SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: cobas® EZH2 Mutation Test

Device Procode: OWD

Applicant's Name and Address: Roche Molecular Systems, Inc.
4300 Hacienda Drive, Pleasanton, CA 94588

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P200014

Date of FDA Notice of Approval: June 18, 2020

II. INDICATIONS FOR USE

The cobas® EZH2 Mutation Test is a real-time allele-specific PCR test for qualitative detection of single nucleotide mutations for Y646N, Y646F or Y646X (Y646H, Y646S, or Y646C), A682G, and A692V of the EZH2 gene in DNA extracted from formalin fixed paraffin embedded (FFPE) human follicular lymphoma tumor tissue specimens. The cobas® EZH2 Mutation Test is intended for the identification of follicular lymphoma patients with an EZH2 mutation for treatment with TAZVERIK™ tazemetostat, in accordance with the approved therapeutic product labeling.

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

III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the cobas® EZH2 Mutation Test product labeling.

V. DEVICE DESCRIPTION

The cobas® EZH2 Mutation Test is designed to detect specific somatic mutations in the enhancer of zeste homolog 2 protein (EZH2) gene and consists of two reagent kits and a system platform:

- The cobas® DNA Sample Preparation kit provides reagents for manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tissue (FFPE).
- The cobas® EZH2 Mutation Test kit provides reagents for automated real-time PCR amplification and detection of the EZH2 mutations. Each kit contains sufficient reagents for 24 tests.

Mutation detection is achieved through PCR analysis with the cobas z 480 analyzer. A mutant control and negative control are included in each run to confirm the validity of the run.

The mutations detected by the cobas® EZH2 Mutation Test are listed in Table 1.

Table 1: List of Intended Target Mutations for the EZH2 Test

Exon	EZH2 Mutation	Nucleotide Nomenclature	Protein Nomenclature	COSMIC ID
16	Y646N	c.1936T>A	p.Tyr646Asn	COSM37031
16	Y646H	c.1936T>C	p.Tyr646His	COSM37030
16	Y646F	c.1937A>T	p.Tyr646Phe	COSM37028
16	Y646S	c.1937A>C	p.Tyr646Ser	COSM37029
16	Y646C	c.1937A>G	p.Tyr646Cys	COSM37032
18	A682G	c.2045C>G	p.Ala682Gly	COSM220386
18	A692V	c.2075C>T	p.Ala692Val	COSM220529

A. Manual Specimen Preparation

The cobas® DNA Sample Preparation Kit is used to manually process FFPE specimens and isolate genomic DNA based on nucleic acid binding to glass fibers. A deparaffinized 5-µm section of an FFPE specimen is lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The bound nucleic acids are washed and then eluted with an aqueous solution. The amount of genomic DNA is spectrophotometrically determined and

adjusted to a fixed concentration of 2 ng/µL with 25 µL used in the amplification and detection mixture.

B. PCR Amplification and Detection

The cobas® EZH2 Mutation Test uses allele-specific PCR (AS-PCR) chemistry for amplification and detection. The AS-PCR primers specifically amplify the targeted mutant sequences over the wild-type sequences and/or other human genomic DNA. The cobas® EZH2 Mutation Test is designed to use three master mix (MMx) reagents which are run in 3 separate wells. The number and types of primers and probes are designed for the particular target(s). Table 2 describes the targets and fluorescent dyes for each master mix.

Table 2: Distribution of Mutations Targeted by the EZH2 Test per Master Mix

Master Mix	Target Group	Dye Name	Exon of EZH2 Gene
1	Y646N	FAM	16
	A692V	HEX	18
	IC	CY5.5	11
2	Y646F	FAM	16
	A682G	JA270	18
	IC	CY5.5	11
3	Y646X (X=H, S, C)	FAM	16
	IC	CY5.5	11

Note: IC = Internal Control

The PCR reaction mixture is heated to denature the genomic DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The DNA polymerase extends each annealed primer 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 EZH2 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® EZH2 Mutation Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP).

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, the 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 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. Three different reporter dyes are used to label the mutations targeted by the test. Amplification of the targeted EZH2 sequences

are detected independently across three reactions by measuring fluorescence at the three characteristic wavelengths in dedicated optical channels.

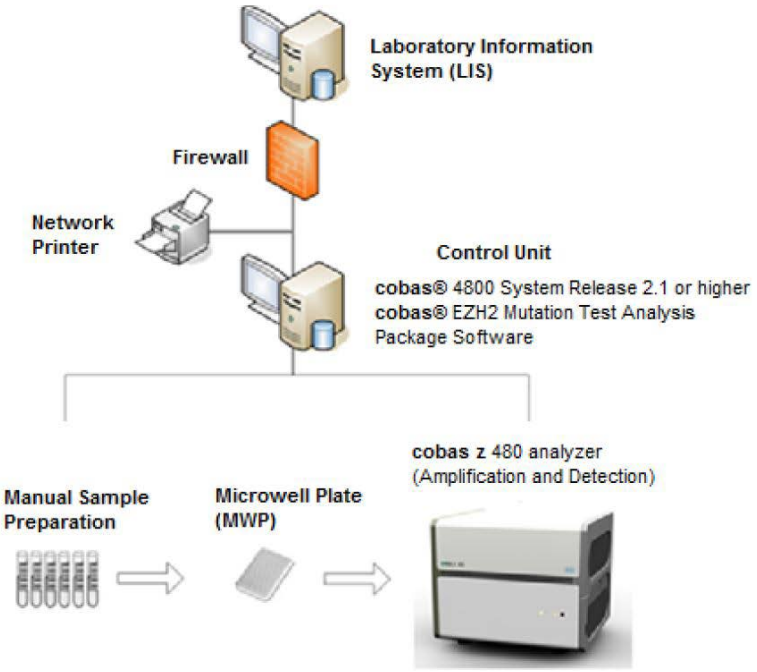
C. Instrument and Software

The cobas® 4800 system is controlled by the cobas® 4800 system release (SR) 2.1 software which provides the core software engines and user interfaces. This core system software was designed to allow multiple assays to be performed on the system using analyte specific analysis package software (ASAP). The cobas® 4800 system software includes a validated cobas® 4800 EZH2 Analysis Package (AP) software. 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 computer also stores the test results in a controlled database. The software provides the results to the user in three formats; a printable PDF results report, a GUI based result viewer and a result export to the LIS. A dedicated Control Unit computer runs the cobas® 4800 SR2.1 software and provides an interface to the cobas z 480 and Laboratory Information System (LIS). The complete system allows a user to create a test work order for each specimen manually.

The main cobas® 4800 system hardware and software components are described below and illustrated in Figure 1.

- cobas z 480 analyzer with real time detection capabilities
- cobas® 4800 96-well microwell plate with transparent sealing film
- cobas® 4800 System Release 2.1 software
- cobas® 4800 control unit
- cobas® EZH2 Mutation Test analysis package software

Figure 1: cobas® 4800 System Hardware Components



D. Interpretation of Results

If the run is valid, then the cycle threshold (Ct) and 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 mutation's observed Ct and the corresponding Internal Control (IC) Ct value from the same Master Mix. Ct values are not available to the user. The thresholds used to determine the yield a mutation detected result are shown in Table 3 below:

Table 3: Thresholds for mutation detected vs mutation not detected

Master Mix and Channel	Mutation or Internal Control	CtR Min	CtR Max	Ct Min	Ct Max
MMX-1 FAM	Y646N	-5	9.0	15	45.0
MMX-1 HEX	A692V	-5	6.5	15	42.5
MMX-1 Cy5.5	Internal Control	N/A	N/A	20	36.0
MMX-2 FAM	Y646F	-5	8.0	15	44.0
MMX-2 JA270	A682G	-5	6.0	15	42.0
MMX-2 Cy5.5	Internal Control	N/A	N/A	20	36.0
MMX-3 FAM	Y646X	-5	9.0	15	45.0
MMX-3 Cy5.5	Internal Control	N/A	N/A	20	36.0

The result output for the assay is Mutation Detected (MD) or No Mutation Detected Table 4 summarizes the various results.

