitivity - Limit of Blank (LoB)**
To assess performance of the **cobas**® KRAS Mutation Test in the absence of template and to ensure that a blank sample does not generate an analytical signal that might indicate a low concentration of mutation, samples without DNA template and those with DNA extracted from CRC FFPET KRAS wild-type specimens were evaluated.

i. **Limit of Blank (LoB) no template** – The LoB was determined using DNA Specimen Diluent reagent, which contains no target (as part of no DNA template control, 0 ng/PCR level, in Limit of Detection study described below). Out of 168 replicates of no DNA template control tested, none of the replicates yielded a "Mutation Detected" result across each sample panel and reagent lot.

ii. **Limit of Blank (LoB) FFPET Specimens** – Five (5) CRC FFPET specimens with tumor content ranging from 40% to 65% that were wild-type for KRAS mutation for codons 12 and 13 were tested using 50 ng DNA per amplification. Only "No Mutation Detected" results were reported in the presence of KRAS wild-type DNA isolated from CRC FFPET specimens.

b. **Analytical Sensitivity - Limit of Detection (LoD)**
Replicate **cobas**® KRAS Mutation Test measurements were performed on dilution panel members that contained various amounts of genomic DNA and various percentages of the KRAS mutation, which bracketed the expected analytical sensitivity of **cobas**® KRAS Mutation Test. The study was performed by testing dilution panels prepared from FFPET specimen blends.

Specimen FFPET blends – Multiple CRC FFPET specimen DNA extracts for specific codon 12 mutants (G12A, G12C, G12D, G12S, G12V, G12R) and a codon 13 mutant (G13D) were blended with KRAS wild-type FFPET specimen DNA extracts to achieve mutant sequences at approximately 10%, 5%, 2.5%, and 1.25% mutation levels. After blending, the final percent mutant sequences of each blend were verified by a MPS method, which was validated for detecting the specific codon 12 and 13 mutants in the specimen FFPET blends. Each specimen blend was diluted to 2ng/µL at the time of testing (50.0 ng per 25 µL reaction). Serial dilutions of each specimen blend were prepared and 8 replicates of each panel member were run using each of 3 **cobas**® KRAS Mutation Test kit lots (n=24 replicates per panel member). The sensitivity of each sample

was determined by the lowest amount of DNA that yielded a KRAS "Mutation Detected" result in at least 95% of the replicates, as shown in Table 5. This study demonstrates that the **cobas**® KRAS Mutation Test can detect KRAS codon 12/13 mutations at approximately 5% mutant sequences using the standard input of 2 ng/µL.

Table 5: Sensitivity of the cobas® KRAS Mutation Test using CRC Specimen FFPET Blends

KRAS Mutation	Codon 12						Codon 13
	G12A	G12C	G12D	G12R	G12S	G12V	G13D
Targeted Level	2.5%	2.5%	1.25%	5.0%	2.5%	2.5%	1.25%
LoD	2.93%	2.61%	1.64%	5.78%	2.55%	2.48%	1.67%

c. **Analytical Sensitivity – Assay Cut-off Validation**

Data from the feasibility studies were used to determine the cutoff values for peak height (PH) and melting temperature (Tm) parameters. A total of 2,578 individual results were analyzed for codon 12/13: 509 results from FFPET samples (221 mutant, 281 wild-type, and 7 invalid results), 295 results for the Calibrator, 374 results for the Mutant Control, 219 results for the Negative Control, and 1,181 results for various cell line and plasmid dilutions. The final and locked Tm and PH parameters were set in order to achieve the **cobas**® KRAS Mutation Test product requirements listed below.

- Analytical sensitivity shall be at or below 5% mutant sequences (by 95% correct call) when 50 ng of genomic DNA is tested.
- Analytical specificity shall be at least 97% when 50 ng of genomic DNA is tested.

For the verification of Tm and PH cut-off values for codon 12/13, results from the 188 specimens and 14 sets of run controls from the "Correlation to Reference Method" study were used. In total, there were 42 results from the run controls (14 Calibrator, 14 Mutant Control, and 14 Negative Control results) and 375 results from the 188 FFPET specimens tested by two KRAS kit lots. No discrepant results between the **cobas**® KRAS Mutation Test and sequencing results (Sanger and MPS) were observed for codon 12/13. Based on this analysis, the ranges of valid Tm and PH values for codon 12/13 were verified.

3. **Analytical Sensitivity – Genomic DNA Input Range**

Various genomic DNA input amounts may result from DNA quantitation errors and/or variation in the amount of degraded DNA. To evaluate the effects of various genomic DNA input amounts, genomic DNA samples from 5 KRAS wild-type and 5 KRAS mutant (three codon 12 and two codon 13) CRC FFPET specimens were evaluated at 8 DNA input concentrations of 100, 50, 25, 12.5, 6.3, 3.1, 1.6, and 0.78 ng per amplification reaction. This study used one **cobas**® DNA Sample Preparation Kit lot, one **cobas**® KRAS Mutation Test kit lot, and two **cobas z** 480 analyzers. The study results supported the recommended DNA input of 50 ng per PCR reaction for the **cobas**® KRAS Mutation Test.

4. **Analytical Sensitivity - Minimum Tumor Content**

Two CRC FFPET mutant specimens (one codon 12, G12V, and one codon 13, G13D) with tumor content ranging from approximately 35% to 40% were selected for this study, due to the lack of specimens with naturally occurring 10% tumor content. Macrodissection was used for achieving approximately 10% tumor content. Seven (7) adjacent 5-micron sections from each CRC FFPET specimen were mounted onto seven slides. One slide was stained to determine the percent tumor content and to outline the tumor within the tissue section by a pathologist. Using the stained slide as a guide, the remaining six sections were macrodissected to remove a portion of the tumor tissue in order to achieve approximately 10% tumor content for each specimen.

DNA from each of the sub-sections was then extracted, quantified and diluted to 2 ng/µL. The DNA was further diluted to eight (8) DNA input levels: 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, and 0.4 ng/PCR reaction. Each diluted sample was tested in six (6) replicates, resulting in a total of forty-eight (48) replicates per specimen. One **cobas**® DNA Sample Preparation Kit lot, one **cobas**® KRAS Mutation Test kit lot, and six **cobas z** 480 analyzers were used in this study. The lowest DNA input level at which the cobas KRAS Mutation Test had an observed "Mutation Detected" hit rate of at least 95% was determined for each specimen. The study results indicated that the **cobas**® KRAS Mutation Test was able to detect KRAS mutant sequences in CRC FFPET specimens with approximately 10% tumor content at DNA input levels of $\geq$ 3.1ng per PCR reaction with observed hit rate of 100%, for the mutations studied (i.e., G12V and G13D).

5. **Analytical Specificity**

a. **Primer and Probe Specificity**

Basic Local Alignment Search Tool (BLAST) search was conducted on the primers and probes included in the test. The primer and probe sequences included in the **cobas**® KRAS Mutation Test and the amplicons generated by the test were each queried using NCBI BLAST for "Genome (All Assemblies)". The BLAST analysis of the primers and probes was last updated in 2012 using assembly GRCh37 and did not identify any

additional sequences. The BLAST analysis for the codon 12/13 oligonucleotides against the human genomic database identified one non-targeted genomic region with significant homology to KRAS. This region is the KRAS pseudogene, KRASP1. The amplicon sequences for codon 12/13 were also analyzed by BLAST. The pseudogene, KRASP1, was again identified as having significant sequence similarity. In addition, the NRAS and HRAS genes were found to have high similarity for the amplicon sequences. No other genomic sequences with significant similarity to the KRAS codon 12/13 sequences were found.

