SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Real-time PCR test
Device Trade Name:	**cobas**® EGFR Mutation Test v2
Device Procode:	OWD
Applicant's Name and Address:	Roche Molecular Systems, Inc. (RMS) 4300 Hacienda Drive Pleasanton, CA 94588-2722
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P150047
Date of FDA Notice of Approval:	June 1, 2016
Priority Review:	Granted priority review status on December 9, 2015 because the device addresses an unmet medical need, as demonstrated by significant clinically meaningful advantage.

The **cobas**® EGFR Mutation Test v2 (P120019/S007) was approved on November 13, 2015 for the qualitative detection of defined mutations in the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded tumor tissue (FFPET) from non-small cell lung cancer (NSCLC) patients. The indications for use of the device as approved in P120019/S007 is to aid in identifying patients with NSCLC whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established shown in the table below. The SSED to support the FFPET indications are available on the CDRH website.

Table 1. Previously Approved cobas® EGFR Mutation Test v2 Indications for FFPET Specimens.

Drug	Mutations
TARCEVA® (erlotinib)	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M

The current PMA was submitted to add an indication for the **cobas**® EGFR Mutation Test v2 to identify NSCLC patients with Exon 19 deletions and L858R substitution mutations from cell-free DNA (cfDNA), also referred to as circulating tumor DNA (ctDNA), isolated from plasma, for treatment with TARCEVA® (erlotinib).

II. INDICATIONS FOR USE

The **cobas**® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) patients. Defined EGFR mutations are detected using DNA isolated from formalin-fixed paraffin-embedded tumor tissue (FFPET) or circulating-free tumor DNA (cfDNA) from plasma derived from EDTA anti-coagulated peripheral whole blood.

The test is indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 below in accordance with the approved therapeutic product labeling:

Table 1.

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	Exon 19 deletions and L858R	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M	

Patients with positive **cobas**® EGFR Mutation Test v2 test results using plasma specimens for the presence of EGFR exon 19 deletions or L858R mutations are eligible for treatment with TARCEVA® (erlotinib). Patients who are negative for these mutations by this test should be reflexed to routine biopsy and testing for EGFR mutations with the FFPET sample type.

Drug safety and efficacy have not been established for the following EGFR mutations listed in Table 2 below that are also detected by the **cobas**® EGFR Mutation Test v2:

Table 2.

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q	G719X, exon 20 insertions, T790M, S768I and L861Q
TAGRISSO™ (osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q	G719X, exon 19 deletions, L858R, exon 20 insertions, T790M, S768I, and L861Q

For manual sample preparation, FFPET specimens are processed using the **cobas**® DNA Sample Preparation Kit and plasma specimens are processed using the **cobas**® cfDNA Sample Preparation Kit. The **cobas z** 480 analyzer is used for automated amplification and detection.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the **cobas**® EGFR Mutation Test v2 labeling.

V. DEVICE DESCRIPTION

The **cobas**® EGFR Mutation Test v2 is based on two major processes: (1) manual sample preparation to obtain DNA from FFPET or plasma; and (2) PCR amplification and detection of target DNA using complementary primer pairs and oligonucleotide probes labeled with fluorescent dyes.

The **cobas**® EGFR Mutation Test v2 is comprised of the following:

1. The **cobas**® EGFR Mutation Test v2 kit provides reagents for automated real-time PCR amplification and detection of the EGFR mutations.
2. The **cobas**® DNA Sample Preparation Kit provides reagents for manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tumor tissue (FFPET).
3. The **cobas**® cfDNA Sample Preparation Kit provides reagents for manual specimen preparation to obtain cfDNA from plasma.

Two external run controls are provided and the EGFR exon 28 wild-type (WT) allele serves as an internal, full process control.

A. Specimen Preparation – Plasma

Plasma specimens are processed and cfDNA is isolated using the **cobas**® cfDNA Sample Preparation Kit, a generic manual specimen preparation based on nucleic acid binding to glass fibers. The components of the **cobas**® cfDNA Sample Preparation Kit are identical to those of the **cobas**® DNA Sample Preparation Kit, with the exception of the High Pure Extension Assembly (HPEA) units.

Two milliliters (mL) of plasma are processed with a protease and chaotropic lysis/binding buffer that protects the cfDNA from DNases. Subsequently, isopropanol is added to the binding mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the cfDNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution.

B. PCR Amplification and Detection

Target Selection and Amplification

The **cobas**® EGFR Mutation Test v2 kit uses primers that define specific base-pair sequences for each of the targeted mutations. For the exon 19 deletion mutations, sequences ranging from 125 to 141 base pairs are targeted; for the L858R substitution mutation in exon 21, a 138 base pair sequence is targeted; for the T790M substitution mutation in exon 20, a 118 base pair sequence is targeted; for the G719X substitution mutation in exon 18, sequences ranging from 104 to 106 base pairs are targeted; for the S768I substitution mutation in exon 20, a 133 base pair sequence is targeted; for the exon 20 insertion mutations, sequences ranging from 125 to 143 base pairs are targeted; for the L861Q substitution mutation in exon 21, a 129 base pair sequence is targeted; for the internal control in exon 28, an 87 base pair sequence is targeted. Amplification occurs only in the regions of the EGFR gene between the primers; the entire EGFR gene is not amplified.

The **cobas**® EGFR Mutation Test v2 uses allele-specific PCR (AS-PCR) chemistry for amplification and detection. The selected AS-PCR primers specifically amplify the targeted mutant sequences over the WT sequences and/or other human DNA. The **cobas**® EGFR Mutation Test v2 is designed to use three master mix (MMx) reagents which are run in three separate wells. The number and types of primers and probes differ based on the particular target(s). The **cobas**® EGFR Mutation Test v2 detects the following EGFR mutations in exons 18, 19, 20, and 21:

Table 2. EGFR Mutations Detected by the cobas® EGFR Mutation Test v2

Exon	EGFR Mutation	EGFR Nucleic Acid Sequence	COSMIC ID[1]
Exon 18	G719X	2156G>C	6239
		2155G>A	6252
		2155G>T	6253
Exon 19	Ex. 19del	2240_2251del12	6210
		2239_2247del9	6218
		2238_2255del18	6220
		2235_2249del15	6223
		2236_2250del15	6225
		2239_2253del15	6254
		2239_2256del18	6255
		2237_2254del18	12367
		2240_2254del15	12369
		2240_2257del18	12370
		2239_2248TTAAGAGAAG>C	12382
		2239_2251>C	12383
		2237_2255>T	12384
		2235_2255>AAT	12385
		2237_2252>T	12386
		2239_2258>CA	12387
		2239_2256>CAA	12403
		2237_2253>TTGCT	12416
		2238_2252>GCA	12419
		2238_2248>GC	12422
		2237_2251del15	12678

Exon	EGFR Mutation	EGFR Nucleic Acid Sequence	COSMIC ID[1]
		2236_2253del18	12728
		2235_2248>AATTC	13550
		2235_2252>AAT	13551
		2235_2251>AATTC	13552
		2253_2276del24	13556
		2237_2257>TCT	18427
		2238_2252del15	23571
		2233_2247del15	26038
Exon 20	S768I	2303G>T	6241
	T790M	2369C>T	6240
	Ex. 20ins	2307_2308ins9GCCAGCGTG	12376
		2319_2320insCAC	12377
		2310_2311insGGT	12378
		2311_2312ins9GCGTGGACA	13428
		2309_2310AC>CCAGCGTGGAT	13558
Exon 21	L858R	2573T>G	6224
		2573_2574TG>GT	12429
	L861Q	2582T>A	6213

[1]Catalogue of Somatic Mutations in Cancer (COSMIC), 2011, v.51. http://www.sanger.ac.uk/genetics/CGP/cosmic.

MMx1 (first amplification reaction) contains:

- Fourteen AS-PCR primers, one common primer, and one common probe are used to detect the Exon 19 deletion and complex (defined as the combination of a deletion and an insertion) mutations.
- One AS-PCR primer, one common primer, and one common probe are used to detect the S768I mutation.

MMx2 (second amplification reaction) contains:

- One AS-PCR primer, one common primer, and one common probe are used to detect the L858R mutation.
- One AS-PCR primer, one common primer, and one common probe are used to detect the T790M mutation.

MMx3 v2 (third amplification reaction) contains:

- Three AS-PCR primers, one common primer, and one common probe are used to detect G719X mutations.
- Three AS-PCR primers, one common primer, and one common probe are used to detect Exon 20 insertion mutations.
- One AS-PCR primer, one common primer, and one common probe are used to detect the L861Q mutation.

A derivative of *Thermus* species Z05-AS1 DNA polymerase is utilized for target amplification. The PCR reaction mixture is heated to denature the DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream

primers anneal to the target DNA sequences. The Z05-AS1 DNA polymerase, in the presence of 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 base-pair regions of the EGFR 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 acid from the specimen is achieved in the **cobas**® EGFR Mutation Test v2 by the use of AmpErase® (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP), which are included in the Master Mix reagents. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is always present in the amplicons due to the use of dUTP as one of the nucleotide triphosphates in the Reaction Mix reagent; therefore, only the amplicons contain deoxyuridine. 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 Detection

The **cobas**® EGFR Mutation Test v2 utilizes real-time PCR technology. Each target-specific, oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher molecule that absorbs (quenches) fluorescent emissions from the reporter dye within an intact probe. During each cycle of amplification, a probe complementary to the single-stranded DNA sequence in the amplicon binds and is subsequently cleaved by the 5′ to 3′ nuclease activity of the Z05-AS1 DNA polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, fluorescence of a characteristic wavelength can be measured when the reporter dye is excited by the appropriate spectrum of light. Two different reporter dyes are used to label the mutations targeted by the test. Amplification of the targeted EGFR sequences are detected independently across three reactions by measuring fluorescence at the two characteristic wavelengths in dedicated optical channels.

Instrument and Software

The **cobas**® 4800 system is controlled by the **cobas**® 4800 system 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 assay specific analysis package software (ASAP). The **cobas**® z480 analyzer component of the test system also has its own internal instrument control software, which is driven by the core software.

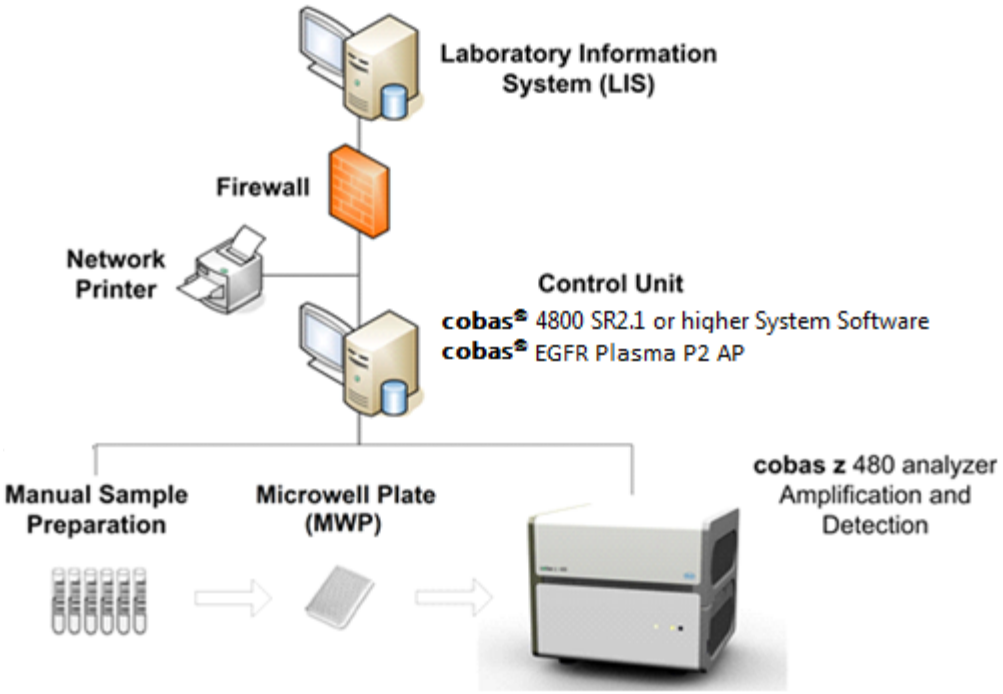
A dedicated Control Unit computer runs the **cobas**® 4800 system software and provides an interface to the **cobas**® **z** 480 and Laboratory Information System (LIS). The computer also processes the fluorescent signals with the assay specific analysis

package (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 either manually or automatically when connected to a LIS. 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 barcode 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 software 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 GUI based result viewer and a result export file that can be exported to the LIS.

The **cobas**® 4800 system software includes the assay-specific **cobas**® 4800 EGFR Analysis Package (AP) software, which contains an algorithm to determine sample results and run validity. The analysis package used to analyze the EGFR results from cfDNA specimens isolated from plasma differs from the one used to analyze EGFR results from DNA specimens isolated from FFPET specimens. The overall **cobas**® 4800 system components are shown in the diagram below:

Figure 1. **cobas**® 4800 System



The final version of the **cobas**® 4800 core system software is v2.1.0 or v2.2.0 and the ASAP software used to analyze all studies in this PMA was EGFR Plasma P2 AP

v1.0.1.1555. The final commercial ASAP software is EGFR Plasma P2 Analysis Package v1.0.1.1567.

Interpretation of Results

If the run is valid, then the cycle threshold (Ct) or CtR (relative cycle threshold) values for each sample will be evaluated against acceptable ranges for each channel. The CtR value is determined by calculating the difference between the mutations observed Ct and the corresponding Internal Control (IC) Ct value from the same Master Mix. Ct values are not available to the user. The tables below summarize how the individual amplification Master Mix results are combined to provide an overall result.

Table 3. Individual Amplification Master Mix Results to Overall Reported Results.

MMx1 Result	MMx2 Result	MMx3 v2 Result	Reported Result
Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected
Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, No Mutation Detected	Valid, No Mutation Detected	Invalid	Invalid
Valid, No Mutation Detected	Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, No Mutation Detected	Valid, Mutation Detected	Invalid	Invalid
Valid, No Mutation Detected	Invalid	Valid, No Mutation Detected	Invalid
Valid, No Mutation Detected	Invalid	Valid, Mutation Detected	Invalid
Valid, No Mutation Detected	Invalid	Invalid	Invalid
Valid, Mutation Detected	Valid, No Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, No Mutation Detected	Invalid	Invalid
Valid, Mutation Detected	Valid, Mutation Detected	Valid, No Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected	Valid, Mutation Detected
Valid, Mutation Detected	Valid, Mutation Detected	Invalid	Invalid

MMx1 Result	MMx2 Result	MMx3 v2 Result	Reported Result
Valid, Mutation Detected	Invalid	Valid, No Mutation Detected	Invalid
Valid, Mutation Detected	Invalid	Valid, Mutation Detected	Invalid
Valid, Mutation Detected	Invalid	Invalid	Invalid
Invalid	Valid, No Mutation Detected	Valid, No Mutation Detected	Invalid
Invalid	Valid, No Mutation Detected	Valid, Mutation Detected	Invalid
Invalid	Valid, No Mutation Detected	Invalid	Invalid
Invalid	Valid, Mutation Detected	Valid, No Mutation Detected	Invalid
Invalid	Valid, Mutation Detected	Valid, Mutation Detected	Invalid
Invalid	Valid, Mutation Detected	Invalid	Invalid
Invalid	Invalid	Valid, No Mutation Detected	Invalid
Invalid	Invalid	Valid, Mutation Detected	Invalid
Invalid	Invalid	Invalid	Invalid

Table 4. Result Interpretation of the cobas® EGFR Mutation Test v2

Test Result	Mutation Result**	Interpretation
Mutation Detected (MD)	Ex.19del L858R T790M *S768I* *G719X* *Ex. 20ins* *L861Q* (More than one mutation may be present)	Mutation detected in specified targeted EGFR region.
No Mutation Detected* (NMD)	N/A	No mutation detected in targeted EGFR regions
Invalid	N/A	Specimen result is invalid.
Failed	N/A	Failed run due to hardware or software failure.