Table 4: Result Interpretation for the cobas® EZH2 Mutation Test

Test Result	Mutation Result	Interpretation
Mutation Detected (MD)	Y646N Y646F Y646X (X=H, S, C) A682G A692V (More than one mutation may be present)	Mutation detected in specified targeted EZH2 region.
No Mutation Detected (NMD)	N/A	Mutation not detected in targeted EZH2 regions.
Invalid	N/A	Specimen result is invalid. Repeat the testing of specimens with invalid results following the instructions outlined in the "Retesting of Specimens with Invalid Results" section.
Failed	N/A	Failed run due to user abort, hardware, or software failure. Contact your local Roche office for technical assistance.

E. Test Controls

One EZH2 mutant control and one EZH2 negative control are included in each run. The EZH2 wild-type allele on exon 11 serves as an internal full process control:

1. *EZH2 Mutant Control*

The mutant Control is a blend of 5 DNA plasmids containing specified EZH2 mutation sequences and cell line DNA that is wild-type for EZH2. 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 the internal control (IC), exon 16 mutation and exon 18 mutation within the acceptable ranges for the run to be considered valid.

2. *EZH2 Negative Control*

The Negative Control is a full process contamination control for a given test batch of specimens. NO reagent is provided for the Negative Control, instead a blank vial containing no specimen is processed through specimen preparation and the resulting eluate is subsequently diluted and amplified and detected. The Negative Control Ct values must be either not detected or greater than the pre-established Ct maximum value for the exon 16 and 18 mutation groups and the Internal Control for the run to be considered valid.

3. *Internal Control*

The Internal Control in exon 11 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 FDA-cleared or approved alternatives for testing formalin-fixed, paraffin-embedded non-Hodgkin's Lymphoma (NHL) tissue for EZH2 mutation status in the selection of patients who are eligible for treatment with tazemetostat.

VII. MARKETING HISTORY

The cobas® EZH2 Mutation Test has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As with any in vitro diagnostic test, potential risks are associated with an incorrect test result or result interpretation and not with the device directly. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect cobas® EZH2 Mutation Test results and subsequently improper patient management decisions in patient treatment.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

For the non-clinical studies described below, percentage of tumor was assessed by pathology review. Next-generation sequencing (NGS) was used to select the specimens for testing. Follicular lymphoma is a type of Non-Hodgkin lymphoma (NHL). Due to the rarity of specimens, other types of NHL specimens were used to supplement the study. Percentage of mutation in the NHL FFPET specimens was determined using an NGS method. The cobas® DNA Sample Preparation Kit was used to obtain extracted DNA for the analytical validations studies.

1. Correlation with Reference Method

The analytical accuracy of the cobas EZH2 Mutation Test was assessed by evaluating the positive and negative percent agreement between the **cobas**® EZH2 Mutation Test and a validated next generation sequencing (NGS) method for the detection of mutations within codons 646, 682, and 692 (Y646F, Y646N, Y646S, Y646H, Y646C, A682G, A692V) of the EZH2 gene, using specimens from patients. Two studies were performed: one study with specimens from patients enrolled into multiple clinical trials as well as the follicular lymphoma patients and second with procured clinical specimens.

a) Study 1: Clinical Trial Specimens

Approximately 900 patients for all cohorts in the E7438-G000-101 study underwent testing of their tumor DNA with the cobas EZH2 Test to determine mutation status of the EZH2 gene. Tumor tissue from biopsies collected from patients (FFPE) were sent to designated clinical trial laboratories for DNA extraction and prospective testing with the

cobas EZH2 Test. Residual extracted DNA from patient screening for the clinical trial sample and/or tissue samples were banked for future retrospective testing with the validated comparator method.

A subset of 378 samples screened for the therapeutic trial was selected for sequencing. The subset included:

- all specimens from enrolled patients regardless of EZH2 mutation status (i.e., both mutation detected and no mutation detected)
- all specimens from screening patients with invalid results
- all specimens from patients with mutation detected but were not enrolled for other reasons

Results

There were 378 samples selected for NGS sequencing, and 341 were sequenced. The 37 samples not sequenced were due to insufficient sample remaining for sequencing. Samples with valid results from both the cobas® EZH2 Mutation Test and the NGS method were evaluable for calculating agreement for mutation detected (MD) or no mutation detected (NMD). The PPA and NPA, as well as the corresponding 95% CIs, are shown in Table 5, with a PPA of 98.3% (95% CI: 94.2%, 99.5%) and an NPA of 98.6% (95% CI: 95.9%, 99.5%). The study acceptance criteria were met.

Table 5: Agreement Between the cobas® EZH2 Mutation Test and Comparator NGS Method for Evaluable Specimens

		NGS Method					Total
		Sequenced			Not Sequenced		
		MD	NMD	Invalid	Selected for NGS Testing	Not Selected for NGS Testing	
cobas EZH2 Test	MD	119	3	0	5	1*	128
	NMD	2	208	3	31	517	761
	Invalid	0	3	3	1	0	7
	Total	121	214	6	37	518	896
Agreement Statistics	PPA (95% CI)	98.3% (94.2%, 99.5%)					
	NPA (95% CI)	98.6% (95.9%, 99.5%)					

MD = mutation detected; NMD = no mutation detected; NGS = next generation sequencing; NPA = negative percent agreement; PPA = positive percent agreement
Note: All CI (confidence interval) are calculated by Wilson score method.
* Not selected due to patient's withdrawal of the consent for further testing.

There were 3 samples with NMD by cobas EZH2 Test which were invalid by NGS and 3 samples invalid by cobas test determined to be wild-type by NGS. The invalid by results

were assumed to be discordant and included in a sensitivity analysis. Invalid results by both tests were not included. The PPA and NPA in this worst case scenario were 96.0% (119/124) and 97.2% (208/214), respectively, as shown in Table 6. The PPA and NPA in the worst case scenario also met the acceptance criteria of ≥95%.

Table 6: Agreement between the cobas® EZH2 Mutation Test with Comparator NGS Method Including Invalids as Discordant Results)

		NGS (Reference Method)					Total
		Sequenced			Not Sequenced		
		MD	NMD	Invalid	Selected for NGS Testing	Not Selected for NGS Testing	
cobas EZH2 Test	MD	119	3	0	5	1*	128
	NMD	2	208	3	31	517	761
	Invalid	0	3	3**	1	0	7
	Total	121	214	6	37	518	896
Agreement Statistics with Invalid as Discordant	PPA (95% CI)	96.0% [=119/(121+3), 95% CI: 90.9%, 98.3%]					
	NPA (95% CI)	97.2% [=208/214, 95% CI: 94.0%, 98.7%]					

MD = mutation detected; NMD = no mutation detected; NGS = next generation sequencing; NPA = negative percent agreement; PPA = positive percent agreement
Note: All CI (confidence interval) are calculated by Wilson score method.
* Not selected due to patients withdraw the consent for further testing.
** Invalid by both tests were not included in worst case scenario analysis.

The accuracy for individual mutations was also evaluated. Results are shown in Table 7 for single mutation. For 7 samples, two mutations were detected in each sample. All samples but 3 were confirmed by the comparator; one cobas Y646N and 2 Y646X yielded both mutants in each of the 3 samples by the NGS method (data not shown).

Table 7: Per Allele Comparison of the cobas® EZH2 Mutation Test vs. NGS

cobas® EZH2	NGS Sequencing						
cobas EZH2 Mutation Test	A682G	A692V	Y646F	Y646N	Y646X	WT	Total
A682G	5	0	0	0	0	1	6
A692V	0	2	0	0	0	1	3
Y646F	0	0	38	0	0	0	38
Y646N	0	0	0	36	0	0	36

Y646X	0	0	0	0	31	1	32
WT	0	0	1	1	0	208	210
Total	5	2	39	37	31	211	325

Note: co-mutation detected by either test is not included in the table.

