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:	P150044
Date of FDA Notice of Approval:	September 28, 2016
Priority Review:	Granted priority review status on December 15, 2015 because the device addresses an unmet medical need, as demonstrated by significant clinically meaningful advantage.

The **cobas**® EGFR Mutation Test v2 was originally approved as a prior version for the qualitative detection of defined mutations in the epidermal growth factor receptor (EGFR) gene in 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 and associated therapeutic indications. The indications for use of the device as previously approved were to aid in identifying patients with non-small cell lung cancer (NSCLC) whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established as shown in the table below. The SSEDs to support the previously approved FFPET and plasma indications are available on the CDRH website.

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

Drug	FFPET Specimen Type		Plasma Specimen Type	
	Submission # Approval Date (Test version)	**Specific EGFR Mutations**	**Submission # Approval Date (Test version)**	**Specific EGFR Mutations**
TARCEVA® (erlotinib)	P120019 May 14, 2013 (v1)	Exon 19 deletions and Exon 20 (L858R)	P150047 June 1, 2016 (v2)	Exon 19 deletions and Exon 20 (L858R)

Drug	FFPET Specimen Type		Plasma Specimen Type	
	Submission # Approval Date (Test version)	**Specific EGFR Mutations**	**Submission # Approval Date (Test version)**	**Specific EGFR Mutations**
		substitution mutations		substitution mutations
TAGRISSO™ (osimertinib)	P120019/S007 November 13, 2015 (v2)	Exon 20 T790M substitution mutation	Current submission	Exon 20 T790M substitution mutation

The current PMA was submitted to add an additional indication for the **cobas**® EGFR Mutation Test v2 to identify NSCLC patients with the exon 20 T790M substitution mutation from circulating-free tumor DNA (cfDNA) isolated from plasma, for treatment with TAGRISSO™ (osimertinib).

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	T790M*

Patients with positive **cobas**® EGFR Mutation Test v2 test results using plasma specimens for the presence of the EGFR mutations listed above are eligible for treatment with the corresponding drug as indicated in Table 1 (see Note* for T790M). 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.

*The efficacy of TAGRISSO™ (osimertinib) has not been established in the EGFR T790M plasma-positive, tissue-negative or unknown population and clinical data for T790M plasma-positive patients are limited; therefore, testing using plasma specimens is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained.

Drug safety and efficacy have not been established for the 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, 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

Exon	EGFR Mutation	EGFR Nucleic Acid Sequence	COSMIC ID[1]
		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
		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.

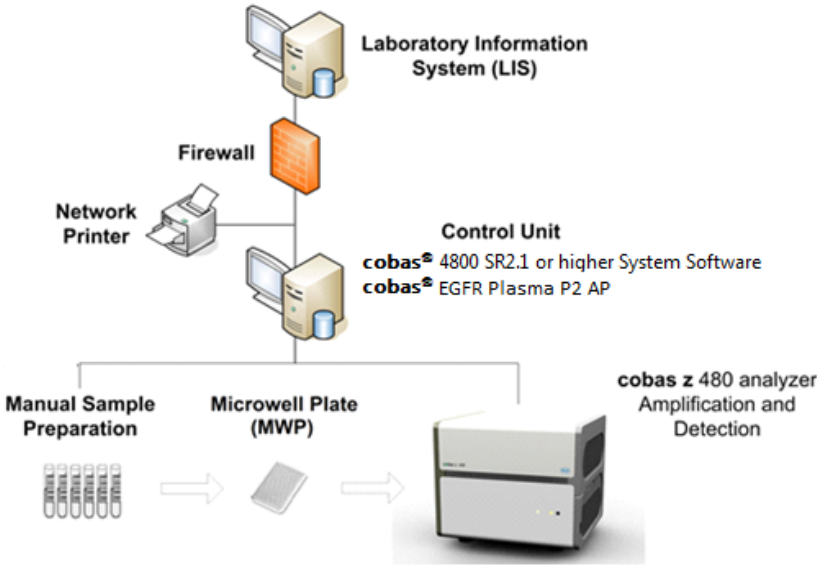
A dedicated Control Unit computer runs the **cobas**® 4800 system software and provides an interface to the **cobas**® **z** 480 and Laboratory Information System (LIS). The computer also processes the fluorescent signals with the assay specific analysis package (ASAP) and stores the test results in 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 Figure 1 below.

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.

Fig 1. **cobas**® 4800 System



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. Tables 3 and 4 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

MMx1 Result	MMx2 Result	MMx3 v2 Result	Reported Result
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
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	Mutation detected in specified targeted EGFR region.

Test Result	Mutation Result**	Interpretation
	S768I *G719X* *Ex. 20ins* *L861Q* (More than one mutation may be present)	
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 for EGFR mutation status in the selection of patients who are eligible for treatment with 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. The **cobas**® EGFR Mutation Test v2 for use with plasma specimens to select patients for treatment with TARCEVA® (erlotinib) was approved on June 1, 2016 and is available in the US.

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

1. Correlation with Reference Method Using Clinical Samples

The analytical accuracy of the **cobas**® EGFR Mutation Test v2 using plasma specimens (**cobas**® EGFR Plasma Test v2) was assessed by comparing its results to a validated quantitative next generation sequencing (NGS) method using banked clinical samples from the AURA2 clinical study. The percent mutation present in specimens was determined for all specimens used to demonstrate performance. Plasma specimens from a second clinical study, AURA1 Extension (AURA1 Ext), were used to supplement the AURA2 study specimens to establish the detection of rare EGFR mutations.

a. Specimen Processing, Disposition, and Testing
Plasma specimens were sent to one of the participating central laboratories after written informed consent was confirmed. cfDNA was isolated manually according to the Instructions for Use for the **cobas**® cfDNA Sample Preparation Kit. Testing was performed according to the Instructions for Use for the **cobas**® EGFR Plasma Test v2. Tissue specimens were processed according to the Instructions for Use for the **cobas**® DNA Sample Preparation Kit.