*A No Mutation Detected (NMD) result does not preclude the presence of a mutation in the targeted EGFR regions, because results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.

**Italicized mutation results consist of mutations that are intended for analytical detection only for both FFPET and plasma specimens and the T790M mutation is for analytical detection only when identified in plasma specimens.

Test Controls

One EGFR mutant control and one EGFR negative control are provided. The EGFR WT allele located on exon 28 serves as an internal, full process control.

1. *EGFR Mutant Control:* The Mutant Control is a blend of seven DNA plasmids containing specified EGFR mutation sequences and cell line DNA that is WT for EGFR. The Mutant Control is composed of plasmids representing the most frequently observed mutation for each mutation class detected by the test. The Mutant Control will be included in every run and will serve as a process control for amplification and detection. The Mutant Control must yield Cycle threshold (Ct) values for all targeted mutations and the Internal Control (IC) within the respective acceptable ranges for the run to be considered valid.

2. *EGFR Negative Control:* The Negative Control is a full process contamination control for a given test batch of specimens. The Negative Control consists of a blank vial containing no specimen (specimen diluent only) which is processed through specimen preparation and the resulting eluate is subsequently used for amplification and detection. The Negative Control Ct values must be either not detected or greater than the pre-established Ct maximum value for all targeted mutation groups and the IC for the run to be considered valid.

3. *EGFR WT Internal Control (IC):* The Internal Control in EGFR exon 28 from test specimens serves as a full process control. This control ensures that every step of the process from specimen preparation to amplification and detection has been completed successfully.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or -approved alternatives for the testing of plasma (or formalin-fixed, paraffin-embedded NSCLC tissue) for EGFR mutation status in the selection of patients who are eligible for treatment with TARCEVA® (erlotinib) or TAGRISSO™ (osimertinib).

VII. MARKETING HISTORY

The **cobas**® EGFR Mutation Test (v1) was introduced into the United States starting on May 14, 2013. The **cobas**® EGFR Mutation Test (v1) is commercially available in the following countries: Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Estonia, Finland, France, Germany, Greece, Guatemala, Hong Kong, Hungary, Iceland, India, Indonesia, Ireland, Italy, Japan, Korea, Latvia, Liechtenstein, Lithuania, Luxembourg, Malaysia, Malta, Mexico, Netherlands, New Zealand, Nicaragua, Norway, Pakistan, Panama, Peru, Philippines, Poland, Portugal, Romania, Singapore, Slovakia,

Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, United Arab Emirates, United Kingdom, United States, Uruguay, Venezuela, Vietnam.

The **cobas**® EGFR Mutation Test (v1) was replaced by the **cobas**® EGFR Mutation Test v2, which was introduced into the United States starting on November 13, 2015 for use with FFPET specimens. The **cobas**® EGFR Mutation Test v2 for use with FFPET and plasma specimens is commercially available in Australia, Austria, Belgium, Bolivia, Bulgaria, Colombia, Croatia, Cyprus, Czechia, Denmark, Ecuador, Estonia, Finland, France, Germany, Greece, Guatemala, Hong Kong, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, New Zealand, Norway, Panama, Paraguay, Poland, Portugal, Romania, Singapore, Slovakia, Slovenia, Spain, Sweden, Switzerland, Thailand, Turkey, United Arab Emirates, United Kingdom, Uruguay, and Vietnam. The **cobas**® EGFR Mutation Test v2 for use with FFPET specimens is also commercially available in Japan.

Neither version of the **cobas**® EGFR Mutation Test for either specimen type, has been withdrawn from the market for reasons related to safety and effectiveness.

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 **cobas**® EGFR Mutation Test v2 results and subsequently improper patient management decisions in NSCLC treatment. No adverse events were reported in connection with the studies used to support this PMA as the studies were performed retrospectively using banked samples.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

For the non-clinical studies described below, the percentage of mutation present in the prepared plasma specimens was assessed using a validated next generation sequencing (NGS) method. Due to the lack of sufficient volume of clinical specimens, some of the studies used contrived samples, which consisted of sheared cell line DNA diluted into healthy donor (HD) EDTA plasma. A commutability study was conducted to demonstrate comparable performance of sheared cell line DNA diluted in HD plasma as compared to NSCLC EDTA plasma. Clinical specimens or clinical specimen pools were used to assess analytical accuracy, confirm the estimated limit of detection (LoD) and evaluate assay reproducibility.

Contrived samples were prepared using cell line DNA stocks, purchased from a commercial source, sheared to an average size of approximately 220 bp using focused-ultrasonication. DNA fragment sizes were confirmed by an orthogonal method. The sheared cell line DNA stocks were then quantitated against a plasmid-based standard curve in order to dilute each EGFR mutation to pre-specified input cp/mL levels for each of the studies. The cell lines used include the predominant

mutation for each mutation group detected by the **cobas**® EGFR Mutation Test v2 using plasma specimens (**cobas**® EGFR Plasma Test v2).

The sheared cell line DNA with multiple mutation targets were combined to allow for the evaluation of more than one EGFR mutation target per sample. The combinations included in each sample are shown in the table below. In each non-clinical study, contrived samples at approximately 3x LoD (in cp/mL) per mutation were evaluated unless otherwise specified.

Table 5. EGFR Mutation Combinations for Contrived Samples

	First EGFR Mutation	Second EGFR Mutation
Contrived Sample 1	Ex. 19del	Ex. 20ins
Contrived Sample 2	G719X	S768I
Contrived Sample 3	L861Q	T790M
Contrived Sample 4	L858R	None

1. Correlation with Reference Method Using Clinical Samples

The analytical accuracy of the **cobas**® EGFR Plasma Test v2 was assessed by comparing its results to a validated quantitative NGS method using banked clinical samples. Percent mutation present in specimens was determined for all specimens used to demonstrate performance.

In this study, plasma specimens collected at baseline screening from patients from three different studies in stage IIIb/IV NSCLC patients: the ASPIRATION (ML25637/NCT01310036)[1], MetMab (Genentech study GO27821/NCT01496742), and MetLung (GO27761/NCT01456325) studies. The available specimens from these studies (hereafter referred to as the ASPIRATION cohort) were tested by both the **cobas**® EGFR Plasma Test v2 and a validated NGS method. The specimens from the MetMab and MetLung studies were included to supplement the samples from the ASPIRATION study to ensure a sufficient number of samples for statistical analysis. All patients from the MetMab study were NMD by the **cobas**® EGFR Tissue Test v1 and were used to supplement the tissue NMD study population.

Specimen Processing, Disposition, and Testing

FFPET specimens were processed according to the standard protocol. In the ASPIRATION study, patients were screened based on an EGFR clinical trial assay and not all samples were re-tested by the **cobas**® EGFR Tissue Test v1 during the study. Tissue samples from MetLung and MetMab were tested with the **cobas**® EGFR Tissue Test v1 during the studies and results were not used to enroll the patients. No additional tissue samples were tested under this study protocol.

1 (Study sponsor's protocol/ClinicalTrials.gov registration number)

Plasma specimens were sent to one of the participating central laboratories after written informed consent was confirmed. cfDNA was isolated manually using **cobas**® cfDNA Sample Preparation Kit. Testing was performed according to the instructions for use for the **cobas**® EGFR Plasma Test v2.

Testing was performed using two different lots of the **cobas**® cfDNA Sample Preparation Kit and one lot of the **cobas**® EGFR Mutation Test v2 Kit on three separate **cobas z** 480 Analyzers.

Accountability of the ASPIRATION Cohort

A total of 411 plasma samples from the ASPIRATION cohort were tested by the **cobas**® EGFR Plasma Test v2. The specimens included 231 specimens from the ASPIRATION study, 110 specimens from the MetMab study, and 70 specimens from the MetLung study. Due to different study designs, the **cobas**® EGFR Tissue Test v1 results were only available for 340/411 patients with plasma samples. However, only 128 samples with valid paired **cobas**® EGFR Tissue Test v1 result had a plasma volume of 2.0 mL. Since the **cobas**® EGFR Plasma Test v2 is validated for 2.0 mL plasma samples, the primary analysis focused on only those samples with a 2.0 mL volume.

Agreement to Reference Method:

Unadjusted Agreement:

Based on the plasma samples tested from the ASPIRATION Cohort (with 2.0 mL plasma volume), the agreements of the **cobas**® EGFR Plasma Test v2 and the NGS method for detection of EGFR Ex. 19del and L858R mutations are presented in the tables below. Among 128 samples with a 2.0 mL plasma volume, a total of 32 samples had MD and 95 had NMD results by the NGS method. The tables below summarize agreement calculations with associated sensitivity analyses.

Table 6. Unadjusted Agreement between cobas® EGFR Plasma Test v2 and NGS for Detection of EGFR Mutations* in Aggregate with 2.0 mL Plasma Volume

	NGS (Plasma)				
cobas® EGFR Plasma Test v2	**MD**	**NMD**	**Invalid**	**Not Tested**	**Total**
MD	28	3	0	0	31
NMD	4	92	1	0	97
Invalid	0	0	0	0	0
Total	32	95	1	0	128
With only Valid Result					
PPA (95% CI)	87.5% (71.9%, 95.0%)				
NPA (95% CI)	96.8% (91.1%, 98.9%)				
PPV (95% CI)	90.3% (75.1%, 96.7%)				
NPV (95% CI)	95.8% (89.8%, 98.4%)				

Sensitivity Analysis with Invalid Result as Discordant

	NGS (Plasma)				
cobas® EGFR Plasma Test v2	**MD**	**NMD**	**Invalid**	**Not Tested**	**Total**
PPA (95% CI)	84.8% (69.1%, 93.3%)				
NPA (95% CI)	96.8% (91.1%, 98.9%)				
PPV (95% CI)	90.3% (75.1%, 96.7%)				
NPV (95% CI)	94.8% (88.5%, 97.8%)				

*Detection of either or both Ex. 19del and L858R Mutations
PPA = Positive Percent Agreement; NPA = Negative Percent Agreement;
PPV = Positive Predictive Value; NPV = Negative Predictive Value

Table 7. Unadjusted Agreement between cobas® EGFR Plasma Test v2 and NGS for Detection of Ex. 19del and L858R Mutations (with 2.0 mL Plasma Volume)

	NGS (Plasma)									
	Ex. 19del					L858R				
cobas® EGFR Plasma Test v2	**MD**	**NMD**	**Invalid**	**Not Tested**	**Total**	**MD**	**NMD**	**Invalid**	**Not Tested**	**Total**
MD	17	2	0	0	19	11	1	0	0	12
NMD	2	106	1	0	109	2	113	1	0	116
Invalid	0	0	0	0	0	0	0	0	0	0
Total	19	108	1	0	128	13	114	1	0	128
With only Valid Result										
PPA (95% CI)	89.5% (68.6%, 97.1%)					84.6% (57.8%, 95.7%)				
NPA (95% CI)	98.1% (93.5%, 99.5%)					99.1% (95.2%, 99.8%)				
PPV (95% CI)	89.5% (68.6%, 97.1%)					91.7% (64.6%, 98.5%)				
NPV (95% CI)	98.1% (93.5%, 99.5%)					98.3% (93.9%, 99.5%)				

Sensitivity Analysis with Invalid Result as Discordant		
PPA (95% CI)	85.0% (64.0%, 94.8%)	78.6% (52.4%, 92.4%)
NPA (95% CI)	98.1% (93.5%, 99.5%)	99.1% (95.2%, 99.8%)
PPV (95% CI)	89.5% (68.6%, 97.1%)	91.7% (64.6%, 98.5%)
NPV (95% CI)	97.2% (92.2%, 99.1%)	97.4% (92.7%, 99.1%)

Adjusted Agreement:

Since the plasma samples tested in this accuracy study were not representative of the intended use population due to enrichment of patients with a tissue MD result as compared to patients with tissue NMD result, the agreement statistics were recalculated based on different tissue MD prevalences (for Ex. 19del and/or L858R mutations) and the results are shown in the table below. PPA ranged from 76.6% to 83.7% and NPA ranged from 97.9% to 98.2% based on 10 – 20% tissue EGFR prevalence in a Caucasian population. Based on 30 – 40% tissue EGFR prevalence in an Asian population, the PPA ranged from 86.3% to 87.7% and NPA ranged from 97.3% to 96.6%, respectively.

Table 8. Agreement Between cobas® EGFR Plasma Test v2 vs. NGS for Detection of EGFR* in Aggregate Based on Different Tissue Mutation Prevalence (with 2.0 mL Plasma Sample) for the ASPIRATION Cohort

Tissue Mutation Prevalence	Agreement between cobas® EGFR Plasma Test v2 and NGS			
	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
10%	76.6 (65.2, 89.2)	98.2 (97.1, 99.3)	78.3 (67.9, 90.8)	98.0 (96.9, 99.1)
20%	83.7 (76.1, 91.6)	97.9 (96.5, 99.0)	86.7 (79.2, 93.8)	97.3 (96.0, 98.6)
30%	86.3 (79.8, 92.4)	97.3 (95.7, 98.7)	89.2 (83.2, 94.4)	96.5 (94.7, 98.1)
40%	87.7 (82.6, 92.7)	96.6 (94.8, 98.2)	90.5 (85.7, 95.0)	95.5 (93.7, 97.3)
50%	88.6 (84.5, 92.8)	95.8 (93.9, 97.7)	91.3 (87.4, 95.3)	94.3 (92.2, 96.5)

* Detection of Ex. 19del and/or L858R substitution mutations

Comparison of the ENSURE and the ASPIRATION Cohorts

Plasma specimens from patients enrolled in ENSURE were used to demonstrate the clinical utility of the **cobas**® EGFR Plasma Test v2 as a CDx for TARCEVA® (see Section X). The **cobas**® EGFR Tissue Test v1 was used to enroll patients in ENSURE. Due to insufficient plasma volumes from patients screened for ENSURE, plasma specimens from the ASPIRATION cohort were used to demonstrate analytical accuracy of the **cobas**® EGFR Plasma Test v2.

The transportability of analytical accuracy from the ASPIRATION cohort to ENSURE was established by a) demonstrating that the baseline demographics and clinical characteristics information are similar among the enrolled patients who were positive by the **cobas**® EGFR Tissue Test v1 in the two studies; b) evaluating whether the PPA and NPA between **cobas**® EGFR Plasma Test v2 vs. **cobas**® EGFR Tissue Test v1 results (using tissue result as reference) were comparable between samples from the two studies; and c) demonstrating that the propensity score is comparable for enrolled patients in the ENSURE and the ASPIRATION cohorts.

a. Patient Demographics

Demographics and clinical characteristics were compared among the enrolled patients with a plasma sample available between the ENSURE and ASPIRATION cohorts by a propensity score method. The propensity score is the conditional probability that a patient is assigned to the ENSURE trial given the specific characteristic of that patient. The stage of disease and histology result was not included as an independent variable, because only stage III patients were enrolled in ENSURE. Similarly, the propensity was compared between:

- Enrolled patients in ENSURE for whom plasma samples were available and for whom plasma samples were not available;
- Enrolled patients in ASPIRATION cohort for whom plasma samples were available and for whom plasma samples were not available;
- Enrolled patients with available plasma samples in the ENSURE and ASPIRATION cohorts.

