510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
ASSAY AND INSTRUMENT COMBINATION

A. 510(k) Number:

K181661

B. Purpose for Submission:

New Device

C. Measurand:

BCR-ABL1 and ABL1 transcripts

D. Type of Test:

Reverse transcription, quantitative, digital droplet polymerase chain reaction (ddPCR) based nucleic acid amplification

E. Applicant:

Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:

Trade Name: QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System
Common Name: BCR-ABL1 Digital PCR Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.6060
   21 CFR 862.2570

2. Classification:

   Class II

3. Product code:

   OYX

   PHG

4. Panel:

   88 – Pathology

H. Indications for Use:

1. Indications for use:

   The QXDx™ 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 QXDx BCR-ABL %IS Kit is a reverse transcription-quantitative PCR performed on the Bio-Rad QXDx™ AutoDG™ ddPCR System 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 QXDx BCR-ABL %IS Kit is intended for use only on the Bio-Rad QXDx AutoDG ddPCR System.

   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:

   QXDx Automated Droplet Generator
   QXDx Droplet Reader
   QXDx Software 1.0

I. Device Description:

The QXDx BCR-ABL %IS Kit components include the BCR-ABL1 Major (p210) and reference gene assays, iScript reverse transcription reagents, and QXDx reagents which are provided to the end user in a reagent pack. Each reagent pack includes supplies sufficient for approximately 96 results including calibrator checks and controls. A description of the reagents provided with the kit are described below in Table 1.

Table 1. Reagents in the QXDx BCR-ABL %IS KIT

Item	Description	Use
QXDX™ BCR-ABL primers & probes	Deoxyoligonucleotide primers and dye- and quencher- conjugated probes.	Provides primers and probes for ddPCR amplification and detection of target sequences.
QXDx™ Nuclease Free Water	Nuclease Free Water	Adjust volume of RT & ddPCR reactions
QXDX™ iScript Advanced Reverse Transcriptase	Reverse Transcriptase	Generate cDNA from RNA template
QXDx™ 5x iScript Select Reaction Mix	Buffer for Reverse Transcriptase with salts, dNTPs	Reaction mix component of the RT reaction to generate cDNA from RNA template
QXDx™ RT Primers	Reverse Transcriptase Primers	Random Primers used to prime the RT reaction to generate cDNA from RNA template
QXDX™ 2X ddPCR™ Supermix	DNA polymerase, salt buffer, dNTPs	Catalyzes the amplification of primers hybridized to templates from the cDNA. Enzyme exonuclease activity degrades hybridized probes to release fluorescence for detection of amplicons in each PCR cycle.
QXDx™ BCR-ABL ~0.1%IS	BCR-ABL and ABL RNA formulated to approximately 0.10% BCR-ABL/ABL	Per run controls to check against acceptance criteria for use of electronic WHO-IS CF factor and reporting of WHO-IS value results
QXDx™ BCR-ABL ~10%IS	BCR-ABL and ABL RNA formulated to approximately 10% BCR-ABL/ABL	Per run controls to check against acceptance criteria for use of electronic WHO-IS CF factor and reporting of
QXDx™ BCR-ABL Neg-CTRL	ABL RNA	Control used to ensure that RT and ddPCR steps performed properly and identify falsely positive results due to contamination
QXDx™ BCR-ABL H-CTRL	BCR-ABL and ABL RNA formulated to approximately 18% BCR-ABL/ABL	Control used to ensure that RT and ddPCR steps performed properly by generating expected MR value
QXDx™ BCR-ABL L-CTRL	BCR-ABL and ABL RNA formulated to approximately .03% BCR-ABL/ABL	Control used to ensure that RT and ddPCR steps performed properly by generating expected MR value

Instrument
The QxDx AutoDG Dx Digital Droplet ddPCR System consists of two instruments, the QxDx Automated Droplet Generator and the QxDx Droplet Reader, and their associated consumables.

The QXDx Automated Droplet Generator partitions samples into approximately 20,000 nanoliter-sized droplets and, after PCR on a thermal cycler; droplets (PCR-positive and PCR-negative) from each sample are counted and analyzed individually on the QXDx Droplet Reader to provide direct quantification of nucleic acid in digital form.

