EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR QUANTIDEX QPCR BCR-ABL IS KIT DECISION SUMMARY

A. DEN Number:

DEN160003

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the QuantideX qPCR BCR-ABL IS Kit

C. Measurand:

BCR-ABL1 and ABL1 transcripts

D. Type of Test:

Reverse transcription, quantitative, polymerase chain reaction (qPCR) based nucleic acid amplification

E. Applicant:

Asuragen

F. Proprietary and Established Names:

Trade Name: QuantideX qPCR BCR-ABL IS Kit
Common Name: BCR-ABL1 RT-qPCR Test

G. Regulatory Information:

1. Regulation section:

21 CFR 866.6060

2. Classification:

Class II (Special Controls)

3. Product code:

OYX

4. Panel:

88 − Pathology

H. Indications For Use:

1. Indications for Use

The QuantideX qPCR BCR-ABL IS Kit is an in vitro nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2. The QuantideX qPCR BCR-ABL IS Kit is a reverse transcription-quantitative PCR performed on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument and is intended to measure BCR-ABL1 to ABL1, expressed as a log molecular reduction (MR value) from a baseline of 100% on the International Scale, in t(9;22) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs).

The test does not differentiate between e13a2 or e14a2 fusion transcripts and does not monitor other rare fusion transcripts resulting from t(9;22). This test is not intended for the diagnosis of CML.

2. Special conditions for use statement(s):

For *in vitro* diagnostic use only.

For prescription use only.

3. Special instrument requirements:

Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument using System Sequence Detection Software v1.4.1.

I. Device Description:

The QuantideX qPCR BCR-ABL IS Kit reagents are adapted for use on the ABI 7500 Fast Dx Real-Time PCR Instrument. The assay includes reagents sufficient for 60 reactions. A description of the reagents provided is described below in Table 1.

Table 1: Components of the QuantideX Assay

Item	Description	Use
QuantideX qPCR BCR-ABL IS Reagents (Box 1 of 2)		
BCR-ABL IS Diluent	Nuclease-free water	Adjust volume of RT reactions
BCR-ABL IS RT Buffer	Deoxynucleotides (dNTPs) and degenerate deoxyoligonucleotides in a buffered salt solution	Random priming of RNA templates and primer extension to generate cDNA from RNA templates
BCR-ABL IS RT Enzyme Mix	Reverse transcriptase	Enzyme component of the RT reaction to generate cDNA from RNA template
BCR-ABL IS qPCR Buffer	Deoxynucleotides (dNTPs) in a buffered salt solution	Provides dNTPs for primer extension to generate DNA amplicons from cDNA templates

BCR-ABL IS Primer Probe Mix	Deoxyoligonucleotide primers, dye- and quencher- conjugated deoxyoligonucleotide probes, and passive reference dye	Provides primers and probes for PCR amplification and detection of target sequences. Passive reference dye is used by the instrument software to normalize fluorescence throughout the PCR
BCR-ABL IS Enzyme Mix	Thermostable DNA polymerase	Catalyzes the amplification of templates hybridized to templates in the cDNA. Enzyme exonuclease activity degrades hybridized probes to release fluorescence for detection of amplicons in each PCR cycle.
QuantideX qPCR BCR-ABL IS Calibrators and Controls (Box 2 of 2)		
BCR-ABL IS CAL1	BCR-ABL and ABL armored RNA formulated to approximately MR 1.0	Generate calibration curve for calculation of MR values for controls and patients specimens
BCR-ABL IS CAL2	BCR-ABL and ABL armored RNA formulated to approximately MR 2.0	
BCR-ABL IS CAL3	BCR-ABL and ABL armored RNA formulated to approximately MR 3.0	
BCR-ABL IS CAL4	BCR-ABL and ABL armored RNA formulated to approximately MR 4.0	
BCR-ABL IS CONH	BCR-ABL and ABL armored RNA formulated to approximately MR 1.5	Control used to ensure that RT and PCR steps performed properly by generating expected MR value
BCR-ABL IS CONL	BCR-ABL and ABL armored RNA formulated to approximately MR 3.5	
BCR-ABL IS CONN	ABL armored RNA	Control used to ensure that RT and PCR steps performed properly and protect against contamination and falsely positive samples to due contamination by generating a results of "Negative (Sufficient ABL1)"

Additional materials required but not provided with the QuantideX qPCR BCR-ABL IS Kit:

1. Reagents for total RNA isolation
2. Applied Biosystems 7500 Fast Dx Real-Time PCR instrument

J. Substantial Equivalence Information:

1. Predicate device name(s) and DEN number(s):
   Not applicable.

2. Comparison with predicate:
Not applicable.

K. Standards/Guidance Documents Referenced:

- Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submission for Software Contained in Medical Devices
- General Principles of Software Validation; Final Guidance for Industry and FDA Staff
- Guidance for Industry, FDA Reviewers and Compliance on Off-the-Shelf Software Use in Medical Devices
- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures, A Statistical Approach
- CLSI EP07-A2, Interference Testing in Clinical Chemistry
- CLSI EP25-A, Evaluation of Stability on In Vitro Diagnostic Reagents

L. Test Principle:

The QuantideX qPCR BCR-ABL IS Kit is a nucleic acid amplification test for the quantitation of BCR-ABL1 RNA. The assay provides simultaneous amplification and detection of two BCR-ABL1 fusion transcripts (e13a2 and e14a2) and ABL1 (an endogenous control) using total RNA extracted from human white blood cells enriched from EDTA whole blood. The test uses multiplex reverse transcription-PCR (RT-PCR) in combination with real-time hydrolysis probe technology. BCR-ABL1 translocations and ABL1 mRNA are simultaneously reverse-transcribed, amplified, detected, and quantified in a single reaction well. b(4) This design is diagrammed below.

Figure 1: Primer and Probe Design for the QuantideX Assay

b(4)

The RNA quantity of BCR-ABL is assessed relative to ABL1 expression within each reaction well, and is computed two ways: (1) as the specimen percent IS value (the percent ratio of BCR-ABL1 to ABL1 expressed on the International Scale), and (2) as a molecular reduction value (MR, a logarithmic decrease from the common baseline of 100% IS or MR 0). ABL1 also serves as a control for assessment of RNA quality for the specimen.

The Assay Calibrator set includes external calibrators built with Armored RNA® Quant (ARQ) technology to generate traceable %IS values (transformed into the primary measurement of a molecular reduction (MR) from the common baseline of 100%IS) from a single calibration curve (provided). Calibrators are run in duplicate and all other specimens and controls may be run in singlicate. The time required from the RT set-up step through the generation of the last test result is ≤ 4 hours for a full kit.

M. Interpretation of Results

The numerical value of the World Health Organization (WHO) International Scale is % IS, the ratio expressed as a percentage of BCR-ABL1 expression to the expression of a control gene (ABL1 in this instance). The International Scale (%IS) is a geometric progression and therefore repeated measurements of a sample are non-normally distributed about the mean. %IS values require log-transformation prior to performing any statistical analyses that require normally-distributed data.