The alignments between the KRAS codon 12/13 amplicon and the pseudogene, HRAS, and NRAS were provided to indicate nucleotide positions under the primers and probes that are mismatched to KRAS. Functional testing of primer and probe specificity was further investigated using CRC FFPET specimens and plasmid blends. The study included multiple mutations in codon 12/13 and other mutations in flanking sequences that are under the probe binding region. Specifically, multiple mutations in codon 12/13 include samples with single base mutations other than those in codons 12 and 13. Other mutations in flanking sequences included complex/double mutations (i.e., deletion and insertion mutations). The results showed that though rare (<0.2%), some complex and multiple mutations of codon 12/13 may result in failure to detect the presence of a mutation (results of "No Mutation Detected") whereas mutations flanking codon 12/13 on exon 2 (e.g., deletion and insertion mutations) may cross-react with the **cobas**® KRAS Mutation Test (results of "Mutation Detected").

b. **Cross Reactivity**

The **cobas**® KRAS Mutation Test has been shown to cross-react with the mutations shown in Table 6. Plasmid constructs (n=4) and CRC FFPET specimen (n=1) containing the rare mutations for codon 13 were blended with wild-type genomic DNA to create approximately 5% mutant samples. Results demonstrated that the **cobas**® KRAS Mutation Test cross-reacts to these mutations at a 100% hit rate. Analytical performance of the **cobas**® KRAS Mutation Test in detecting these mutations has not been evaluated.

Table 6: Mutations Determined to Cross-React with the cobas® KRAS Mutation Test

Mutation	AA Change	COSMIC ID
c.37G>T	**13C**	527
c.37G>A	**13S**	528
c.37G>C	13R	529
c.38G>C	**13A**	533
c.38G>T	**13V**	534

Bold = Tested with plasmids

c. **Microorganisms and KRAS Homologs**

Specificity of the **cobas**® KRAS Mutation Test was evaluated by determining whether or not presence of KRAS silent mutation plasmids or KRAS homolog plasmids or colon-related microorganisms interfere with detection of KRAS codon 12/13 mutations.

i. **KRAS homologs and silent mutations** – The KRAS gene belongs to a family of the RAS proteins. In humans, three RAS genes encode four distinct but highly homologous ~21 kDa RAS proteins: HRAS, NRAS, KRAS4A and KRAS4B (KRAS4A and KRAS4B are alternative splice variants of the KRAS gene). To test specific sequences for cross-reactivity, three plasmids containing homologous RAS gene sequences in exon 2 and six plasmids containing silent mutations in codon 12 or 13 were used in this study.

Each of the three plasmids with homologous RAS gene sequences (i.e., KRAS codon 12/13 pseudogene, NRAS exon 2, and HRAS exon 2) was blended with KRAS wild-type DNA (K562 cell line genomic DNA) and a plasmid containing KRAS codon 12 or codon 13 mutations. Nine (9) different DNA blends were formulated for testing. Each of the six plasmids containing silent mutations was blended with KRAS wild-type DNA and KRAS mutant codon 12 or 13 plasmid DNA to evaluate exclusivity and interference, respectively. Twelve (12) different DNA blends were formulated for testing. The DNA blends were made based on the assumptions that there are 16,000 copies in 50 ng of genomic DNA and that each copy of genomic DNA contains one copy of a homolog and one copy of the KRAS gene. Therefore, each DNA blend contained 16,000 copies/PCR of a homologous RAS gene with and without 5% codon 12 and 13 mutant.

There were no invalid runs for this study. All of the samples with homologous RAS gene sequences or silent mutation sequences yielded "No Mutation Detected" results in wild-type background DNA, indicating that the RAS homologous sequences and the silent mutation sequences do not cross-react with the cobas® KRAS Mutation Test. For samples containing 5% KRAS mutant sequences, all of the samples with homologous RAS gene sequences or silent mutations yielded "Mutation Detected" results. The results indicated that the RAS homologous sequences or silent mutation sequences do not interfere with the detection of 5% KRAS mutant sequences by the cobas® KRAS Mutation Test.

ii. **Testing of colon-related microorganisms –** *Bacteroides caccae, Prevotella intermedia,* and *Escherichia coli (E. coli)* at 1×10^6 colony forming units (CFU) did not cross-react or interfere with the **cobas**® KRAS Mutation Test when added to six KRAS codon 12,

one codon 13, and one wild-type specimens during the tissue lysis step.

6. **Interference – Effects of Necrotic Tissue**

To evaluate the potential interference of high necrotic tissue content in CRC FFPET specimens using the **cobas**® KRAS Mutation Test, twenty (20) CRC FFPET specimens, including nine (9) KRAS codon 12/13 mutant specimens and eleven (11) KRAS wild-type specimens were tested. Percent necrosis from all tested specimens ranged from 5% to 70%, as provided by the specimen vendors and confirmed by a pathologist.

DNA isolated from two 5-micron sections for each of the 20 CRC FFPET specimens was run in duplicates. One lot of the **cobas**® DNA Sample Preparation Kit, one lot of the **cobas**® KRAS Mutation Test kit, and two **cobas z** 480 analyzers were used in the study. Results were considered acceptable if specimen results matched the expected result based on sequencing results. All observed results matched the expected results for all the specimens tested. The data supported that necrotic tissue content up to 50% in KRAS mutant and up to 70% in KRAS wild-type CRC FFPET specimens does not interfere with the call results for the **cobas**® KRAS Mutation Test.

7. **Interference – Triglycerides or Hemoglobin**

To evaluate the potential interference of triglycerides and hemoglobin on the performance of the **cobas**® KRAS Mutation Test, four conditions were tested for each of 8 CRC FFPET specimens:

- Control-1: Tissue lysis buffer/Proteinase K (TLB/PK) only (blank control)
- Control-2: TLB/PK spiked with saline (solvent control)
- Hemoglobin: TLB/PK spiked with hemoglobin at 2 mg/mL
- Triglycerides: TLB/PK spiked with triglycerides at 37 mM

Four 5-micron sections were obtained from each of the 8 CRC KRAS FFPET specimens (one wild-type and 7 KRAS mutant specimens). The levels of triglycerides (37 mM) and hemoglobin (2 mg/mL) were equal to the levels recommended to be tested by the Clinical and Laboratory Standards Institute (CLSI) guideline EP7-A2, "Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition". The above described four (4) testing solutions were prepared by spiking either the appropriate amount of control material or potentially interfering substance into the TLB/PK solutions. Each of the eight (8) FFPET specimens were extracted using 0.25 mL of each of the 4 testing solutions for a total of thirty-two (32) extractions. This study used one lot of **cobas**® DNA Sample Preparation Kit, one lot of **cobas**® KRAS Mutation Test kit, one **cobas z** 480 analyzer, and one operator. One KRAS test run was performed in the study, and the run and all results were valid.

Four (4) of the seven (7) mutant specimens (i.e., G12C, G12D, G12V, G13D) had percent mutant sequences near 3X LoD of the KRAS test (i.e., 15% mutation), allowing for assessment of the interference at ~3-fold analytical sensitivity. All observed results matched the expected results at the levels of triglycerides and hemoglobin tested, indicating that triglycerides and hemoglobin do not interfere with the performance of the **cobas**® KRAS Mutation Test.