The baseline demographic and clinical covariates of patients with a MD result by the **cobas**® EGFR Tissue Test v1 and a plasma sample of 2mL from the ENSURE and ASPIRATION cohorts were compared. There were some covariates which were statistically significantly different between the two studies in univariate analysis, including age; smoking history; BMI; tumor stage; histology; and ECOG score. Due to the imbalance of some baseline covariates between the two studies, a propensity score method (logistic regression model) was used by including all seven baseline covariates except the disease stage in the model, and propensity scores were generated for each patient. It was found that none of the covariates was different between the two studies at a significance level of 0.05 after adjusting the propensity score quintiles, indicating that each covariate was balanced within the propensity score strata.

Table 9. Demographics and Baseline Clinical Characteristics of Tissue EGFR Mutation Positive Patients with Plasma Specimen of 2.0 mL from ENSURE and ASPIRATION Cohort

Characteristics	ENSURE (n=180)	ASPIRATION Cohort (n=48)*	*p*-value	
			Before	After
Age (Years)				
<65 Years	143 (79.4%)	25 (52.1%)	0.0001	0.4783
≥ 65 Years	37 (20.6%)	23 (47.9%)		
Sex (%)				
Male	67 (37.2%)	22 (45.8%)	0.2772	0.8422
Female	113 (62.8%)	26 (54.2%)		
Smoking History (%)				
Never Smoked	129 (71.7%)	23 (47.9%)	0.0019	0.9885
Past/Current Smoker	51 (28.3%)	25 (52.1%)		
BMI (Kg/m²) (Mean ± SD)	22.9 ± 3.6	24.3 ± 3.6	0.0261	0.5946
Tumor Stage (%)				
Stage IIIB	15 (8.3%)	0 (0.0 %)	0.0038	N/A
Stage IV	165 (91.7%)	46 (95.8%)		
Missing	0 (0.0 %)	2 (4.2%)		
Histology (%)				
Adenocarcinoma	170 (94.4%)	45 (93.8%)	0.0328	0.9260
Other	10 (5.6%)	1 (2.1%)		
Missing	0 (0.0 %)	2 (4.2%)		
ECOG Score (%)				
0	26 (14.4%)	20 (41.7%)	0.0006	0.5502
1	142 (78.9%)	26 (54.2%)		
2	9 (5.0%)	1 (2.1%)		
Missing	3 (1.7%)	1 (2.1%)		
EGFR Mutation (%) by Tissue				
Ex. 19del	98 (54.4%)	29 (60.4%)	0.4592	0.9678

Characteristics	ENSURE (n=180)	ASPIRATION Cohort (n=48)*	*p*-value	
			Before	After
L858R	82 (45.6%)	19 (39.6%)		

* The 48 patients include 39 patients from MetLung study and 9 from the ASPRIATION study.
Note: Tissue EGFR Mutation positive referred as mutation detected for either Ex. 19del or L858R mutation as determined by **cobas**® EGFR Tissue Test v1.
Note: N/A = Not Applicable.
Note: Patients with missing covariate information were not used in propensity score analysis.
Note: Covariate tumor stage was not included in the propensity score analysis as stage III patients were only enrolled in the ENSURE study.
Note: p-value was calculated before and after adjusting the propensity score quintiles.

b. Agreement between plasma and tissue samples

The agreement between plasma and tissue samples by the **cobas**® EGFR Plasma Test v2 and the **cobas**® EGFR Tissue Test v1 was performed for detection of exon 19 deletion and L858R mutations in aggregate (for plasma samples with 2.0 mL volume) and a three-way agreement between the two specimen types and NGS using plasma samples. These comparisons are presented in the table below.

Table 10. Agreement between cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 for Detection of EGFR Mutations (with 2.0 mL Plasma Sample)

cobas® Plasma Test v2	cobas® EGFR Tissue Test v1		Total
	MD	NMD	
MD	30	1	31
NMD	18	79	97
Total	48	80	128
PPA (95% CI)	62.5% (48.4%, 74.8%)		
NPA (95% CI)	98.8% (93.3%, 99.8%)		
PPV (95% CI)	96.8% (83.8%, 99.4%)		
NPV (95% CI)	81.4% (72.6%, 87.9%)		

A three-way agreement was provided to compare the EGFR mutation status in aggregate between the **cobas**® EGFR Tissue Test v1, the **cobas**® EGFR Plasma Test v2, and NGS. These results are shown in the table below.

Table 11. Three-way Agreement among cobas® EGFR Tissue Test v1, cobas® EGFR Plasma Test v2, and NGS for Detection of EGFR Mutations (with 2.0 mL Plasma Sample) from the ASPIRATION Cohort

cobas® Tissue Test v1	cobas® EGFR Plasma Test v2	NGS (Plasma)			
		MD	NMD	Invalid	Total
MD	MD	28	2	0	30
	NMD	3	14	1	18
	Invalid	0	0	0	0
	Total	31	16	1	48

cobas® Tissue Test v1	cobas® EGFR Plasma Test v2	NGS (Plasma)			
		MD	NMD	Invalid	Total
NMD	MD	0	1	0	1
	NMD	1	78	0	79
	Invalid	0	0	0	0
	Total	1	79	0	80

c. Comparison of Agreements Between ASPIRATION and ENSURE Cohorts
The agreements, NPA and PPA, were compared between the tissue and plasma results for the patients from each study cohort and p-values calculated. The p-value for PPA was also adjusted based on the calculated propensity scores and are shown below.

Table 12. Comparison of NPA and PPA of cobas® EGFR Plasma Test v2 vs. cobas® EGFR Tissue Test v1 (with 2.0 mL Plasma Sample)

Sample Source	Total Tissue NMD Patients with Plasma Samples	Results by cobas® EGFR Plasma Test v2			NPA (%)	p-value	
		MD	NMD	Invalid			
ENSURE	229	4	217	8	98.2% (95.4%, 99.3%)	1.0000	
ASPIRATION Cohort	80	1	79	0	98.8% (93.3%, 99.8%)		
	Total Tissue MD Patients with Plasma Samples				PPA (%)	Before*	After*
ENSURE	212	161	49	2	76.7% (70.5%, 81.9%)	0.043	0.206
ASPIRATION Cohort	48	30	18	0	62.5% (48.4%, 74.8%)		

* p-value was calculated before and after adjusting the propensity score quintiles.

After adjusting based on propensity scores for those in which a mutation was detected, there is no significant difference in the populations based on the p-values.

d. Rare Mutations**:**
All five rare mutations [in tyrosine kinase inhibitor (TKI)-naïve patients], targeted by the **cobas**® EGFR Plasma Test v2 (Ex. 20ins, L861Q, S768I, G719X, and T790M) were detected and confirmed by the validated NGS method using plasma samples from the ASPIRATION Cohort and four rare mutations targeted by the assay (Ex. 20ins, S768I, G719X, and T790M) were detected in the ENSURE clinical study.

- ASPIRATION Cohort
  - Three Ex. 20ins mutations were detected by the **cobas**® EGFR Plasma Test v2. Two were confirmed by NGS using plasma samples, but the

remaining result could not be confirmed due to the lack of residual sample remaining for NGS testing.
    - Two G719X mutations were detected by the **cobas**® EGFR Plasma Test v2. One sample was confirmed with G719X mutation while the other was not confirmed by NGS.
        - G719X was detected in four other samples but not detected by the **cobas**® EGFR Plasma Test v2; due to the percent mutation of the samples being below the assay cut-off.
    - One L861Q and one S768I were mutations detected by the **cobas**® EGFR Plasma Test v2 were confirmed by NGS.
    - Six T790M mutations were detected by **cobas**® EGFR Plasma Test v2. Four were confirmed by NGS using plasma samples while the other two were not confirmed by NGS due to the percent mutation of the samples being below the NGS cut-off.

- ENSURE Study
    - Three Ex. 20ins; three G719X; five S768I; one T790M; and eight L861Q mutations were detected by the **cobas**® EGFR Plasma Test v2; however, they could not be confirmed due to the lack of sufficient plasma available for NGS testing.

2. <u>Contrived Sample Comparison</u>

A study was performed to compare the performance of the **cobas**® EGFR Plasma Test v2 with intact cell line DNA and mechanically sheared cell line DNA (to an average size of approximately 220 bp) diluted in both HD EDTA plasma and NSCLC EGFR WT EDTA plasma. In two separate experiments, four panels were created with a combination of cell line DNA from Ex. 19del, L858R, and T790M mutations and tested over a total of eight levels each (10x, 5x, 1x (twice), 0.75x, 0.5x. 0.2x, 0.1x , and 0.03x LoD). A total of 20 replicates of each panel member were run and tested with each of two lots of the **cobas**® cfDNA Sample Preparation Kit in combination with two lots of the **cobas**® EGFR Mutation Test v2 kit. Ten replicates were split across ten runs to include one replicate of each level and condition per run. A total of 20 runs were performed with five operators and 18 **cobas z** 480 analyzers. Each experiment was reported separately prior to pooling. The hit rates for each specimen were determined and potential bias estimated using linear regression.

Table 13. Hit Rates for the Contrived Sample Comparison Study (n=20) with Pooled Results

Target	DNA Type	Plasma Source	5x LoD (Round 1)	1x LoD (Round 1)	1x LoD (Round 2)	0.75x LoD (Round 2)	0.5x LoD (Round 1)	0.2x LoD (Round 2)	0.1x LoD (Round 2)	0.03x LoD (Round 2)
Ex. 19del	Intact	NSCLC	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	75% (50.9-91.3)
		HD	100%	95%	100%	100%	100%	100%	90%	80%

Target	DNA Type	Plasma Source	5x LoD (Round 1)	1x LoD (Round 1)	1x LoD (Round 2)	0.75x LoD (Round 2)	0.5x LoD (Round 1)	0.2x LoD (Round 2)	0.1x LoD (Round 2)	0.03x LoD (Round 2)
			(83.2-100)	(75.1-99.9)	(83.2-100)	(83.2-100)	(83.2-100)	(83.2-100)	(68.3-98.8)	(56.3-94.3)
	Sheared	NSCLC	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	65% (40.8-84.6)
		HD	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	80% (56.3-94.3)	70% (45.7-88.1)
L858R	Intact	NSCLC	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	95% (75.1-99.9)	85% (62.1-96.8)	75% (50.9-91.3)	25% (8.7-49.1)
		HD	100% (83.2-100)	80% (56.3-94.3)	100% (83.2-100)	100% (83.2-100)	75% (50.9-91.3)	95% (75.1-99.9)	45% (23.1-68.5)	0% (0-16.8)
	Sheared	NSCLC	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	100% (83.2-100)	85% (62.1-96.8)	90% (68.3-98.8)	80% (56.3-94.3)	20% (5.7-43.7)
		HD	95% (75.1-99.9)	85% (62.1-96.8)	100% (83.2-100)	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	70% (45.7-88.1)	5% (0.1-24.9)
T790M	Intact	NSCLC	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	35% (15.4-59.2)	20% (5.7-43.7)	0% (0-16.8)
		HD	100% (83.2-100)	95% (75.1-99.9)	100% (83.2-100)	100% (83.2-100)	80% (56.3-94.3)	20% (5.7-43.7)	0% (0-16.8)	0% (0-16.8)
	Sheared	NSCLC	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	100% (83.2-100)	85% (62.1-96.8)	25% (8.7-49.1)	10% (1.2-31.7)	0% (0-16.8)
		HD	100% (83.2-100)	85% (62.1-96.8)	100% (83.2-100)	100% (83.2-100)	80% (56.3-94.3)	40% (19.1-64.0)	15% (3.2-37.9)	0% (0-16.8)

10x LoD values not included as all were 100% (83.2 – 100).

Table 14. R^2, Slope, and Intercept for the Contrived Sample Comparison with Pooled Results

Target	DNA Type	Donor Type	R^2	Slope	95% CI	p-value of t-test for Slope*	Intercept	95% CI	p-value of t-test for Intercept*
Ex 19del	Intact	NSCLC	0.93	-3.51	-3.65 to -3.37	0.74	39.40	39.14 - 39.65	0.71
		HD	0.90	-3.45	-3.62 to -3.28	0.87	39.39	39.08 - 39.70	0.74
	Sheared	NSCLC	0.92	-3.47	-3.62 to -3.31	N/A	39.31	39.02 - 39.60	N/A
		HD	0.91	-3.27	-3.44 to -3.11	0.23	38.91	38.61 - 39.22	0.20
L858R	Intact	NSCLC	0.88	-3.11	-3.28 to -2.94	0.90	42.92	42.58 - 43.25	0.42
		HD	0.83	-2.89	-3.09 to -2.69	0.29	42.80	42.40 - 43.19	0.67
	Sheared	NSCLC	0.86	-3.09	-3.28 to -2.90	N/A	42.66	42.28 - 43.04	N/A

Target	DNA Type	Donor Type	R^2	Slope	95% CI	p-value of t-test for Slope*	Intercept	95% CI	p-value of t-test for Intercept*
		HD	0.85	-3.01	-3.20 to -2.82	0.59	42.53	42.16 - 42.90	0.67
T790M	Intact	NSCLC	0.92	-4.34	-4.54 to -4.14	0.46	41.77	41.38 - 42.15	0.17
		HD	0.91	-4.46	-4.68 to -4.24	0.97	42.45	42.02 - 42.89	0.77
	Sheared	NSCLC	0.92	-4.47	-4.67 to -4.27	N/A	42.36	41.97 - 42.74	N/A
		HD	0.88	-4.35	-4.59 to -4.11	0.52	42.06	41.58 - 42.53	0.44

* Comparison to sheared cell line DNA in NSCLC EDTA plasma;
N/A = not applicable.
Note: p-value analysis is based on predicted values determined from the pooled regression analysis

Based on these analyses, the results demonstrate that the performance of the **cobas**® EGFR Plasma Test v2 is equivalent when using contrived specimens consisting of sheared or intact cell line DNA in a NSCLC or HD EDTA plasma background.

3. Analytical Sensitivity

a. Analytical Sensitivity – Limit of Blank (LoB)

A panel of 33 unique EGFR WT EDTA HD plasma specimens, tested in duplicate, with each of three separately manufactured reagent lots, was used to establish the LoB. The LoB was defined as the 95th percentile of the Ct or CtR (depending on the specific mutation) results obtained with a blank sample.

LoB was determined using the nonparametric option as prescribed in Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2 for the reported CtR or Ct values of each lot and all lots combined for the EGFR WT samples. Any Ct result that was Not a Number (NaN) was converted to a value of 55 prior to calculating the CtR. The value of 55 corresponds to the total number of cycles in the PCR profile and is considered equivalent to a NaN result. For each lot and for all lots combined, the 5th percentile of the reported values for each channel was determined.

There were no invalid runs out of 146 total runs and 7 replicates gave invalid results due to either the IC not being detected or the Ct value being out of range. Of the invalid replicates, only three had sufficient residual plasma to allow for repeat testing and generated valid results upon repeat. The LoB was determined to be zero for all mutations tested.

b. Analytical Sensitivity - Limit of Detection (LoD)

The study was performed by testing dilution panels constructed from cell line DNA that was sheared to ~220 bp, quantified against a plasmid standard curve, and diluted into EGFR WT EDTA plasma from HDs. Specimen blend

panels were constructed and studied for the predominant mutations for Ex. 19del, L858R, T790M, Ex. 20ins, S768I, G719X, and L861Q mutations.

Six panels were created of two combinations of mutant DNA co-diluted in three separate dilution series. Combination 1 consisted of sheared cell line DNA for Ex. 19del, L858R, and T790M. Combination 2 consisted of sheared cell line DNA for S768I, L861Q, G719X, and Ex. 20ins. Three lots each of the **cobas**® cfDNA Sample Preparation Kit and the **cobas**® EGFR Mutation Test v2 kit were used in the study. Twenty-four replicates of each sample at each level were tested per lot for a total of 72 replicates. An extra replicate was tested at one level for one lot with Combination 2 resulting in a total of 73 total replicates.