Software:
QXDx Software is used to analyze all test results. This software is provided with the QXDx AutoDG ddPCR System.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   QuantideX qPCR BCR-ABL IS Kit
   Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software

2. Predicate 510(k) number(s):

   DEN160003
   K141220

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate
Intended Use	The QXDx™ 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 QXDx BCR-ABL %IS Kit is a reverse transcription-quantitative PCR	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

Similarities		
Item	New Device	Predicate
	performed on the Bio-Rad QXDx™ AutoDG™ ddPCR System 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.	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.
Measurement Type	Quantitative	Same
Measuring Range	MR 0.3 to MR 4.7	Same
Specimen Type	RNA from whole blood (EDTA)	Same
Anti-coagulant	EDTA	Same
Traceability	1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR	Same
Reporting Units	Both %IS and Molecular Response (MR)	Same

Differences		
Item	Device	Predicate
RNA Input	1000 ng	RNA input range of 1 to 5µg

Differences		
Item	Device	Predicate
Calibrators	Two levels formulated at 0.1% and 10% BCR-ABL/ABL.	Four levels formulated to MR1.0, 2.0, 3.0, 4.0.
Quality Controls	3 levels of external control RNA High (%IS of 18) RNA Low (%IS of 0.03) RNA Negative	3 levels of external control reagents: RNA High (MR 1.5) RNA Low (MR 3.5) ABL armored RNA RNA Negative
Fundamental Technology	Digital PCR	Real-Time PCR
Instrument	Bio-Rad QXDx™ AutoDG™ ddPCR System	Applied Biosystems 7500 Fast Dx Real Time PCR Instrument
Instrument Computer Operating System	Microsoft Windows 10	Microsoft Windows 7
Degree of Automation	Same, except amplification functionality is not included	Requires manual transfer of amplification mixture to amplification/detection instrument. Automated control of amplification, detection and data analysis
Primary Operational Amplification and Detection Components	Amplification functionality is not included. Nanoliter droplet fluorimeter for walk away PCR detection.	Integrated thermal cycler and microvolume fluorimeter for walk away PCR amplification and detection
Amplification Reaction Volume	20-25 μL in 96-well Bio-Rad PCR plates	10-30 μL in 96-well Fast PCR plates

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP7-A2, Interference Testing in Clinical Chemistry
CSLI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

L. Test Principle:

The QXDx™BCR-ABL %IS test uses random primed reverse transcription in combination with Droplet Digital PCR technology to quantify BCR-ABL fusion transcripts e13a2 (b2a2) and e14a2 (b3a2) and ABL transcripts in total RNA extracted from human peripheral blood. Testing begins with total RNA purified from EDTA-anticoagulated peripheral whole blood

specimens. The RNA sample and iScript reverse transcription reagents are combined to produce complementary DNA (cDNA), which is then added to the ddPCR Supermix to prepare the PCR-ready sample. BCR-ABL primers and probes are designed for the detection of BCR-ABL p210 (b2a2 and b3a2) major breakpoint translocation and the ABL primers are designed for the detection of the ABL sequence.

A total of 25 microliters of the PCR-ready sample is loaded into a 96-well PCR plate. The plate, as well as required consumables (Automated Droplet Generation Oil for Probes, DG32 Cartridges w/ Gaskets and ddPCR Pipet Tips) are loaded into the QXDx Automated Droplet Generator. The QXDx Automated Droplet Generator uses microfluidics to combine oil and aqueous sample to generate the nanoliter-sized droplets required for ddPCR analysis. The 96-well PCR plate containing droplets from the QXDx Automated Droplet Generator is sealed with foil and a plate sealer, and thermal cycled to end point (~40 cycles) using a thermal cycler. Each plate must contain 4 run controls and two %IS calibrator checks. The thermal cycled plate is then loaded into the QXDx Droplet Reader. The Droplet Reader singulates the droplets and streams them in single file past a two-color detector. The detector reads the droplets to determine which contain target (positive) and which do not (negative) and measure the positive and negative droplets in the FAM and HEX fluorescent channels. The ddPCR is performed in duplicate on all samples, controls and calibrator checks.