8. <u>Reproducibility</u>

An external study was performed to assess the reproducibility of the **cobas**® KRAS Mutation Test with a 15-member panel of DNA samples extracted from CRC FFPET sections of KRAS wild-type or mutant specimens, tested in duplicate per run, across 3 testing sites with 2 operators per site, 3 reagent lots and 5 non-consecutive testing days. The panel included six codon 12 mutations and one codon 13 mutation along with a wild-type DNA sample. Each mutation was represented in duplicate at LoD and 3x LoD levels. Two replicates of the panel at desired concentrations were used in each run. Panels were provided to each of the 3 testing sites in a blinded fashion. Operators performed amplification and detection with the **cobas z** 480 instrument using the **cobas**® KRAS Mutation Test kits provided by RMS. One panel tested by a given operator at a given site was considered one run. Each operator completed 5 valid runs with each of 3 reagent lots for a total of 15 non-consecutive days of testing, and operators performed runs separately from the other operator at the site. Testing was completed with one lot before testing began with another lot.

Overall 97.8% of runs (90/92) were valid. A total of 3,060 tests were performed in 90 valid runs. From the valid runs, 99.6% of test results (3,048/3,060) were valid. There were 'No Mutation Detected' results in 175 valid tests for the wild-type panel member with 100% agreement. For the codon 12 and 13 panel members at both 1x LoD and 3x LoD, agreement was 100%. Results by overall agreement are presented in Table 8 and Table 9 below.

Table 7: Overall Estimates of Agreement by Panel Member

Panel Member	Number of Valid Tests	Agreement N	Agreement % (95% CI)*
Wild Type	175	175	100.0 (97.9, 100.0)
Codon 12 - Mutation 12D - LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12V - LOD	178	178	100.0 (97.9, 100.0)
Codon 12 - Mutation 12C - LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12A - LOD	179	179	100.0 (98.0, 100.0)
Codon 12 - Mutation 12S - LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12R - LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12D - 3 x LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12V - 3 x LOD	179	179	100.0 (98.0, 100.0)
Codon 12 - Mutation 12C - 3 x LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12A - 3 x LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12S - 3 x LOD	180	180	100.0 (98.0, 100.0)
Codon 12 - Mutation 12R - 3 x LOD	180	180	100.0 (98.0, 100.0)
Codon 13 - Mutation 13D - LOD	180	180	100.0 (98.0, 100.0)
Codon 13 - Mutation 13D - 3 x LOD	180	180	100.0 (98.0, 100.0)

Note: Results are included as agreement when a valid test of Mutant Type panel member has a result of 'mutation detected' or when a valid test of Wild Type panel member has a result of 'no mutation detected'.
* 95% CI = 95% exact binomial confidence interval.

The total precision mean, standard deviation, and coefficient of variation (CV, %) of mean PH attributed to lot, site/instrument, operator, day, and within-run by panel member are summarized below. Across all variance components (i.e., lot, site/instrument, operator, day, and within run), the overall CV ranged from 5.9% to 13.3% across all panel members.

Table 8: Mean, Standard Deviation, and Coefficient of Variation (%) for PH by Panel Members

			Standard Deviation (SD) and Percent Coefficient of Variation											
			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	N	Mean PH	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Wild Type	175	0.047	0.001	1.6	0.002	3.4	0.001	2.4	0.005	11.4	0.002	5.2	0.006	13.3
Codon 12 - Mutation 12D - LOD	180	0.039	0.001	1.6	0.000	0.0	0.003	7.7	0.001	3.8	0.002	5.8	0.004	10.5
Codon 12 - Mutation 12V - LOD	178	0.040	0.001	2.9	0.001	1.7	0.002	5.5	0.001	3.6	0.002	5.8	0.004	9.4
Codon 12 - Mutation 12C - LOD	180	0.039	0.000	0.4	0.000	0.0	0.003	7.4	0.001	2.7	0.002	6.0	0.004	9.9
Codon 12 - Mutation 12A - LOD	179	0.041	0.000	0.4	0.000	0.0	0.002	5.5	0.002	3.8	0.002	4.7	0.003	8.2
Codon 12 - Mutation 12S - LOD	180	0.037	0.001	1.6	0.000	0.0	0.003	7.0	0.001	3.9	0.002	6.5	0.004	10.5
Codon 12 - Mutation 12R - LOD	180	0.027	0.000	0.4	0.000	0.0	0.001	5.1	0.001	2.4	0.002	7.2	0.002	9.1
Codon 12 - Mutation 12D - 3 x LOD	180	0.073	0.001	1.2	0.000	0.0	0.003	4.2	0.002	3.3	0.002	2.5	0.004	6.0
Codon 12 - Mutation 12V - 3 x LOD	179	0.070	0.000	0.4	0.000	0.0	0.003	4.9	0.001	1.6	0.002	3.4	0.004	6.2
Codon 12 - Mutation 12C - 3 x LOD	180	0.065	0.001	1.3	0.000	0.0	0.003	5.4	0.002	2.8	0.002	3.3	0.005	7.1
Codon 12 - Mutation 12A - 3 x LOD	180	0.067	0.000	0.6	0.000	0.0	0.003	4.7	0.002	2.9	0.005	7.0	0.006	8.9
Codon 12 - Mutation 12S - 3 x LOD	180	0.064	0.001	1.3	0.000	0.0	0.004	6.3	0.002	3.5	0.002	3.9	0.005	8.3
Codon 12 - Mutation 12R - 3 x LOD	180	0.049	0.000	0.7	0.000	0.0	0.002	4.4	0.001	1.5	0.002	3.6	0.003	5.9
Codon 13 - Mutation 13D - LOD	180	0.039	0.000	0.4	0.000	0.0	0.003	7.1	0.000	1.1	0.002	6.3	0.004	9.6
Codon 13 - Mutation 13D - 3 x LOD	180	0.069	0.001	1.6	0.000	0.0	0.004	5.4	0.002	3.1	0.002	3.2	0.005	7.2

Note: SD = standard deviation; CV = coefficient of variation; PH = melting curve peak height.

9. Repeatability

Repeatability of the **cobas**® KRAS Mutation Test was assessed using eight CRC FFPET specimens, including two codon 12, two codon 13 KRAS mutant specimens, and four KRAS wild-type specimens. The specimens were tested in duplicate by two operators, using two different reagent lots and four **cobas z** 480 analyzers over four days (n=32/specimen) at one site. The **cobas**® KRAS Mutation Test had a correct call accuracy of 100% (256/256) for all days, specimens, replicates, operators and reagent lots combined.

10. Lot-to-Lot Reproducibility

The **cobas**® KRAS Mutation Test utilizes two separate kits: (1) the DNA Sample Preparation kit for isolation of DNA from CRC FFPET specimens, and (2) the **cobas**® KRAS Mutation Test kit for the amplification and detection of KRAS mutation status. The objective of this study was to determine the effects of using different lot combinations of the **cobas**® DNA Sample Preparation kit

and the **cobas**® KRAS Mutation Test kit on the resulting mutation status of CRC FFPET specimens.

Eight CRC FFPET specimens were tested with nine combinations of three lots of the **cobas**® DNA Sample Preparation kit and three lots of the **cobas**® KRAS Mutation Test kit. The specimens were selected for mutation status based on Sanger and/or MPS sequencing results. Of the eight (8) specimens used in this study, six (6) specimens were codon 12 mutants (G12A, G12C, G12D, G12S, G12V, G12R), one specimen was codon 13 mutant (G13D) and one specimen was wild-type. Four (4) of the seven (7) mutant specimens (G12C, G12D, G12V, and G13D) had percent mutant sequences near 3x LoD of the **cobas**® KRAS Mutation Test, i.e., 15%. The test results were analyzed for each specimen, and all observed results matched expected results (100% correct calls). The mean PH, mean Tm and % CV values for PH and Tm were summarized across lots and no trend in PH and Tm values was observed.