Another value commonly reported in the literature is the Molecular Reduction, or MR value. The MR value is traditionally written as $MR^{x.x}$. However, for simplicity and legibility, the QuantideX assay will report the value as MRx.x. The MR value is the $\log_{10}$ reduction from the internationally standardized baseline, defined as 100% IS. Therefore,

$$MRx.x = log_{10}\left(\frac{100}{\%IS}\right) = log_{10}(100) - log_{10}(\%IS) = 2 - log_{10}(\%IS)$$

The test uses MR values for the calibration standards as well as the primary specimen output, with %IS also reported. MR values with their corresponding %IS values are shown below in Table 2.

Table 2: MR Values and Corresponding %IS Values

MR	%IS
0.0	100
0.5	32
1.0	10
1.5	3.2
2.0	1
2.5	0.32
3.0	0.1
3.5	0.032
4.0	0.01
4.5	0.0032
4.7	0.002
5.0	0.001

N. Performance Characteristics:

1. Analytical Performance:

a. Precision:

i. *Within-laboratory Precision:* Five human RNA specimens positive for BCR-ABL1 were diluted into RNAs from human CML-negative blood to formulate 25 samples ranging from MR 1.0 to MR 4.0, encompassing both transcript types (e13a2 and e14a2). Testing spanned 3 lots, 3 operators, 20 runs, and 3 instruments. The acceptance criteria for this study were that the standard deviations satisfy the criteria in Table 3. The results of the precision study are shown in Table 4. The range of observed standard deviations at each MR level across the 5 specimens is summarized in Table 5. The maximum observed standard deviation was 0.134. The acceptance criteria for this study were met supporting the conclusion that the assay has acceptable repeatability ≤ MR 4.0.

Table 3: Acceptance Criteria for Reproducibility/Precision Studies

Log Scale		%IS	
MR Value	**SD Criteria**	**%IS Value**	**%CV Criteria**
< 3.5	≤ 0.21	> 0.0316	≤ 50
3.5-4.25	≤ 0.29	0.0316-0.0056	≤ 75
> 4.25	≤ 0.36	< 0.0056	≤ 100

Table 4: Precision Study Results

Target MR	Sample	Lot	Operator	Instrument	Run	Within	Total
		SD	SD	SD	SD	SD	SD
1	PA1S01	0.049	0.000	0.000	0.000	0.010	**0.059**
	PA1S06	0.003	0.028	0.000	0.046	0.016	**0.093**
	PA1S11	0.000	0.020	0.057	0.000	0.014	**0.091**
	PA1S16	0.036	0.000	0.065	0.000	0.027	**0.128**
	PA1S21	0.000	0.026	0.023	0.000	0.027	**0.076**
2	PA1S02	0.033	0.010	0.000	0.000	0.011	**0.054**
	PA1S07	0.015	0.015	0.033	0.021	0.022	**0.105**
	PA1S12	0.027	0.000	0.000	0.012	0.019	**0.058**
	PA1S17	0.000	0.000	0.051	0.000	0.023	**0.074**
	PA1S22	0.000	0.000	0.021	0.000	0.010	**0.031**
3	PA1S03	0.006	0.025	0.000	0.000	0.025	**0.056**
	PA1S08	0.000	0.000	0.017	0.000	0.018	**0.035**
	PA1S13	0.032	0.000	0.000	0.000	0.038	**0.070**
	PA1S18	0.000	0.000	0.022	0.000	0.028	**0.050**
	PA1S23	0.000	0.028	0.000	0.000	0.019	**0.047**
3.5	PA1S04	0.000	0.000	0.000	0.000	0.046	**0.046**
	PA1S09	0.000	0.000	0.000	0.000	0.047	**0.047**
	PA1S14	0.000	0.000	0.000	0.053	0.058	**0.111**

Target MR	Sample	Lot	Operator	Instrument	Run	Within	Total
		SD	SD	SD	SD	SD	SD
	PA1S19	0.016	0.000	0.000	0.000	0.035	**0.052**
	PA1S24	0.018	0.000	0.053	0.000	0.031	**0.103**
4	PA1S05	0.000	0.000	0.000	0.000	0.074	**0.074**
	PA1S10	0.037	0.021	0.024	0.000	0.052	**0.134**
	PA1S15	0.000	0.000	0.000	0.000	0.071	**0.071**
	PA1S20	0.000	0.000	0.000	0.000	0.040	**0.040**
	PA1S25	0.000	0.067	0.020	0.000	0.024	**0.111**

Table 5: Minimum and Maximum Standard Deviations Observed by MR Level

Target MR	SD Range	
	Min	Max
MR1	0.059	0.128
MR2	0.031	0.105
MR3	0.035	0.070
MR3.5	0.046	0.111
MR4	0.040	0.134

ii. *Site-to-Site Reproducibility:* The same 25 sample pool described above was evaluated at 4 sites by multiple operators over multiple non-consecutive days for a total of 1200 measurements. This dataset was analyzed using a nested random effect analysis of variance. The acceptance criteria were the same as those described in Table 3 above. The results are summarized in Table 6 below. The acceptance criteria were met supporting the overall conclusion that the assay is reproducible within all variables tested >MR 1.0 ≤ 4.0.

Table 6: Summarized Variance for all Samples and Pools in Multi-Site Reproducibility

Targeted MR	Sample	Site	Day	Operator	Within	Total
		SD	SD	SD	SD	SD
MR1	pA1s01	0.008	0.000	0.012	0.028	0.049
	pA1s06	0.009	0.014	0.000	0.021	0.044
	pA1s11	0.024	0.017	0.014	0.022	0.077
	pA1s16	0.017	0.020	0.000	0.019	0.056
	pA1s21	0.013	0.021	0.011	0.020	0.064
MR2	pA1s02	0.000	0.021	0.000	0.068	0.089
	pA1s07	0.000	0.022	0.004	0.017	0.044
	pA1s12	0.032	0.009	0.026	0.021	0.087
	pA1s17	0.012	0.016	0.009	0.019	0.055
	pA1s22	0.012	0.014	0.000	0.031	0.057
MR3	pA1s03	0.039	0.024	0.005	0.036	0.104
	pA1s08	0.000	0.023	0.009	0.028	0.059
	pA1s13	0.039	0.026	0.000	0.052	0.117
	pA1s18	0.018	0.000	0.009	0.033	0.059

	pA1s23	0.036	0.019	0.000	0.032	0.087
MR3.5	pA1s04	0.061	0.023	0.026	0.037	0.147
	pA1s09	0.013	0.042	0.010	0.043	0.108
	pA1s14	0.038	0.039	0.041	0.051	0.169
	pA1s19	0.026	0.010	0.000	0.035	0.071
	pA1s24	0.050	0.027	0.000	0.032	0.109
MR4	pA1s05	0.054	0.027	0.032	0.053	0.167
	pA1s10	0.029	0.000	0.000	0.067	0.095
	pA1s15	0.014	0.037	0.000	0.070	0.122
	pA1s20	0.042	0.000	0.000	0.079	0.121
	pA1s25	0.026	0.028	0.005	0.063	0.123