The QXDx Droplet Reader connects to a laptop computer running QXDx Software. The software provides measured levels of BCR-ABL and reference gene, to calculate the(BCR-ABL/ABL concentration ratio, as well as check the values for acceptable limits for controls and the %IS calibrator checks.

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 QXDx BCR-ABL %IS Kit will report the value as MRx.x. The MR value is the log10 reduction from the internationally standardized baseline, defined as 100% IS. Therefore,

$$MRx.x = \log10(100/\%IS) = \log10(100) - \log10(\%IS) = 2 - \log10(\%IS)$$

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 show below:

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

The results are interpreted automatically by the QXDx Software from measured droplet counts, fluorescent signals, and embedded calculation algorithms that report out BCR-ABL and ABL copies and displayed in the "View Results" window with MR, %IS, and copies. An indication of sample suitability is indicated as the ABL1 copies Sufficient to MRx.x column.

There are 7 possible statuses for results as identified below:

ABL1	**BCR-ABL1**	**Result Reporting**
Sufficient to MRx.x	Positive, above range	BCR-ABL detected above LOQ
Sufficient to MRx.x	Positive, in range	BCR-ABL detected at x.xxxx %IS Molecular Response Level (Log10) = x.x Sufficient ABL1 copies for MR x.x
Sufficient to LoD	Positive, below LoQ	BCR-ABL detected below LOQ Sufficient ABL1 copies for MR x.x
Sufficient to LoD	Undetected	BCR-ABL Not Detected Sufficient ABL1 Detected to LOD
Sufficient to MRx.x	Undetected	BCR-ABL Not Detected Sufficient ABL1 Copies for MR x.x
Insufficient	Any	Fail Insufficient ABL1 Copies
Undetected	Any	Fail No ABL1 copies detected

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision and Reproducibility were assessed using 2 panels of 6 test samples each plus 2 controls (18% and 0.03% IS) tested in 36 replicates. The samples were prepared by mixing 12 independent pools of RNA isolated from BCR-ABL negative whole blood specimens with 6 independent pools of RNA isolated from BCR-ABL positive whole blood specimens, representing both e13a2 and e14a2 variants, in BCR-ABL:ABL ratios at MR1.0, MR2.0, MR2.5, MR3.0, MR3.5, and MR4.0. The control samples were prepared by mixing RNA isolated from a BCR-ABL negative cell line and RNA isolated from a BCR-ABL positive cell line (e13a2 variant).

Samples were assayed in 2 replicates per run for 2 runs per day for 3 non-consecutive days at 3 sites (one instrument at each site) with one reagent lot for a total of 36 replicates. Each run was performed by an independent operator (2 operators per site). At MR3.0, %CV was required to be <15% for each precision study (i.e., Within run, Between instrument, Between day, Between operator), and for other levels, total CV must be:

- LoQ: ≤50%
- MR0.3-2.49: ≤ 10%
- MR2.5-3.49: ≤ 20%
- MR3.5-4.0: ≤ 50%

A total of 576 observations were included in a variance components analysis with random effects for site, day, and run (operator) to assess repeatability, within-day precision, within-site precision, and reproducibility of measured MR level. Results indicated very low variability, including within sites, and all acceptance criteria were satisfied (all CVs were < 15%). Total MR and %IS precision were calculated for the assay (Table 2) and in-kit calibrators and controls (Table 3). The acceptance criteria were met.[1]

Table 2. Reproducibility Variance Components Modeling

MR Bin	Target MR	Variant	Variance Components			Total Variance	Mean MR	Within-Run SD	Within-Day SD	Within-Site SD	Between-Site SD
			Site	Day	Run(Op)						
1.0	0.7	e13a2	0.000	0.000	0.000	0.000	0.70	0.000	0.000	0.000	0.000
	1.0	e13a2	0.000	0.000	0.001	0.002	0.98	0.037	0.044	0.044	0.044
	1.0	e14a2	0.000	0.000	0.001	0.002	1.03	0.037	0.046	0.046	0.047
	1.2	blended	0.002	0.000	0.000	0.004	1.35	0.037	0.043	0.043	0.061
2.0	2.0	blended	0.000	0.000	0.000	0.001	1.99	0.022	0.022	0.022	0.024
	2.0	e14a2	0.000	0.000	0.001	0.002	2.08	0.037	0.044	0.044	0.045
	2.4	e13a2	0.000	0.000	0.001	0.002	2.42	0.037	0.047	0.047	0.047

[1] Precision below MR4.0 cannot be assured. The assay is not indicated for discontinuation from TKIs or for monitoring after discontinuation.