11. <u>Specimen Handling – Curl Versus Slide Equivalency</u>

To evaluate the equivalence of using DNA extracted from 5-micron unmounted CRC FFPET sections (FFPET "curls") and DNA extracted from CRC FFPET sections mounted on slides (FFPET "slides"), 50 CRC FFPET specimens (30 wild-type and 20 KRAS codon 12/13 mutant specimens determined by Sanger sequencing) were tested. The mutant specimens included 17 codon 12 and three codon 13 specimens, covering all seven KRAS mutations. Two 5-micron sections were obtained from each CRC FFPET specimen: one FFPET slide and one FFPET curl. Both slide and curl sections were tested using one **cobas**® DNA Sample Preparation Kit lot, one **cobas**® KRAS Mutation Test kit lot and three **cobas z** 480 analyzers. The tumor content ranged from 25% to 80% for mutant specimens and from 50% to 90% for wild-type specimens. The results demonstrated 100% (50/50) agreement between unmounted and mounted CRC FFPET sections.

12. <u>Specimen Handling – Reproducibility</u>

The reproducibility of the **cobas**® DNA Sample Preparation kit was examined using sections taken from 3 CRC FFPET specimen blocks, one containing a codon 12 mutation (G12D), one containing a codon 13 mutation (G13D), and one that is wild-type for KRAS mutation. From each of the three specimens, thirty-six (36) 5-micron sections were obtained. Each of the three external sites tested twelve (12) sections for each specimen over six (6) non-consecutive days. On each test day, one operator from each site isolated, quantified and tested the DNA from two FFPET curl sections for each specimen using one lot of **cobas**® DNA Sample Preparation kit and one lot of the **cobas**® KRAS Mutation Test kit. All testing reactions were performed using **cobas z** 480 analyzer with KRAS Analysis Package. One lot of the **cobas**® KRAS Mutation Test kit reagents was used in this study, in combination with three lots of the **cobas**® DNA Sample Preparation kit at each site. All runs performed at 3 sites were valid. All mutant and wild-type specimen results were valid and yielded the expected call result

(correct call =100%, 36/36 for each specimen), supporting the reproducibility for the **cobas**® KRAS Mutation Test at the pre-analytical step of DNA isolation.

13. Guard Banding

The objective of the guard banding studies was to establish the robustness of the PCR conditions for the **cobas**® KRAS Mutation Test. Guard banding studies were performed on the **cobas**® KRAS Mutation Test Thermal Cycling Profile, PCR reaction mix (RXN), and Proteinase K concentration (for DNA isolation procedure).

a. **Thermal Cycling Profile**

The thermal cycling profile was guard banded by varying both the denaturation and annealing temperatures by ± 1°C. Eight (8) specimens were used: six (6) specimens were codon 12 mutants (G12C, G12V, G12A, G12R, G12D, G12S), one (1) specimen was codon 13 mutant (G13D), and one (1) specimen was wild-type. All seven (7) mutant DNA samples (DNA pools from FFPETs and DNA blends) had percent mutant sequences near 3x LoD of the KRAS Test, i.e., 15%, by MPS. The eight (8) DNA samples were tested in triplicate using nine (9) different PCR profiles, which have ±1°C temperature variation in the standard denaturation and annealing step temperatures, for a total of 216 test results. In addition, six (6) replicates of Mutant Control were also tested for each PCR profile. All reported calls (i.e., Mutation Detected or No Mutation Detected) for the mutant and wild-type specimens were as expected. All Mutant Control results were valid within the pre-specified values for PH. There were no obvious trends for Tm and PH values when denaturing and annealing temperatures were varied. All Tm and PH values of all samples were within software specifications. The results showed that the **cobas**® KRAS Mutation Test is able to tolerate variations of ± 1°C of the PCR thermal cycling profile denaturation and annealing temperatures by generating correct results under all test conditions.

b. **PCR Reaction Mix (RXN)**

The components of the PCR amplification mixture (KRAS RXN, Oligo, MGAC, and Sample) were tested by varying each individual component volumetrically while keeping the other components at the control volumes. Each component volume was tested by varying the volume by ± 6%. A total of 11 conditions were tested, including the target (prescribed) condition and two conditions where three components (KRAS RXN, Oligo and MGAC) had varied volumes by ± 6%. Of the eight (8) specimens used for the study, six (6) specimens were codon 12 mutants (G12C, G12V, G12A, G12R, G12D, G12S), one (1) specimen was codon 13 mutant (G13D), and one (1) specimen was wild-type. All seven (7) mutant DNA samples (DNA pools from FFPETs and DNA blends) had percent mutant sequences near 3x LoD of the KRAS test,

i.e., 15%, by MPS. In addition, five (5) replicates of the KRAS Mutant Control (MC) were also tested using each of the eleven (11) KRAS working master mixes. Three (3) replicates of each specimen pool were tested for each condition. All reported calls (i.e., Mutation Detected or No Mutation Detected) for the mutant and wild-type specimens were as expected. All Mutant Control results were valid within pre-specified values for PH. There were no significant trends observed for Tm1 and PH1 (for the mutant specimens and the Mutant Control), Tm2 and PH2 (for the wild-type specimen and the Mutant Control) and peak height ratio (PHR, PHR=PH1/PH2, for the Mutant Control) when volumes of working master mix components were varied. All Tm and PH values were within software specifications. The results of the procedural guard band study showed that the **cobas**® KRAS Mutation Test is able to generate correct results using various guard band volumes (± 6%) of KRAS Reaction Mix, MGAC, Oligo Mixes and Sample during the PCR reaction setup.

c. **Proteinase K (PK)**

During sample preparation, the CRC FFPET specimen is lysed by incubation at an elevated temperature with a proteinase and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. For each specimen processed by the **cobas**® DNA Sample Preparation kit, 70 µL of PK and 180 µL of DNA Tissue Lysis Buffer are added. The mixture is first incubated at 56°C for 60 minutes and then at 90°C for 60 minutes. Proteinase K was guard banded by varying the PK volume (±20%), the first incubation temperature (±2°C), and the first incubation time (±25%) using the **cobas**® DNA Sample Preparation kit. Of the eight (8) specimens used in this study, six (6) specimens were codon 12 mutant (G12A, G12C, G12D, G12S, G12V, G12R), one (1) specimen was codon 13 mutant (G13D) and one (1) specimen was wild-type. Four (4) of the seven (7) mutant specimens (G12C, G12D, G12V, and G13D) have percent mutant sequences near 3x LoD of the KRAS Mutation Test, i.e., 15%. All reported mutation status calls for the mutant and wild-type specimens were as expected for the nine conditions tested. There were no significant trends observed for Tm1 and PH1 (for the mutant specimens) and Tm2 and PH2 (for the wild-type specimen) when Proteinase K volume, heating temperature, and incubation time were varied. All Tm and PH values were within specifications. The results confirm that the **cobas**® KRAS Mutation Test is able to tolerate differences of ±20% for PK volume, ±2°C for the first incubation temperature, and ±25% for the first incubation time.

14. Cross-Contamination

AmpErase® (Uracil- N-glycosylase) is used in the test to avoid carryover contamination. This study was conducted to evaluate the rate of cross

contamination in the whole workflow (from the sample processing to amplification/detection) for the **cobas**® KRAS Mutation Test using CRC FFPET specimens. Cross contamination with the **cobas**® EGFR Mutation Test was evaluated by co-processing a KRAS mutant specimen with high percent mutation (G12D, 79.9% mutation by MPS, tumor content 70%) and the KRAS Negative Control in a checkerboard pattern. Sixty (60) 5-micron sections were obtained from the CRC FFPET specimen. Twelve (12) sections of the mutant specimen and twelve (12) replicates of the KRAS Negative Control were extracted and amplified/detected using alternating positions (checkerboard pattern) in sample layout for each of five (5) runs according to the **cobas**® KRAS Mutation Test Package Insert. Five (5) runs were performed in this study using one lot of **cobas**®DNA Sample Preparation Kit, one lot of **cobas**®KRAS Mutation Test kit, one operator and one **cobas z** 480 analyzer. All sixty (60) replicates for the high percent mutant sample generated the expected result of "Mutation Detected" (MD). No false positive results were observed for any of the sixty (60) replicates of Negative Control in this study. For the PH1 values of the mutant specimen tested, the SD value and the % CV value were 0.004 and 3.6%, respectively. For the Tm1 values of the mutant specimen tested, the SD value and the % CV value were 0.098 and 0.2%, respectively.