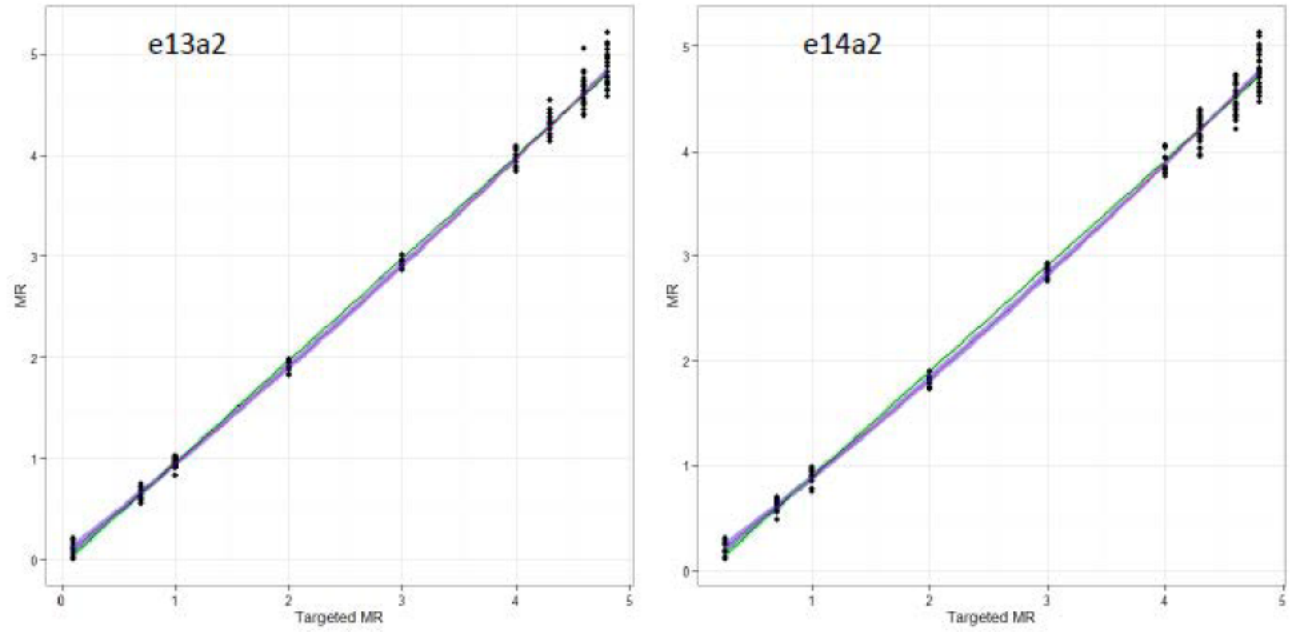
b.Linearity/Reportable Range:

Linearity was estimated by testing 2 separate RNA specimens that were positive for BCR-ABL (one e13a2 and one e14a2). Because an appropriate reference method could not be identified, transcript quantity is relative to the QuantideX assay. Each was diluted into RNAs from CML-negative whole blood to a range of MR 0.1 to MR 4.8. Testing was conducted across 2 lots of assay kits. The acceptance criteria for this testing were the same as those described in Table 3 above.

The linear regression curves demonstrated slopes of 1.01 for both transcripts and intercepts of -0.11 and -0.05 for e13a2 and e14a2, respectively. Transcript e13a2 was linear from MR 0.12 to MR 4.84 with a maximum SD of 0.17 and transcript e14a2 was linear from MR 0-.22 to MR 4.78 with a maximum SD of 0.17.

Second and 3rd order polynomial regressions were also assessed and the analysis supports linearity from at least MR 0.3 (50% IS) to MR 4.7 (0.002% IS). These results support the conclusion that the assay is linear for both transcripts from MR 0.3 (50%IS) to MR4.7 (0.002%IS).

Figure 2: Linearity of e13a2 (left) and e14a2 (right) transcripts



c. Detection Limit:

i. Limit of Blank (LoB):
The LoB was determined by testing 30 non-leukemic human RNA specimens that were presumed negative for BCR-ABL. Testing spanned 3 assay kit lots, 4 operators, 9 runs, 4 calendar days and 4 qPCR instruments for a total of 265 valid measurements.

Out of 265 valid measurements, 263 were undetectable.

ii. Limit of Detection (LoD):
The LoD was determined using 4 separate human RNA specimens that were positive for BCR-ABL1, each serially diluted into RNAs from human CML-negative whole blood to 28 dilution levels. Testing spanned 2 assay kit lots, 4 operators, 15 calendar days, and 4 qPCR instruments. Both e13a2 and e14a2 transcripts were evaluated.

The non-parametric method to determine LoD for quantitative devices was used, keeping the type II error under 5%. The median value of the tested %IS values across all included panel member replicates was determined and defined as the LoD. This analysis yielded a LoD for each transcript of 0.002% IS/MR 4.7. The results are pictured below in Table 7.

Table 7: Determination of LoD for both BCR-ABL Transcripts

Transcript	Specimens	Total Results	Total Positive	Percent Undetected	Median %IS	LOD (MR)
e13a2	pC1s02, pC1s24, pC1s25	179	172	3.9	0.002	4.70
e14a2	pC1s09, pC1s10, pC1s11, pC1s12, pC1s17, pC1s18, pC1s19	420	400	4.8	0.002	4.70

iii. Limit of Quantitation (LoQ):
The LoQ was determined by testing 6 specimens that were derived from 6 human RNA that were positive for BCR-ABL (each diluted into RNA from human CML-negative whole blood to the target of MR 4.7).The results were evaluated against the acceptance criteria of SD ≤ 0.36 at MR 4.5 or greater. Testing generated 120 measurements across 2 lots of kit. MR values ranged from 4.6 to 4.87 with SD values ranging from 0.23 to 0.34. The results indicate that the LoQ is equivalent to the LoD (MR 4.7).

d.Analytical Specificity:

i. Interfering Substances:
Potential interfering substances from both blood sources and RNA extraction sources were tested with specimens at the LoQ in the following concentrations:

- Hemoglobin 200 g/L
- Lipid 5.6 mM
- Albumin 50 g/L
- Conjugated bilirubin 86 mM
- Unconjugated bilirubin 257 µM
- Guanidinium containing lysis buffer 1%
- Ethanol 7%
- Phenol 0.1%
- Assay wash buffer 10%
- Genomic DNA 50 ng/RT

The RNA specimens tested were residual CML-positive blood diluted to approximately MR4.0 into RNA from CML-negative whole blood. Testing was performed in 9 replicates and compared to control samples. The acceptance criteria were that the mean of the test samples was ± 0.5 MR of the control. The acceptance criteria were met, supporting the conclusion that none of the tested agents interfered with the assay at the concentrations listed.

ii. Primer Specificity:
Analytical specificity (i.e., exclusivity) for the BCR-ABL breakpoints e13a2 and e14a2 was tested using 11 leukemic specimens (CML, Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL)) and 2 non-leukemic RNA specimens. Testing generated 117 measurements across 3 lots of assay kit. The acceptance criteria were met in that specimens without a major BCR-ABL1 break point were reported as negative for BCR-ABL1 or below LoD in > 8/9 replicates. The results across the various samples are shown in Table 8. The results support supported the conclusion that the test detects the BCR-ABL1 e13a2 and e14a2 fusion transcripts exclusively.