The lowest level (cp/mL) that yielded at least a 95% MD rate for each mutation was established as the LoD. All mutations were detected at ≤100 cp/mL.

Table 15. Established LoD for each Predominant Mutation using Sheared Cell Line DNA in EDTA HD Plasma

Mutation	Nucleic Acid Sequence	LoD (cp/mL)
Ex. 19del	2235-2249del15	75
L858R	2573 T>G	100
T790M	2369 C>T	100
S768I	2303 G>T	25
L861Q	2582 T>A	30
G719X	2156 G>A	100
Ex. 20ins	2307_2308insGCCAGCGTG	25

c. Analytical Sensitivity – Clinical Specimen Confirmation

An 11-member panel, consisting of pooled residual clinical plasma specimens along with mutant control and negative control samples were tested in duplicate by each of two operators at three separate sites. Three unique reagent lots were used across the three testing sites with two lots per site (i.e., Site 1, lots A and B; Site 2, lots A and C; and Site 3, lots B and C) and each site performed a total of 88 tests. The panel included five different mutations (three Ex. 19del, one L858R, and one T790M) which were run at the estimated LoD and 2x LoD, and one WT panel member.

Table 16. Limit of Detection Confirmation Panel

Panel Member	Description of Mutations in Panel	Target Concentration (copies/mL)	Est. LoD by contrived specimens
1	0	WT	N/A
2	Ex 19del (2235_2249DEL15)	75	75
3		150	
4	Ex 19del (2236_2250DEL15)	75	N/A

Panel Member	Description of Mutations in Panel	Target Concentration (copies/mL)	Est. LoD by contrived specimens
5		150	
6	Ex 19del (2240_2257DEL18)	75	N/A
7		150	
8	T790M	100	100
9		200	
10	L858R	100	100
11		200	

Table 17. Overall Estimates of Agreement by Panel member

Panel Member	Number of Valid Tests	Agreement N	Agreement % (95% CI)[a]
WT – N/A	23	23	100 (85.2, 100.0)
Ex 19del 1 - 1x LoD	24	24	100 (85.8, 100.0)
Ex 19del 1 - 2x LoD	24	24	100 (85.8, 100.0)
Ex 19del 2 - 1x LoD	23	23	100 (85.2, 100.0)
Ex 19del 2 - 2x LoD	24	24	100 (85.8, 100.0)
Ex 19del 3 - 1x LoD	24	24	100 (85.8, 100.0)
Ex 19del 3 - 2x LoD	24	24	100 (85.8, 100.0)
T790M - 1x LoD	24	23	95.8 (78.9, 99.9)
T790M - 2x LoD	24	24	100 (85.8, 100.0)
L858R - 1x LoD	24	24	100 (85.8, 100.0)
L858R - 2x LoD	24	24	100 (85.8, 100.0)

Note: Results are included as agreement when a valid test of Mutant Type panel member has a result of MD or when a valid test of WT panel member has a result of NMD.

[a] 95% CI = 95% exact binomial confidence interval.

All 12 runs were valid with 262/264 valid test results. One replicate representing a WT call and one replicate of Ex. 19del 2 at 1x LoD generated invalid results due to no detection of the internal control or generation of a Ct value greater than the Ct_{max} cut-off specification. These replicates were not repeated and therefore excluded from the study. Among the valid replicates, one replicate for T790M failed to detect the mutation but all other replicates generated correct results. Based on the valid results using pooled clinical samples, the estimated LoDs from the contrived samples were confirmed. The variability observed in this study is summarized in the table below.

Table 18. Overall Mean and Standard Deviations for CtR from Valid Results of Mutant Panel Members

Panel Member	N	Mean CtR	Standard Deviation (SD)		
			Site/ Instrument	Within-Run	Total
Ex 19del 1 - 1x LoD	24	8.40	0.00	0.37	0.37
Ex 19del 1 - 2x LoD	24	6.89	0.14	0.20	0.24
Ex 19del 2 - 1x LoD	23	7.65	0.12	0.34	0.36

Panel Member	N	Mean CtR	Standard Deviation (SD)		
			Site/ Instrument	Within-Run	Total
Ex 19del 2 - 2x LoD	24	7.00	0.10	0.46	0.47
Ex 19del 3 - 1x LoD	24	8.82	0.16	0.60	0.62
Ex 19del 3 - 2x LoD	24	7.63	0.10	0.26	0.28
T790M - 1x LoD	23	8.76	0.18	0.58	0.60
T790M - 2x LoD	24	7.61	0.36	0.34	0.50
L858R - 1x LoD	24	12.48	0.00	0.84	0.84
L858R - 2x LoD	24	11.49	0.00	0.52	0.52

d. Analytical Sensitivity – LoD Verification of Rare Mutations

A study was performed to verify that the **cobas**® EGFR Plasma Test v2 can detect the non-predominant mutations that are part of the assay's design when present in EDTA plasma and to confirm that each of these mutations had a hit rate that is within the lower bound of the 95% CI of the LoD for the respective predominant mutation. If the non-predominant mutation had a hit rate ≥ 85% at the tested level, corresponding to the predominant mutation, then it was considered equivalent, because the upper bound of the 95% CI would overlap with the 95% hit rate. If the level was not equivalent, then concentrations up to 100 cp/mL were tested.

Plasmids containing the non-predominant mutations were diluted in HD EDTA plasma and tested with 21 replicates per level using one lot of **cobas**® EGFR Plasma Test v2 reagents. Plasmid DNA for each of the 35 non-predominant mutation targets was diluted in unique units of EDTA HD plasma to the LoD level of the predominant mutation for the respective mutation, or up to 100 cp/mL.

Fifty-four (54) runs out of the 60 total runs were valid. Four of the invalid runs were due to known operator errors and two were due to run controls: one out of range negative control, and one out of range mutation control. There were 11invalid replicates out of 956 replicates tested, which were due to out of range IC Ct values. All invalids were valid upon repeat testing. A summary of the final LoDs determined for the non-predominant mutations is shown in the table below.

Table 19. Summary of the LoD Hit Rate for EGFR Plasmids in HD Plasma

EGFR Mutation	EGFR Non-predominant Mutation	LoD for Non-Predominant Mutation [cp/mL]	# Hits/21 Valid Replicates	Hit Rate [%]
G719X	G719S	100	21	100
	G719C	100	21	100
Ex 20ins	2319_2320insCAC	80	19	90.5

EGFR Mutation	EGFR Non-predominant Mutation	LoD for Non-Predominant Mutation [cp/mL]	# Hits/21 Valid Replicates	Hit Rate [%]
	2310_2311insGGT	80	20	95.2
	2311_2312ins9 (gcgtggaca)	30	18	85.7
	2309_2310 complex (ac>ccagcgtggat)	30	19	90.5
Ex 19del	2233_2247del15	25	21	100
	2236_2253 del 18	25	21	100
	2236-2250 del15	25	21	100
	2237-2251 del 15	25	21	100
	2237_2254 del 18	25	21	100
	2238_2255 del 18	25	21	100
	2239-2247 del 9	25	21	100
	2239-2253 del 15	25	21	100
	2239-2256 del 18	25	21	100
	2240_2251 del 12	25	20	95.2
	2240-2257 del 18	25	21	100
	2235_2252>AAT	25	20	95.2
	2237-2255 >T	25	20	95.2
	2238_2248>GC	25	18	85.7
	2238_2252>GCA	50	21	100
	2239-2248 TTAAGAGAAG>C	25	20	95.2
	2239_2258>CA	25	21	100
	2239-2251 >C	25	21	100
	2253_2276del24	25	21	100
	2235_2248>AATTC	50	21	100
	2237_2252>T	25	20	95.2
	2238_2252del15	25	21	100
	2235_2251>AATTC	50	21	100
	2235_2255>AAT	25	18	85.7
	2236_2248>AGAC	25	20	95.2
	2237_2253>TTGCT	25	19	90.5
	2237_2257>TCT	25	21	100
	2239_2256>CAA	100	20	95.2
L858R	2573_2574TG>GT	20	20	95.2

4. Analytical Specificity

Studies to assess both Inclusivity/Cross-Reactivity and Exclusivity were not performed for this PMA as there has been no change to the assay's primers, probes, or targeted mutations. (See Summaries of Safety and Effectiveness Data for P120019/S007.)

To identify potential interference of the **cobas**® EGFR Plasma Test v2, one lot of the **cobas**® cfDNA Sample Preparation Kit reagents and one lot of the **cobas**® EGFR Plasma Test v2 kit were used in testing of potentially interfering endogenous and exogenous substances, and microorganism testing.

a. Interference – Endogenous Interferents

To evaluate the potential interference of triglycerides, hemoglobin, albumin, and bilirubin (conjugated bilirubin and unconjugated bilirubin) on the performance of the **cobas**® EGFR Plasma Test v2, three HD plasma units (four for albumin) - each spiked with sheared cell line DNA (~220bp) - were used to create a five-member panel: four contrived mutant samples at approximately 3x LoD for each mutation and one EGFR WT sample. The potential endogenous interferents were spiked into the contrived samples prior to processing with the **cobas**® DNA Sample Preparation Kit, and the results after amplification and detection were compared to a neat (unspiked) sample. The levels of interferents tested were equal to the levels recommended to be tested by the CLSI EP7-A2 and are summarized in the table below.

Table 20. Table Potential Interferent Concentrations

Potential Interferent	Concentration
Hemoglobin*	2.0 g/L
	1.5 g/L
Albumin	60 g/L
Unconjugated Bilirubin	0.2 g/L
Conjugated Bilirubin	0.2 g/L
Triglycerides	37 mM (33 g/L)

The effect of excess hemoglobin was initially tested by spiking an aliquot with approximately 2 g/L. All results matched the expected results for each sample except for one dropout for Ex. 20ins. This replicate was repeated and was positive for Ex. 20ins. The concentration of hemoglobin was decreased to approximately 1.5 g/L of hemoglobin. All results matched the expected results for each sample after the addition of 1.5 g/L of hemoglobin. Excess albumin at a final concentration of approximately 60 g/L was evaluated and all results matched the expected results for each sample except for one G719X mutation sample which failed to identify the mutation.

No interference with the performance of the **cobas**® EGFR Plasma Test v2 was observed from either triglycerides, bilirubin (conjugated and unconjugated). Albumin at a concentration of ≥60 g/L may interfere with the **cobas**® EGFR Test.

b. Interference – Exogenous Interferents

A study was conducted to evaluate the potential interference of therapeutic drugs (TARCEVA® and Neupogen®) and excess EDTA (to represent a short drawn specimen). As above, the potential interferents were spiked into aliquots from a sample panel prior to processing with the **cobas**® DNA Sample Preparation Kit. The levels of interferents tested were based on levels

recommended to be tested by the CLSI EP7-A2 guideline and are summarized in the table below.

Table 21. Potential Interferent Concentrations

Potential Interferent	Concentration
EDTA	9.0 mg/mL
Neupogen®	147 ng/mL
TARCEVA®	90 µg/mL

All runs and sample replicates generated the expected results and demonstrated that EDTA, Neupogen®, and TARCEVA® do not interfere with the performance of the **cobas**® EGFR Plasma Test v2 for use with plasma samples.

c. Interference – Microorganisms

To evaluate the potential interference from contamination by *Staphylococcus epidermidis*, the same sample panel from the endogenous interfering substances study was used to assess $1x10^6$ colony forming units (CFU) of *S. epidermidis*. There were no invalid runs or samples, and the results demonstrated that $1x10^6$ CFU/mL of *S. epidermidis* did not interfere with the **cobas**® EGFR Plasma Test v2.

5. <u>Reproducibility and Precision</u>

The reproducibility of the **cobas**® EGFR Plasma Test v2 for the detection of mutations in exons 18, 19, 20, and 21 of the EGFR gene was evaluated in two studies. The first study used a nine member sample panel consisting of contrived sheared cell line DNA samples spiked into pooled WT NSCLC plasma. The mutant sheared cell line DNAs were combined into 4 double mutant positive combinations (see table below) at concentrations equivalent to approximately 100 copies/mL and 300 copies/mL and a single WT pooled NSCLC plasma sample.

Table 22. Reproducibility Panel

Panel Member	Description of Panel member	Target Conc. (copies/mL)
1	WT	0
2	Ex 19del (2235_2249del15) and T790M	100/100
3	Ex 19del (2235_2249del15) and T790M	300/300
4	S768I and G719X	100/100
5	S768I and G719X	300/300
6	L858R and T790M	100/100
7	L858R and T790M	300/300
8	L861Q and Ex 20ins(2307_2308ins9)	100/100
9	L861Q and Ex 20ins (2307_2308ins9)	300/300

Each of two operators at each site tested the sample panel in duplicate once a day for three non-consecutive days using each of two lots of reagents for a total of 648 tests per panel member. Three unique lots of reagents were included in the study where each lot was tested at two sites.

One run was invalid due to an operator error and required repeating, resulting in 36/37 valid runs. The invalid run was not included in the analysis. Of 648 tests performed in total from all panel members, 646 generated valid results. There were a total of 1220 valid test results. The final results per mutation member are summarized in the tables below.

Table 23. Overall Estimates of Agreement by Mutation Member

Mutation Member	copies/mL	Number of Valid Tests	Agreement N	Agreement % (95% CI)[a]
WT	N/A	72	72	100 (95.0, 100.0)
G719X	100	72	65	90.3 (81.0, 96.0)
Ex 19del (2235_2249del15)	100	72	72	100 (95.0, 100.0)
Ex 20ins (2307_2308ins9)	100	72	72	100 (95.0, 100.0)
S768I	100	72	72	100 (95.0, 100.0)
T790M	100	143	139	97.2 (93.0, 99.2)
L858R	100	71	70	98.6 (92.4, 100.0)
L861Q	100	72	72	100 (95.0, 100.0)
G719X	300	71	70	98.6 (92.4, 100.0)
Ex 19del (2235_2249del15)	300	72	72	100 (95.0, 100.0)
Ex 20ins (2307_2308ins9)	300	72	72	100 (95.0, 100.0)
S768I	300	71	71	100 (94.9, 100.0)
T790M	300	144	142	98.6 (95.1, 99.8)
L858R	300	72	71	98.6 (92.5, 100.0)
L861Q	300	72	72	100 (95.0, 100.0)

Note: Results are included as agreement when a valid test of Mutation member has a result of MD or when a valid test of WT panel member has a result of NMD.
[a] 95% CI = 95% exact binomial confidence interval.

All mutation members demonstrated high agreement (e.g., >97%) except G719X at 100 copies/mL (1x LoD). The lower agreement for this sample was due primarily to multiple missed calls (n = 6/24 replicates combined) occurring at one site (site 3) for both operators with one lot of reagents. One other replicate failed at a second site (site 1) with that same lot; however, the overall data does not support the conclusion that there were any manufacturing problems with that lot. Four replicates failed for the T790M mutation at 100 copies/mL, with three of the four failures occurring at one site.