15. <u>Stability Studies</u>

a. **<u>Clinical Specimens, FFPET Blocks</u>**
The stability of CRC FFPET specimen blocks stored at room-temperature (15ºC to 32ºC) was evaluated using 10 CRC FFPET specimens, including five KRAS wild-type and five KRAS mutant specimens with tumor content ranging from 40% to 75%. The 5 KRAS mutant specimens were G12V, G12D, G12C and two G13 specimens, and the percent mutation ranged from 11.3% to 53.8% by MPS. The specimen blocks were stored at 32°C with testing conducted at 0, 3, 6, 9, and 13 months using two lots of the reagents. Results for nine of the ten specimens matched the expected results up to stability time point at 13 months. One codon 13 mutant specimen gave "No Mutation Detected" results at Month 13 due to the exhaustion of tumor tissue in the FFPET block as confirmed by the pathologist using an adjacent section. The results support that CRC FFPET clinical specimens are stable for up to 13 months when stored at 15-30ºC

b. **<u>Clinical Specimen, Slide-Mounted and Slide Curl</u>**
To demonstrate the stability of sections from CRC FFPET clinical specimens when mounted on a slide ("slide") or when not mounted on a slide ("curl"), 10 CRC FFPET specimens, including five KRAS mutant and five KRAS wild-type specimens were evaluated. One of the mutant specimens (G12D) had percent mutant sequences near 2x LoD of the KRAS test, i.e., ~10% mutation. Multiple 5-micron sections were

obtained from each of the 10 CRC FFPET specimens, mounted or not mounted on slides (one section per slide). The slides and curls were stored at 32°C and tested after 0, 33 (1 month), 61 (2 months) and 94 days (3 months). At each time point, two slides and two curls for each specimen were processed using one lot of the reagent. Observed results for all wild-type and mutant slides and curls of each specimen matched the expected results after storage at 32°C for Month 1, Month 2, and Month 3. These results indicate that sections stored as slides or curls are stable for 3 months at 32°C for use with the **cobas**® KRAS Mutation Test kit.

c. **Extracted DNA From FFPET Specimens**

Stability of DNA extracted from CRC FFPET specimens was evaluated using 10 CRC FFPET specimens, including five KRAS mutant and five KRAS wild-type specimens. The five KRAS mutant specimens were G12V, G12D, G12C and two G13 specimens, and the percent mutation ranged from 11.3% to 53.8% by MPS. DNA extracts obtained from each of the 10 CRC FFPET specimens were tested as follows:

- after storage at -20°C for 15, 31, and 61 days;
- after storage at 2°C to 8°C for 0, 15, 31, and 61 days;
- after storage at 32°C for 15, 31, and 61 days;
- after 0, 1, 2, 3, and 4 freeze-thaw cycles. Each freeze/thaw cycle was defined as a minimum of 24 hours at -20°C followed by thawing and testing.

After storage at -20°C, 2°C to 8°C, or 32°C, results from the ten (10) specimens matched the expected results (based on sequencing results) at all tested time points, indicating that DNA extracted from CRC FFPET specimens using the **cobas**® DNA Specimen Preparation kit is stable up to 2 months when stored at -20°C, 2 to 8°C, or 32°C. The results also indicated that the extracted DNA is stable for up to 4 freeze/thaw cycles when stored at -20°C. Little variation for Tm (less than 1°C), PH (less than 0.1) and PHR values was observed after storage for 61 days at 32°C, including one specimen (G12D) with 11.3% mutation.

d. **Working (Activated) Master Mix**

To evaluate the stability of **cobas**® KRAS Mutation Test Working (activated) Master Mixes stored at 32°C for up to 3 hours, one lot of reagent was used to test eight CRC FFPET specimens, including five KRAS mutant and three KRAS wild-type specimens. Five (5) of the eight (8) specimens were tested as extracted DNA (G12C, G12V, G13D, G12D, and wild-type) while the remaining three (3) specimens (G12A, G12R, and G12S) were tested as DNA blends that were made from extracts of CRC FFPET mutant and wild-type specimens to obtain the desired level of mutant sequences. All seven mutant DNA samples had

percent mutant sequences near 3x LoD, i.e., 15% by MPS. Working (activated) Master Mix was prepared by adding KRAS Master Mix to Magnesium Acetate and Oligo Mix. Working Master Mix stability was tested at five time points (0, 1, 1.5, 2 and 3 hours of incubation at 32°C) using two replicates per time point for each of the eight samples. The observed results matched the expected results when the Working Master Mix was stored for 0, 1, 1.5, 2 or 3 hours at 32ºC prior to the addition of DNA for the **cobas**® KRAS Mutation Test amplification and detection. There were no obvious trends for Tm1 and PH1 for all seven mutant specimens and for Tm2 and PH2 for the one wild-type specimen over the 3 hours tested. Results of the eight specimens showed that values were well within acceptance limits, and that none of the parameters examined would lead to failed results within the 3 hours tested.

e. **Extracted DNA Plus Working (Activated) Master Mix**

To evaluate the stability of the combination of extracted DNA from CRC FFPET specimens and the **cobas**® KRAS Mutation Test Working (activated) Master Mixes when stored at 32°C for up to 3 hours, one lot of reagent was used to test eight (8) CRC FFPET specimens, including seven (7) KRAS mutant and one (1) KRAS wild-type specimens. The specimens used in this study are identical to the ones described above in "Working (Activated) Master Mix". The study tested five different time points, i.e., 0, 1, 1.5, 2 and 3 hours of incubation at 32°C after addition to the working master mix. DNA samples were tested in duplicate along with KRAS Mutant Control (KRAS MC), Calibrator (KRAS CAL), and Negative Control (KRAS NC using DNA SD). The observed results matched the expected results when the extracted DNA plus Working Master Mix was stored for 0, 1, 1.5, 2 or 3 hours at 32ºC prior to **cobas**® KRAS Mutation Test amplification and detection. The results of the study showed that the combination of extracted DNA using the DNA **cobas**® Specimen Preparation kit and the **cobas**® KRAS Mutation Test Working Master Mix is stable for up to three hours when stored at 32°C prior to the start of amplification and detection.

f. **Open Vial, cobas® EGFR Mutation Test**

To determine the open vial stability of the reagents in the **cobas**® KRAS Mutation Test kit, six (6) kits were used to test three (3) CRC FFPET specimens, including one (1) KRAS mutant (G12D) and two (2) KRAS wild-type specimens. Three kits were tested on Days 0, 15, 21, and 33 and three additional kits were tested on Days 0, 48, 61, and 96. The six (6) kits and testing time points were done to demonstrate the open vial stability of the **cobas**® KRAS Mutation Test kit reagents for up to 30 days or 90 days with up to four uses per kit. The kits were stored at 2-8°C between uses. Six (6) 5-micron sections were obtained from each of the three (3) CRC FFPET specimens. The DNA extracts from all sections of each specimen were pooled and frozen in aliquots of 200µL

at -20°C. For each testing time point, the extracted DNA was thawed, diluted, and tested in two replicates. All valid results from the three (3) specimens matched the expected results when the **cobas**® KRAS Mutation Test kit reagents were used four times over 96 days when stored at -20°C between uses. There are no obvious trends for Tm1 and Tm2 for all three specimens over the time periods tested. Results for all Tm and PH parameters for the three specimens tested at all the time points showed values well within acceptance limits. The trend analysis showed that none of the parameters examined would lead to failed results within the time period of 96 days tested. These results indicate that the open vial stability of the **cobas**® KRAS Mutation Test kit reagents is at least 90 days.