Table 8: Specificity in the Presence of other Fusion Transcripts

Leukemia Type	Fusion Transcript	Sample type	Result[2]
CML or ALL	t(9;22) minor breakpoint e1a2 (p190)	Cell line RNA	<LOD (9/9 Und)
CML	t(9;22) micro breakpoint e19a2 (p230)	IVT[1] in Non-leukemic RNA	<LOD (9/9 Und)
AML	t(8;21) AML1/ETO	Cell line RNA	<LOD (9/9 Und)
AML M3 / APL	t(15;17) PML/RARA	Cell line RNA	<LOD (9/9 Und)
AML M4	inv(16) CBFB/MYH11	Cell line RNA	<LOD (9/9 Und)
AML M5	t(9;11) MLLT3/MLL	Cell line RNA	<LOD (9/9 Und)
AML M7	t(1;22) Megakaryoblastic	IVT in Non-leukemic RNA	<LOD (9/9 Und)
ALL	t(12;21) TEL/AML1	Cell line RNA	<LOD (9/9 Und)
ALL	t(1;19) E2A/PBX1	Cell line RNA	<LOD (9/9 Und)
ALL	t(4;11) MLL/AF4 (e9e5)	Cell line RNA	<LOD (8/9 Und)
ALL	t(4;11) MLL/AF4 (e10e4)	Cell line RNA	<LOD (9/9 Und)
Non-leukemic	N/A	Donor blood	<LOD (9/9 Und) across 2 specimens

[1]The in vitro transcript (IVT) for e19a2 did not contain BCR exons e13 or e14. Specimens harboring the native e19a2 breakpoint are expected to contain these exons and therefore may generate some low level positivity.

[2]The specimens listed in the table as "Und" were reported as "Undetected (sufficient ABL1)".

iii. Specimen Carryover Contamination:

Carryover contamination studies were performed on a specimen panel composed of total RNA derived from residual clinical CML-positive blood serially diluted into RNA from CML-negative whole blood. Samples were added to the plate such that 25 wells of high positive (MR 0.8) were alternated with 25 wells of CML-negative specimen in a checkerboard pattern. The acceptance criteria were that > 95% of CML- negative samples tested negative or below LoD.

Testing found that all negative specimens (25/25) were reported as "Negative (sufficient ABL1)". All high positive samples gave an appropriate MR value (mean MR value of 0.79 [16%IS], range of MR0.73 to 0.83 [19 to 15%IS, respectively]). The acceptance criteria were met supporting the conclusion that the test generates no significant carryover between wells on the plate.

e. RNA Isolation and RNA Input:

i. RNA Isolation:

Three commonly used RNA extraction methods were tested using CML-positive white blood cells serially diluted across 4 levels (MR 1-4) into CML-negative human anti-coagulated whole blood. Testing incorporated both transcripts (e13a2 and e14a2). Two lots of assay kits were used in this testing. The acceptance criteria were that each MR value would conform to the SD criteria described in Table 3 above.

All 3 isolation methods gave equivalent results with the standard deviation within each method being less than 0.1 for all levels and lots. Therefore, it is concluded that the test is compatible with generic methods of RNA isolation, and any validated method of RNA extraction and isolation that yields unbiased isolation of total RNA in sufficient quantity and quality may be used with the assay.

ii. RNA Input:

The assay uses an RNA input concentration of 1000 to 5000 ng. To validate that the performance of the assay across the RNA concentrations, an RNA input study was conducted. A total of 30 samples derived from 2 primary RNA samples diluted into non-leukemic human RNA were tested. Samples were set to target MR values of 1, 3, or 4 with target RNA inputs ranging from 250 ng to 6000 ng, and were run in 9 replicates per sample giving a total of 216 evaluable observations. Acceptance criteria were the same as those described in Table 3 above. The results are shown in Table 9 below. The acceptance criteria were met for RNA inputs ranging from 750 to 6000 ng from MR 1 to MR 4.5, supporting the recommended RNA input range of 1000-5000 ng.

Table 9: RNA Input Study Results

Dilution Series	Specimen	RNA Input (ng)	Reps	Percent Positive	Mean MR	SD (MR)	Mean %IS	%CV (%IS)	Series Mean MR	Batch SD (MR)	Batch Mean %IS	Batch %CV (%IS)
1	pR1s01	6000	9	100	0.99	0.06	10.2263	13.3	1.00	0.04	10.1028	9.0
	pR1s02	5000	9	100	1.00	0.03	10.0471	7.9				
	pR1s03	3000	9	100	1.00	0.04	10.0171	9.7				
	pR1s04	1000	9	100	0.98	0.02	10.5391	5.7				
	pR1s05	750	9	100	1.00	0.05	10.0122	10.4				
	pR1s06	250	9	100	1.01	0.02	9.7939	5.1				
2	pR1s07	6000	9	100	3.05	0.06	0.0905	13.6	3.06	0.07	0.0886	16.0
	pR1s08	5000	9	100	3.08	0.06	0.0844	13.5				
	pR1s09	3000	9	100	3.07	0.03	0.0859	6.8				
	pR1s10	1000	9	100	3.05	0.03	0.0897	7.8				
	pR1s11	750	9	100	3.06	0.08	0.0892	17.3				
	pR1s12	250	9	100	3.05	0.13	0.0928	30.0				
3	pR1s13	6000	9	100	4.67	0.23	0.0025	55.7	4.70	0.27	0.0024	68.4
	pR1s14	5000	9	100	4.69	0.27	0.0024	69.7				
	pR1s15	3000	9	100	4.73	0.29	0.0023	72.5				
	pR1s16	1000	9	89	4.67	0.32	0.0028	84.3				
	pR1s17	750	9	89	4.75	0.22	0.0020	54.1				
	pR1s18	250	9	33	4.66	0.52	0.0044	173.1				
5	pR2s01	6000	9	100	4.30	0.09	0.0051	19.8	4.28	0.21	0.0059	50.6
	pR2s02	5000	9	100	4.32	0.21	0.0053	53.0				
	pR2s03	3000	9	100	4.27	0.15	0.0057	35.5				
	pR2s04	1000	9	100	4.32	0.29	0.0060	75.4				
	pR2s05	750	9	100	4.36	0.17	0.0047	40.2				
	pR2s06	250	9	100	4.11	0.22	0.0089	54.7				

f. Traceability and Stability (Reagent and Specimen):

i. Traceability:

The assay calibrators are traceable to the First (1st) WHO International Genetic Reference Panel. White HE, Matejtschuk P, Rigsby P, et al. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111-7. The Value Assignment process for the calibrators is depicted below in Figure 3. Traceability to the 1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR was demonstrated by measuring the WHO Reference Panel with 3 assay kit lots and comparing the measured values to the published values of the panel. Each of the 4 panel members was tested in duplicate across 3 runs (1 run per lot). The MR values for each level of the reference panel were calculated by regression to each lot of the kit calibrator. Measured MR values were compared to published MR values through an additional

regression analysis to determine the slope and intercept. The acceptance criteria for this testing were that the regression model have a slope close to 1 (1.0 to 1.1.) and an intercept close to 0 (0.02-0.11). The results are pictured below in Figure 4. The acceptance criteria were met supporting the conclusion that traceability of the assay calibrators to the WHO Reference Panel has been established.