The adjusted agreement analysis was performed to account for the different proportion of MD and NMD samples (as determined by cobas® EZH2 Mutation Test) selected for sequencing. The projected numbers of MD and NMD by NGS in cobas® EZH2 Mutation Test subgroups MD and NMD (adjusted for selection bias for NGS, as well as taking invalid/failed cobas/NGS results into consideration) with adjusted PPA of 94.7% (95% CI: 86.8%, 100.0%) and NPA of 99.6% (95% CI: 99.0%, 100.0%).

Accuracy for the follicular lymphoma specimens independently was also assessed. The results are consistent with the larger panel demonstrating a PPA of 97.7% and NPA of 96.1%. The results are summarized in Table 8 below.

Table 8: Comparison of the cobas EZH2 Mutation Test with NGS in FL Patients

		NGS (Reference Method)				
		MD	**NMD**	**Invalid**	**Not-Sequenced**	**Total**
cobas EZH2 Mutation Test	**MD**	43	2	0	0	45
	NMD	1	49	0	3	53
	Invalid	0	0	0	1	1
	Total	44	51	0	4	99
Agreement Statistics	**PPA(95% CI)**	97.7% (88.2%, 99.6%)				
	NPA(95% CI)	96.1% (86.8%, 98.9%)				
Note: 1 NMD results was classified as invalid in this table due to unapproved sample type was used in cobas testing						

b) Study 2: Procured FFPE specimens

A total of 104 unique nonhodgkin lymphoma FFPE specimens were tested for the presence of Y646N, Y646F, Y646H, Y646S, Y646C, A682G and A692V mutations with two lots of the cobas® EZH2 Mutation Test Kits and compared with results with a validated NGS method to determine the PPA, NPA and overall percent agreement (OPA) between methods.

Results

There were a total of 3 discordant results between the cobas® EZH2 Mutation Test and NGS. The overall concordance of the cobas® EZH2 Mutation Test vs. MiSeq was 98.1% for lot 1 (102/104) and 99.0% (103/104) for lot 2. The comparisons of individual mutations vs MiSeq are shown for each lot in Table 9.

The comparisons of per variant for each lot are shown in Table 10 and Table 11. The 520 total results represent 5 different mutation results or a WT result for each of the 104 samples used in the study.

Table 9: Summary of Percent Agreement Between cobas® EZH2 Mutation Test and NGS Per Specimen

	Lot 1 vs. NGS	Lot 2 vs. NGS
Positive Percent Agreement	100% (50/50) 95% CI: 92.9%-100%	100% (50/50) 95% CI: 92.9%-100%
Negative Percent Agreement	96.3% (52/54) 95% CI: 87.5%-99.0%	98.1% (53/54) 95% CI: 90.2%-99.7%
Overall Percent Agreement	98.1% (102/104) 95% CI: 93.3%-99.5%	99.0% (103/104) 95% CI: 94.8%-99.8%

Table 10: Per Allele Comparison of the cobas® EZH2 Mutation Test Lot 1 vs. NGS

		NGS						
		Y646N	**A692V**	**Y646F**	**A682G**	**Y646X**	**WT**	**Total**
cobas® EZH2 Mutation Test	**Y646N**	13	0	0	0	0	1	14
	A692V	0	4	0	0	0	0	4
	Y646F	0	0	14	0	0	1	15
	A682G	0	0	0	5	0	0	5
	Y646X	0	0	0	0	19	2	21
	WT	0	0	0	0	0	461	461
	Total	13	4	14	5	19	465	520*

*Note: The 520 total results represent 5 different mutation results or a WT result for each of the 104 samples used in the study.

Table 11: Per Allele comparison of the cobas® EZH2 Mutation Test Lot 2 vs. NGS

		NGS						
		Y646N	**A692V**	**Y646F**	**A682G**	**Y646X**	**WT**	**Total**
cobas® EZH2 Mutation Test	**Y646N**	13	0	0	0	0	1	14
	A692V	0	4	0	0	0	0	4
	Y646F	0	0	14	0	0	1	15
	A682G	0	0	0	5	0	0	5
	Y646X	0	0	0	0	19	0	19
	WT	0	0	0	0	0	463	463
	Total	13	4	14	5	19	465	520*

*Note: The 520 total results represent 5 different mutation results or a WT result for each of the 104 samples used in the study.

2. Analytical Sensitivity

a) Limit of Blank (LoB) using FFPE Specimen Blends

To assess performance of the cobas EZH2 Test in the absence of template and to ensure that a wild-type sample does not generate an analytical signal that might indicate a low concentration of mutation, 195 replicates of NHL FFPE EZH2 wild-type specimens were evaluated using the analysis prescribed in the CLSI EP17-A2 guideline. The assay does not exceed 55 cycles. The LoB was determined to be zero for replicates when the observed CtR result exceeded the mutant CtR threshold. The results displayed an overall false positive rate of 2.6% (5/195). A root cause analysis indicated that the discordant specimens were determined to have low levels of mutant not detected by the sequencing. Due to the uncertainty in the mutant status for clinical specimens, a postmarket study evaluating wild-type cell lines is a condition of approval to provide a more accurate approximation of the LoB. The mean thresholds observed from the LoB studies using clinical specimens for each mutation are listed in Table 12.

Table 12: Observed mean CtRs for Mutants with WT DNA

Targeted Mutation	N	Mean CtR Observed with WT-DNA	MD CtR Cutoff (≤)
Y646N	195	20.9	9.0
A692V	195	10.5	6.5
Y646F	195	21.9	8.0
A682G	195	13.8	6.0
Y646X	195	10.7	9.0

b) Limit of Detection (LoD) using FFPE Specimen Blends

A study was performed to determine the limit of detection (LoD) for the cobas® EZH2 Mutation Test. DNA isolated from 20 NHL FFPE specimens with EZH2 mutations (Y646N, A692V, Y646F, A682G, Y646H, Y646S and Y646C) were blended with DNA isolated from wild-type NHL FFPE samples to achieve 21 unique DNA blends (panel members) targeting 10.0%, 7.5%, 5.0%, 2.5%, and 1.0% mutation levels as determined by an NGS method. Twenty-one (21) replicates of each targeted mutation level using 50ng DNA were tested. Three (3) reagent lots were used for the study. The limit of detection for each sample was determined as the lowest percent mutation that gave an EZH2 "Mutation Detected" rate of at least 95% for the targeted mutation. The data is shown in Table 13. The study demonstrates that the cobas® EZH2 Mutation Test can detect mutations in EZH2 exons 16 and 18 with at least 5% mutation level using the standard input of 50 ng DNA per reaction well. The claimed limit of detection for the assay is 5% based on the highest LoD achieved (Y646H).

Table 13: Limit of Detection for the cobas® EZH2 Mutation Test using FFPE Sample DNA Blends

Exon	AA Change	Nucleic Acid Change	Percent Mutation in the Panel Member to achieve ≥95% "Mutation Detected" Rate with 50 ng DNA input per reaction well (N=21 Valid replicates)
16	Y646N	TAC>AAC	2.1
			2.6
			2.5
18	A692V	GCA>GTA	2.0
			1.6
			2.7
16	Y646F	TAC>TTC	1.6
			2.2
			1.3
18	A682G	GCA>GGA	1.1
			1.8
			2.0
16	Y646H	TAC>CAC	0.9
			1.9
			4.7
16	Y646S	TAC>TCC	2.3
			3.9
			2.5
16	Y646C	TAC>TGC	0.7

Exon	AA Change	Nucleic Acid Change	Percent Mutation in the Panel Member to achieve ≥95% "Mutation Detected" Rate with 50 ng DNA input per reaction well (N=21 Valid replicates)
			2.4
			2.9

c) Limit of Detection Using Cell Line Blends

A study was performed to determine the Limit of Detection (LoD) for the cobas® EZH2 Mutation Test using lymphoma cell lines. Genomic DNA extracted from lymphoma cell lines for EZH2 mutations (Y646N, Y646F, Y646S, Y646C, and A682G) were blended with wild-type cell line DNA to achieve DNA blends (panel members). The blends were tested at Neat, 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 with an additional dilution of 1:128 for Y646C from a homozygous cell line. A total of 21 replicates of each targeted mutation level were tested using one lot of cobas® EZH2 Mutation Test kit. The final percent mutation was determined by NGS. The cobas® EZH2 Mutation Test was able to achieve a ≥ 95% detection rate in the EZH2 gene with a percent mutation level ranging from 1.1% to 2.0% with a 50 ng/PCR DNA input as shown in Table 14. The results support the claimed LoD of 5% for the assay.