g. **cobas® KRAS Mutation Test**

Stability of the components of the **cobas**® KRAS Mutation Test kit were assessed at various time points after storage at -20°C (real time) in upright and inverted orientations using three lots of the kit reagents. The test samples for the stability testing were the KRAS Mutant Control (KRAS MC), KRAS Calibrator (KRAS CAL) and the KRAS NC (DNA SD). Six (6) replicates of the KRAS MC, six (6) replicates of KRAS CAL, and one replicate of the DNA SD (negative control) were tested for each storage condition. Stability was evaluated by performing functional testing at 3, 5, 9, 12, 15, 18, 21, and 25 months. Functional testing had to pass both validation and acceptance criteria to be considered a passing time point. For the KRAS MC as on-test samples, the median results for PH1, PH2 and the Peak Height Ratio (PHR) must fall within specified ranges. To date, stability testing at real time (-20°C) temperature with three kit lots was completed and met the acceptance criteria through 25 months. Thus, stability data generated for these three (3) kit lots support expiry dating of the **cobas**® KRAS Mutation Test for storage at -25°C to -15°C for 25 months.

h. **cobas® DNA Sample Preparation Kit**

Stability of the **cobas**® DNA Sample Preparation Kit, including open-vial stability, was demonstrated in the approved PMA, P110020, for the **cobas**® 4800 BRAF V600 Mutation Test.

i. **Shipping**

To evaluate the tolerance of the **cobas**® KRAS Mutation Test kit to temperature extremes that can occur during shipping of the product, one set of simulated shipping temperature profile was tested as outlined below.

Table 9: Simulated Shipping Temperature Profile

Time	Storage Temperature
	Category B
2 Days	2-8ºC
20 Hours	25ºC
5 Days	15ºC
20 Hours	25ºC
10 Days	15ºC
5 Days	25ºC

The **cobas**® KRAS Mutation Test kit is stored at -25°C to -15°C and shipped at 2°C to 8°C (Category B shipping condition). Six (6) kits of **cobas**® KRAS Mutation Test were subjected to the simulated shipping profile of Category B. At the same time, six (6) additional kits of the same lot were stored as controls at the recommended storage condition (-25°C to -15°C) without going through the simulated shipping profile. At the end of the simulated shipping profile, the stressed kit and the non-stressed control kit were examined for their functional performance using the kit release testing procedure. The remaining stressed and non-stressed kits were stored at the recommended -25°C to -15°C kit storage temperature and were tested again when the kit was at 10 months post-DOM (Date Of Manufacturing).

Functional testing was performed using Mutant Control and Calibrator for codon 12/13. The DNA Specimen Diluent (DNA SD) was used as a Negative Control. There were three runs performed for the simulated shipping study. All runs were valid and all samples in all runs passed the validation criteria. The simulated shipping study for the **cobas**® KRAS Mutation Test under stressed conditions showed that the results from the kit release testing performed at 10 months post-DOM yielded expected results for all parameters for codon 12/13.

To further ensure that temperature extremes above 25°C do not occur during shipping of the **cobas**® KRAS Mutation Test, a calibrated temperature measuring device (data logger) is mandatory for every shipment of product from the manufacturing site to the distribution center. Any temperature excursion experienced during shipment of product (as recorded by the data logger) that exceeds > 0°C and ≤ 15°C is escalated to RMS Quality for evaluation and decision on disposition of the product according to the product specification. The Warehouse and Distribution SOP (Review of T.I.M.S. Shipment Temperature Curves) describes the requirements and the decision matrix for what to do if requirements are not met. If the temperature curve falls within

acceptance requirements, the product is released. If it does not, the product is rejected.

16. Antimicrobial Effectiveness Testing (AET)

To assess the effectiveness of the preservatives in the **cobas**® KRAS Mutation Test kit components, AET was performed on the following kit components: Reaction Mix (KRAS MIX), KRAS Codon 12/13 Oligo Mix (KRAS OM1), Magnesium Acetate (MGAC), Mutant Control (KRAS MC), and the KRAS Calibrator (KRAS CAL). One lot of the **cobas**® KRAS Mutation Test kit was tested and each component from this lot was tested shortly after date of manufacture and at approximately 2, 15, and 29 months. For DNA SD, one lot aged 6 months and another lot aged 29 months were tested separately to demonstrate the antimicrobial effectiveness across the lifetime of this component. A total of five (5) microorganisms (i.e., *Staphylococcus aureus, Candida albicans, Aspergillus niger, Pseudomonas aeruginosa and Escherichia coli*) were individually spiked into each of the seven (7) components on Day 0. The components were tested by determining the colony forming units (cfu) count in log10 on Days 14 and 28. Results demonstrated that the preservatives in the components of the **cobas**® KRAS Mutation Test kit are effective at preventing the growth of the microorganisms tested in this study.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the **cobas**® KRAS Mutation Test was evaluated in a study using retrospective samples from patients with advanced colorectal cancer. This study compared the **cobas**® KRAS Mutation Test with Sanger sequencing and the FDA-approved QIAGEN *therascreen* KRAS RGQ PCR Kit for detection of mutations in codons 12 and 13 of the KRAS gene in CRC tumor specimens using a subset of subjects enrolled in Roche study NO16968. Roche study NO16968 was a randomized phase III trial comparing capecitabine (Xeloda) plus oxaliplatin (XELOX) with bolus fluorouracil/leucovorin (FU/LV) as adjuvant therapy for stage III (Duke's C) colon cancer. Clinical outcomes of patients enrolled in Roche study NO16968 were not reviewed, and were not the basis of this PMA submission. As a supplement to these tumor specimens, a small subset of additional CRC tumor specimens was obtained from other sources. Method comparison data using specimens from this clinical study and predictive values of the **cobas**® KRAS Mutation Test were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

A subset of 398 CRC tumor specimens were obtained from subjects enrolled between April 2003 and October 2004 in Roche study NO16968. In addition, a set of 82 CRC supplemental samples were obtained from commercial tissue repositories, where informed consent allowing additional mutation testing had been obtained from all specimen donors.

The primary objective was to estimate positive percentage agreement (PPA), negative percentage agreement (NPA), and overall percentage agreement (OPA) of the **cobas®** KRAS Mutation Test with Sanger DNA sequencing and the QIAGEN *therascreen* KRAS RGQ PCR Kit, which is an FDA-approved test for detection of codon 12 and 13 mutations (in aggregate) in tumor samples from the population under study.

The secondary objective was to estimate predictive values of the **cobas®** KRAS Mutation Test for the comparator method(s) used in the cetuximab and panitumumab clinical studies [1-4].

1. Clinical Inclusion and Exclusion Criteria

Inclusion Criteria

- Male or female patients ≥18 years of age
- Patients with histologically confirmed late-stage CRC
- Sufficient archival FFPE tumor material for mutation analysis
- Written informed consent obtained from patient or legal guardian

Exclusion Criteria

- Lack of sufficient FFPET material to perform KRAS mutation testing with the **cobas®** KRAS Mutation Test, Sanger sequencing and the FDA-approved test
- Lack of informed consent for mutation testing

2. Follow-up Schedule

Not applicable.