Figure 3: Depiction of the Traceability of the QuantideX Calibrators to the WHO Reference Standard Panel

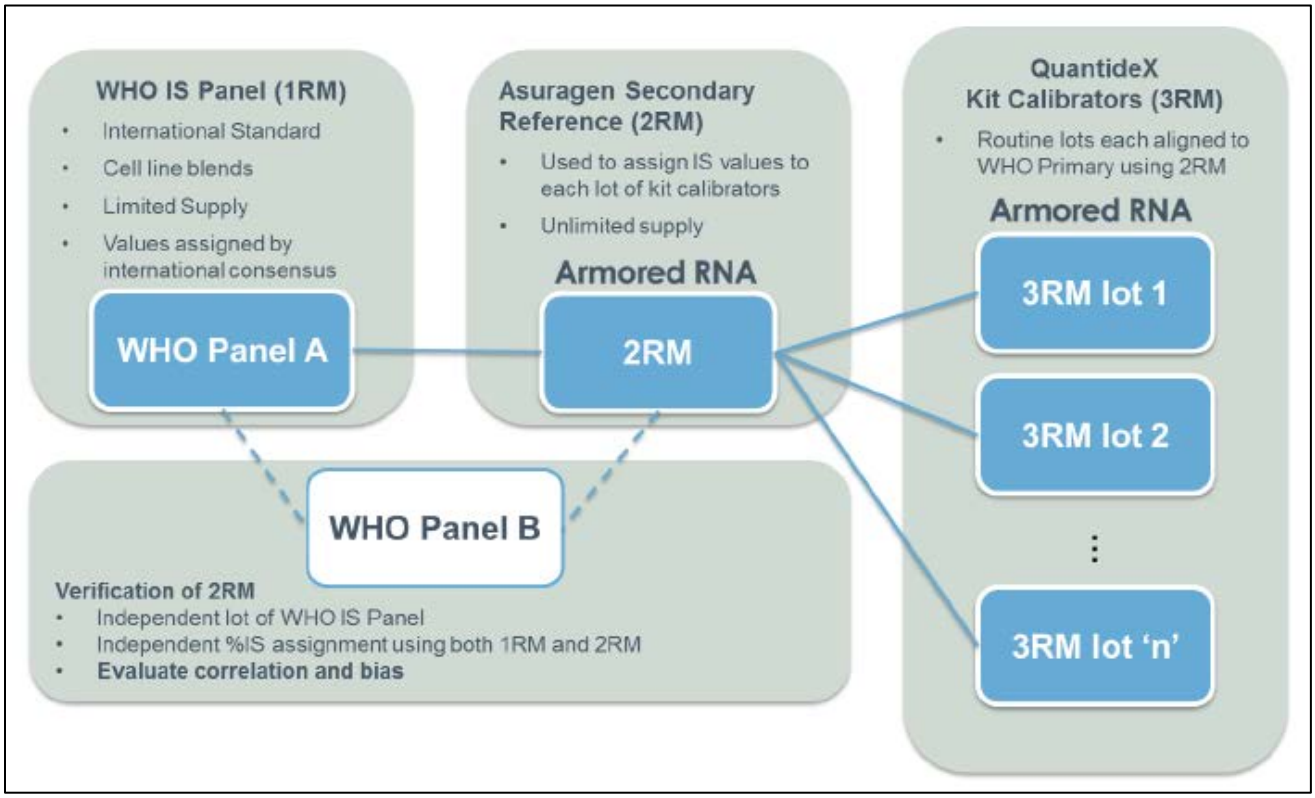
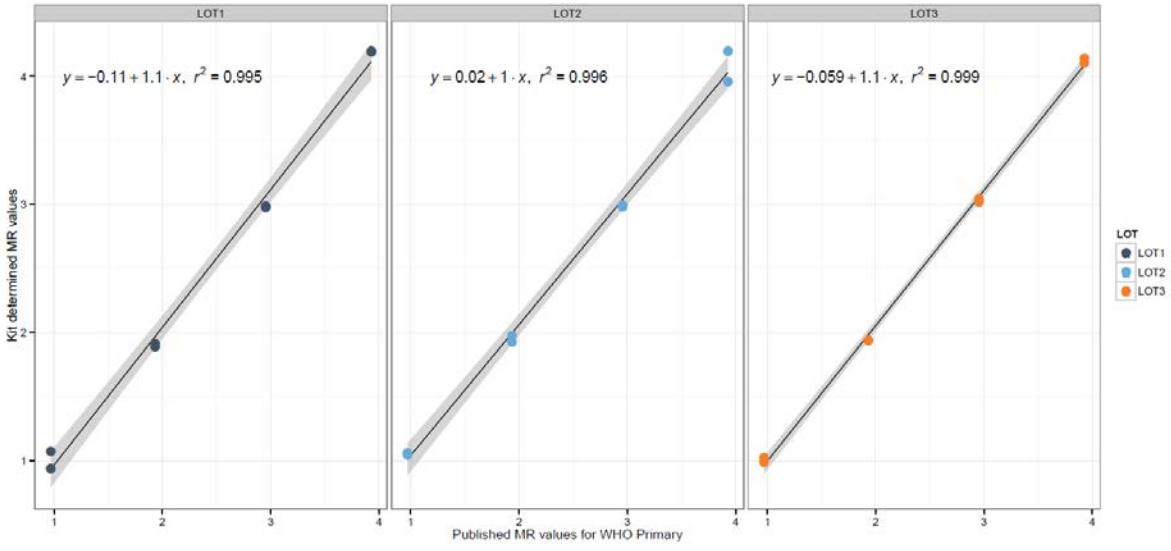


Figure 4: Measured vs. Published Values for WHO Standards by Lot



ii. Reagent Stability:

a. Real-time Stability:
Reagent shelf life stability studies were conducted using 3 lots with testing ongoing at T0, T3, T6, T9, T12, and T13 months. At each time point 15 replicates are tested (5 replicates/lot). Samples are total RNA derived from residual CML positive whole blood serially diluted into total RNA from human CML negative whole blood. The 5 samples are diluted to approximately MR 1, 2, 3, 4, and < LoD. Acceptance criteria for each time point and each panel member are results within ± 0.5 MR of the mean MR value of the same panel member from T0. Controls and calibrators must pass acceptance criteria at each time point. Current stability is 6 months.