Table 14: Limit of Detection for the cobas® EZH2 Mutation Test using cell line DNA

Exon	Mutation	Nucleic Acid Change	Percent Mutation in the Panel Member to achieve ≥95% "Mutation Detected" Rate with 50 ng DNA input per reaction well (N=21 Valid replicates)
16	Y646N	TAC>AAC	1.9
16	Y646F	TAC>TTC	1.1
18	A682G	GCA>GGA	2.0
16	Y646S	TAC>TCC	1.2
16	Y646C	TAC>TGC	1.7

3. Analytical Specificity

a) Cross Reactivity

Sequence homology searches were performed comparing the cobas EZH2 Test primer and probe sequences with known sequence databases using BLAST(Traditional BLAST, Thermo BLAST, and PCR BLAST) against human genomic DNA to assess for potential cross- reactivity of non-targeted DNA sequence that may be present in human genomic DNA. Based on sequence similarities, potential cross-reactivity with an EZH2 pseudogene and an EZH2 paralog (EZH1) was evaluated by spiking EZH2 pseudogene and EZH1

plasmid DNA into FFPE genomic DNA 15 specimens consisting of 5 EZH2 mutant specimens and 10 wild-type specimens, at a ratio of approximately 50% plasmid DNA to 50% amplifiable specimen DNA. This study was conducted by one operator using two cobas z 480 analyzers and one reagent lot of the cobas® EZH2 mutation Test.

All samples demonstrated the expected results except that one wild-type samples spiked with EZH1 gave an unexpected call for Y646X with a CtR value close to the threshold. The cobas EZH2 Test exhibited no cross-reactivity when tested with the DNA sequences of non-targeted mutations (EZH2 Pseudogene and EZH2 Paralog).

b) Cross Contamination

To determine if cross contamination may occur during sample processing and execution, 12 mutant NHL FFPE specimens and 12 EZH2 wild type NHL FFPE specimens were processed in a checkerboard pattern in each of 5 runs. This study was performed by 3 operators using 4 cobas z 480 analyzers and 1 reagent lot.

Out of the 60 wild type specimen results, there were no instances of cross contamination. Out of the 60 EZH2 mutant specimen results, there were 3 instances (3/120) where an unexpected additional mutation result was also observed. A root cause analysis was performed and determined that the unexpected results were not due to instrument cross contamination.

c) Interference – Endogenous Interferents

Two wild type specimens and 15 EZH2 mutant specimens (3 from each mutation group, namely Y646N, A692V, Y646F, A682G and Y646X) were evaluated. Hemoglobin (2 mg/mL, CLSI recommended high concentration) and triglycerides (37 mM, CLSI recommended high concentration) were spiked into the lysis buffer during the specimen preparation procedure and compared to unspiked paired sample. The results demonstrated that there is no interference with the cobas® EZH2 Mutation Test when these potential interfering substances were added to the lysis step during the specimen preparation procedure.

d) Interference – Exogenous Interferents

Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone were tested at concentrations representing 3 times the peak serum concentration (3X Cmax) that a drug achieves after its administration or at a concentration equivalent to 3X therapeutic doses distributed in 5 liters of blood. The tested drugs were shown not to interfere with the cobas® EZH2 Mutation Test when each potential interfering substance was added to the lysis step during the specimen preparation procedure.

e) Interference -Necrotic Tissue

The impact of necrotic tissue on the performance of the cobas EZH2 assay was evaluated using specimens from the accuracy assessment with procured specimens. Necrosis content data was available for 98 out of 104 specimens tested ranging from 0% to 95%. Two FFPET slides were sent to pathologist to determine percent tumor and percent necrosis. In total 67%

of the specimens had ≥50% necrotic tissue. There was 1 discordant specimen (both lots were MD and NGS NMD) at 65% tumor necrosis content results in the 98 samples. The results demonstrated that NHL FFPE specimens with necrotic tissue content from 0% to 95% do not interfere with the call results of the cobas EZH2 Test.

4. Reproducibility Study

a) Precision (Repeatability)

Precision (Repeatability) of the cobas® EZH2 Mutation Test was assessed using five NHL FFPE EZH2 mutant specimens (Y646N, Y646F, Y646X, A682G, and A692V) with a percent mutation ranging from 12% to 26% and 2 EZH2 FFPE wild type specimens. The specimens were tested in duplicate by 2 operators, using 2 different reagent lots and 2 cobas z 480 analyzers over 8 days. A total of 32 replicates of each specimen were evaluated. The cobas® EZH2 Mutation Test had an overall agreement of 100%, as shown in Table 15.

Table 15: Percent Agreement and 95% CI by Specimen and Reagent Lot

Sample ID	Expected Result	Correct Calls Lot 1	% Accuracy Lot 1	Correct Calls Lot 2	% Accuracy Lot 2	Overall 95% two- sided CI for both lots combined
S01	Y646N	16	100%	16	100%	89 - 100%
S02	A692V	16	100%	16	100%	89 - 100%
S03	Y646F	16	100%	16	100%	89 - 100%
S04	A682G	16	100%	16	100%	89 - 100%
S05	Y646H	16	100%	16	100%	89 - 100%
S06	Wild-type	16	100%	16	100%	89 - 100%
S07	Wild-type	16	100%	16	100%	89 - 100%

b) Reproducibility with Clinical Specimens

A reproducibility study was performed to assess the reproducibility of the cobas® EZH2 Mutation Test at 3 testing sites with 2 operators per site, 3 reagent lots, and 5 non-consecutive testing days (per operator), with a 15-member panel of DNA samples extracted from FFPE sections of wild-type (WT) and mutant specimens (Table 16). The panel included mutation-positive specimens representing Y646N, Y646F, Y646H, Y646S and Y646C in exon 16, and A682G and A692G in exon 18. Each mutation-positive specimen was represented at 2 levels of % mutation: near the level of detection (approximately 5% 1X LoD) and 3x LoD. Each sample at each concentration was run in duplicate. Of 91 runs performed, 90 (98.9%) were valid. A total of 2,700 tests were performed from valid runs with 2,690 (99.6%) valid and 10 (0.4%) invalid results. Percent agreement was 100% for all panel members, exceeding the acceptance criteria of ≥ 95% agreement in samples with mutation level ≥ LoD.