b. Accountability of the AURA2 Cohort
Three hundred forty-four (344) of the 383 patients with matching tissue specimens had sufficient plasma samples available for testing by the **cobas**® EGFR Plasma Test v2. Of those, 322 (93.6%) samples had sufficient plasma for testing by the validated NGS reference method (see Table 6 below).

c. Agreement to Reference Method

i. Agreement for T790M Mutation:
Patient enrollment into the AURA2 study was based on FFPET biopsy test results using the **cobas**® EGFR Mutation Test v1 (**cobas**® EGFR Tissue Test v1), which were later bridged to test version 2 (v2). Based on the plasma samples tested from the AURA2 study, the agreements of the **cobas**® EGFR Plasma Test v2 and the NGS method for detection of the T790M mutation are presented in Table 5 below, stratified by the **cobas**® EGFR Tissue Test v1 results. Agreement between the three test methods, when stratified by the **cobas**® EGFR Tissue Test v2 also yielded similar results.

Table 5. Three-Way Agreement Among cobas® EGFR Tissue Test v1, cobas® EGFR Plasma Test v2, and NGS for Detection of T790M

cobas® EGFR Tissue Test v1	**cobas® EGFR Plasma Test v2**	**NGS**			**Total**
		T790M+	**T790M(–)**	**No Plasma Sample**	
T790M+	T790M+	109	13	9	131

cobas® EGFR Tissue Test v1	cobas® EGFR Plasma Test v2	NGS			Total
		T790M+	T790M(–)	No Plasma Sample	
	T790M(–)	8	79	5	92
	Invalid/No Plasma Sample	3	0	7	10
	Total	120	92	21	233
T790M(–)	T790M+	18	3	1	22
	T790M(–)	3	79	7	89
	Invalid/No Plasma Sample	0	0	29	29
	Total	21	82	37	140
Invalid	T790M+	2	0	0	2
	T790M(–)	1	5	0	6
	Invalid/No Plasma Sample	0	0	2	2
	Total	3	5	2	10

T790M+ = T790M mutation detected (MD); T790M(-) = T790M mutation not detected (NMD)

Percent agreements and their 95% 2-sided confidence intervals (CIs) for the T790M mutation between the **cobas**® EGFR Plasma Test v2 and the NGS comparator method are shown in Table 6. A sensitivity analysis was conducted for the worst case scenario where invalid or missing results were treated as discordant results between the **cobas**® EGFR Plasma Test v2 and the NGS method. The estimates are shown in Table 6.

Table 6. Agreement between cobas® EGFR Plasma Test v2 and NGS for Detection of T790M Mutation

		NGS				Total
		T790M+	T790M(–)	Invalid	No Plasma Sample	
cobas® EGFR Plasma Test v2	**T790M(+)**	129	16	0	10	155
	T790M(–)	12	163	0	12	187
	Invalid	2	0	0	0	2
	Total	143	179	0	22	344
Valid Results Only (Total N=320)	**PPA (95% CI)**	91.5% (85.7%, 95.1%)				
	NPA (95% CI)	91.1% (86.0%, 94.4%)				
	PPV (95% CI)	89.0% (82.8%, 93.1%)				
	NPV (95% CI)	93.1% (88.4%, 96.0%)				
Sensitivity Analysis (Worst Case Scenario) (Total N=344)	**PPA (95% CI)**	83.2% (76.6%, 88.3%)				
	NPA (95% CI)	86.3% (80.6%, 90.4%)				
	PPV (95% CI)	83.2% (76.6%, 88.3%)				
	NPV (95% CI)	86.3% (80.6%, 90.4%)				

ii. Agreement for Ex. 19del and L858R Mutations:

As part of the enrollment criteria into the AURA2 study, all patients were required to demonstrate the presence of an activating mutation in addition to T790M. Based on the plasma samples tested from the AURA2 study, 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 Table 7 below.

Table 7. Agreement between cobas® EGFR Plasma Test v2 and NGS for Detection of Individual Ex 19del and L858R Sensitizing Mutations

cobas® EGFR Plasma Test v2	NGS Method								
	Ex. 19del				L858R				
	MD	NMD	No Sample	Total	MD	NMD	No Sample	Invalid	Total
MD	137	12	16	165	83	11	0	3	97
MND	11	160	6	177	3	222	1	19	245
Invalid	1	1	0	2	1	1	0	0	2
Total	149	173	22	344	87	234	1	22	344
PPA (95% CI)	92.6% (87.2%, 95.8%)				96.5% (90.2%, 98.8%)				
NPA (95% CI)	93.0% (88.2%, 96.0%)				95.3% (91.7%, 97.3%)				

The agreement between the **cobas**® EGFR Plasma Test v2 and the NGS method for the detection of mutations in exons 18, 19, 20 and 21 in aggregate in the EGFR gene are shown in Table 8 below.

Table 8. Agreement between cobas® EGFR Plasma Test v2 and NGS for Detection of All EGFR Mutations

		NGS				Total
		EGFRm (+)	EGFRm (–)	Invalid	No Plasma Sample	
cobas® EGFR Plasma Test v2	**EGFRm(+)**	232	16	0	21	269
	EGFRm (–)	15	57	0	1	73
	Invalid	2	0	0	0	2
	Total	249	73	0	22	344
Valid Results Only (Total N=320)	**PPA (95% CI)**	93.9% (90.2%, 96.3%)				
	NPA (95% CI)	78.1% (67.3%, 86.0%)				
	PPV (95% CI)	93.5% (89.8%, 96.0%)				
	NPV (95% CI)	79.2% (68.4%, 86.9%)				
Sensitivity Analysis (Worst Case Scenario) (Total N=344)	**PPA (95% CI)**	92.8% (88.9%, 95.4%)				
	NPA (95% CI)	60.6% (50.5%, 69.9%)				
	PPV (95% CI)	86.2% (81.6%, 89.9%)				
	NPV (95% CI)	76.0% (65.2%, 84.2%)				

EGFRm(+) = EGFR mutation detected (MD), EGFRm(-) = EGFR mutation not detected (NMD)

iii. *Rare Mutations:*

All four rare mutations that the **cobas**® EGFR Plasma Test v2 was designed to detect (i.e., Ex. 20ins, L861Q, S768I, and G719X) were detected and confirmed by the validated NGS method using plasma samples from the AURA1 Ext and AURA2 studies. The results are summarized in Table 9.