Table 24. Overall Mean and SD for CtR from Valid Results of Mutation Members

				Standard Deviation (SD) Observed					
Mutation Member	Copies /mL	N	Mean CtR	Lot	Site/ Instrument	Operator	Day	With-in Run	Total
				SD	SD	SD	SD	SD	SD
G719X	100	65	9.08	0.38	0.00	0.07	0.42	0.39	0.69

Mutation Member	Copies /mL	N	Mean CtR	Standard Deviation (SD) Observed					
				Lot	Site/ Instrument	Operator	Day	With-in Run	Total
				SD	SD	SD	SD	SD	SD
Ex 19del (2235_2249del15)	100	72	7.34	0.00	0.09	0.00	0.00	0.33	0.35
Ex 20ins (2307_2308ins9)	100	72	4.77	0.12	0.20	0.00	0.46	0.33	0.61
S768I	100	72	4.33	0.09	0.00	0.13	0.11	0.32	0.37
T790M	100	139	8.55	0.50	0.11	0.00	0.00	0.67	0.84
L858R	100	70	11.93	0.28	0.14	0.10	0.48	0.63	0.86
L861Q	100	72	5.75	0.29	0.23	0.00	0.42	0.22	0.60
G719X	300	70	7.44	0.10	0.00	0.00	0.34	0.43	0.56
Ex 19del (2235_2249del15)	300	72	5.69	0.05	0.11	0.10	0.00	0.24	0.29
Ex 20ins (2307_2308ins9)	300	72	4.07	0.00	0.12	0.00	0.15	0.43	0.47
S768I	300	71	3.24	0.00	0.00	0.00	0.11	0.23	0.26
T790M	300	142	6.73	0.38	0.13	0.00	0.15	0.25	0.49
L858R	300	71	10.37	0.00	0.00	0.05	0.00	0.56	0.57
L861Q	300	72	4.98	0.22	0.14	0.00	0.25	0.15	0.39

The second reproducibility study consists of the LoD confirmation study which is described in Section IX.A.3.c., above.

6. Lot-to-Lot Reproducibility

To evaluate lot interchangeability of the **cobas**® EGFR Plasma Test v2 and the **cobas**® cfDNA Sample Preparation Kit, a study was performed using 12 contrived EGFR mutant samples, covering each of the mutations detected by the assay, consisting of sheared cell line DNA diluted in HD plasma and one EGFR WT sample. One replicate of each sample at each of three concentrations was tested with each of the lots of the **cobas**® EGFR Plasma Test v2 kit tested in combination with three lots of the **cobas**® cfDNA Sample Preparation Kit. A 100% correct call rate for each specimen with each combination was observed, supporting that each of the two kits can be used in different lot combinations.

7. Specimen Handling

Summary specimen handling information was provided based on the original clinical study protocols. After collection of the EDTA blood samples, plasma was separated by centrifugation within four (4) hours of collection and stored at ≤ -70°C until cfDNA isolation.

An additional study is planned based on a submitted protocol to support sample handling (i.e., initial sample processing and storage) and specimen stability studies. See Section XIV, below.

8. Guard banding

The objective of the guard banding studies was to establish the robustness of the PCR conditions for the **cobas**® EGFR Plasma Test v2. Guard banding studies

were performed on the **cobas**® EGFR Plasma Test v2 Thermal Cycling Profile and Proteinase K concentration and incubation time (for DNA isolation procedure). Each guardband study used one lot of the **cobas**® cfDNA Sample Preparation Kit and one lot of the **cobas**® EGFR Plasma Test v2 kit. Three replicates of each test sample were evaluated by three operators and three **cobas**® **z** 480 analyzer.

Two contrived mutant samples, consisting of sheared cell line DNA spiked into pooled HD plasma at approximately 5x LoD for each mutation, and one pooled EGFR *wild-type* HD plasma specimen were tested. The contrived mutant specimens were pooled to include three and four different mutations each (C1: Ex. 19del, L858R, and T790M mutations and C2: S768I, L861Q, G719X, and Ex. 20ins mutations).

a. Thermal Cycling Profile

Part 1 tested the thermal cycling temperature with ±1°C to the control thermal cycling conditions outlined in the instructions for use. Three replicates of the samples described above were tested in three runs on one instrument. For each condition, all runs and results were valid and the results from each of the 27 replicates were correct.

b. Proteinase K (pK) Volumes

Part 2 tested the volume of the Proteinase K (pK) reagent used in the incubation step of DNA isolation. Three replicates from each mutant and WT sample were extracted according to the instructions for use with 250 µL ±20% of the normal pK reagent volume. The results from each extraction were compared to the third control replicate. For each condition, all runs and replicates were valid. All replicates gave the expected result except for one missed G719X result from a replicate which had 20% additional pK reagent, resulting in a total of 26/27 correct calls (96.3%).

c. pK Incubation Times

Part 3 tested the time of sample incubation with pK reagent. Three replicates from each mutant and WT sample were extracted according to the instructions for use but included incubating the replicates for ±5 minutes. For each condition, all runs and results were valid, and the results from each of the 27 replicates were correct.

Results from the guard-banding study showed that the **cobas**® EGFR Plasma Test v2 is robust in regard to incubation time of ±5 min, pK volume of ±20% and thermal cycling temperatures of ±1°C.

9. Stability Studies

a. cobas® EGFR Mutation Test v2 (Shelf-Life)

A study was performed to confirm the stability of the existing **cobas**® EGFR Plasma Test v2 kit amplification and detection reagents. The **cobas**® EGFR Plasma Test v2 kit recommended storage temperature is 2-8°C. The kit contains six components:

- EGFR MMx1
- EGFR MMx2
- EGFR MMx3 v2
- Magnesium Acetate (MGAC)
- EGFR Mutant Control (EGFR MC)
- DNA Specimen Diluent (DNA SD)

In addition to the EGFR MC and DNA SD, this study included samples composed of eluates from six EDTA plasma samples generated using the **cobas**® cfDNA Sample Preparation Kit. All eluates were frozen until testing at each stability time point. The six plasma samples consisted of the following: four contrived samples created by spiking combinations of non-sheared cell line DNA for the seven predominant EGFR mutations detected by the assay into HD pooled plasma; one NSCLC EDTA plasma sample positive for the EGFR T790M mutation diluted into HD WT pooled plasma; and one HD EDTA plasma pool (EGFR WT).

Stability was evaluated by performing functional testing at day 0, 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months, 13 months, 18 months, and 19 months. The eluates for the plasma samples were tested at day 0, 6 months, 13 months, and 19 months. For a given storage condition to pass, the EGFR MC and DNA SD results must be within the pre-specified Ct ranges (which are identical to the Ct values for assessing validity of a run). At time points where the plasma samples were tested, the plasma samples had to yield the expected mutation result. To date, real-time stability testing for the **cobas**® EGFR Plasma Test v2 kit has been completed through 19 months (relative to Day 0 of the stability study).

b. cfDNA Sample Preparation Kit

A study was conducted to confirm the stability of the **cobas**® cfDNA Sample Preparation Kit reagents. The **cobas**® cfDNA Sample Preparation Kit recommended storage temperature is 15-30°C. The real-time temperature used for the stability study was 32°C. In addition to a sample containing EGFR WT cell line DNA, a panel consisting of 3 clinical samples including two EGFR WT HD plasma specimens and one NSCLC WT plasma pool were used.

All kit components are identical to that approved under P120019/S007, except for the High Pure Extension Assembly Unit, of the **cobas**® cfDNA Sample Preparation kit. The stability of the **cobas**® cfDNA Sample Preparation Kit was assessed at various time points after storage at 32°C in upright orientation using three lots of the kit reagents. Stability was evaluated by performing functional testing at day 0, 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months, 13 months, 18 months, and 19 months.. To date, real-time stability testing for the **cobas**® EGFR Plasma Test v2 kit has been completed through 19 months (relative to Day 0 of the stability study).

c. Prepared Specimen Stability

A study was performed to assess the stability of prepared specimens. In this study, 4 contrived EGFR mutant samples, consisting of sheared cell line DNA at three different concentrations diluted in pooled plasma, and one WT HD plasma sample were tested. On Day 0, the samples were processed and the eluates were pooled to create 5 pooled samples (4 mutant and one WT) which were then made into aliquots with sufficient volume for one time point. The pooled eluates were then stored at 32°C, 2-8°C, and -20°C (including freeze-thaw cycles) and tested in duplicate at pre-specified time points for each temperature storage condition as listed in the table below.

Table 25. Time Points and Conditions for the Prepared Specimen Stability Study

Time Point	Temperature
Day 0	N/A
Day 2	32°C
Day 8	2-8°C
	32°C
Day 22	2-8°C
Day 31	-20°C (Freeze/thaw cycle 1 and 2)
Day 61*	-20°C (Freeze/thaw cycle 1 and 2)

* One invalid run was successfully repeated (corresponding to the sample panel tested on day 63).

At the pre-specified time points for each temperature storage condition, eluates were tested. One lot each of the **cobas**® cfDNA Sample Preparation Kit and **cobas**® EGFR Mutation Test v2 kit were used in the study. One out of 26 runs was invalid due to an out of range negative control. All replicates for each of the valid runs yielded correct calls.

The cfDNA extracted from specimens was stable when stored for up to 21 days at 2-8°C, up to 60 days and two freeze-thaw cycles at -20°C, and up to 7 days at 32°C.

d. Open Container Stability

Two stability studies were performed to demonstrate the stability of opened reagents for the **cobas**® cfDNA Sample Preparation Kit and the **cobas**® EGFR Mutation Test v2 Kit.

cobas® cfDNA Sample Preparation Kit Open Container Stability

The first study evaluated the **cobas**® cfDNA Sample Preparation Kit reagents in two parts. Part A tested four uses (i.e., container opening, removal of reagents, container closure, and storage) of the **cobas**® cfDNA Sample Preparation Kit reagents over a period of 33 days (with testing at Days 0, 15, and 33, and volume removed at Day 21 [no testing]) and Part B tested four uses of the **cobas**® cfDNA Sample Preparation Kit reagents over a period of 91 days (with testing on Days 47, 61, and 91, and volume removed on Day 0 [used Day 0 results from Part A were used as Day 0 for Part B]).

The samples used in this study were 4 contrived EGFR mutant samples, covering each of the mutations detected by the assay, consisting of sheared cell line DNA at three different concentrations diluted in HD plasma and one EGFR WT sample. The samples were stored ≤-70 °C until the start of the study. At each designated time point, the samples were thawed, processed in duplicate, and tested. The **cobas**® cfDNA Sample Preparation Kit was stored at 32°C.

In Part A, all replicates produced the expected results except that one mutant at Day 15 and one at Day 33. One replicate for G719X at 3x LoD, which reported the expected mutation result at Day 15, but had a high Ct value when compared to all other replicates, tested to date. The sample was retested in duplicate and the resulting Ct values were within the range of the other replicates. On Day 33, one L858R replicate at 3x LoD reported the expected mutation result, but also had a Ct value that was considered high compared to all other replicates tested to date. The sample was re-tested in duplicate and both replicates gave correct calls and had Ct values in the expected range. In Part B, all runs and results were valid and correct.

Based on the study results, open reagent vials of the **cobas**® cfDNA Sample Preparation Kit are stable and are usable up to four times in a period of 90 days when stored at 15-30°C.

cobas® EGFR Mutation Test v2 Kit Open Container Stability

The second study evaluated the **cobas**® EGFR Mutation Test v2 kit reagents in two parts. Part A tested four uses (i.e., container opening, removal of reagents, container closure, and storage) of the **cobas**® EGFR Mutation Test v2 kit reagents over a period of 31 days (with testing at Days 0, 15, 21 and

31). Part B tested four uses of the **cobas**® EGFR Mutation Test v2 kit reagents over a period of 91 days (with testing on Days 47, 61, and 91).

In this study, 4 contrived EGFR mutant samples, consisting of sheared cell line DNA at three different concentrations diluted in pooled plasma, and one WT HD plasma sample were tested. Prior to Day 0, the samples were processed and the eluates were pooled to create 5 pooled samples (4 mutant and one WT) which were then made into aliquots with sufficient volume for one time point. The pooled eluates were then stored at -20°C. At each designated time point, the eluates were thawed, and tested in duplicate. The **cobas**® EGFR Mutation Test v2 reagents were stored at 2-8°C.

In Parts A and B, all runs and results were valid and as expected except one replicate for Exon 19 deletion/Exon 20 Insertion at 3xLoD on Day 0 and one replicate for L861Q/T790M at 3x LoD on Day 61. The replicate at Day 0 for Exon 19 Deletion had a high Ct value when compared to all Exon 19 Deletion replicates tested through Day 46 and was excluded from the analysis. The replicate on Day 61 was retested in duplicate and both replicates gave correct calls and had Ct values in the expected range. All runs and results were valid and correct.

Based on the study results, open reagent vials of the **cobas**® EGFR Mutation Test v2 kit are stable and are usable up to four times in a period of 90 days when stored at 2-8°C.

e. Working (Activated) Master Mix

A study was performed to assess the stability of Working (Activated) Master Mix. A panel of 13 samples was used to conduct this study: 12 contrived EGFR mutant samples consisting of sheared cell line DNA at three different concentrations were diluted in HD pooled plasma, and one wild-type HD plasma sample. The samples were processed and the eluates were pooled for each sample and then stored at -20°C for two days prior to starting the study. The pooled eluates for each sample were tested in duplicate with freshly activated EGFR MMx and with activated EGFR MMxs stored for two hours at 2-8°C or 32°C. One lot each of the **cobas**® cfDNA Sample Preparation Kit and **cobas**® EGFR Mutation Test v2 kit were used in the study.

Before the start of testing, the sample replicates were processed through sample preparation. Once sample preparation was complete, the eluates for each panel member were pooled and stored at -20ºC for up to two days prior to the start of the study. Two replicates were tested for each time point.

All replicates for each of the valid runs yielded correct calls. The Working (Activated) Master Mix Stability demonstrated that that the activated MMxs

are stable for up to 125 minutes (including approximately 5 minutes for assay preparation) when stored at 2-8°C or 32°C.

f. Prepared Specimen Plus Working (Activated) Master Mix

A study was performed to assess the stability of Prepared Specimen plus Working (Activated) Master Mix. A 13 member panel, consistent with the one described previously was used in this study. The samples were processed and the eluates were pooled for each sample and then stored at 2-8°C for five days prior to starting the study. The pooled eluates for each sample were mixed with Working (Activated) Master Mix and tested in duplicate after the mixtures were stored for two hours at 2-8°C or 32°C.

One of the ten runs was invalid due to an out of range negative control, but produced valid and correct calls upon repeat. There were no invalid results out of 104 replicates tested in the 10 valid runs and all yielded correct calls. A prepared specimen and activated MMxs are stable for up to 120 minutes when stored at 2-8°C or 32°C.

g. Shipping

All components of the **cobas**® EGFR Mutation Test v2 kit and the **cobas**® EGFR Mutation Test v1 are the same, with the exception of the MMX-3 v2 reagent and the critical components of the **cobas**® cfDNA Sample Preparation kit and the **cobas**® DNA Sample Preparation Kit are also identical. Shipping stability was established under the **cobas**® EGFR Mutation Test v1 and was not expected to be different.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

Roche Molecular Systems, Inc. conducted a bridging study to support the safety and effectiveness of the **cobas**® EGFR Plasma Test v2 to select patients for treatment with erlotinib by detecting the presence of specific EGFR mutations (Ex. 19del or L858R) in EDTA plasma specimens. In the bridging study, patients' available plasma samples were evaluated for the presence of EGFR mutations using the **cobas**® EGFR Plasma Test v2. The results were bridged to the **cobas**® EGFR Tissue Test v1 results to estimate progression-free survival (PFS) for patients with a MD result based on the **cobas**® EGFR Plasma Test v2. The ENSURE study (YO25121/ NCT01342965), conducted in Asia,

Malaysia, and the Philippines, was a multicenter Phase III, randomized, open-label study to evaluate the safety and efficacy of erlotinib vs. cisplatin in combination with gemcitabine (chemotherapy) as first-line therapy for patients with stage IIIb/IV NSCLC. In the clinical study, treatment-naïve NSCLC patients' FFPET specimens were screened for enrollment using the **cobas**® EGFR Tissue Test v1 using FFPET specimens. Patients whose tumor specimens were positive for EGFR activating mutations were randomized to treatment with either TARCEVA® or chemotherapy. Plasma specimens were collected from all patients at initial screening for participation in the clinical study, and were subsequently tested in the bridging study.