Table 16: Overall Estimates of Agreement by Panel Member

Panel Member	Number of Valid Tests	Agreement Number	Agreement % (95% CI)[a]
Wild -Type	180	180	100 (98.0, 100.0)
Ex16 Y646N LoD	179	179	100 (98.0, 100.0)
Ex16 Y646F LoD	179	179	100 (98.0, 100.0)
Ex16 Y646H LoD	179	179	100 (98.0, 100.0)
Ex16 Y646S LoD	179	179	100 (98.0, 100.0)
Ex16 Y646C LoD	180	180	100 (98.0, 100.0)
Ex18 A682G LoD	180	180	100 (98.0, 100.0)
Ex18 A692V LoD	180	180	100 (98.0, 100.0)
Ex16 Y646N 3xLoD	180	180	100 (98.0, 100.0)
Ex16 Y646F 3xLoD	179	179	100 (98.0, 100.0)
Ex16 Y646H 3xLoD	178	178	100 (97.9, 100.0)
Ex16 Y646S 3xLoD	180	180	100 (98.0, 100.0)
Ex16 Y646C 3xLoD	179	179	100 (98.0, 100.0)
Ex18 A682G 3xLoD	179	179	100 (98.0, 100.0)
Ex18 A692V 3xLoD	179	179	100 (98.0, 100.0)

Note: Results are included as agreement when a valid test of MT panel member has a result of 'Mutation Detected'; or when a valid test of Wild Type panel member has a result of 'No Mutation Detected'.

[a] 95% CI = 95% exact binomial CI, CI = confidence interval; LoD = limit of detection; WT = wild-type

The between-lot, between-site, between-operator, between-day/run and within-run precision of the cycle threshold ratios for specimens is shown in Table 17. The data summarizes overall mean, SD, and CV (%) for Ct values from samples with correct test results from valid runs for each panel member. Overall, CV (%) ranged from 1.5% to 1.9% across all panel members. Within each component, CV (%) ranged from 0.0% to 1.6% across all panel members.

Table 17: Overall Mean, Standard Deviation and Coefficient of Variation (%) for Cycle Threshold (Ct) Results per Panel Member

			Standard Deviation (SD) and Percent Coefficients of Variation											
			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	N	Mean Ct	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Ex16 Y646N LoD	179	33.46	0.17	0.5%	0.13	0.4%	0.12	0.4%	0.32	1.0%	0.31	0.9%	0.51	1.5%

Ex16 Y646F LoD	179	31.50	0.08	0.3%	0.00	0.0%	0.15	0.5%	0.15	0.5%	0.40	1.3%	0.46	1.5%
Ex16 Y646H LoD	179	33.70	0.30	0.9%	0.00	0.0%	0.19	0.6%	0.13	0.4%	0.44	1.3%	0.58	1.7%
Ex16 Y646S LoD	179	35.25	0.19	0.5%	0.00	0.0%	0.17	0.5%	0.00	0.0%	0.49	1.4%	0.55	1.6%
Ex16 Y646C LoD	180	34.08	0.28	0.8%	0.00	0.0%	0.14	0.4%	0.00	0.0%	0.51	1.5%	0.60	1.8%
Ex18 A682G LoD	180	32.38	0.21	0.6%	0.00	0.0%	0.13	0.4%	0.00	0.0%	0.48	1.5%	0.54	1.7%
Ex18 A692V LoD	180	31.18	0.07	0.2%	0.00	0.0%	0.19	0.6%	0.30	1.0%	0.37	1.2%	0.52	1.7%
Ex16 Y646N 3xLoD	180	30.93	0.20	0.7%	0.00	0.0%	0.14	0.5%	0.27	0.9%	0.42	1.3%	0.55	1.8%
Ex16 Y646F 3xLoD	179	29.68	0.13	0.5%	0.09	0.3%	0.14	0.5%	0.00	0.0%	0.39	1.3%	0.44	1.5%
Ex16 Y646H 3xLoD	178	32.51	0.27	0.8%	0.00	0.0%	0.13	0.4%	0.10	0.3%	0.41	1.3%	0.51	1.6%
Ex16 Y646S 3xLoD	180	33.51	0.17	0.5%	0.00	0.0%	0.11	0.3%	0.20	0.6%	0.45	1.3%	0.53	1.6%
Ex16 Y646C 3xLoD	179	32.67	0.24	0.7%	0.00	0.0%	0.11	0.3%	0.00	0.0%	0.52	1.6%	0.58	1.8%
Ex18 A682G 3xLoD	179	30.67	0.25	0.8%	0.00	0.0%	0.13	0.4%	0.01	0.0%	0.36	1.2%	0.46	1.5%
Ex18 A692V 3xLoD	179	29.07	0.10	0.3%	0.00	0.0%	0.21	0.7%	0.31	1.0%	0.41	1.4%	0.56	1.9%

c) Lot-to-Lot Reproducibility

A study was performed to demonstrate the interchangeability of various lots of cobas® DNA Sample Preparation kit with various lots of cobas® EZH2 Mutation Test. Five (5) mutant specimens (Y646N, A692V, Y646F, A682G and Y646S, representing the Y646X group), and 4 wild type specimens were used for this study. Nine (9) lot combinations were created by crossing 3 lots of the cobas® DNA Sample Preparation Kit with 3 lots of the cobas® EZH2 Mutation Test. The testing was conducted using 3 operators, and 5 cobas z 480 analyzers. One (1) wild type sample generated a mutation call for the Y646X mutation, resulting in the negative percent agreement not meeting the study acceptance criteria of having a point estimate of 100% (point estimate for the data is 97.2%). All other sample results and positive percent agreement met the acceptance criteria. The positive, negative and overall percent agreements over all lot combinations are shown in Table 18. A root cause analysis was performed and determined that the unexpected result was sample related and not due to the performance of the reagents.

Table 18: Lot Interchangeability Percent Agreement

	MiSeq MD	MiSeq NMD	Total
cobas EZH2 MD	45	1	46
cobas EZH2 NMD	0	35	35
Total	45	36	81
% agreement	100%	97.2%	98.8
95% Confidence Interval	92.1% – 100%	85.5%– 99.9%	93.3% – 100%

5. Robustness Studies -Guard Banding

Guard Band studies were performed to establish the robustness of the PCR conditions for the cobas EZH2 Mutation Test.

a) Thermal Cycling Profile

A study was performed to determine if the cobas® EZH2 Mutation Test would generate results consistent with the control (target) condition when the thermal cycler annealing and denaturing temperature varied up to ±2°C. Two wild type and five mutant specimens from each mutation group, namely Y646N, A692V, Y646F, A682G, and Y646X, were used for the study and 3 replicates were tested for each temperature condition. Some differences in Ct and CtR values can be observed when PCR temperatures vary by as little as +/- 1°C. However, all sample results were called correctly, indicating that sample test result interpretations are generally robust to minor changes (+/- 1°C) in PCR temperatures. Invalid runs may occur occasionally when thermocycling temperatures are higher than expected.

b) Working (Activated) Master Mix Stability

A study was performed to assess the stability of Working (Activated) Master Mix. A panel of seven NHL FFPE specimens, consisting of 5 blended mutant specimens (Y646N, A692V, Y646F, A682G and Y646H) and 2 wild-type specimens, were used in the study. DNA extracted from the 5 mutant specimens was blended with wild-type sample DNA to prepare EZH2 mutant samples to levels near the assay limit of detection (~1.5 to 3X LOD). The 7 samples used in the study were tested with activated Master Mixes that were prepared by adding magnesium acetate to each EZH2 Master Mix and then incubated at 32°C for 0, 35, 65, and 125 minutes. For a time point to pass, the blended FFPE samples had to yield the expected mutation result. The observed results for each of the 5 tested mutant blends and wild type specimens were as expected for all time points. Based upon the results of this study, Working (Activated) Master Mix is stable for up to 125 minutes when stored at 32°C.

c) Prepared Specimen Plus Working (Activated) Master Mix Stability

A study was performed to assess the stability of Prepared Specimen plus Working (Activated) Master Mix. A panel of seven NHL FFPE specimens, consisting of 5 blended

mutant specimens (Y646N, A692V, Y646F, A682G and Y646H) and 2 wild-type specimens, were used in the study. DNA extracted from the five mutant specimens was blended with wild-type sample DNA to prepare EZH2 mutant samples to levels near the assay limit of detection (~1.5 to 3X LOD). The 7 samples used in the study were mixed with Working (Activated) Master Mix and incubated at 32ºC for 0, 35, 65, and 125 minutes prior to testing. For a time point to pass, the blended FFPE samples had to yield the expected mutation result. The observed results for each of the 5 tested mutant blends and wild type specimens were as expected for all time points. Based upon the results of this study, a prepared specimen and Working (Activated) Master Mix is stable for up to 125 minutes when stored at 32°C.

d) **Proteinase K**

Proteinase K studies were not completed. A postmarketing study will be conducted to assess the robustness of the assay for different proteinase K digestion times.