b. Freeze-thaw Stability:
Total RNA derived from residual BCR-ABL positive whole blood serial diluted into RNA from BCR-ABL negative was used to evaluate the impact of freeze-thaw on reagent performance. Stability testing was performed on 3 assay kit lots, 5 kits from each lot, 1 kit tested per cycle (per calendar week). The acceptance criteria were results within ± 0.5 MR value of time 0 (testing began from a frozen reagent) for up to 5 freeze-thaw cycles for all panel members (MR 1, 2, 3, 4, and negative). The testing met the acceptance criteria and will be labeled for stable up to 4 uses (including freeze-thaw between uses).

c. Shipping Stability:
Three shipping conditions were examined, (1) stable shipping at -15 to -30˚C (2) shipping stress with minimum load with temperature recorders and (3) shipping stress with maximum load with temperature recorders. Samples were the same panel as described in the freeze-thaw study. Shipping stress conditions were as follows:

- 22˚C± 3˚C, 4 hrs then
- 35˚C± 3˚C, 6 hrs then
- 30˚C± 3˚C, 56 hrs then
- 35˚C± 3˚C, 6 hrs then
- Returned to -15 to -30˚C until testing.

Acceptance criteria were the average MR value of each panel member from the shipping stress conditions were within ± 0.5 MR of the same panel member from the stable storage condition. All acceptance criteria were met. The assay is stable following shipment of up to 72 hr on dry ice.

iii. Specimen Stability (Whole Blood Stability):
Testing was performed on human CML positive clinical whole blood specimens. Time points were calculated from time of blood draw.

Receipt time of specimens was used as the baseline time point. RNA was isolated after receipt and accessioning and at T48, T60, and T≥72 hrs from whole blood stored at 2-8˚C. Additionally RNA from CML-negative blood was isolated at T4 and again at T24, T48, T60, and T72. Multiple replicates of 13 CML positive specimens were tested at 4 time points. Three CML negative specimens were tested at 5 time points. The acceptance criteria were that all results be within 0.5 MR units from the baseline result. The negative sample was evaluated in terms of the variability of the ABL1 transcript. The acceptance criteria were met supporting that the test may be used with whole blood collected in EDTA and stored at 2-8˚C for up to 72 hrs.

2. Clinical Performance:
To support the indicated use of measuring BCR-ABL1 to ABL1, expressed as a log molecular reduction (MR value) from a baseline of 100% on the International Scale, the sponsor performed a study demonstrating the clinical performance of the QuantideX qPCR BCR-ABL IS Kit in t(9;22) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs). This approach is consistent with multiple studies cited in guidelines reporting the significance of MR status after TKI treatment for the monitoring of CML.

A retrospective, multi-center clinical outcome study was conducted at 3 US sites to provide evidence of the clinically validity of MR3.0 (0.1% IS) as a threshold for event status at 36 months in CML patients. A total of 139 samples from 98 patients were collected at 2 clinical sites according to protocols approved by the Institutional Review Boards (IRBs). Of those enrolled, a total of 137 evaluable samples were available from 96 subjects. Sample inclusion criteria included the following:

- Samples must be in the 12-18 month time frame after the patient started on original or new TKI therapy
- Samples from be from adults diagnosed with CML and started with first line or new TKIs
- After the date of the sample and through to 32-40 months clinical treatment failure and disease progression status information for event determination is required.

The demographic information regarding the 96 subjects is shown in Table 10 below.

Table 10: Demographics of the Clinical Study Population

Statistic	Value
Number of Samples in Analysis Population	137
Age(years)	
N	96
Mean (SD)	46.5 (13.8)
Minimum	19
Median	47.5
Maximum	75

Statistic	Value
Race (n[%])	
American Indian or Alaska Native	1 (1.0%)
Asian	5 (5.2%)
Black or African American	3 (3.1%)
White	83 (86.5%)
Other	1 (1.0%)
Unknown	3 (3.1%)
Gender (n[%])	
Male	53 (55.2%)
Female	43 (44.8%)
Ethnicity (n[%])	
Hispanic or Latino	3 (3.1%)
Not Hispanic or Latino	92 (95.8%)
Unknown	1 (1.0%)
State or Province of Residency	
AK	1 (1.0%)
AZ	1 (1.0%)
BC	1 (1.0%)
CA	6 (6.3%)
CO	1 (1.0%)
FL	3 (3.1%)
GA	1 (1.0%)
IA	1 (1.0%)
ID	4 (4.2%)
MD	1 (1.0%)
MO	1 (1.0%)
NJ	7 (7.3%)
NY	1 (1.0%)
OR	42 (43.8%)
PA	9 (9.4%)
TN	1 (1.0%)
WA	15 (15.6%)
Total	96 (100%)

Outcome evaluation was based on determination of event-free survival at 32-40 months on CML patients tested 12-18 months after starting TKI medication.

The device performance was assessed by the probability of at least one event by the endpoint 32-40 months after initiation of TKI treatment as estimated from the Kaplan-Meier survival function. Acceptance criteria were that these probabilities be statistically significantly different and have point estimates differing by at least 10 percentage points.

The definition of an event included:

- Death by any cause
- The development of accelerated-phase or blast crisis CML

- Loss of complete hematologic response
- Loss of complete cytogenetic response
- Appearance of mutation
- Change in TKI treatment not due to toxicity and not due to the results of another BCR-ABL assay

Results are shown below. Figure 5 shows the event free survival (EFS) curves of subjects by MR status based on clinical decision point: MR < 3 (top line) and MR ≥ 3 (bottom line). Figure 6 shows the EFS curves for all subjects < MR 3 (solid line) with the 95% confidence intervals captured in dotted lines. Figure 7 shows the EFS curves for all subjects ≥ 3 (solid line) with the 95% confidence intervals captured in dotted lines.