3. Clinical Endpoints

Not applicable.

B. Accountability of PMA Cohort

A total of 480 tumor samples were available for the study, with 398 from the Roche study cohort and 82 supplemental samples. Nineteen samples from the Roche study cohort were not eligible for KRAS mutation testing. Of them, 15 were found to have no tumor content and 4 were determined not to be CRC, as confirmed by pathology evaluation. A total of 461 samples were eligible and tested for KRAS mutations by the three methods.

The **cobas**® KRAS Mutation Test results are shown in Table 10. Of the 437 samples with valid test results, 163 (37.3%) were Mutation Detected (MD, positive) and 274 (62.7%) were No Mutation Detected (NMD, wild-type, or negative).

Table 10: Specimen Accountability

	Total	**Roche Study Sample**	**Supplemental Sample**
Total Tumor Samples Available	480	398	82
No Tumor Content	15	15	0
Not colon/colorectal cancer	4	4	0
Samples Eligible for cobas Test	461	379	82
Invalid **cobas**® KRAS Mutation Test result	24 (5.2 %)	16 (4.2 %)	8 (9.8 %)
Valid **cobas**® KRAS Mutation Test result	437 (94.8 %)	363 (95.8 %)	74 (90.2 %)
Mutation Detected (Positive)	163 (37.3 %)	128 (35.3 %)	35 (47.3 %)
No Mutation Detected (Wild Type, Negative)	274 (62.7 %)	235 (64.7 %)	39 (52.7 %)

C. Study Population Demographics and Baseline Parameters

Demographics of the enrolled patients and their corresponding tumor characteristics are presented in Table 11. The median age of patients enrolled in this study was 61 years old, with a range from 26 to 89 years old. Approximately 89% of patients were Caucasian and 54% were male. Most patients had an ECOG score of 0 or 1(95%) and were Stage III (88.8%). Most tumor samples (97%) were primary tumors and the remaining 3% were metastatic. Most tumor samples (86%) had tumor content ≥20%, and more than half (54%) had no tumor necrosis present.

Table 11: Demographics and Baseline Clinical Characteristics and Specimen Availability for the cobas® KRAS Mutation Test

Characteristics	Statistic	Overall	Roche Study Sample	Supplemental Sample
Total		480	398	82
Age (years)				
	Mean ± SD	61 ± 10.699	60 ± 10.319	63 ± 12.134
	Median	61	61	64
	Range	26 - 89	26 - 83	32 - 89
Sex				
Male	n (%)	257 (53.5%)	228 (57.3%)	29 (35.4%)
Female	n (%)	223 (46.5%)	170 (42.7%)	53 (64.6%)
Race/Ethnicity				
Caucasian	n (%)	428 (89.2%)	363 (91.2%)	65 (79.3%)
Others	n (%)	52 (10.8%)	35 (8.8%)	17 (20.7%)
ECOG Score				
0	n (%)	312 (65.0%)	298 (74.9%)	14 (17.1%)
1	n (%)	140 (29.2%)	99 (24.9%)	41 (50.0%)
2	n (%)	11 (2.3%)	0 (0%)	11 (13.4%)
Unknown	n (%)	17 (3.5%)	1 (0.3%)	16 (19.5%)
TNM Disease Stage				
I	n (%)	10 (2.1%)	0 (0%)	10 (12.2%)
II	n (%)	36 (7.5%)	1 (0.3%)	35 (42.7%)
III	n (%)	408 (85.0%)	397 (99.7%)	11 (13.4%)
IV	n (%)	18 (3.8%)	0 (0%)	18 (22.0%)
Unknown	n (%)	8 (1.7%)	0 (0%)	8 (9.8%)
Tumor Type				
Primary	n (%)	463 (96.5%)	394 (99.0%)	69 (84.1%)
Metastatic	n (%)	12 (2.5%)	0 (0%)	12 (14.6%)
Unknown	n (%)	5 (1.0%)	4 (1.0%)	1 (1.2%)
Tumor Content				
<10%	n (%)	26 (5.4%)	25 (6.3%)	1 (1.2%)
10-20%	n (%)	42 (8.8%)	38 (9.5%)	4 (4.9%)
>20%	n (%)	412 (85.8%)	335 (84.2%)	77 (93.9%)
Necrosis				
No Necrosis	n (%)	261 (54.4%)	225 (56.5%)	36 (43.9%)
<10%	n (%)	76 (15.8%)	59 (14.8%)	17 (20.7%)
≥10%	n (%)	143 (29.8%)	114 (28.6%)	29 (35.4%)

TNM is cancer staging system which is based on the size and/or extent of the primary tumor (T), the amount of spread to nearby lymph nodes (N), and the presence of metastasis (M) to other parts of the body.

D. Safety and Effectiveness Results

1. Safety Results

As an *in vitro* diagnostic test, the **cobas**® KRAS Mutation Test involves testing on FFPE human CRC tissue sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of CRC by pathologists. Removal of these tissues therefore, presents no additional safety hazard to the patient being tested.

Adverse event information for cetuximab and the panitumumab are available in the cetuximab and the panitumumab clinical studies [1-4]. Most common adverse reactions (≥ 25%) in clinical trials of Erbitux (cetuximab) are: cutaneous adverse reactions (including rash, pruritus, and nail changes), headache, diarrhea, and infection. Most common adverse reactions (≥ 20%) in clinical trials of Vectibix (panitumumab) in combination with oxaliplatin-containing chemotherapy are diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, dermatitis acneiform, pruritus, and dry skin.

2. Effectiveness Results

KRAS Mutation Results by Different Test Methods

All of the 461 eligible samples were tested by the **cobas**® KRAS Mutation Test, Sanger sequencing and the FDA-approved test (Table 12). If the initial test result was invalid by any of the three tests, re-testing by the **cobas**® KRAS Mutation Test and the FDA-approved test was performed up to two times. However, for Sanger sequencing, the number of attempts were not limited unless there was insufficient remaining sample quantity for re-testing. The **cobas**® KRAS Mutation Test had an invalid rate of 5.2%. Sanger sequencing had an invalid rate of 2.6% and the FDA-approved test had the invalid rate of 2.8% for mutation testing in samples qualified in the DNA assessment step (i.e., 8% of did not qualify in DNA sample assessment step). Among the samples with valid results, the **cobas**® KRAS Mutation Test and the FDA-approved test had similar KRAS mutation-positive rates (37.3% vs 36.3%, respectively), while Sanger sequencing had a lower positive rate (28.5%).

Table 12: KRAS Mutation Result by Different Testing Methods

	cobas® KRAS Mutation Test	**FDA-approved Test**	**Sanger Sequencing**
Number of Samples Tested	461	461	461
Invalid result	24 (5.2 %)	50 (10.8 %)	12 (2.6 %)
Valid result	437 (94.8 %)	411 (89.2 %)	449 (97.4 %)
No Mutation Detected	274 (62.7 %)	262 (63.7 %)	321 (71.5 %)
Mutation Detected	163 (37.3 %)	149 (36.3 %)	128 (28.5 %)

Note: 37 samples with tumor content ≥20% failed sample assessment for the FDA-approved test. These samples did not go through the next step of mutation detection and were counted as invalid results.