A summary of the clinical study is presented below.

A. Study Design

The ENSURE study was a multicenter Phase III, randomized, open-label, two-arm study which was conducted by Hoffmann-La Roche/Genentech to evaluate the safety and efficacy of erlotinib vs. cisplatin in combination with gemcitabine as first-line therapy for patients with stage IIIb/IV NSCLC. The study was conducted in Asia and patients were enrolled based on the identification of EGFR activating mutations detected by the **cobas**® EGFR Tissue Test v1 in FFPET tissue specimens. Patients were enrolled in the study between 2011 and 2012.

In the bridging study, after informed consent was confirmed, patients' frozen plasma specimens were tested at one of two central laboratories with the **cobas**® EGFR Plasma Test v2, regardless of available volume.

1. Inclusion/Exclusion Criteria

a. Inclusion/Exclusion Criteria for the ENSURE study

Patients were enrolled into the ENSURE study and continued on treatment until the occurrence of disease progression (based on RESIST 1.1) or unacceptable toxicity. The inclusion/exclusion criteria for the ENSURE study are summarized below.

Inclusion Criteria:
1) Adult patients, ≥ 18 years of age
2) Locally advanced or recurrent (stage IIIB) or metastatic (stage IV) NSCLC
3) Presence of EGFR activating mutations (Ex. 19del or L858R) in tumors
4) Measurable disease according to RECIST criteria
5) European Cooperative Oncology Group (ECOG) performance status ≤ 2

Exclusion criteria:
1) Prior exposure to agents directed at the human epidermal receptor (HER) axis (e.g. but not limited to erlotinib, gefitinib, cetuximab, trastuzumab)

2) Prior chemotherapy or systemic anti-neoplastic therapy for advanced disease
3) Lack of physical integrity of the upper gastrointestinal tract, or malabsorption syndrome, or inability to take oral medication, or active gastroduodenal ulcer disease
4) Any inflammatory changes of the surface of the eye
5) ≥ grade 2 peripheral neuropathy
6) History of any other malignancies within 5 years, except for adequately treated carcinoma in situ of the cervix or basal or squamous cell skin cancer
7) Brain metastasis or spinal cord compression that has not yet been definitely treated with surgery and/or radiation, or treated but without evidence of stable disease for at least 2 months
8) HIV infection
9) Pregnant, nursing or lactating women

b. Additional Inclusion/Exclusion criteria for the Bridging Study

Patients from ENSURE study were enrolled into the bridging based on the following criteria.

Inclusion Criteria:
1) Samples from subjects who were screened using the **cobas**® EGFR Tissue Test for FFPET.

Exclusion Criteria:
1) All samples available from the studies chosen were included for testing, regardless of volume.

2. Follow-up Schedule

As the bridging study was conducted retrospectively to establish safety and effectiveness for selecting patients using plasma samples, no follow-up was conducted.

3. Clinical Endpoints

The primary drug efficacy of TARCEVA® based on the **cobas**® EGFR Plasma Test v2 was evaluated by progression-free survival analysis (PFS) by investigators' assessment and Independent Review Committee assessment (IRC-assessed PFS). PFS was defined as the time between randomization and the first occurrence of progressive disease or death from any cause, whichever occurs first. The primary analysis of PFS used evidence of objective progression according to RECIST 1.1 criteria.

4. Bridging Study

A total of 647 patients with NSCLC were screened for the ENSURE study using the **cobas**® EGFR Tissue Test v1 in FFPET, and, 601 patients had valid results. Among them, 517 had available plasma samples and 441 of them had samples with ≥ 2.0 mL of plasma. Of the patients enrolled, 98.6% (214/217) had a plasma sample available for testing, 84% (180/214) with ≥ 2.0 mL and 94% (202/214) with ≥ 1.5 mL. Of the 180 enrolled patients who had ≥ 2.0 mL plasma samples available for testing with the **cobas**® EGFR Plasma Test v2, 179 patients had valid plasma test results.

The distribution of plasma samples by volume, tumor tissue results, and by study arm are shown in the following two tables.

Table 26. Plasma Samples by Volume and cobas® EGFR Tissue Test v1 Result from ENSURE Screened Patients

Valid cobas® EGFR Tissue Test v1 Result	Total (N=517)	Plasma Volume		
		2.0 mL (n=441)	1.5 - 1.9 mL (n=44)	< 1.5 mL (n=32)
Mutation Detected	251 (48.5%)	212 (48.1%)	22 (50.0%)	17 (53.1%)
No Mutation Detected	266 (51.5%)	229 (51.9%)	22* (50.0%)	15 (46.9%)

*One patient with Ex. 20ins (NMD for Ex. 19del or L858R substitution mutation) was randomized in error and was treated as MD.

Table 27. Results of the cobas® EGFR Plasma Test v2 in the ENSURE Enrolled Patients by Sample Volume and Treatment Arm

Plasma Volume	Treatment Arm	cobas® EGFR Plasma Test v2 Result (no. of patients)		
		MD	NMD	Invalid
2.0 mL (N = 180)	Erlotinib	68	22	1
	Chemotherapy	69	20	0
1.5 - 1.9 mL (N = 22)	Erlotinib	6	8	0
	Chemotherapy	4	2	2
< 1.5 mL (N = 12)	Erlotinib	1	3	0
	Chemotherapy	5	3	0

The **cobas**® EGFR Plasma Test v2 was validated for use with 2.0 mL volume of plasma; therefore while all patients who had available plasma specimens were tested, only those who had a plasma volume of ≥ 2.0 mL were included in the agreement and efficacy analysis.

Table 28. The Agreement of cobas® EGFR Tissue Test v1 and cobas® EGFR Plasma Test v2 for Detection of EGFR Mutations* (for samples with volume of 2.0mL and Valid Test Results)

		cobas® EGFR Tissue Test v1		Total
		MD	NMD	
cobas® EGFR Plasma Test v2	MD	161	4	165
	NMD	49	217	266

		cobas® EGFR Tissue Test v1		Total
		MD	NMD	
	Invalid Test Result	2	8	10
	Invalid Based on Plasma Volume <2mL	39	37	76
	No Plasma Sample	9	75	84
	Total	260	341	601
With only Valid Result	**PPA (95% CI)**	76.7% (70.5%, 81.9%)		
	NPA (95% CI)	98.2% (95.4%, 99.3%)		
	PPV (95% CI)	97.6% (93.9%, 99.1%)		
	NPV (95% CI)	81.6% (76.5%, 85.8%)		

* Ex. 19del and L858R in aggregate.

Since the cobas® Plasma Test v2 test is intended to be used for initial screening, and for patients where no mutation is detected plasma specimens should be reflexed to having EGFR status determined from FFPET tissue specimen, PPV estimate was deemed critical. The data showed that PPV was 97.6%.

A sensitivity analysis was performed to account for invalid results, and an estimation of predictive values, adjusted for mutation prevalence based on mutation prevalence in tissue samples, are summarized in the tables below.

Table 29. Sensitivity Analysis of Agreement between the cobas® EGFR Tissue Test v1 and Plasma test v2 (Sample Volume of 2.0 mL)

	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
With Only Invalid Test Results as Discordant	75.9% (69.8%, 81.2%)	94.8% (91.1%, 97.0%)	93.1% (88.3%, 96.0%)	81.0% (75.8%, 85.2%)
With All Invalids (Due to Test Result and Sample Volume <2mL) as Discordant	64.1% (58.0%, 69.8%)	81.6% (76.5%, 85.8%)	76.7% (70.5%, 81.9%)	70.7% (65.4%, 75.5%)
With All Invalids and no sample as Discordant	61.9% (55.9%, 67.6%)	63.6% (58.4%, 68.6%)	56.5% (50.7%, 62.1%)	68.7% (63.4%, 73.5%)
With Parametric Bootstrap Method for All Invalids and no sample	71.5% (60.0%, 82.7%)	82.1% (63.9%, 98.5%)	74.9% (59.0%, 97.3%)	78.9% (70.4%, 86.5%)

Table 30. Estimated Predictive Values of the cobas® EGFR Tissue Test v1 and cobas® EGFR Plasma Test v2 (Patients with Plasma Sample Volumes 2.0 mL) Based on Differing EGFR Mutation Tissue Prevalence

Assumed EGFR Prevalence Based on Tissue Samples	PPV	NPV
10%	82.8% (71.3%, 93.7%)	97.4% (96.2%, 98.7%)
15%	88.6% (79.7%, 96.9%)	96.0% (94.3%, 97.6%)
20%	91.6% (85.0%, 97.8%)	94.4% (92.3%, 96.3%)
30%	94.8% (90.0%, 98.6%)	90.9% (88.4%, 93.4%)
40%	96.8% (93.0%, 99.4%)	86.4% (83.3%, 89.4%)
50%	97.8% (95.0%, 100.0%)	80.8% (77.4%, 84.8%)

The result of 79 samples with a volume of < 2.0 mL were treated as invalid in this analysis

* PPV and NPV were calculated using a bootstrap method based on different population prevalence, based on tissue samples.

The agreements between the tissue and plasma results for each of the EGFR Ex. 19del and L858R activating mutations are summarized in the tables below.

Table 31. Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 for Detection of Ex. 19del (Patients with Valid Tissue v1 Result and with Plasma Sample Volume of 2.0 mL)

		cobas® EGFR Tissue Test v1		Total
		MD	NMD	
cobas® EGFR Plasma Test v2	MD	97	4	101
	NMD	23	307	330
	Invalid Test Result	1	9	10
	Invalid Based on Plasma Volume	22	54	76
	No Plasma Sample	5	79	84
	Total	148	453	601
With only Valid Result	PPA (95% CI)	80.8% (72.9%, 86.9%)		
	NPA (95% CI)	98.7% (96.7%, 99.5%)		
	PPV (95% CI)	96.0% (90.3%, 98.4%)		
	NPV (95% CI)	93.0% (89.8%, 95.3%)		

Table 32. Sensitivity Analysis of Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 for Detection of Ex. 19del (Patients with Valid Tissue v1 Result and with Plasma Sample Volume of 2.0 mL)

	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
With Only Invalid Test Results as Discordant	80.2% (72.2%, 86.3%)	95.9% (93.2%, 97.6%)	88.2% (80.8%, 93.0%)	92.7% (89.4%, 95.1%)
With All Invalids (Due to Test Result and Sample Vol.) as Discordant	67.8% (59.8%, 74.9%)	82.1% (77.9%, 85.6%)	59.1% (51.5%, 66.4%)	87.0% (83.1%, 90.1%)
With All Invalids and No Sample as Discordant	65.5% (57.6%, 72.7%)	67.8% (63.3%, 71.9%)	39.9% (34.0%, 46.2%)	85.8% (81.8%, 89.0%)
With Parametric Bootstrap Method for All Invalids and No Sample	75.0% (62.8%, 85.8%)	83.1% (67.8%, 98.7%)	59.3% (42.6%, 94.9%)	91.2% (86.6%, 95.0%)

Table 33. The Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 for Detection of L858R (Patients with Valid Tissue v1 Result and 2.0 mL Plasma Sample Volume)

		cobas® EGFR Tissue Test v1		Total
		MD	NMD	
cobas® EGFR Plasma Test v2	MD	61	3	64
	NMD	29	338	367
	Invalid Test Result	1	9	10
	Invalid Based on Plasma Volume <2mL	17	59	76

		cobas® EGFR Tissue Test v1		Total
		MD	NMD	
	No Plasma Sample	4	80	84
	Total	112	489	601
With only Valid Result	**PPA (95% CI)**	67.8% (57.6%, 76.5%)		
	NPA (95% CI)	99.1% (97.4%, 99.7%)		
	PPV (95% CI)	95.3% (87.1%, 98.4%)		
	NPV (95% CI)	92.1% (88.9%, 94.4%)		

Table 34. Sensitivity Analysis of Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 for Detection of L858R (Patients with Valid Tissue v1 Result and 2.0 mL Plasma Sample Volume)

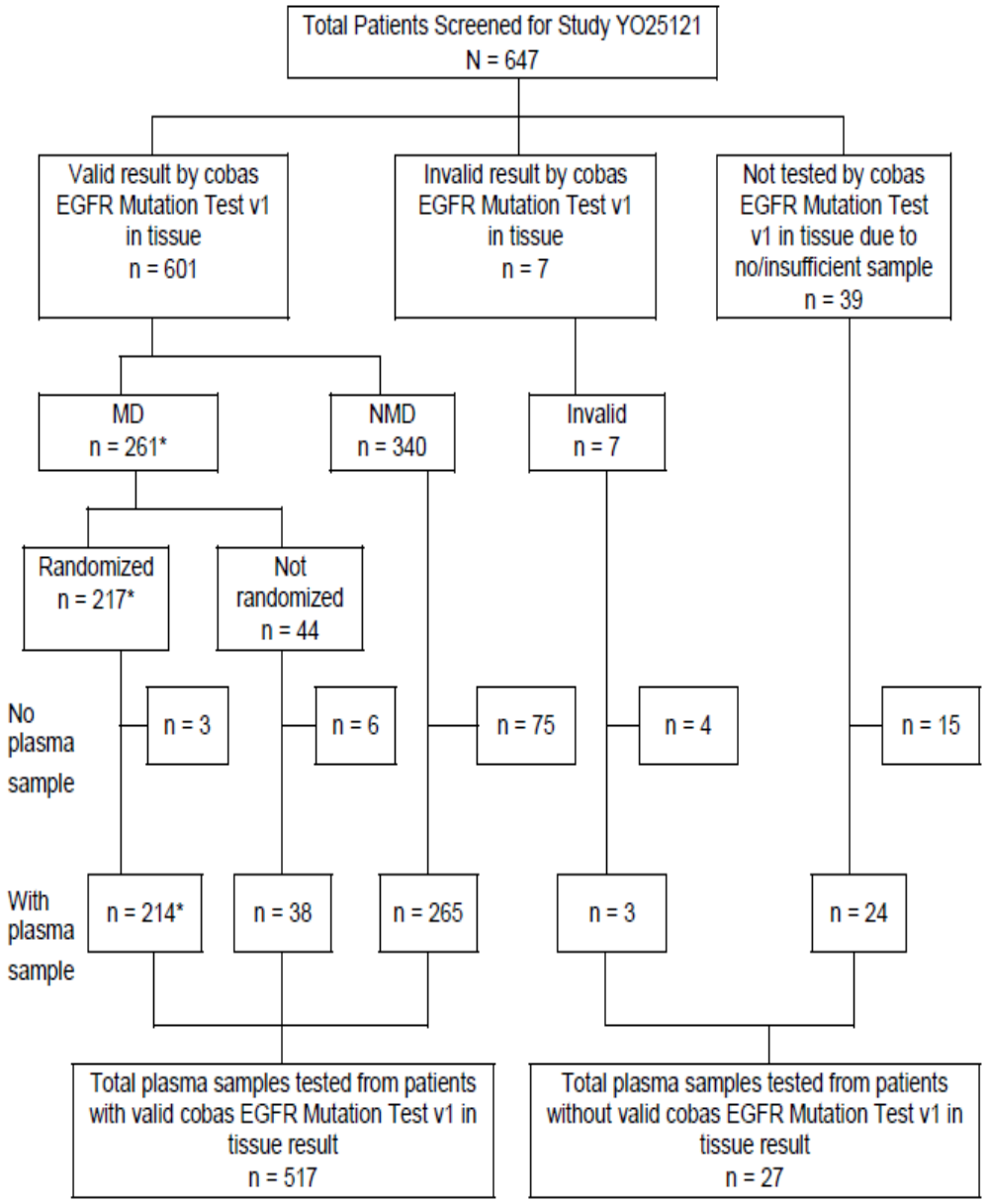
	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
With Only Invalid Test Results as Discordant	67.0% (56.9%, 75.8%)	96.6% (94.1%, 98.0%)	83.6% (73.4%, 90.3%)	91.8% (88.6%, 94.2%)
With All Invalids (Due to Test Result and < 2mL Sample Vol.) as Discordant	56.5% (47.1%, 65.4%)	82.6% (78.7%, 86.0%)	46.2% (37.9%, 54.7%)	87.8% (84.1%, 90.7%)
With All Invalids and No Sample as Discordant	54.5% (45.2%, 63.4%)	69.1% (64.9%, 73.1%)	28.8% (23.1%, 35.2%)	86.9% (83.2%, 89.9%)
With Parametric Bootstrap Method for All Invalids and No Sample	64.3% (50.9%, 77.7%)	84.7% (69.3%, 98.8%)	48.6% (30.7%, 91.7%)	91.1% (87.3%, 94.6%)

B. Accountability of PMA Cohort

A total of 647 patients were screened for the ENSURE study and based on results of the **cobas**® EGFR Tissue Test v1 and other inclusion and exclusion criteria, 217 patients were enrolled and randomized to treatment with erlotinib (n = 110) or chemotherapy (n = 107).).