Table 9. Agreement of Rare Mutations between cobas® EGFR Plasma Test v2 and NGS.

Study	Rare Mutations	MD by cobas® EGFR Plasma Test v2, NMD by NGS	MD by both cobas® EGFR Plasma Test v2 and NGS	NMD by cobas® EGFR Plasma Test v2, MD by NGS	PPA %
Total	G719X	1	10	6	62.5
	Ex 20ins	0	0	1	0
	S768I	0	5	1	83.33
	L861Q	0	4	0	100

In four of five (4/5) of the missed calls by the **cobas**® EGFR Plasma Test v2 for G719X from AURA2, percent mutation in the specimens were above the NGS LoD (0.18%) but below the mutation cut-off for the **cobas**® EGFR Plasma Test v2. For the fifth G719X and the one S768I mutations, which were missed by the **cobas**® EGFR Plasma Test v2, the specimens' Ct cut-off values exceeded the maximum Ct cut-offs for the respective mutations by the **cobas**® EGFR Plasma Test v2. The Ex. 20ins mutation that was not detected by the **cobas**® EGFR Plasma Test v2was not a variant that the test was designed to detect. No other Ex. 20ins mutations were detected by either the **cobas**® EGFR Plasma Test v2or NGS.

2. Contrived Sample Comparison

   See Summary of Safety and Effectiveness Data for P150047.

3. Analytical Sensitivity

   See Summary of Safety and Effectiveness Data for P150047.

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 Summary of Safety and Effectiveness Data for P120019/S007.)

Studies to assess interference by potentially interfering endogenous and exogenous substances, and microorganism testing are summarized in the Summary of Safety and Effectiveness Data for P150047.

5. Reproducibility and Precision

   See Summary of Safety and Effectiveness Data for P150047.

6. Lot-to-Lot Reproducibility

   See Summary of Safety and Effectiveness Data for P150047.

7. Specimen Handling

   See Summary of Safety and Effectiveness Data for P150047.

8. Guard banding

   See Summary of Safety and Effectiveness Data for P150047.

9. Stability Studies

   See Summary of Safety and Effectiveness Data for P150047.

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 osimertinib by detecting the presence of the EGFR T790M substitution mutation in EDTA plasma specimens. The specimens used in the bridging study consisted of plasma specimens, collected at baseline, from patients with NSCLC who progressed following prior EGFR tyrosine kinase inhibitor (EGFR-TKI) therapy.

Patient specimen cohorts from two AstraZeneca clinical studies, AURA1 Ext (Protocol D5160C00001) and AURA2 (Protocol D5160C00002), were utilized to support this PMA submission. The AURA1 Ext Phase 1 study and the AURA2 Phase II study enrolled patients with advanced or metastatic NSCLC who had progressed following an EGFR-TKI for second line treatment with osimertinib along with patients who

progressed after treatment with an EGFR-TKI and at least one platinum-based doublet chemotherapy. The patients were enrolled based on an FFPET specimen positive EGFR sensitizing mutation and a T790M substitution mutation result using the **cobas**® EGFR Mutation Test v1. Enrollment for both studies was conducted at approximately 50 sites worldwide. These clinical studies were used to support accelerated approval of TAGRISSO (osimertinib) under NDA 208065. The AURA1 Ext study specimens were used only to supplement specimens with rare EGFR mutations (see Section IX.A.1.c.*iii,* above). The AURA2 study supported the approval of the **cobas**® EGFR Mutation Test v2 using FFPET specimens under P120019/S007.

A. Study Design

The study design for the AURA2 study describing the enrollment of patients based on testing of FFPET specimens is available in the Summary of Safety and Effectiveness for P120019/S007.

1. Inclusion/Exclusion Criteria

   Patients were enrolled into the AURA2 study and continued on treatment until the occurrence of disease progression (based on RESIST 1.1) or unacceptable toxicity. For the inclusion/exclusion criteria for the original AURA2 study and the bridging study between the first and second versions of the **cobas**® EGFR Tissue Test, see the Summary of Safety and Effectiveness for P120019/S007.

   The inclusion/exclusion criteria for inclusion into the bridging study between the **cobas**® EGFR Tissue Test and the **cobas**® EGFR Plasma Test v2 are summarized below:

   Inclusion Criteria:
   Samples from subjects who were eligible for screening under the AURA1 Ext and AURA2 study protocols with a valid EGFR mutation result were eligible for this protocol. The criteria also indicated that:
   - An FFPET NSCLC biopsy following progression of disease on the latest line of therapy must have been available for mutation analysis.
   - A concurrently obtained plasma sample must have been available for analysis.

   Exclusion criterion:
   Samples were excluded if there was insufficient plasma volume for testing.

2. Follow-up Schedule

   See the Summary of Safety and Effectiveness for P120019/S007.

3. Clinical Endpoints

   See the Summary of Safety and Effectiveness for P120019/S007.

4. Bridging Study

In this bridging study, plasma specimens were collected at baseline screening from patients enrolled in the AURA2 clinical study. The available specimens from the study were tested by both the **cobas**® EGFR Plasma Test v2 and a validated NGS method. Agreements between the detection of EGFR T790M mutation status and EGFR mutations in exons 18, 19, 20 and 21, in aggregate, between the patients' plasma and tissue specimen results are shown in the Tables 10 and 12 below, respectively. Sensitivity analyses to account for missing data were also performed and the results are included in Tables 11 and 13 below.