6. FFPE Specimen Processing and Stability

a) Macrodissection

Specimens with less than 15% tumor content should be macrodissected as part of the sample preparation. A study was performed to determine whether macrodissection of FFPE specimens will improve the detectability of EZH2 mutations using the cobas EZH2 Mutation Test. Twenty (20) FFPE NHL specimens were selected (10 mutant and 10 wild-type) based on the percent tumor content as determined from the examination of specimen slides by a licensed pathologist. The percent tumor of the tested specimens ranged from 15% - 85%. For each selected specimen, 8 slides were prepared. Four (4) of the slides were processed (2 for macrodissection and 2 without macrodissection). The study was conducted by 2 operators using 2 analyzers and 1 lot each of the cobas DNA sample preparation kit and the EZH2 mutation test. The results demonstrated that all specimens were concordant with and without macrodissection. Comparison of CtR indicating a minor improvement in detection for macrodissected specimens (<0.5 decrease in CtR value).

a) Slide-Mounted and Curl

A study was performed to assess the stability of FFPE specimens after sectioning, either mounted on a slide or unmounted as a curl. In this study, 8 NHL FFPE specimens (6 mutants and 2 wild type specimens) were selected. Six (6) specimens were EZH2 mutants (1 each representing targeted mutation group Y646N, Y646F, A682G, A692V, and 2 from the Y646HSC group) and 2 specimens were wild-type. Mounted and unmounted sections were stored at 32°C and tested at 0, 31, 63, 183, 387 and 741 days using the cobas+ EZH2 Mutation Test. For a time point to pass, the FFPE samples had to yield the expected mutation result. Based upon the results of this study, NHL FFPE specimen sections, when stored at room temperature (32°C) in test tubes (i.e., as a “curl”) and when mounted on a microscope slide, are stable for up to 741 days (24 months) when tested with the cobas® EZH2 Mutation Test.

b) Processed Specimen Stability

A study was performed to assess the stability of processed specimens. In this study, 8 NHL FFPE specimens (6 mutants and 2 wild type specimens) were selected. Six specimens were EZH2 mutants (one each representing targeted mutation group Y646N, Y646F, A682G, A692V, and two from the Y646HSC group) and two specimens were wild type. On Day 0, DNA was extracted from multiple 5-µm curls for each FFPE specimen using 1 lot of cobas® DNA Sample Preparation Kit lot. For each specimen, eluate (i.e., processed specimen) from the curls were pooled, diluted, and aliquoted in vials with sufficient processed specimen volume for testing of two replicates at each time point. Aliquots were stored at three temperatures (32°C, 2–8°C, and -20°C) and tested at the following time points:

- After storage at -20°C for 15, 31, 61, 123, 394, or 742 days
- After storage at 2–8°C for 0, 15, 31, 61, 123, or 394 days
- After storage at 32°C for 0, 1, 4, or 9 days
- The eluates stored at -20°C were also used to test up to 6 freeze-thaw cycles.

For a time point to pass, the FFPE samples had to yield the expected mutation result. The observed mutation results for all samples at all storage temperatures were as expected for all time points tested. Based on the results of this study, processed specimens are stable for at least 12 months at 2–8°C, and at least two years when stored at -20°C with up to 6 freeze-thaw cycles. When stored at room temperature (32°C), processed specimens are stable for at least 8 days.

7. Reagent Stability

a) cobas® EZH2 Mutation Test (Shelf Life)

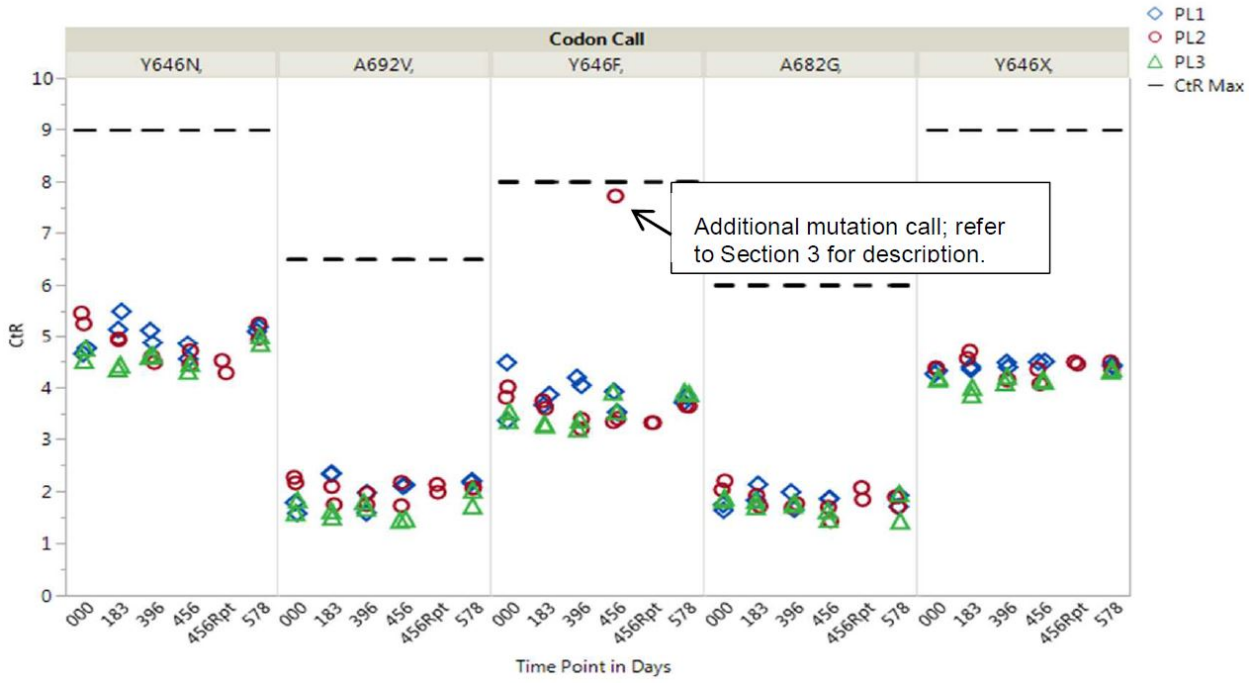
Two (2) studies were performed to establish the stability of the cobas® EZH2 Mutation Test amplification and detection reagents. The cobas® EZH2 Mutation Test kit recommended temperature is 2–8°C.

In the first study, stability of the kit was assessed at various time points after storage at 2-8°C in upright and inverted orientations using 3 lots of kit reagents. The test samples were the EZH2 Mutant Control (EZH2 MC) and the DNA Specimen Diluent (DNA SD), which serve as a positive control and negative control, respectively, in the cobas® EZH2 Mutation Test. Eight replicates of the EZH2 MC and DNA SD were tested at each storage condition. Stability was evaluated by performing functional testing at Day 0, 6 months, 13 months, 15 months, and 17 months. For a time point to pass, the EZH2 MC and DNA SD results must be within the pre-specified Ct ranges. Real time stability testing for this study passed 15 months storage for all three lots of kit reagents.

In the second study, samples composed of eluates from FFPE samples generated using the cobas® DNA Sample Preparation Kit were used. All eluates were frozen until testing at each stability time point. The 7 FFPE samples consisted of 5 NHL FFPE EZH2 mutant specimens (Y646N, Y646F, Y646X, A682G, and A692V) and two EZH2 FFPE wild-type specimens. Stability was evaluated by testing eluates at Day 0, 6 months, 13 months, 15 months, and 19 months. For a time point to pass, the FFPE samples had to yield the

expected mutation result. Figure 2 shows that each sample for each kit lot passed stability at each tested time point and that CtR values met prespecified criteria. Real time stability from this study passed 19 months for all 3 lots of kit reagents. Both studies will support the initial shelf life of 12 months.