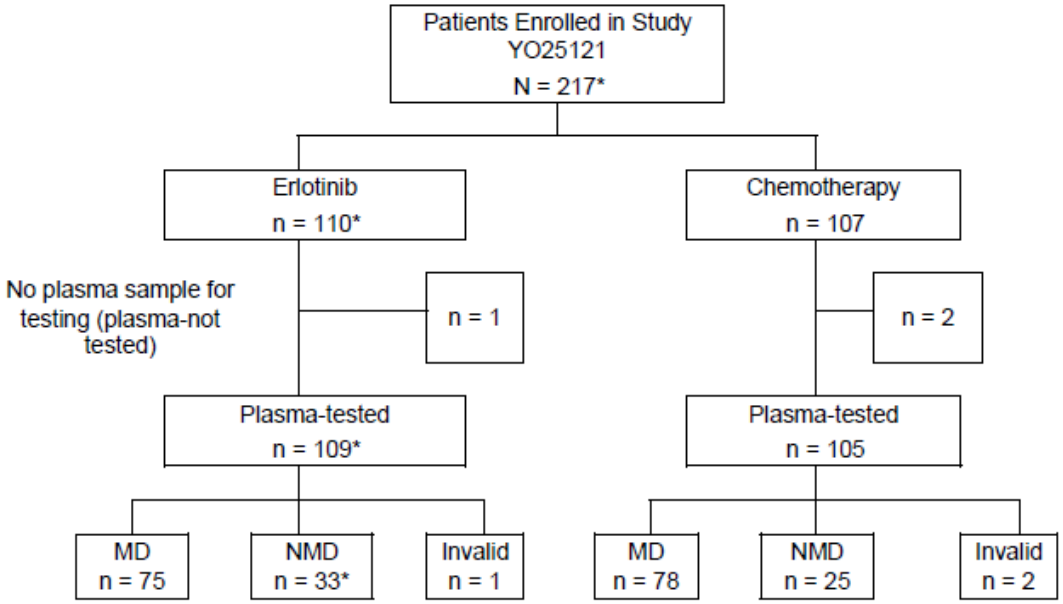
The final Plasma Full Analysis Set (FAS) was defined as the patients in the Tissue FAS who were also EGFR MD (Ex. 19del and/or L858R mutation) by the **cobas**® EGFR Plasma Test v2. The disposition of samples for patients in the ENSURE study is shown in the figure below.

Figure 2. Patient/Sample Disposition for the cobas® EGFR Plasma Test v2



*One patient with Ex. 20ins (NMD for e Ex. 19del or L858R) was randomized in error and was treated as MD in the ENSURE study. This patient had a NMD test result by the **cobas**® EGFR Plasma Test v2 but had a plasma volume of less than 2.0 mL, and was therefore not included in the clinical efficacy analysis.

Figure 3. Disposition of Enrolled Patients in the ENSURE study by Availability for the cobas® EGFR Plasma Test v2



*One patient with Ex. 20ins (NMD for Ex. 19del or L858R) was randomized in error and was treated as MD in the ENSURE Study. This patient had a NMD test result by the **cobas**® EGFR Plasma Test v2, but had a plasma volume of less than 2.0 mL and was therefore not included in the clinical efficacy analysis.

C. Study Population Demographics and Baseline Parameters

Of the 179 enrolled patients with a plasma sample of 2mL and a valid result by the **cobas**® EGFR Plasma Test v2, the median age was 57 years, with patients being slightly younger in the NMD population. The prevalence of mutations in the Asian population is higher than that seen in the US population (40-50% vs. 10-20%, respectively); therefore, efficacy, agreement, and predictive value calculations were adjusted for the prevalence differences. A justification for the acceptance of foreign data was provided. A summary of the patient demographics and baseline characteristics is in the tables below.

Table 35. Demographics and Baseline Characteristics of ENSURE Enrolled Patients among Patients with a Valid Test Result by the cobas® EGFR Plasma Test v2 (sample volume of 2.0 mL)

Characteristics	cobas® EGFR Plasma Test v2 Result		
	Overall (N=179)	MD (n=137)	NMD (n=42)
Age (years)			
Mean ± SD	56 ± 10.6	57 ± 10.2	55 ± 11.6
Median	57	58	55
Range	32 – 79	32 – 79	33 - 76

Characteristics	cobas® EGFR Plasma Test v2 Result		
	Overall (N=179)	MD (n=137)	NMD (n=42)
Age (years)			
< 65 Years	142 (79.3%)	107 (78.1%)	35 (83.3%)
≥ 65 Years	37 (20.7%)	30 (21.9%)	7 (16.7%)
Sex (%)			
Male	37 (20.7%)	51 (37.2%)	16 (38.1%)
Female	112 (62.6%)	86 (62.8%)	26 (61.9%)
Smoking History (%)			
Never Smoked	128 (71.5%)	97 (70.8%)	31 (73.8%)
Past/Current Smoker	51 (28.5%)	40 (29.2%)	11 (26.2%)
BMI (Kg/m²)	23 ± 3.6	23 ± 3.4	24 ± 4.3
Tumor Stage (%)			
Stage IIIB	15 (8.4%)	12 (8.8%)	3 (7.1%)
Stage IV	164 (91.6%)	125 (91.2%)	39 (92.9%)
Histology (%)			
Adenocarcinoma	169 (94.4%)	128 (93.4%)	41 (97.6%)
Other [a]	10 (5.6%)	9 (6.6%)	1 (2.4%)
ECOG Score (%)			
0 – 1	170 (95.0%)	128 (93.4%)	42 (100.0%)
2+	9 (5.0%)	9 (6.6%)	0 (0.0 %)
Tissue EGFR Mutation (%)			
Ex. 19del	98 (54.7%)	82 (59.9%)	16 (38.1%)
L858R	81 (45.3%)	55 (40.1%)	26 (61.9%)

[a] Other includes bronchioalveolar carcinoma, large cell carcinoma, mixed with predominantly adenocarcinoma component, squamous cell carcinoma and other without specification.

The baseline demographic and disease characteristics of plasma FAS were, in general, balanced between erlotinib and chemotherapy arms and consistent with the overall ENSURE tissue FAS as shown in table below.

Table 36. Demographics and Baseline Characteristics of ENSURE Enrolled Patients versus Patients with a Mutation Detected by the cobas® EGFR Plasma Test v2 (2.0 mL Sample Volume)

Characteristics	cobas® EGFR Tissue Test v1 ENSURE FAS (Tissue FAS)		cobas® EGFR Plasma Test v2 Mutation Detection Population (Plasma FAS)	
	Erlotinib (n=110)	Chemotherapy (n=107)	Erlotinib (n=68)	Chemotherapy (n=69)
Age (years)				
Mean ± SD	56.7 ± 10.4	55.8 ± 10.4	58 ± 10.2	56 ± 10.1
Median	58	56	60	56
Range	33 – 79	30 – 78	34 – 79	32 – 78
Age (years)				

Characteristics	**cobas® EGFR Tissue Test v1 ENSURE FAS (Tissue FAS)**		**cobas® EGFR Plasma Test v2 Mutation Detection Population (Plasma FAS)**	
	Erlotinib (n=110)	**Chemotherapy (n=107)**	**Erlotinib (n=68)**	**Chemotherapy (n=69)**
< 65 Years	87 (79.1%)	85 (79.4%)	50 (73.5%)	57 (82.6%)
≥ 65 Years	23 (20.9%)	22 (20.6%)	18 (26.5%)	12 (17.4%)
Sex (%)				
Male	42 (38.2%)	42 (39.3%)	26 (38.2%)	25 (36.2%)
Female	68 (61.8%)	65 (60.7%)	42 (61.8%)	44 (63.8%)
Smoking History (%)				
Never Smoked	79 (71.8%)	74 (69.2%)	49 (72.1%)	48 (69.6%)
Past/Current Smoker	31 (28.2%)	33 (30.8%)	19 (27.9%)	21 (30.4%)
Tumor Stage (%)				
Stage IIIB	10 (9.1%)	7 (6.5%)	5 (7.4%)	7 (10.1%)
Stage IV	100 (90.9%)	100 (93.5%)	63 (92.6%)	62 (89.9%)
Histology (%)				
Adenocarcinoma	104 (94.5%)	101 (94.4%)	63 (92.6%)	65 (94.2%)
Other [a]	6 (5.5%)	6 (5.6%)	5 (7.4%)	4 (5.8%)
ECOG Score (%)				
0 – 1	103 (93.6%)	101 (94.4%)	64 (94.1%)	64 (92.8%)
2+	7 (6.4%)	6 (5.6%)	4 (5.9%)	5 (7.2%)
Tissue EGFR Mutation (%)				
Ex. 19del	57 (52.3%)	61 (57.0%)	35 (51.5%)	47 (68.1%)
L858R	52 (47.7%)	46 (43.0%)	33 (48.5%)	22 (31.9%)

[a] Other includes bronchioalveolar carcinoma, large cell carcinoma, mixed with predominantly adenocarcinoma component, squamous cell carcinoma and other without specification.

D. Safety and Effectiveness Results

1. Safety Results

   The safety with respect to treatment with erlotinib was addressed in the original drug approval and was summarized as part of the approval of the **cobas**® EGFR Tissue Test v1. See Summary of Safety and Effectiveness Data for P120019.

2. Effectiveness Results

   The multi-center, randomized Phase III ENSURE study tested the administration of erlotinib versus gemcitabine plus cisplatin as first-line treatment for stage IIIB/IV NSCLC in patients with activating EGFR mutations (Ex. 19del or L858R substitution mutations) as determined by the **cobas**® EGFR Tissue Test v1. The **cobas**® EGFR Plasma Test v2 was developed after patients had been screened and enrolled in the ENSURE study using the **cobas**® EGFR Tissue Test v1. Retrospective testing of plasma specimens from patients screened from the ENSURE study was performed using the **cobas**® EGFR Plasma Test v2.

Of the 217 patients enrolled (i.e., those with an Ex. 19del or L858R mutation detected in a tissue sample by the **cobas**® EGFR Tissue Test v1), 214 (98.6%) had plasma samples available and 180 patients of them had plasma sample of at least 2.0 mL. Of the 180 plasma samples with a volume of 2.0 mL tested by **cobas**® EGFR Plasma Test v2, 137 had a MD result for an Ex. 19del and/or an L858R mutation (68 patients from the erlotinib arm, and 69 patients from the chemotherapy arm).

For the clinical outcome analyses, the groups were defined as summarized below.

Table 37. Clinical Analysis Dataset

Analysis Populations	Description
ENSURE Tissue Full Analysis Set (FAS) (tEGFR+) (N = 217)	All patients who had an EGFR MD by the **cobas**® EGFR Tissue Test v1 and were randomized in the ENSURE study
Tissue MD and Plasma-tested Population (tEGFR+∩ pEGFR tested) (N = 180)	Patients in the Tissue FAS who were tested for an EGFR mutation by the **cobas**® EGFR Plasma Test v2
Tissue MD and Plasma MD/NMD Population (tEGFR+∩ pEGFR+/-) (N = 179)	Patients in the Tissue FAS who had a valid result by the **cobas**® EGFR Plasma Test v2 (MD/NMD)
Tissue MD and Plasma MD Population (tEGFR+∩ pEGFR+) (N = 137)	Patients in the Tissue FAS who were EGFR MD by the **cobas**® EGFR Plasma Test v2
Tissue MD and Plasma NMD Population (tEGFR+∩ pEGFR-) (N = 42)	Patients in the Tissue FAS who were EGFR NMD by the **cobas**® EGFR Plasma Test v2
Plasma MD Population (pEGFR+)	Patients in the Tissue FAS who had an EGFR MD by the **cobas**® EGFR Plasma Test v2 and tissue NMD patients who are MD by the **cobas**® EGFR Plasma Test v2 [Note: Tissue NMD patients were, by definition, not enrolled in the ENSURE study. Efficacy was estimated by imputation methods.]
EGFR MD population (EGFR+)	Patients in the Tissue FAS and tissue NMD patients who were MD by the **cobas**® EGFR Plasma Test v2 [Note: Tissue NMD patients were, by definition, not enrolled in the ENSURE study. Efficacy was estimated by imputation methods.]

At the cutoff date for the final PFS analysis, November 19, 2012, among patients enrolled in the ENSURE study with a plasma sample volume of 2.0 mL and who had an EGFR mutation detected by the **cobas**® EGFR Plasma Test v2 (Plasma FAS), the investigator-assessed PFS was prolonged for patients treated with erlotinib compared to patients treated with chemotherapy. The results are summarized in the table below. A total of 60 patients (87.0%) in the chemotherapy arm and 41 patients (60.3%) in the erlotinib arm had a PFS event. Median PFS in the chemotherapy arm was 4.6 months compared to 8.3 months in the erlotinib arm and the risk of a PFS event (progression or death, whichever

occurs first) was reduced by 71% (HR = 0.29; 95% CI: 0.19, 0.45; log-rank p-value < 0.0001) for patients in the erlotinib arm.

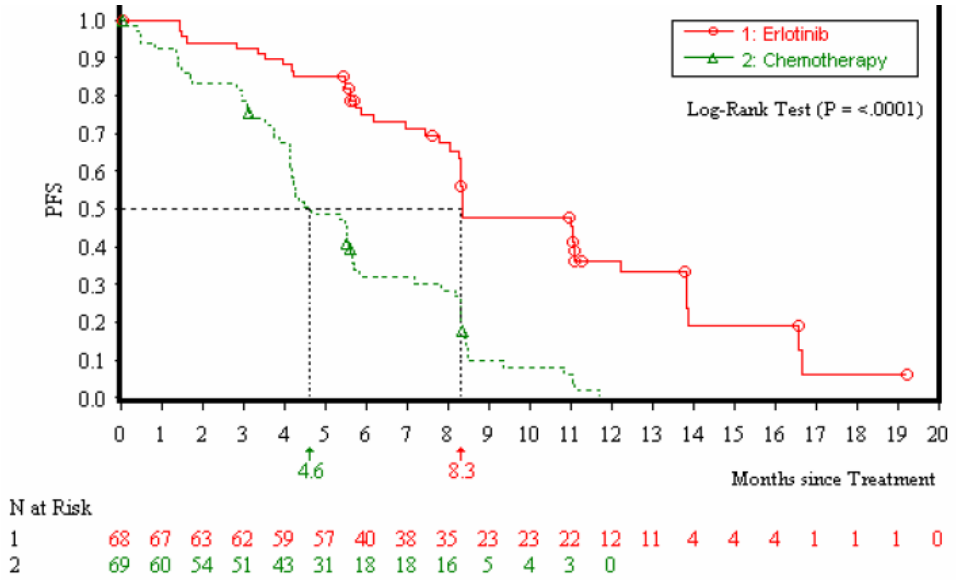
Table 38. Summary of Investigator-Assessed PFS for the ENSURE Study Enrolled Patients with a MD by the cobas® EGFR Plasma Test v2 (2.0 mL Sample Volume)

	Chemotherapy (N = 69)		**Erlotinib (N = 68)**
Patients with event	60 (87.0%)		41 (60.3%)
Patients without event [a]	9 (13.0%)		27 (39.7%)
Time to event (months)			
Median#	4.6		8.3
95% CI for Median [b]	[4.1; 5.6]		[8.3; 11.1]
25% and 75%-ile [b]	3.2; 8.3		5.9; 13.8
Range [c]	0.0 to 11.7		0.0 to 19.2
p-Value (Log-Rank Test)		<.0001	
Hazard Ratio		0.29	
95% CI		[0.19; 0.45]	

Event: disease progression or death, whichever occurred earlier.
[a] censored.
[b] Kaplan-Meier estimates.
[c] including censored observations.