Table 10. Agreement between cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1 and v2 for Detection of T790M

cobas® EGFR Plasma Test v2	cobas® EGFR Tissue Test v1				cobas® EGFR Tissue Test v2			
	T790M+	T790M (–)	Invalid	Total	T790M+	T790M (–)	Invalid	Total
T790M+	131	22	2	155	128	22	5	155
T790M(–)	92	89	6	187	91	90	6	187
Invalid	2	0	0	2	2	0	0	2
No Plasma Sample	8	29	2	39	10	27	2	39
Total	233	140	10	383	231	139	13	383
PPA (95% CI)	58.7% (52.2%, 65.0%)				58.4% (51.8%, 64.8%)			
NPA (95% CI)	80.2% (71.8%, 86.5%)				80.4% (72.0%, 86.7%)			
PPV (95% CI)	85.6% (79.2%, 90.3%)				85.3% (78.8%, 90.1%)			
NPV (95% CI)	49.2% (42.0%, 56.4%)				49.7% (42.5%, 56.9%)			

Table 11. Sensitivity Analysis of Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v2 in Detecting T790M Mutation

	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
With invalid as discordant result	56.4% (49.9%, 62.7%)	76.9% (68.5%, 83.6%)	82.6% (75.8%, 87.7%)	47.6% (40.6%, 54.7%)
With invalid & no samples as discordant result	54.0% (47.6%, 60.2%)	62.5% (54.4%, 70.0%)	70.3% (63.3%, 76.5%)	45.2% (38.5%, 52.2%)
Verification bias adjusted for invalid and no sample w/ bootstrap method for CI	58.4% (51.9%, 64.6%)	80.7% (74.2%, 86.8%)	83.1% (78.0%, 88.0%)	54.3% (49.6%, 59.3%)

Table 12. Agreement between cobas® EGFR Plasma Test v2 and Tissue Test v2 for Overall EGFR Mutation Detection

cobas® EGFR Plasma Test v2	cobas® EGFR Tissue Test v2			Total
	EGFRm+	EGFRm(-)	Invalid	
EGFRm+	257	2	10	269
EGFRm(-)	62	10	1	73

cobas® EGFR Plasma Test v2	cobas® EGFR Tissue Test v2			Total
	EGFRm+	EGFRm(-)	Invalid	
Invalid	2	0	0	2
No Plasma Sample	32	5	2	39
Total	353	17	13	383
PPA (95% CI)	80.6% (75.9%, 84.5%)			
NPA (95% CI)	83.3% (83.3%, 95.3%)			

EGFRm+ = EGFR mutation detected (MD); EGFRm(-) = EGFR mutation not detected (NMD)

Table 13. Sensitivity Analysis of Agreement of cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v2 for Overall EGFR Mutation

Scenario	PPA (95% CI)	NPA (95% CI)
With Invalid as discordant result	79.8% (75.1%, 83.8%)	45.5% (26.9%, 65.3%)
With Invalid and no samples as discordant result	72.6% (67.7%, 77.0%)	37.0% (21.5%, 55.8%)
Verification Bias Adjusted for invalid and no samples with Bootstrap method for confidence intervals	80.6% (76.4%, 84.9%)	83.3% (65.2%, 96.3%)

B. Accountability of PMA Cohort

A total of 472 patients were screened for AURA2; 383 patients had tissue samples tested by the **cobas**® EGFR Tissue Test v1 and were eligible for the study. Of those eligible patients, 344 (89.9%) had a plasma sample tested by the **cobas**® EGFR Plasma Test v2. Of the eligible patients with a valid tissue test (v1) result (n = 373), 233 were T790M+ and 140 were T790M(–) as shown in the Table 14 below. Of 233 patients who were T790M+ (by v1 of the tissue test), 210 were enrolled in the AURA2 study and received at least one dose of osimertinib and were considered as the tissue full analysis set (tFAS). Of the 210 tFAS patients enrolled and treated in the AURA2 study, 207 (98.6%) had a plasma sample and were tested by **cobas**® EGFR Plasma Test v2. Sample availability and accountability are described further in Figure 2 and Table 14 below.