MR Bin	Target MR	Variant	Variance Components			Total Variance	Mean MR	Within-Run SD	Within-Day SD	Within-Site SD	Between-Site SD
			Site	Day	Run(Op)						
	2.5	e13a2	0.000	0.000	0.000	0.000	2.50	0.017	0.017	0.017	0.017
3.0	2.7	blended	0.000	0.000	0.000	0.002	2.78	0.033	0.037	0.043	0.043
	3.1	e14a2	0.001	0.000	0.001	0.004	3.14	0.047	0.055	0.055	0.062
	3.2	blended	0.000	0.000	0.000	0.004	3.29	0.064	0.064	0.064	0.064
3.5	3.7	e13a2	0.000	0.000	0.000	0.005	3.44	0.074	0.074	0.074	0.074
	3.5	e14a2	0.000	0.000	0.001	0.005	3.54	0.062	0.073	0.073	0.073
	3.4	blended	0.000	0.000	0.000	0.009	3.62	0.091	0.093	0.093	0.093
	3.7	e14a2	0.001	0.002	0.000	0.010	3.70	0.084	0.084	0.092	0.098
4.0	4.0	e14a2	0.000	0.009	0.000	0.026	4.14	0.132	0.132	0.162	0.162
	4.0	blended	0.000	0.000	0.000	0.038	4.16	0.195	0.195	0.195	0.195
	4.2	e13a2	0.003	0.000	0.005	0.042	4.23	0.183	0.197	0.197	0.205

Table 3. QXDx™ BCR-ABL %IS Kit Precision Data – Calibrator Checks and Controls

Sample ID	Target MR Level	N	MR (Observed)			Target Level (% IS)	% IS (Observed)		
			Mean	SD	% CV		Mean	SD	% CV
H-CTRL	1.0	40	0.70	0.0000	0	20	19.1925	0.6773	3.5
~10% IS	1.0	38	1.13	0.0460	4.1	10	7.2887	0.3801	5.2
~0.1% IS	3.0	38	3.09	0.0578	1.9	0.1	0.0827	0.0087	10.6
L-CTRL	3.5	40	3.50	0.0733	2.1	0.03	0.0321	0.0048	15.0
Neg-CTRL	<LOD	40	n/a*			0	0	0	n/a
NTC	N/A	40	n/a			n/a	n/a		
* Log of 0%IS cannot be calculated									

Lot-to-lot Precision

Sixteen (16) BCR-ABL1 negative whole blood and 100 BCR-ABL1 positive RNA samples representing both the e13a2 and e14a2 variants were procured from commercial vendors. The positive RNA samples were pooled to create 6 positive patient RNA pools. Each positive pool was then used to create a minimum of 15 samples each of which was unique to one pool. Specimens were evaluated at MR1.0, MR2.0, MR2.5, MR3.0, MR3.5, and MR4.0. Testing spanned 3 kit lots, 2 instruments, 2 operators, 3 replicates over 3 days for 108 data points per sample. The mean, standard deviation (SD) and %CV were calculated for each sample according to CLSI EP5-A2 prescribed methods for data analysis. Calibrators and controls were run singly on each plate but generated a total of

36 replicates. At MR3.0, %CV was required to be <15% for each precision study (i.e., Within run, Between instrument, Between day, Between operator), and for other levels, total CV must be:

- LoQ: ≤50%
- MR0.3-2.49: ≤ 10%
- MR2.5-3.49: ≤ 20%
- MR3.5-4.0: ≤ 50%

• Reagent lots must be interchangeable and yield BCR-ABL ratios within precision requirement for each level

Table 4 below shows that precision was acceptable for each precision study and for each BCR-ABL level based on both %CV and SD. Table 5 shows precision for the calibrator checks.