The Kaplan-Meier curves for the investigator-assessed PFS are shown in the figure below for patients with either an Ex. 19del or L858R mutation in plasma. The curves start to separate after approximately 1.5 months and remain well separated over the course of the observation period (p value < 0.001). The patients in the erlotinib arm had a longer PFS compared to patients in the chemotherapy arm, showing substantial benefit to therapy with erlotinib in patients with detectable EGFR activating mutations.

Figure 4. Kaplan-Meier Plot of Investigator-assessed PFS for the ENSURE Enrolled Patients with a MD by the cobas® EGFR Plasma Test v2 (sample volume of 2.0 mL)



The results of the IRC-assessed PFS analysis were consistent with the investigator-assessed PFS analysis. Based on the IRC analysis, a total of 41 (59.4%) patients in the chemotherapy arm and 33 (48.5%) patients in the erlotinib arm had a PFS event. Median PFS was 5.6 months in the chemotherapy arm compared to 11.0 months in the erlotinib arm (HR = 0.32; 95% CI: 0.19, 0.53; log-rank p < 0.0001).

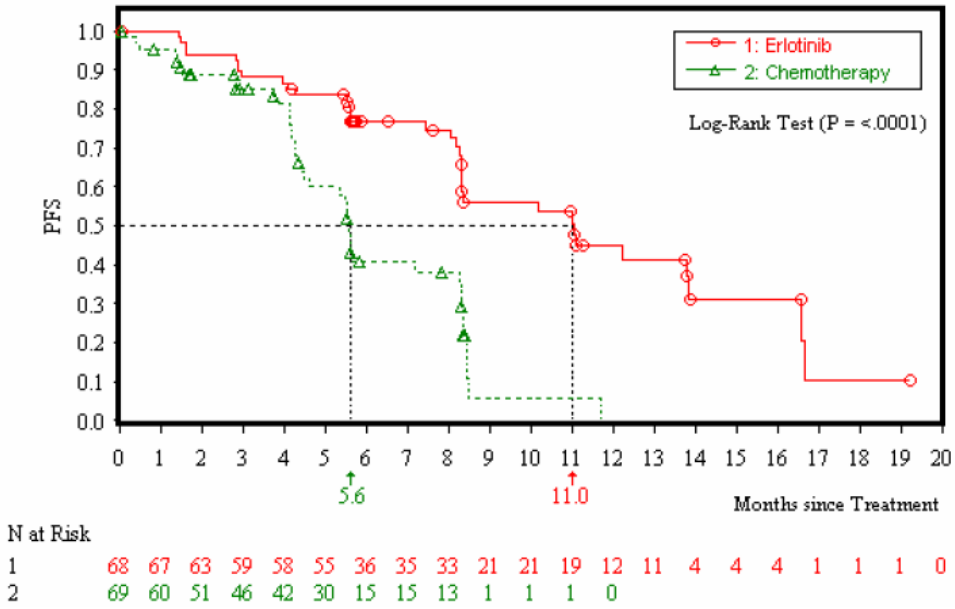
Table 39. Summary of IRC-Assessed PFS for the ENSURE Study Enrolled Patients with a MD by the cobas® EGFR Plasma Test v2 (2.0 mL Sample Volume)

	Chemotherapy (N = 69)		Erlotinib (N = 68)
Patients with event	41 (59.4%)		33 (48.5%)
Patients without event a	28 (40.6%)		35 (51.5%)
Time to event (months)			
Median#	5.6		11.0
95% CI for Median b	[4.5; 8.2]		[8.3; 13.8]
25% and 75%-ile b	4.2; 8.3		7.4; 16.6
Range c	0.0 to 11.7		0.0 to 19.2
p-Value (Log-Rank Test)		< 0.0001	
Hazard Ratio		0.32	
95% CI		[0.19; 0.53]	

Event: disease progression or death, whichever occurred earlier.
[a] censored.
[b] Kaplan-Meier estimates.
[c] including censored observations.
Cut-off for statistical analysis: November 19, 2012

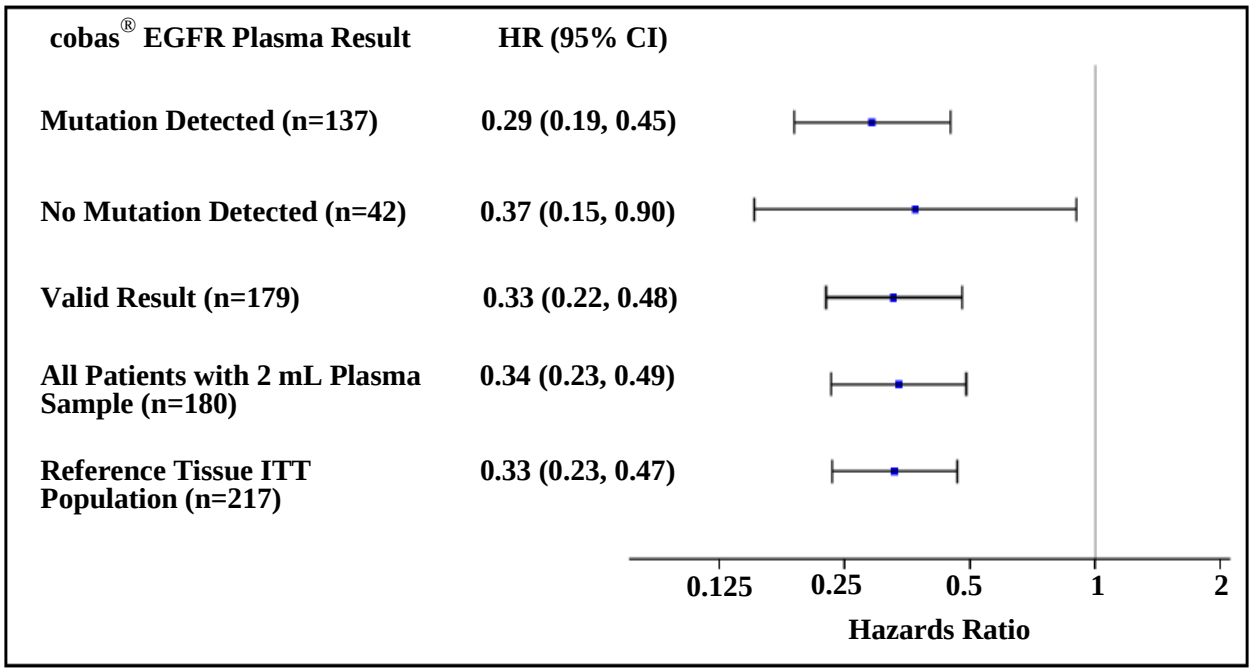
The Kaplan-Meier curves for the IRC-assessed PFS are shown in the figure below for patients with either an Ex. 19del or L858R mutation in plasma. The patients in the erlotinib arm had a longer PFS compared to patients in the chemotherapy arm and the two curves were well separated from approximately 4 months through the course of the observation period (p value < 0.001) showing substantial benefit to therapy with erlotinib in patients with detectable EGFR activating mutations.

Figure 5. Kaplan-Meier Plot of IRC-assessed PFS for the ENSURE Enrolled Patients with a MD by the cobas® EGFR Plasma Test v2 (2.0 mL Sample Volume)



The Forest plot shown below compares the hazard ratios for investigator-assessed PFS together with their corresponding 95% confidence intervals for: 1) all patients with a plasma sample volume of 2.0 mL (n = 180); 2) all patients with a plasma sample volume of 2.0 mL and a valid plasma test result (n = 179); 3) all patients with a plasma sample volume of 2.0 mL and a MD plasma test result (n = 137); and 4) all patients with a plasma sample volume of 2.0 mL and a NMD plasma test result (n = 42), as shown in the figure below. The HR for PFS for the ITT population of the ENSURE study, based on the **cobas**® EGFR Tissue Test v1, is included as the reference.

Figure 6. Forest Plot of Investigator-assessed PFS in Sensitivity Analysis Populations (2.0 mL Sample Volume)



E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included two investigators. Neither 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

Paired tissue and EDTA plasma results from an independent cohort were provided from 18 NSCLC patients, including 15 with EGFR mutations and 3 WT specimens as reported by the **cobas**® EGFR Tissue Test v2. The results span at least four years from the initial FFPET results, determined to the last time at which the EDTA plasma specimens were tested, in February 2016 (final plasma testing). Stability of the plasma specimens were traced to the original FFPET results through interim plasma-serum blend (50:50) results obtained in December, 2012. The interim results were used to confirm that the mutation was present in plasma at the time of tissue sampling. Of the originally identified 18 specimens, 6 were excluded from the analysis, because there were no corresponding FFPET results by the **cobas**® EGFR Tissue Test v1.

The plasma and serum specimens were stored at ≤ -70ºC from the time of draw until final plasma testing in February, 2016 under conditions consistent with the ENSURE and

ASPIRATION study specimens. The plasma specimen age range for the ENSURE study was from 30 – 47 months and for the ASPIRATION study was from 33 – 48 months.

The **cobas**® EGFR Plasma Test v2 is validated for a 2.0 mL plasma volume, this volume was not available for all specimens included in this study. In addition to 2.0 mL, the residual plasma volumes for each specimen were estimated at the final time point and ranged from 0.5 – 1.8 mL. Initial testing of the FFPET specimens was conducted using the **cobas**® EGFR Tissue Test v1 whereas the plasma specimens were tested with the **cobas**® EGFR Plasma Test v2 using the current cut-off values. One specimen contained an L861Q mutation which was manually verified based on the **cobas**® EGFR Tissue Test v1 raw data (the **cobas**® EGFR Tissue Test v1 did not report L861Q). Additionally, one NMD specimen gave a positive Ex. 19del result at the final time point.

Eleven of the twelve specimens (91.67%), included in the stability study, yielded mutation results which were identical between the final plasma and original FFPET results. The twelfth result had a low-positive Ex. 19del mutation result for the final plasma testing that was not present in the FFPET or interim plasma/serum mutation results; this call was likely a false positive result, which was considered not likely due to specimen storage. Similarly, an additional Ex. 20ins mutation call was observed in another specimen at the interim testing time point (from the plasma/serum blend). This additional mutation was not observed in the final plasma result or the FFPET result. This false positive result may have been due to specimen contamination rather than due to specimen storage.

Despite the residual specimen volume being below the 2.0 mL validated volume, the data from this stability study does support the validity of the data from the ENSURE clinical study as the specimens in the ENSURE study were handled and stored according to similar protocols.

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 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.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the **cobas**® EGFR Plasma Test v2 in the detection of the Ex. 19del and L858R mutations in cfDNA extracted from plasma was demonstrated in retrospective analyses for patients enrolled in the Phase III ENSURE study as summarized in Tables 28, 30, 31, 33, 38, and 39. Analytical performance studies with

the **cobas**® EGFR Plasma Test v2, when used according to the instructions for use, demonstrate the ability to detect the Ex. 19del and L858R mutations with an analytical sensitivity of 75 cp/mL and 100 cp/mL, respectively, of cfDNA extracted from NSCLC patient plasma.

The safety and effectiveness of TARCEVA® (erlotinib) has not been established in patients whose plasma specimens have G719X, Ex. 20ins, T790M, S768I or L861Q mutations, which are also detected by the **cobas**® EGFR Plasma Test v2.

B. Safety Conclusions

As a diagnostic test, the **cobas**® EGFR Plasma Test v2 involves testing on plasma isolated from EDTA anti-coagulated peripheral blood collected from NSCLC patients. The risks of the **cobas**® EGFR Plasma 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 EGFR test results, and consequently improper patient management decisions in NSCLC treatment. Since a patient with a negative result (including a false negative result) from the **cobas**® EGFR Plasma Test v2 will be reflexed to having their EGFR status determined from their FFPET tissue specimen, the risks of the **cobas**® EGFR Plasma Test are largely associated with a false positive result in a patient, who may then undergo treatment with erlotinib with inappropriate expectation of therapeutic benefit and experience side effects.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in the ENSURE study, which were used to support PMA approval as described in Tables 38 – 39, and Figures 4 – 6, above. The clinical benefit of the **cobas**® EGFR Plasma Test v2 was demonstrated in a retrospective analysis of efficacy and safety data obtained from a Phase III, multicenter, open-label, two arm study in which erlotinib demonstrated a median investigator-assessed PFS of 8.3 months vs. 4.6 months when treated by chemotherapy. The risk of a PFS event (progression or death, whichever occurs first) in these tissue and plasma positive patients was reduced by 71% (HR = 0.29; 95% CI: 0.19, 0.45; log-rank p-value < 0.0001) for patients in the erlotinib arm.

The risks of the **cobas**® EGFR Plasma Test v2 are associated with the potential mismanagement of patients resulting from erroneous test results. The device is a key part of diagnostic evaluation for non-small cell lung cancer in decisions regarding treatment with erlotinib. There is currently no FDA approved test for the selection of candidate metastatic NSCLC patients for treatment with erlotinib based on identification of Ex. 19del or L858R activating mutations from patient's plasma specimens.

In conclusion, given the available information above, the data support the use of the

cobas® EGFR Plasma Test v2 as an aid in selecting NSCLC patients for erlotinib treatment based on a **cobas**® EGFR Plasma Test v2 "Mutation Detected" result for EGFR Ex. 19del or L858R mutations when detected in plasma, and the probable benefits outweigh the probable risks.

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the Phase III ENSURE clinical study support the utility of the **cobas**® EGFR Plasma Test v2 as an aid in selecting patients with advanced NSCLC for whom erlotinib, an EGFR TKI, is indicated. Erlotinib demonstrated clinical benefit in terms of PFS that appears to be robust and of a magnitude to reasonably predict clinical benefit for erlotinib in patients identified with the **cobas**® EGFR Plasma Test v2.

XIV. CDRH DECISION

CDRH issued an approval order on June 1, 2016. The final conditions of approval cited in the approval order are described below.

Limited specimen handling and specimen storage stability information was included in the PMA application. Additional testing is needed to determine the robustness and consistency of assay performance in regard to both pre-analytical and storage conditions. Successful completion of an agreed upon study protocol is needed to validate and support claims regarding whole blood and plasma sample handling and storage stability for the **cobas**® EGFR Mutation Test v2 using clinical plasma samples. The final study data, study conclusions, and labeling revisions should be submitted within 2 years of the PMA approval date.

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, Warnings, Precautions, and Limitations in the device labeling. Refer to the drug label for TARCEVA® (erlotinib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.