Fig. 2. Patient Accountability for Plasma Test from AURA2

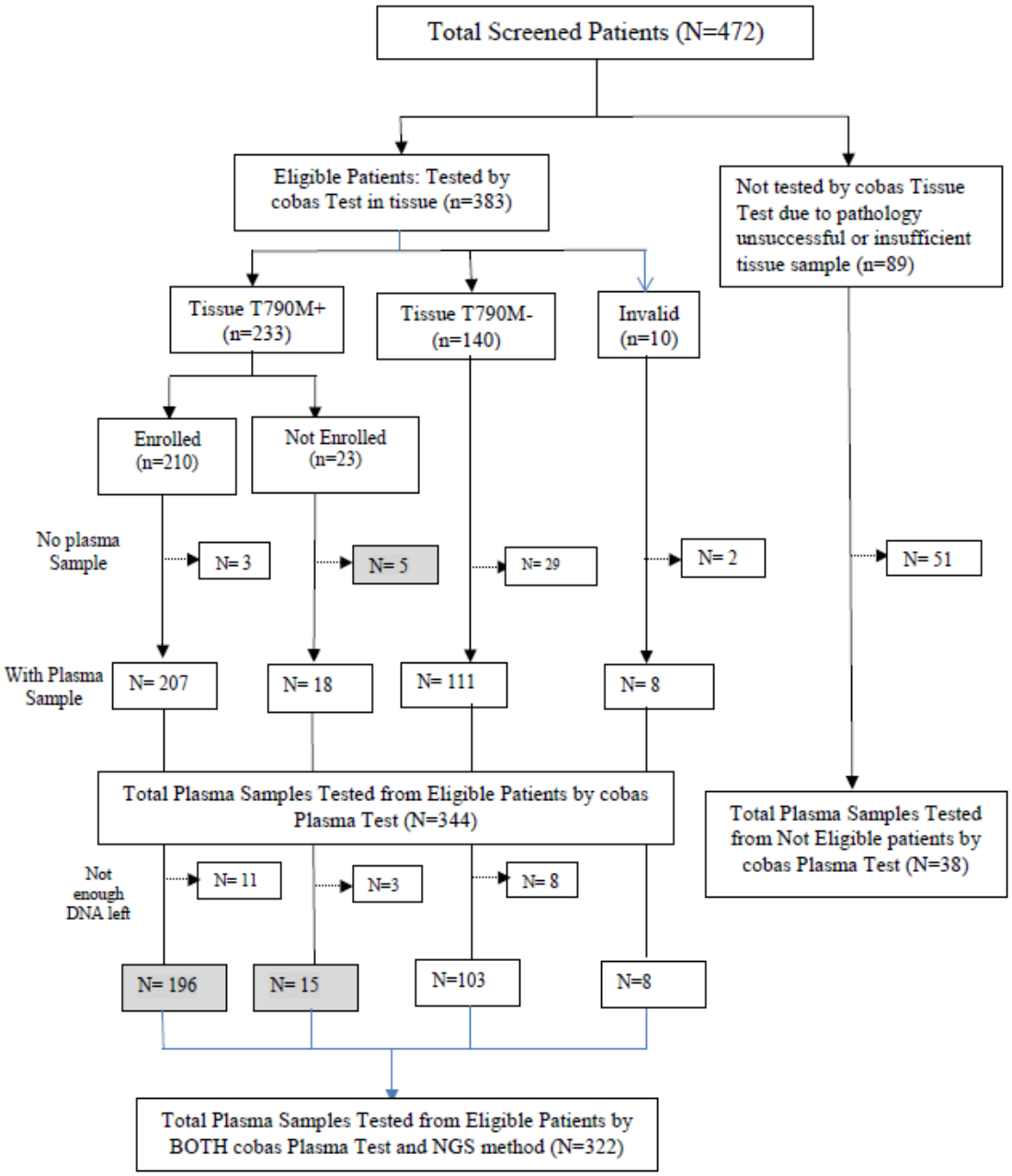


Table 14. Plasma Sample Availability from AURA2 by Category

Category	Total	Plasma Sample n(%)
Total screened patients	472	382 (80.9%)
Tested by **cobas**® Tissue Test v1 (Eligible)	383	344 (89.8%)
T790M+	233	225 (96.6%)
Enrolled and Treated with Tagrisso™	210	207 (98.6%)

Category	Total	Plasma Sample n(%)
T790M(–)	140	111 (79.3%)
Invalid	10	8 (80.0%)
Not Tested by **cobas**® EGFR Tissue Test v1	89	38 (42.7%)
Pathology Unsuccessful*	34	25 (73.5%)
Without Tumor Tissue	55	13 (23.6%)

* Tissue samples from 4 patients with tissue pathology that were 'unsuccessful' were mistakenly tested by the **cobas**® EGFR Tissue Test v1. The tissue results were treated as 'not tested' by the **cobas**® EGFR Tissue Test v1.

C. Study Population Demographics and Baseline Parameters

The average age for the 383 eligible patients is 62.6 years, ranging from 33 to 88 years old, approximately 68% were female, 96.6% were not Hispanic or Latino, 65% were Asian, and 73% never smoked. Approximately 78% of patients were classified as metastatic/stage IV cancer and approximately 95% had a histologic diagnosis of adenocarcinoma (NOS). The demographics and baseline clinical characteristics are shown in Table 15 below.

Table 15. Demographics and Baseline Clinical Characteristics of All Screened Patients by Plasma Specimen Availability

Characteristics		Overall (n=383)	Available for Plasma Test		
			Yes (n=344)	No (n=39)	p-value
Age (Years)					
	Mean ± SD	62.6±10.7	62.6±10.8	62.1±9.9	0.7852
	Median (Range)	63.0 (33, 88)	64.0 (33, 88)	63.0 (37, 85)	
Sex, n (%)					
	Male	122 (31.9%)	111 (32.3%)	11 (28.2%)	0.6058
	Female	261 (68.1%)	233 (67.7%)	28 (71.8%)	
Race, n (%)					
	White	125 (32.6%)	103 (29.9%)	22 (56.4%)	0.0002
	Asian	248 (64.8%)	234 (68.0%)	14 (35.9%)	
	Other	10 (2.6%)	7 (2.0%)	3 (7.7%)	
Ethnic Origin, n (%)					
	Hispanic Or Latino	11 (2.9%)	10 (2.9%)	1 (2.6%)	0.0001
	Not Hispanic Or Latino	370 (96.6%)	334 (97.1%)	36 (92.3%)	
	Missing	2 (0.5%)	0 (0.0%)	2 (5.1%)	
Region, n (%)					
	Asia	215 (56.1%)	207 (60.2%)	8 (20.5%)	<0.0001
	Europe	67 (17.5%)	60 (17.4%)	7 (17.9%)	
	North America	101 (26.4%)	77 (22.4%)	24 (61.5%)	
Smoking Status, n (%)					
	Never	279 (72.8%)	246 (71.5%)	33 (84.6%)	0.0812
	Current/Former	104 (27.2%)	98 (28.5%)	6 (15.4%)	
AJCC Disease Stage, n (%)					
	I/II/III	76 (19.8%)	70 (20.3%)	6 (15.4%)	<0.0001
	IV	298 (77.8%)	270 (78.5%)	28 (71.8%)	

Characteristics		Overall (n=383)	Available for Plasma Test		
			Yes (n=344)	No (n=39)	p-value
	Missing	9 (2.3%)	4 (1.2%)	5 (12.8%)	
Overall Disease Classification					
	Primary	216 (56.4%)	187 (54.4%)	29 (74.4%)	0.0568
	Metastatic	166 (43.3%)	156 (45.3%)	10 (25.6%)	
	Tumor (not specified)	1 (0.3%)	1 (0.3%)	0 (0.0%)	
Histology Type, n (%)					
	Adenocarcinoma (NOS)	363 (94.8%)	328 (95.3%)	35 (89.7%)	0.0001
	Other	18 (4.7%)	16 (4.7%)	2 (5.1%)	
	Missing	2 (0.5%)	0 (0.0%)	2 (5.1%)	
cobas® EGFR Tissue Test v1 T790M Result					
	T790M+	233 (60.8%)	225 (65.4%)	8 (20.5%)	<0.0001
	T790M(–)	140 (36.6%)	111 (32.3%)	29 (74.4%)	
	Invalid	10 (2.6%)	8 (2.3%)	2 (5.1%)	

Note: In Race, category 'Other' combines Black or African American, Native Hawaiian or Other Pacific Islander and Other.