Table 4. QXDx™ BCR-ABL %IS Kit and System Precision Data – Patient and Control Samples – MR Analysis

Sample ID	N	% Positive	Mean MR	Within Run		Operator		Day		Lot		Instrument	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
MR1	108	100	1.40	0.022	1.60	0.013	0.94	0.010	0.70	0.017	1.20	0.007	0.53
MR2	108	100	2.47	0.038	1.56	0.006	0.23	0.011	0.45	0.027	1.11	0.004	0.19
MR2.5	108	100	2.80	0.046	1.65	0.008	0.30	0.003	0.01	0.013	0.46	0.000	0.00
MR3	108	100	3.31	0.080	2.42	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00
MR3.5	108	100	3.63	0.103	2.83	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00
MR4	108	99	4.13	0.162	3.94	0.011	0.29	0.000	0.00	0.000	0.00	0.012	0.28
MR4-5	108	89	4.65	0.242	5.22	0.000	0.00	0.000	0.00	0.042	0.90	0.000	0.00
IHC 17%	108	100	0.73	0.068	0.93	0.000	0.00	0.000	0.00	0.018	2.40	0.057	0.78

Table 5. QXDx™ BCR-ABL %IS Kit Precision Data – Calibrator Checks and Controls

Sample ID	Target MR	N	Specifi-cation	Mean MR Value	MR Total Precision			Target % BCR-ABL
					SD	%CV	Status	
BCR-ABL 0.1%IS	3	36	≤ 20%	3.07	0.06	2.09	Pass	0.1
BCR-ABL 10%IS	1	36	≤ 10%	1.11	0.04	3.65	Pass	10
BCR-ABL H-CTRL	1	36	≤ 10%	0.70	0.02	2.19	Pass	20
BCR-ABL L-CTL	3.5	36	≤ 20%	3.47	0.07	2.11	Pass	0.03
BCR-ABL Neg-CTRL	<LOD	N/A	N/A	Cannot log transform 0 value				0

**%CV calculations done with full precision*

Extraction Method Comparison
The performance and sensitivity of the QXDx™ BCR-ABL %IS Test is dependent on the RNA quantity, purity and integrity. The purpose of this study was to demonstrate equivalency of three (3) commercial RNA extraction methods for use with the QXDx™ BCR-ABL %IS Kit. Samples were extracted using these 3 methods must yield mean BCR-ABL ratios within 95% CI of ≤ 0.5 log difference. A single positive CML blood sample arrived untested within 24 hrs of draw with the starting MR level unknown. Four dilutions were created in an attempt to capture the assay range, above and below the clinical decision point of MR3. One (1) BCR-ABL1 positive whole blood CML patient sample was mixed with one BCR-ABL negative whole blood patient sample and diluted four (4) logs. Each test sample was divided into 3 aliquots and assigned to one of three (3) extraction methods.

The three extraction kits demonstrated equivalent performance of the extraction methods for use in the QXDx™ BCR-ABL %IS Kit when following the target specifications below:

RNA Specifications

Category	Target Specification
Whole Blood Volume	5 – 10 mL
Nucleated Cell Count	≥ 1E+07
Concentration of isolated RNA	~ 100 ng/µL
Yield of isolated RNA	≥ 1000 ng
RNA Purity	$A_{260}/A_{280} > 1.6$ $A_{260}/A_{230} > 1.2$

b. Linearity/assay reportable range:
Two (2) positive BCR-ABL RNA patient pools were prepared by mixing RNA extracted from BCR-ABL positive whole blood. The positive specimens were diluted in a pool of 15 negative specimens to create 9 levels ranging from MR0.3 to MR4.7 with 4-8 replicates each. Pool 1 contained RNA from five patients positive for the E13a2 variant and Pool 2 contained RNA from five patients positive for the E14a2 variant. The slope was required to be 0.8-1.2 and the correlation coefficient had to be 0.97 to 1.0.

The QXDx™ BCR-ABL %IS Kit is linear throughout the measuring range of 50%-0.002% in %IS ratio, and MR0.3-MR4.7 in log-space. Variant e13a2 had a measured range of MR0.3 to MR5.32 with a maximum SD of 0.21. Variant e14a2 had a measured range of MR0.3 to MR4.73 with a maximum SD of 0.13. Additionally, 2nd and 3rd-order polynomial regression fits were assessed. Deviation from first order linearity was within acceptable limits. Linearity of MR result was demonstrated from at least MR 0.3 (50%IS) to MR 4.7 (0.002%IS). Results are shown in Table 6.