Figure 5: EFS by MR Status – MR <3 (top) and MR ≥3 (bottom)

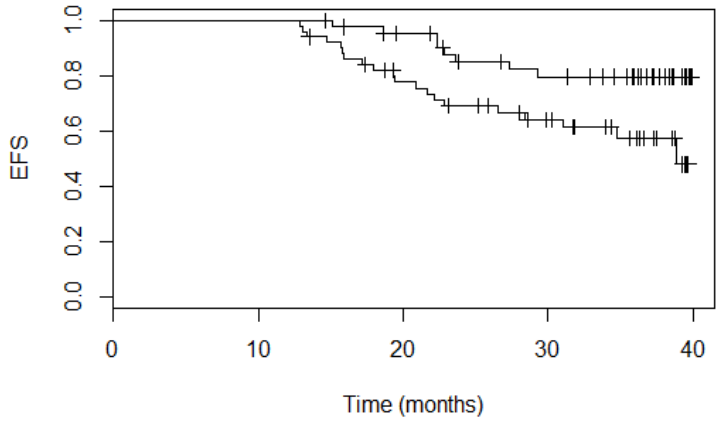


Figure 6: EFS for subjects with MR < 3 (solid line) and 95% CI (dotted lines)

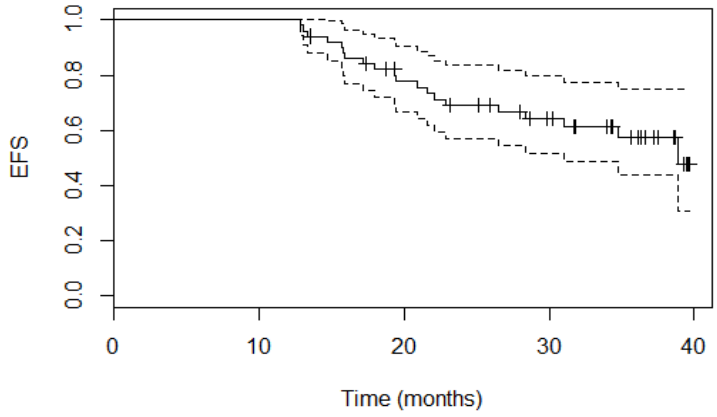


Figure 7: EFS for subjects with MR ≥ 3 (solid line) and 95% CI (dotted lines)

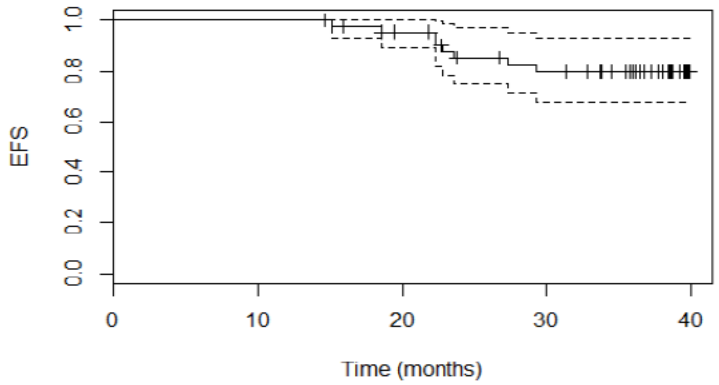


Table 11 below summarizes the EFS estimate difference around MR 3, and demonstrates that the acceptance criteria for the study were met.

Table 11: EFS Estimate Difference by MR 3

	Estimate	se	Z	P	Low CI	High CI
EFS difference	22.2%	10.1%	2.20	0.0279	2.0%	42.4%

An analysis was conducted to compare patients whose event status was known at 36 months versus those who were not. The acceptance criteria were that a 95% Wilson score confidence interval for the difference would be above 0, which were met.

To support the quantitative claim, the association of event hazard was also assessed using the Cox Proportional Hazards (PH) model. The 5 specimens with non-detectable MR readings were assigned either the value of MR 5 or a random value. Both analyses yielded the same results. Table 12 below summarizes the results obtained.

Table 12: Cox PH Model

Coefficient	SE	Hazard rate	Z	P	LR
-0.642	0.175	0.526	-3.68	0.0002	14.58 (P=0.0001)

Finally, results were analyzed using all of the cut-points in addition to the clinical decision cut-point of MR3. Table 13 below summarizes the results for MR 1, 2, 4, and 4.5, in addition to the cut-point MR3. All but the 2 highest thresholds were significant. In summary, the data support the conclusion that the assay is appropriate for use in BCR-ABL (e13a2 and e14a2) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs) around a threshold of MR3 (the pre-specified threshold for clinical use). The clinical validity of thresholds other than MR3 has not been established.

Table 13: EFS Rate by MR Threshold

Split	Chi-squared	P	n	EFS	SE
MR < 1	39.9	0.0000	12	15.6%	13.7%
MR ≥ 1			84	74.4%	5.2%
MR < 2	11.6	0.0007	28	50.1%	9.9%
MR ≥ 2			68	76.0%	5.7%
MR < 3	6.0	0.0139	51	57.5%	7.8%
MR ≥ 3			45	79.7%	6.4%
MR < 4	2.1	0.1520	79	64.8%	6.0%
MR ≥4			17	82.4%	9.3%
MR < 4.5	1.05	0.3050	86	66.9%	5.6%
MR ≥ 4.5			10	80.0%	12.7%

O. Instrument Name: Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument

P. System Descriptions:

1. Modes of Operation:
   The Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument using System Sequence Detection Software v1.4.1is designed to accept 96-well microtiter plates. The instrument outputs quantitative results based on the measured fluorescence signals.

2. Software:
   FDA has reviewed the applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No _____

3. Calibration and Quality Controls:
   See discussion of Traceability in section N.1.f.i above

Q. Other Supportive Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

1. Evidence Supporting the Prognostic Significance of MR 3.0 (0.1% IS) in CML:
   The evidence supporting the prognostic significance of molecular response to TKI therapy in CML is discussed in the NCCN Guidelines Version 1.2016 Chronic Myelogenous Leukemia (https://www.nccn.org/patients/guidelines/cml/files/assets/common/downloads/files/cml.pdf) as outlined below:
   - Page MS-23 Prognostic Significance of Molecular Response to First-line TKI Therapy - Several studies have reported that achievement of a major

molecular response (MMR) after treatment with imatinib is associated with durable long-term cytogenetic remission and a lower rate of disease progression.

- Page MS-26 Prognostic Significance of Molecular Response to Second-line TKI Therapy - The 3-month molecular response after initiation of second-line TKI therapy has also been reported to be a predictor of OS and EFS in patients who are still in chronic phase resistant to imatinib.

2. Verification of ABL1 as Endogenous Control:

Two human RNA specimens serially were diluted 2-fold into water to create a sample set ranging from ~8-2,000 ng/RT. Two lots of assay were used in this testing. Data showed that RNA at approximately 125 ng was the inflection point for assay reporting "Negative (sufficient ABL)" and "Fail (low ABL1)". The acceptance criteria were that the results of testing would all fall into 1 of these 2 categories. The data support the claim that samples with undetectable BCR-ABL1 but with acceptable ABL1 levels may be reported as "Negative (sufficient ABL1)" and that ABL1 can serve as an endogenous internal control.

Table 14: ABL Control Verification

Member #	Predominant BCR-ABL1 Breakpoint	RNA ng/RT	Result of "Negative (sufficient ABL1)" (n)	Result of "Fail (low ABL1)" (n)
1	RNA 1: N/A	2000	6	0
2		1000	6	0
3		500	6	0
4		250	6	0
5		125	3	3
6		63	0	6
7		31	0	6
8		16	0	6
9		8	0	6
10	RNA 2: N/A	2000	6	0
11		1000	6	0
12		500	6	0
13		250	6	0
14		125	4	1
15		63	0	6
16		31	0	6
17		16	0	6
18		8	0	6

R. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the special controls for this device type.