All the variables other than age and sex are significantly different for the patients with and without plasma samples while smoking status and overall disease classification were considered of borderline significance. A multiple logistic regression model with stepwise selection was used to compare the patient demographics and clinical characteristics within each T790M status [T790M+, T790M(–), and T790M status unknown by the **cobas**® EGFR Tissue Test v1]. Study participants' region (i.e., location), smoking status, and tissue T790M status, were related to the availability of plasma samples. The impact of variables (region of study participants and smoking) were further investigated on the overall agreement (concordant or discordant) as well as the 3-group agreement (positive concordant, negative concordant and discordant) of T790M status between tissue and plasma for those with valid results from both tests by a propensity score method. The equal distribution across the T790M subgroups indicates that those variables did not affect the agreement between the plasma and tissue tests.

To further ensure that the impact of the identified covariates to the agreement estimates were minor, the distributions of the covariates conditioned on each assay agreement status were assessed. Chi-square tests reported for each subgroup indicated that neither the smoking status nor the region of the study participants has significant connection with the **cobas**® EGFR Plasma Test v2results provided the **cobas**® EGFR Tissue Test v1 result (smoking status p-values = 0.9825 – 0.9868 and region of the study participants p-values = 0.9795 – 09803).

D. Safety and Effectiveness Results

1. Safety Results

   The safety with respect to treatment with osimertinib was addressed in the original drug approval and was summarized as part of the original approval of the

cobas® EGFR Tissue Test v2. See Summary of Safety and Effectiveness Data for P120019/S007. No adverse events were reported in connection with the studies used to support this PMA as the studies were performed retrospectively using banked samples.

2. Effectiveness Results

The objective response rate (ORR) from the AURA2 study based on enrollment with a T790M+ tissue result using the **cobas**® EGFR Tissue Test v1 was 61.0% (128/210; 95% CI: 57.0%, 70.8%) in the tFAS population. There were 198 patients in the tFAS who also had measurable disease at baseline according to blinded independent central review (BICR) and these were considered as the **cobas**® EGFR Tissue Test v1 tissue Evaluable for Response Analysis Set (tERAS). The ORR was 64.1% (127/198; 95% CI: 54.0%, 67.6%) in the tERAS.

Only three (1.4%) of the 210 tFAS patients did not have a plasma sample. Of the 207 tFAS patients with a plasma sample, 117 were T790M+ (i.e., T790M+ by both the **cobas**® EGFR Tissue Test v1 and **cobas**® EGFR Plasma Test v2) and 89 were T790M(–) (i.e., T790M+ by the **cobas**® EGFR Tissue Test v1 and T790M(–) by the **cobas**® EGFR Plasma Test v2), and one had an invalid result by the **cobas**® EGFR Plasma Test v2.

Table 16 summarizes the number of patients with a T790M+ plasma positive result from the original AURA2 tFAS population.

Table 16. Patient Enrollment Status by cobas® EGFR Plasma Test v2 and cobas® EGFR Tissue Test v1

		Enrollment Based on cobas® EGFR Tissue Test v1 Result		
		Enrolled (tFAS)	**Not Enrolled T790M(–)**	**Total**
cobas® EGFR Plasma Test v2	**T790M+**	117	22	139
	T790M(–)	89	89	178
	Invalid	1	0	1
	No Plasma Sample	3	29	32
	Total	210	140	350

To estimate the drug efficacy for patients who were T790M+ by the **cobas**® EGFR Plasma Test v2 was calculated by assuming the ORR to be 48.8% (80% of ORR in tFAS) to 0% (no drug efficacy) among those patients who were T790M+ by the **cobas**® EGFR Plasma Test v2 and T790M(–) by the **cobas**® EGFR Tissue Test v1. The estimates are shown in Table 17, below. To compare efficacy estimates, ORR data (based on 3 months after the enrollment) for patients who were T790M(–) in tissue from a Phase I study group (cohort 3) from the AURA study was provided. In this study group, both EGFR T790M+ and T790M(–) NSCLC patients were enrolled based on the **cobas**® EGFR Tissue Test v1 and received the 80mg dose of TAGRISSO™ (osimertinib). Based on the 20.7% ORR observed in the cohort 3 patients who were T790M(–) by tissue, the estimated

ORR for those patients who were T790M+ by the **cobas**® EGFR Plasma Test v2 was 54.6%. These results indicated that the drug efficacy was robust in patients who were T790M+ by the **cobas**® EGFR Plasma Test v2.

Table 17. ORR (BICR) for T790M+ by the cobas® EGFR Plasma Test v2

Patients Not in FAS [cobas® EGFR Tissue Test v1 T790M(–) and cobas® EGFR Plasma Test v2 T790M+] Assumed Response Rate (%)	**All Patients Plasma T790M+ ORR (95 % CI)**
48.8%	59.0% (53.3%, 64.7%)
36.6%	57.1% (51.2%, 62.9%)
30.5%	56.1% (50.2%, 62.1%)
18.3%	54.2% (48.0%, 60.4%)
6.1%	52.3% (45.7%, 58.8%)
0%	51.3% (44.5%, 58.1%)
20.7% (AURA cohort 3)	54.6% (48.1%, 61.1%)

A total of 111 tERAS patients were T790M+ by the **cobas**® EGFR Plasma Test v2. The ORR for this subset was 64.9% (95% CI: 52.1%, 70.4%), which is similar to the 64.1% observed ORR in the tERAS population. The ORRs for patients who were T790M(–) by the **cobas**® EGFR Plasma Test v2 and T790M+ by the **cobas**® EGFR Tissue Test v1 were also similar to the observed ORRs in FAS and ERAS population. These data are summarized in Table 18, below.