Figure 2: CtR Plot for Each Kit Lot Across Time Points



b) Open Reagent Stability

A study was performed to assess the stability of opened reagents for the cobas® EZH2 Mutation Test. In this study, two EZH2 wild-type specimens and five low percent DNA blends with Y646N, A692V, Y646F, A682G and Y646H mutations were tested. For each mutation, the NHL FFPE DNA stocks were blended with wild-type DNA stocks to create low percent (~1.5-3X Limit of Detection - LOD) mutant samples. The mutation status and percent mutation of blends were determined by a validated NGS sequencing method. The cobas® EZH2 Mutation Test reagents were tested at Day 0 (first use/opening), Day 31 (second use/opening), Day 63 (third use/opening), and Day 91 (fourth use/opening). For a time point to pass, the blended FFPE samples had to yield the expected mutation result. The observed results for each of the five tested mutant blends and wild type specimens were as expected for all time points. Based upon the results of this study, opened cobas® EZH2 Mutation Test kit reagents, when stored at 2–8°C between uses, are stable for at least 90 days with a total of four uses.

c) Shipping Stability

A study was performed to assess the stability of the cobas® EZH2 Mutation Test kit during simulated shipping conditions. In this study, kits were either stored at the recommended storage temperature (2–8°C) throughout or stressed with higher or lower temperature profiles for different periods of time (Table 19) prior to functional testing at 3 months and

13 months (one month post kit expiration). To pass shipping stability, kits stored at both stressed conditions had to pass functional testing at each time point, with the EZH2 MC and DNA SD results within the pre-specified Ct ranges. The results of this study show after 13 months, the temperature stressed kit is stable during the simulated shipping conditions tested.

Table 19: Simulated Shipping Temperature Profile

Stress Profile with 37°C Maximum		**Stress Profile with -20°C Minimum**	
Time	**Temp**	**Time**	**Temp**
2 days	2-8°C	2 days	2-8°C
20 hrs	32°C	20 hrs	32°C
5 days	15°C	5 days	15°C
20 hrs	32°C	20 hrs	32°C
10 days	15°C	10 days	15°C
5 days	37°C	5 days	-20°C
until kit expires	2–8°C or indicated storage temp	until kit expires	2–8°C or indicated storage temp

X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the cobas EZH2 Mutation Test for detecting EZH2 mutations in FFPE specimens from patients with relapsed/refractory follicular lymphoma (FL) who may benefit from treatment with TAZVERIK (tazemetostat), was established with clinical data generated in a clinical trial. The study enrolled patients into one of two cohorts: those with EZH2 mutations detected by the Roche cobas EZH2 Mutation Test and those without mutations detected by the test. The major efficacy outcome measure was overall response rate (ORR) as determined by an independent review committee (IRC). ORR was 69% in the EZH2 mutant positive patient population. The accuracy of the mutation results were determined using a validated sequencing method and met prespecified agreement >95%. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

The Epizyme Phase 1/2 study was an open-label, multi-center study (Study E7438-G000-101, NCT01897571) investigating the safety and efficacy of TAZVERIK treatment in patients with relapsed or refractory follicular lymphoma (R/R FL) with 2 prior systemic therapies, and whose tumor tissue harbored an EZH2 mutation detected by the Roche cobas EZH2 Mutation Test. The study initially started as a non-registrational clinical trial in July 2015 and the cobas® EZH2 Mutation Test was utilized to select patients. In November 2016 and April 2017, the FDA granted fast track designation to Epizyme for the first-in-class experimental drug in FL, respectively, and the regulatory status of the E7438-G000-101 trial was changed to a registrational study.

The cobas® EZH2 Mutation Test CTA used in the Epizyme clinical study E7438-G000-101 had the same configuration as the final assay, the cobas® EZH2 Mutation Test IVD. Therefore, CTA results were considered the same as the IVD results and no bridging study was needed. Efficacy results are based on data entered into the study database through 09 August 2019 for subjects with FL enrolled as of 24 May 2019. All safety data entered through 24 May 2019 from all subjects was summarized. The major efficacy outcome measures were ORR and DOR according to the International Working Group Non-Hodgkin Lymphoma (IWG-NHL) criteria as assessed by Independent Review Committee. Median duration of follow-up was 22 months (range 3 month to 44 months).

Patients were screened by the cobas EZH2 Test to determine the mutation status of the EZH2 gene in their tumor tissue. Patients with EZH2 gene mutation-positive hotspots (Y646F, Y646N, Y646S, Y646H, Y646C, A682G, A692V) are reported as Mutation Detected (MD) by the cobas EZH2 Test.

1. Clinical Inclusion and Exclusion Criteria

a) Inclusion Criteria:

Subjects must have histologically confirmed FL (all grades) with R/R disease following at least 2 standard prior systemic treatment regimens where at least 1 anti-CD20-based regimen was used.

Other main entrance criteria included requirement for sufficient archival tumor tissue that had been successfully tested for EZH2 mutation status and cell of origin (COO) (DLBCL only); measurable disease by IWG-NHL criteria, ECOG performance status of $\leq$2, and adequate renal, hepatic, bone marrow and cardiac function.

b) Exclusion Criteria:

Subjects who met any of the following criteria were not permitted to be enrolled in this study:

- Prior exposure to tazemetostat or other inhibitor(s) of EZH2
- Subjects with known or prior history of leptomeningeal or brain metastasis, history of T-cell lymphoblastic lymphoma/T-cell acute lymphoblastic leukemia (T-LBL/T-ALL), active infection (including hepatitis B or C virus), major surgery within 4 weeks, or known to be immunocompromised.

2. Follow-up Schedule

Subjects were screened for determination of eligibility for the study within 28 days of the first dose of study treatment. A signed, written informed consent form (ICF) was obtained prior to any study-specific assessments or procedures being performed. Subjects were required to undergo EZH2 mutation testing of their tumor tissue prior to the conduct of other screening procedures. Disease assessment and other clinical assessments were conducted according to protocol as part of the screening assessment and monitoring during the trial.

Following screening, subjects attended study visits on Days 1 and 15 of Cycles 1 and 2 and Day 1 of every cycle thereafter until termination of treatment. Starting at Cycle 3, Day 15 assessment was conducted by telephone contact; for these visits, laboratory assessments were required only if medically indicated and could be conducted at either the study site laboratory or a local laboratory. A post-treatment visit was conducted within 30 (±3) days of the last dose of study treatment or prior to the start of alternate anticancer therapy. Subjects with AEs ongoing at that time were followed until the AE had resolved or was deemed to be continuing indefinitely.

3. Clinical Endpoints

The primary efficacy endpoint is ORR, defined as the percentage of subjects with a response of complete response (CR) or partial response (PR). Subjects with a best overall response (BOR) of stable disease (SD) or progressive disease (PD), or with non-evaluable, unknown, or missing response, were included as non-responders. The ORR was summarized using number and percent, including the exact 95% binomial confidence interval (CI) around the ORR. The concordance rate of IRC and investigator assessments was summarized for ORR and DOR.

Efficacy endpoints were evaluated in the intent-to-treat (ITT) population which included all subjects with R/R FL who received at least 1 dose of tazemetostat.

Safety endpoints included AEs and concomitant medications, physical examinations, vital signs, clinical laboratory assessments, and 12-lead ECGs. Safety endpoints were evaluated in the safety population which included all subjects with DLBCL and FL who received at least 1 dose of tazemetostat.