Agreement between the Three Test Methods: 3-way Method Comparison
The agreement of the **cobas**® KRAS Mutation Test using Sanger sequencing and an FDA-approved test as the comparator methods for detection of mutations in codon 12/13 is presented in Table 13. The PPA between the **cobas**® KRAS Mutation Test and Sanger sequencing was 96.9% (95% CI: 92.2% to 98.8%), and the NPA was 88.7% (95% CI: 84.7% to 91.8 %). The PPA between the **cobas**® KRAS Mutation Test and the FDA-approved test was 93.3% (95% CI: 88.1% to 96.3%), and the NPA was 96.5% (95% CI: 93.5% to 98.1%).

Table 13: Comparison of the cobas® KRAS Mutation Test with Comparator Methods for Detection of KRAS Mutations in Codon 12/13

cobas® KRAS Mutation Test	Comparator Method							
	Sanger Sequencing				FDA-approved Test			
	Mutation Detected	No Mutation Detected	Invalid	Total	Mutation Detected	No Mutation Detected	Invalid	Total
Mutation Detected	124	34	5	163	139	9	15	163
No Mutation Detected	4	268	2	274	10	248	16	274
Invalid	0	19	5	24	0	5	19	24
Total	128	321	12	461	149	262	50	461
PPA (95% CI)	96.9% (92.2%, 98.8%)				93.3% (88.1%, 96.3%)			
NPA (95% CI)	88.7% (84.7%, 91.8%)				96.5% (93.5%, 98.1%)			
OPA (95% CI)	91.2% (88.1%, 93.5%)				95.3% (92.8%, 97.0%)			

*Predictive Values of the **cobas**® KRAS Mutation Test*
The predictive values of the **cobas**® KRAS Mutation Test were calculated by combining the PPA and NPA of the **cobas**® KRAS Mutation Test relative to a comparator method together with the prevalence of a KRAS "Mutation Detected" result by the comparator method in the published clinical studies for cetuximab or panitumumab [1-4]. The positive and negative predictive values (PPV and NPV) of the **cobas**® KRAS Mutation Test refer to the predictive values of **cobas**® "Mutation Detected" and "No Mutation Detected" results for the comparator method, respectively [5]. The clinical performance was summarized by the quantity of PPV + NPV – 1; this quantity has been called the *attenuation factor* [5].

Table 14: Attenuation Factors for the cobas® KRAS Mutation Test

Data Source [Ref]	Comparator Method	PPV (95% CI)	NPV (95% CI)	Attenuation Factor (95% CI)
Cetuximab [1]	Sanger Sequencing	0.858 (0.811, 0.902)	0.975 (0.946, 0.994)	83.3% (77.7, 88.3)
Cetuximab [6]	FDA-approved Test	0.957 (0.927, 0.981)	0.945 (0.909, 0.978)	90.2% (85.6, 94.4)
Panitumumab [4, 7]	FDA-approved Test	0.949 (0.914, 0.977)	0.956 (0.927, 0.981)	90.4.% (86.1, 94.4)

Ref: refer to references listed in section XVI

E. Financial Disclosures

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

To support the clinical performance of the **cobas**® KRAS Mutation Test, a statistical model was used to project the performance of **cobas**® KRAS Mutation Test in clinical studies for cetuximab and panitumumab [1-4], without actually having the samples/specimens from these studies tested with the **cobas**® KRAS Mutation Test. The projection is based on agreement of the **cobas**® KRAS Mutation Test with a comparator method that was used in the clinical study. The agreement data are from the 3-way method comparison study, which used samples external to the clinical studies for cetuximab and panitumumab [1-4].

Model projections indicate that for cetuximab or panitumumab, clinical outcomes will improve in the KRAS "No Mutation Detected" population, whereas the clinical outcomes will not improve in the KRAS "Mutation Detected" population, if the study population would have been determined by the **cobas**® KRAS Mutation Test results. These conclusions are based on the following argument. Under the model's assumptions, the difference in therapeutic efficacy between the **cobas**® "No Mutation Detected" and "Mutation Detected" populations is projected to be attenuated relative to the corresponding difference for the comparator method by the factor PPV + NPV – 1. Attenuation was projected to be 83.3-90.4%, depending on the comparator method and the clinical study (Table 14).

The model makes two key assumptions. First, the **cobas**® KRAS Mutation Test result is conservatively assumed to be non-differential for clinical efficacy (cetuximab or panitumumab) given the comparator method result (i.e., non-differential measurement error, or NDME [8]). That is, given the comparator method result, clinical efficacy is assumed not to depend on the **cobas**® KRAS Mutation Test result. Second, the 3-way method comparison study for the **cobas**® KRAS Mutation Test with the comparator method(s) is assumed to be *transportable* to the clinical study. That is, the data from the two studies is assumed to be poolable. These statistical assumptions were challenged as the follows. To relax the model assumption of transportability, clinical performance was re-evaluated after adjustment for covariates that were significantly associated with agreement of the **cobas**® KRAS Mutation Test with the comparator method. To evaluate robustness of clinical performance of the **cobas**® KRAS Mutation Test, *k* negative concordant results and *k* positive concordant results were changed to discordant results [9]. The number *k* is defined as 1 to *n*, where *n* is the total number of discordant results. The number *k* was increased until a tipping point was reached at which the result of clinical performance was changed. Additionally, variation in the clinical outcomes was increased beyond observed variation to evaluate the robustness of the **cobas**® KRAS Mutation Test. To evaluate the impact of missing/invalid test results for either the **cobas**® KRAS Mutation Test or the comparator method(s), various scenarios were created to impute the missing results as discordant between the tests. These additional analyses demonstrated that the projected clinical performance of the **cobas**® KRAS Mutation Test was robust and remained comparable to the FDA-approved *therascreen* KRAS RGQ PCR Kit.

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the **cobas**® KRAS Mutation Test kit was assessed by the agreement rates between the **cobas**® KRAS Mutation Test and the comparator methods (i.e., *therascreen* KRAS RGQ PCR Kit and Sanger sequencing). The totality of the preclinical and clinical data demonstrated that the **cobas**® KRAS Mutation Test appropriately and reproducibly detects KRAS mutations in codons 12 and 13 in CRC and can aid in identification of CRC patients being considered for cetuximab or panitumumab therapy.

B. Safety Conclusions

The adverse effects of the device are extrapolated on outcome data collected in clinical trials for cetuximab and panitumumab [1-4]. As an *in vitro* diagnostic test, the **cobas**® KRAS Mutation Test involves testing on FFPE human CRC tissue sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of CRC by pathologists. The test, therefore, presents no additional safety hazard to the patient being tested. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect KRAS test results, and consequently improper treatment decisions for CRC patients (see Benefit-Risk Conclusion below).

C. Benefit-Risk Conclusions

The probable benefits of the **cobas**® KRAS Mutation Test are based on evaluation that the test performs consistently and provides clinically relevant results for assessing patients with CRC being considered for cetuximab or panitumumab therapy. The risks of the **cobas**® KRAS Mutation Test are associated with the potential mismanagement of patients resulting from false results of the test. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect KRAS test results, and consequently improper treatment decisions for CRC patients. A false positive test result may lead to treatment being withheld from a patient who might have benefitted. A false negative test result may lead to treatment being administered to a patient who is not expected to benefit, and potentially any adverse side effects associated with treatment. The device is a key part of diagnostic evaluation for CRC in decisions regarding treatment with cetuximab or panitumumab.

In conclusion, the data support the use of the **cobas**® KRAS Mutation Test as an aid in selecting CRC patients for cetuximab or panitumumab based on a **cobas**® KRAS Mutation Test "No Mutation Detected" result, and the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The provided studies support use of the **cobas**® KRAS Mutation Test as an aid in selecting patients with CRC for treatment with Erbitux (cetuximab) or Vectibix (panitumumab).

XIV. CDRH DECISION

CDRH issued an approval order on May 7, 2015.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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