Table 18. ORR Rate by Plasma Result Status among Enrolled Patients with Confirmed Responses

Analysis Population	**Plasma Test Result**	**N Enrolled**	**# of Patients with ORR**	**ORR (95% CI)**
tFAS	**T790M+**	117	72	61.5% (55.2%, 73.7%)
	T790M(–)	89	53	59.6% (51.3%, 73.0%)
	Overall	210	128	61.0% (57.0%, 70.8%)
tERAS	**T790M+**	111	72	64.9% (52.1%, 70.4%)
	T790M(–)	83	52	62.7% (48.6%, 69.8%)
	Overall	198	127	64.1% (54.0%, 67.6%)

Drug efficacy for T790M+ patients based on the **cobas**® EGFR Plasma Test v2 was established by bridging the efficacy results from the **cobas**® EGFR Tissue Test v1. Some of the T790M(–) patients by the **cobas**® EGFR Tissue Test v1 were T790M+ by the **cobas**® EGFR Plasma Test v2. These patients were not enrolled/dosed (by design) in the clinical trial and thus no drug efficacy data is available for these patients. To estimate the drug efficacy for all patients T790M+ by the **cobas**® EGFR Plasma Test v2, the ORR for this subset of patients was assumed to be: 1) a proportion (c = 0.8, 0.6, 0.5, 0.3, 0.1, 0) of the drug efficacy (ORR = 61%) in the tFAS population, and 2) equal to the ORR reported for the tissue T790M(–) patients with unknown plasma results from cohort 3 of the

AURA study, i.e., 20.7% with 95% CI of 8.0% to 39.7%. Furthermore, the different proportions of plasma testing between the tFAS population and the T790M(–) (by the **cobas**® EGFR Tissue Test v1) screen fail population was also adjusted in drug efficacy for T790M+ by the **cobas**® EGFR Plasma Test v2. The weighted average was used for drug efficacy estimation in this scenario.

The 210 patients in tFAS population along with the estimated 28 patients who would be T790M+ by **cobas**® EGFR Plasma Test v2 and T790M(–) by **cobas**® EGFR Tissue Test v1 represented the screening population of patients with T790M mutation by either the **cobas**® EGFR Tissue Test v1 or **cobas**® EGFR Plasma Test v2. A sensitivity analysis based on a different assumed drug efficacy on these 28 patients was performed. An assumed number of responders and response rates among the 28 patients who would be T790M+ by **cobas**® EGFR Plasma Test v2 and T790M(–) by **cobas**® EGFR Tissue Test v1 ranged from 16 (57.1%) to 0 (0.0%). The ORRs for patients who are T790M+ by either the **cobas**® EGFR Plasma Test v2 or **cobas**® EGFR Tissue Test v1 (Triage FAS) are summarized in Table 20 below.

A total of 209 patients would be enrolled as tFAS if the enrollment were based on **cobas**® EGFR Tissue Test v2. Of those, 128 were categorized as a responder (ORR = 61.2%). The assumed number of responders and response rates [17 (56.7%) to 0 (0.0%)] amongst the 30 patients with T790M mutation by the **cobas**® EGFR Plasma Test v2 and who were T790M(–) or invalid by the **cobas**® EGFR Tissue Test v2 are summarized in Table 19.

Table 19. Comparison of ORRs for T790M+ Patients Based on Different Versions of Tissue Test for Triage Algorithm (FAS Population)

T790M+ Based on	Enrolled tFAS (Responders, ORR)	Not Enrolled (projected T790M- by tissue and T790M+ by plasma)		Total Triage FAS	
		N	Assumed ORR	N	ORR
cobas® EGFR Tissue Test v2 or Plasma Test v2	209 (128, 61.2%)	30	56.7% to 0%	239	60.7% (54.1%, 67.3%) to 53.6% (46.8%, 60.4%)
cobas® EGFR Tissue Test v1 or Plasma Test v2	210 (128, 61%)	28	57.1% to 0%	238	60.5% (53.9%, 67.1%) to 53.8% (47.1%, 60.5%)

As shown in Table 19, the ORR results for the Triage FAS patients by the **cobas**® EGFR Tissue Test v2 or the **cobas**® EGFR Plasma Test v2 (T790M+ based on the **cobas**® EGFR Tissue Test v2 or **cobas**® EGFR Plasma Test v2) are similar to those for Triage FAS patients by **cobas**® EGFR Tissue Test v1 or **cobas**® EGFR Plasma Test v2 (T790M+ based on **cobas**® EGFR Tissue Test v1 or **cobas**® EGFR Plasma Test v2). This demonstrates that in patients with a known T790M substitution mutation in a FFPET specimen, which also had a T790M positive

plasma result had clinical benefit similar to those treated with TAGRISSO™ (osimertinib) based on a tissue result.

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

E. Financial Disclosure

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

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Additional data from paired tissue and EDTA plasma results from an independent cohort were provided to support specimen handling and storage conditions based on similar protocols. See Summary of Safety and Effectiveness Data for P150047.

Very preliminary and limited data were also provided from a small cohort of patients from the AURA study and from an ongoing independent external study demonstrating the efficacy of TAGRISSO™ (osimertinib) in EGFR T790M mutation positive patients selected using the **cobas**® EGFR Plasma Test v2. In this study patients may also be enrolled based on a T790M mutation positive result in a FFPET specimen; however for those enrolled based on a plasma specimen result, the presence of the mutation in tissue may be unknown.

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.

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 T790M substitution mutation in cfDNA extracted from plasma, where the mutation was known to be present in NSCLC tissue specimens, was demonstrated in

retrospective analyses of patients enrolled in the Phase II AURA2 study as summarized in Tables 16 - 19. Analytical performance studies with the **cobas**® EGFR Plasma Test v2, when used according to the Instructions for Use, demonstrate the ability to detect the T790M substitution mutation with an analytical sensitivity of 100 cp/mL of cfDNA extracted from NSCLC patient plasma.