S. Patient Perspectives:

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

T. Identified Risks and Required Mitigations:

Identified Risks to Health	Required Mitigations (See Section V below for Special Controls)
False negative results	Special Controls (1) and (2)
False positive results	Special Controls (1) and (2)
Lack of traceability of results	Special Control (3)

U. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	The QuantideX qPCR BCR-ABL IS Kit is an in vitro nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2. The QuantideX qPCR BCR-ABL IS Kit is a reverse transcription-quantitative PCR performed on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument and is intended to measure BCR-ABL1 to ABL1, expressed as a log molecular reduction (MR value) from a baseline of 100% on the International Scale, in t(9;22) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs). The test does not differentiate between e13a2 or e14a2 fusion transcripts and does not monitor other rare fusion transcripts resulting from t(9;22). This test is not intended for use in the diagnosis of CML. This kit has significant benefit in that it allows for quantification of the BCR-ABL1 transcript in CML patients with the e13a2 or e14a2 fusion during treatment. Performance was assessed by the probability of at least one event by the endpoint 36 months after initiation of their current TKI treatment as estimated from the Kaplan Meier survival function. A total of 139 samples from 98 patients were collected and enrolled in the clinical trial at 2 clinical sites (OHSU and Hospital of the University of Pennsylvania). Performance of the test was assessed by the probability of at least one event by the endpoint 36 months after initiation of TKI treatment as estimated from the Kaplan Meier survival function. The primary endpoint set the null hypothesis as the event probabilities of the group attaining MR3.0 and of the group not attaining MR3.0 is equal. The alternative is that the 36-month event-free probability of the group attaining MR3.0 is greater than those not attaining MR3.0. The success criteria for the primary endpoint were a difference whose estimate exceeded 10 percent, and which was significantly different from

	zero at the one-sided 2½ % level. Both requirements have been met: the EFS difference was 22.2% (95% CI: 2.0%-42.4%) and the p-value was 0.0279.
Summary of Risk(s)	There is minimal potential risk associated with use of this device given the combination of required general controls and special controls. This device is not a qualitative device, but a quantitative device for the levels of BCR-ABL1 and ABL1 transcripts. There is a possibility that an individual with a low MR value (i.e. MR 4.5) would be assigned a higher MR value (i.e. MR 1.0). False results would lead to inappropriate conclusions about the patient status; however, in this case, it is likely that the patient would get repeat testing for BCR-ABL1, either immediately or during longitudinal monitoring; in either case, this scenario would not affect the patient receiving therapy. Additionally, the patient would possibly get testing for mutations in BCR-ABL1 to determine if increasing BCR-ABL transcripts is due the development of resistance markers associated poor response to the TKI therapy. Another possibility is that an individual with a high MR value (MR 1.0) would be assigned a low MR value (i.e. MR 4.0). In this case, false results may lead to conclusions that the patient is responding to therapy. However, patients are retested at regular intervals. Additionally, the patient may miss the opportunity to get tested for mutations in BCR-ABL1. In addition, there are special controls in place based on the analytical performance of the device and traceability to international standards to mitigate these possibilities. Finally, the test may yield no result due to presence of the wrong translocation for this assay. In this case, the clinician would order a FISH assay and the translocation would be appropriately typed and the disease would be followed by other assays. In addition, special controls, in particular labeling requirements, mitigate these possibilities.
Summary of Other Factors	The ordering physician should be certain that the patient has the e13a2 or e14a2 fusion transcripts before ordering this test.
Conclusions Do the probable benefits outweigh the risks?	Yes, the probable benefits of this device, which allows for quantitation of the BCR-ABL1 and ABL1 transcripts, outweigh the potential risks, given that the combination of required general controls and special controls established for this device.

V. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.6060. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code:	OYX
Device Type:	BCR-ABL Quantitation Test
Class:	II (special controls)
Regulation:	21 CFR 866.6060

a. *Identification*. A BCR-ABL Quantitation Test is an reverse transcription-quantitative polymerase chain reaction (RT-qPCR) test for the quantitation of BCR-ABL1 expressed on the International Scale and control transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs). This test is not intended for the diagnosis of CML.

b. *Classification*. Class II (special controls). A BCR-ABL Quantitation Test must comply with the following special controls:
    1. Premarket notification submissions must include the following information:
        i. The indication for use must indicate the variant(s) for which the assay was designed and validated, for example BCR-ABL e13a2 and/or e14a2.
        ii. A detailed description of all components in the test, including the following:
            (A) A detailed description of the test components, all required reagents, instrumentation and equipment, including illustrations or photographs of non-standard equipment or methods.
            (B) Detailed documentation of the device software including, but not limited to, standalone software applications and hardware-based devices that incorporate software.
            (C) Methodology and protocols for control procedures for the assay to allow reporting on the International Scale.
            (D) A description of the result outputs, analytical sensitivity of the assay, and the range of values that will be reported.
            (E) A description of appropriate internal and external controls that are recommended or provided. The description must identify those control elements that are incorporated into the testing procedure.
        iii. Information that demonstrates the performance characteristics of the test, including:
            (A) For indications for use based on a threshold established in a predicate device of this generic type, device performance data from either a method comparison study to the predicate device or through a clinical study demonstrating clinical validity using well-characterized prospectively or retrospectively obtained clinical specimens, as appropriate, representative of the intended use population.
            (B) For indications for use based on a threshold not established in a predicate device of this generic type, device performance data from a clinical study demonstrating clinical validity using well characterized prospectively or

retrospectively obtained clinical specimens, as appropriate, representative of the intended use population.

(C) Device reproducibility data generated, using a minimum of three sites, of which at least two sites must be external sites, with two operators at each site. Each site must conduct a minimum of 3 runs per operator over non-consecutive days evaluating a minimum of 5 different BCR-ABL concentrations that span and are well distributed over the measuring range and include MR3 (0.1% IS). Results shall be reported as the standard deviation and percentage coefficient of variation for each level tested. Pre-specified acceptance criteria must be provided and followed.

(D) Device precision data using clinical samples to evaluate the within-lot, between-lot, within-run, between run, and total variation.

(E) Device linearity data using a dilution panel created from clinical samples.

(F) Device analytic sensitivity data, including limit of blank, limit of detection, and limit of quantification.

(G) Device specificity data, including interference and cross-contamination.

(H) Device stability data, including real-time stability of samples under various storage times, temperatures, and freeze-thaw conditions

iv. Identification of risk mitigation elements used by your device, including a detailed description of all additional procedures, methods, and practices incorporated into the instructions for use that mitigate risks associated with testing using your device.

2. Your 21 CFR 809.10 compliant labeling must include the following:
   i. The intended use in your 21 CFR 809.10(a)(2) and 21 CFR 809.10(b)(2) complaint labeling must include an indication for use statement that reads “This test is not intended for the diagnosis of CML.”
   ii. A detailed description of the performance studies conducted to comply with section b.1.iii. and a summary of the results.
3. Your device output must include results on the International Scale (%IS) and your assay must include multi-point calibration controls traceable to a relevant international reference panel (e.g., the World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL mRNA).