B. Accountability of PMA Cohort

A total of 45 FL patients with EZH2 mutations were enrolled based on results of cobas EZH2 Mutation Test and other inclusion and exclusion criteria. Patients were allowed to enroll on archived tumor specimen. If archived specimens were not available, new specimens were obtained. Specimens with EZH2 gene mutation-positive hotspots (Y646F, Y646N, Y646S, Y646H, Y646C, A682G, A692V) are reported as Mutation Detected (MD) by the cobas EZH2 Test. The distribution of the FL mutations is shown below in Table 20.

Table 20: Distribution of EZH2 Mutations as Determined by cobas EZH2 Mutation Test (FL Positive Patients)

Mutation Status	Count	Proportion (%)	95% CI of Proportion (%)
A682G	4	8.89	(3.51, 20.73)
A682G; Y646X	1	2.22	(0.39, 11.57)
A692V	1	2.22	(0.39, 11.57)
Y646F	12	26.67	(15.96, 41.04)
Y646N	11	24.44	(14.24, 38.67)

Mutation Status	Count	Proportion (%)	95% CI of Proportion (%)
Y646N; Y646F	1	2.22	(0.39, 11.57)
Y646X	15	33.33	(21.36, 47.93)
Total	45		

Note: All CI (confidence interval) are calculated by Wilson score method.
Note: The proportion for each mutation type are calculated among the population of Mutation Detected by cobas EZH2 Mutation Test.

C. Study Population Demographics and Baseline Parameters

Demographics and specimen characteristics for the 45 patients are shown summarized in Table 21. The median age was 62 years (range 38 to 80 years), 58% were female, 42% had early progression following front-line therapy (POD24), and all had an ECOG PS of 0 or 1. The median number of lines of prior systemic therapy was 2 (range 1 to 11) with 49% refractory to rituximab, and 49% refractory to their last therapy.

Table 21: Demographics and Baseline Characteristics for Eligible Patients Screened by cobas EZH2 Test (FL Positive Cohort)

Characteristics		Total (n=45)
Age	Mean ± SD	61.8 ± 9.0
	Median (Range)	62 (38, 80)
Age (Years)	<65 Years	25 (55.6%)
	≥65 Years	20 (44.4%)
Sex	F	26 (57.8%)
	M	19 (42.2%)
Histology	Follicular Lymphoma	42 (93.3%)
	Other	3 (6.7%)
Source of Tissue Collection	Biopsy	30 (66.7%)
	Core Needle Biopsy	3 (6.7%)
	Excision	3 (6.7%)
	Resection	3 (6.7%)
	Other	6 (13.3%)
Tumor Type	Primary	8 (17.8%)
	Metastatic	2 (4.4%)
	Not Available	35 (77.8%)

B. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with TAZVERIK was addressed during the review of the NDA and is not addressed in detail in this SSED. Refer to Drugs@FDA for safety information on TAZVERIK.

The cobas EZH2 Mutation Test involves using FFPE FL tumor tissue specimens. These specimens are routinely removed as part of the practice of medicine for the diagnosis of FL of pathologists. No adverse events were observed due to the device use.

2. Effectiveness Results

The efficacy results presented in this report are based on data from a total of 42 instead of 45 subjects with EZH2 MT disease because 3 patients did not receive 2 prior systemic therapies and were excluded from the efficacy data analysis. The population enrolled was representative of subjects with R/R FL receiving 2L+ treatment, a group of subjects with limited treatment options.

Results for the primary efficacy endpoint of ORR and DOR were summarized based on independent review committee (IRC) assessed responses, and the results are shown in Table 21.

Table 21. Efficacy Results for Patients with EHZ2 Mutant Relapsed or Refractory Follicular Lymphoma

Efficacy Endpoints	**TAZVERIK™ EZH2 MD FL Patients**
Overall Response Rate (95% CI)*	69% (53%, 82%)
Complete Response	12%
Partial Response	57%
Duration of Response	
Median (95% CI) in months	10.9 (7.2, NE)
Range in months	0.0+, 22.1+

CI: Confidence Interval; NE = not estimable.

* Median time to response for patients with EZH2 MD follicular lymphoma was 3.7 months (range 1.6 to 10.9).

3. Subgroup Analyses

Subgroup analyses was not performed for EZH2 Mutation Test results.

4. Pediatric Extrapolation

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

C. 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 3 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The cobas® EZH2 Mutation Test analytical data demonstrated a robust and reliable assay performance for the detection of defined EZH2 mutations in patients with follicular lymphoma. Clinical reproducibility and accuracy data demonstrated the clinical validity of the cobas® EZH2 Mutation Test to inform the treatment with tazemetostat in FL patients.

The clinical effectiveness of the Roche cobas EZH2 Mutation Test, was demonstrated in a single-arm, multi-center international clinical trial of TAZVERIK (tazemetostat) in patients with relapsed/refractory follicular lymphoma patients. Of the 45 patients, the ORR was 69%. The data demonstrates that the assay is effective for determining of FL patients who may benefit from TAZVERIK therapy.

B. Safety Conclusions

The probable risks of the Roche EZH2 Mutation Test are associated with the potential mismanagement of patients resulting from false results. The safety of the device is based on the robust accuracy, precision and analytical sensitivity of the test as demonstrated in the analytical validation studies as well as data collected in a clinical study conducted to support PMA approval.

C. Benefit-Risk Determination

Treatment with tazemetostat provides meaningful clinical benefit to relapsed/refractory follicular lymphoma patients with EZH2 mutations, as measured by ORR demonstrated in the Epizyme Phase 1/2, open-label, multi-center trial (Study E7438-G000-101, NCT01897571). The ORR was 69% (95% CI: 53-82%). 12% of the patients had a complete response and 57% of patients had a partial response. Given the available information, the data supports the conclusion that cobas EZH2 Mutation Test has probable benefit in selecting EZH2 mutant follicular lymphoma patients for treatment with tazemetostat.

There is potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, and failure to provide a result and 2) incorrect interpretation of test results by the user. The risks of the cobas EZH2 Mutation Test for selection of follicular lymphoma patients with EZH2 mutations for treatment with tazemetostat are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The risks of erroneous results are partially mitigated by the analytical performance of the device.

The likelihood of false results was assessed by an analytical accuracy study that performed specifically to evaluate the concordance between the cobas EZH2 Mutation Test and a validated NGS method. Out of 341 patients sequenced, the PPA was 98.3% (95% CI: 94.2%, 99.5%) and NPA was 98.6% (95% CI: 95.9%, 99.5%). The analytical performance of the device, partially mitigates the risks associated with the device.

In summary, the balance of probable benefits to probable risks for the use of the cobas EZH2 Mutation Test to select follicular lymphoma with EZH2 mutations for treatment with tazemetostat is favorable, when considering the factors outlined above.

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 cobas® EZH2 Mutation Test when used in accordance with the indications for use.

The results of this Method Comparison (Accuracy) Study support the intended use of the cobas EZH2 Test for diagnostic purposes as an aid in the clinical management of patients with FL eligible for treatment with tazemetostat.

XIII. CDRH DECISION

CDRH issued an approval order with conditions for approval on June 18, 2020. The final conditions of approval cited in the approval order are described below.

1. The applicant must provide robust and high confidence data demonstrating the limit of blank (LoB) by evaluating 60 replicates of DNA from wild-type cell lines and summarize the background cycle threshold and cycle threshold ratios for each mutation reaction for each of the replicates and submit updated labeling. The data from this study must be adequate to conclude that the thresholds used for discriminating wild-type from mutant are supported.

2. The applicant must provide robust and high confidence data to demonstrate the robustness of the assay to decreased and increased volume and incubation times for

Proteinase K in cobas EZH2 Mutation Test. The samples tested must represent the 3 codons. The study design must be adequate to demonstrate that reasonable deviations in proteinase K digestion do not lead to incorrect results, or the labeling must be updated to inform the user when such deviations will potentially lead to incorrect results.

The final study data and labeling revisions should be submitted within 1 year 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).

XIII. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.