Very preliminary and limited data were provided demonstrating the efficacy of TAGRISSO™ (osimertinib) in EGFR T790M mutation positive patients selected using the **cobas**® EGFR Plasma Test v2 but in whom the patient's tumor tissue may be T790M mutation negative or unknown. The data provided however was not sufficient to draw conclusions regarding the effectiveness of the **cobas**® EGFR Plasma Test v2 in the plasma-positive,

The safety and effectiveness of TAGRISSO™ (osimertinib) has not been established in patients whose plasma specimens have Ex. 19del, L858R, G719X, Ex. 20ins, 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 an FFPET specimen, the risks of the **cobas**® EGFR Plasma Test v2 are largely associated with a false positive result in a patient, who may then undergo treatment with TAGRISSO™ (osimertinib) with inappropriate expectation of therapeutic benefit and experience side effects.

The patient population included in the study used to support the safety and effectiveness of the **cobas**® EGFR Plasma Test v2 for selecting patients for treatment with TAGRISSO™ (osimertinib) was originally selected using FFPET specimens. Clinical data for the EGFR T790M plasma-positive, tissue-negative (or unknown) population is limited, and therefore additional clinical data is needed to establish the clinical performance of the test in this population (see Section XIV. below). Given the lack of data, testing with the **cobas**® EGFR Plasma Test is considered most appropriate for patients from whom a tumor biopsy cannot be obtained

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in the AURA2 study, which were used to support PMA approval as described in Tables 16 – 19 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 global Phase II, open-label, single-arm study, in patients with locally advanced or metastatic NSCLC (Stage IIIB-IV) and a positive T790M result in tumor specimens, who had progressed following prior therapy with an approved EGFR TKI agent. TAGRISSO™ (osimertinib) demonstrated an ORR of 61.5% (72/117; 95% CI: 55.2%, 73.7%) in all patients with positive T790M results in tumor specimens and 64.9%% (72/111; 95% CI: 52.1%, 70.4%) in a subset of these patients whose plasma specimens also demonstrated a T790M positive result. This demonstrated that in patients with a known T790M substitution mutation in a FFPET specimen in which also had a T790M positive plasma result had clinical benefit similar to those treated with TAGRISSO™ (osimertinib) based on a tissue result.

The risks associated with use of the **cobas**® EGFR Plasma Test v2 are mitigated in the description of the performance observed in the Instructions for Use which also supports the accompanying update to the TAGRISSO™ (osimertinib) prescribing information which was reviewed by CDER. In particular, the efficacy of TAGRISSO™ (osimertinib) has not been established in the EGFR T790M plasma-positive, tissue-negative or unknown population and for which clinical data for T790M plasma-positive patients are limited. Prospective clinical data for patients with EGFR positive metastatic NSCLC who are T790M plasma-positive (but tissue-negative or unknown) to further characterize outcomes with TAGRISSO™ (osimertinib) therapy from real world patients who have been selected for treatment on the basis of an EGFR T790M mutation positive result from plasma (cfDNA) using the **cobas**® EGFR Mutation Test v2 is planned as a condition of approval.

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 NSCLC in decisions regarding treatment with TAGRISSO™ (osimertinib). There is currently no FDA approved test for the selection of candidate metastatic NSCLC patients for treatment with TAGRISSO™ (osimertinib) based on identification of a T790M substitution mutation from patient's plasma specimens.

In conclusion, given the available information above, the data supports the use of the **cobas**® EGFR Plasma Test v2 as an aid in selecting NSCLC patients for TAGRISSO™ (osimertinib) treatment based on a **cobas**® EGFR Plasma Test v2 "Mutation Detected" result for EGFR T790M mutations when detected in plasma, that the probable benefits outweigh the probable risks. However because the outcome of patients with a plasma T790M+ result, but who are T790M(–) or unknown by tissue, who are treated with TAGRISSO™ (osimertinib) is uncertain, testing using the **cobas**® EGFR Plasma Test v2 is most appropriate for patients from whom a tumor biopsy cannot be obtained.

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 AURA2 Phase II clinical study support the utility of the **cobas**® EGFR Plasma Test v2 as an aid in selecting patients with advanced NSCLC and T790M mutation for whom TAGRISSO™ (osimertinib), an EGFR TKI, is indicated. Osimertinib demonstrated clinical benefit in terms of ORR that appears to be robust and of a magnitude to reasonably predict clinical benefit for osimertinib in patients with T790M mutation identified with the **cobas**® EGFR Plasma Test v2.

XIV. CDRH DECISION

CDRH issued an approval order on September 28, 2016. The final conditions of approval cited in the approval order are described below.

1. The data provided in the PMA application demonstrates that the **cobas**® EGFR Mutation Test v2 using plasma can identify patients who will benefit from osimertinib therapy; however the trial was conducted in patients previously demonstrated to have the EGFR T790M mutation in tumor samples. Limited data was provided for patients who are EGFR T790M mutation positive in plasma (cfDNA) but whose FFPET specimens were EGFR T790M mutation negative or unknown. Therefore, as part of the agreed upon post-market commitment (PMC #3119-1) to NDA 208065, additional data regarding overall response rate is needed for from NSCLC patients treated with osimertinib from "real-world" cohorts of patients who have been selected for treatment on the basis of an EGFR T790M mutation positive result from plasma using the **cobas**® EGFR Mutation Test v2. Please also provide duration of response data and, if available, the tissue EGFR T790M status on these patients.

2. Limited specimen handling and specimen storage stability information was included in the PMA application. Additional testing and data are required to establish robustness in regard to the pre-analytical specimen handling instructions to users to ensure consistent performance of the **cobas**® EGFR Plasma Test v2 with clinical plasma specimens.

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 TAGRISSO™ (osimertinib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.