Table 6. Linearity/Dynamic Range for %BCR-ABL/ABL

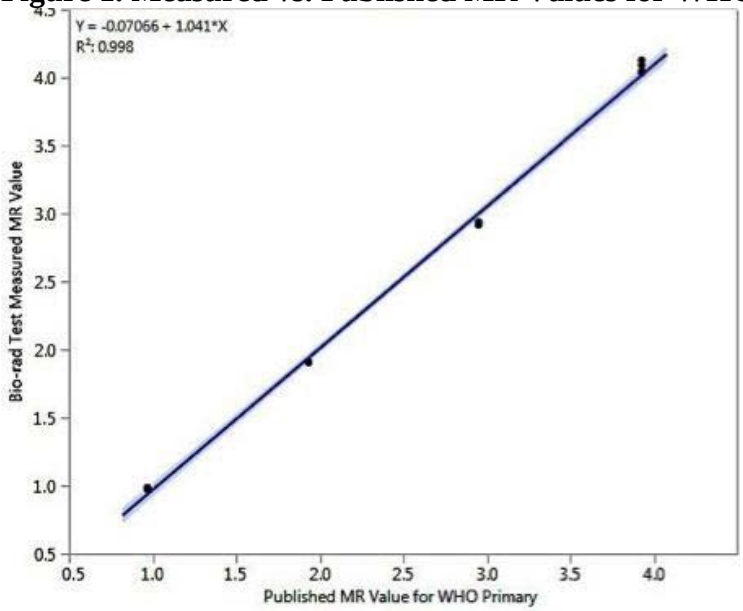
Breakpoint	Linearity/ Dynamic Range	Linearity/ Dynamic Range	Slope (m)	Intercept (y)	R^2 - Value
e13a2	50% IS to 0.002% IS	MR 0.3 – MR 4.7	1.04	0.058	0.996
e14a2	50% IS to 0.002% IS	MR 0.3 – MR 4.7	1.01	0.177	0.993

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Verification of WHO Standard Quantification

Traceability to the 1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RT-qPCR was demonstrated by measuring the WHO Reference Panel with seven independent QXDx™ BCR-ABL %IS Kit lots and comparing the measured values to the values published in the Reference Panel's Instructions for Use. Each of the 4 WHO Reference Panel members was tested in 4 replicates across 8 runs (1 run per lot for 6 lots and two runs per lot for one lot). The measured MR values for each level of the WHO Reference Panel were adjusted by a common derived correction factor, CF=0.93. The measured MR values were compared to the published MR values through a regression analysis to determine slope and intercept values. The analysis showed correlation with R2 values of 0.992-0.999. The slope of the regression lines varied between 0.978 and 1.04, and the intercepts were between -0.07 and 0.059. An example of one kit lot is shown in Figure 1.

Figure 1. Measured vs. Published MR Values for WHO International Standard



d. Detection limit:

Limit of Blank

The limit of blank was determined by testing 36 independent BCR-ABL negative blood samples by 1 operator using 2 kit lots. Out of 144 tests, 141 had no detectable BCR-ABL values. Three had measurements below the LoD of the test and were reported as "Detected below LLoQ".

Limit of Detection/Limit of Quantification

Three (3) positive BCR-ABL RNA patient sample pools were prepared Pool 1 used a mix of 15 patients positive for the e13a2 and/or e14a2 variants. Pool 2 contained 5 patients positive for the e13a2 variant and Pool 3 contained 5 patients positive for the e14a2 variant. One negative BCR-ABL RNA sample pool was prepared and used in the dilution of the positive patient sample pools. In addition, two (2) contrived positive BCR-ABL RNA samples were prepared. Pool 1 contained five (5) patients positive for the e13a2 variant and Pool 2 contained five (5) patients positive for the e14a2 variant. Both pools were diluted with the same negative pool containing 9 BCR-ABL negative patients.

The calculated LoD was found to be 0.002% IS BCR-ABL or MR 4.7. In addition to the LoD analysis, the LoQ range was tested by calculating %CV for each variant and lot. The %CV values were sorted by target IS concentration and the lowest %IS ratio with a CV less than the minimum cut-off of 76% was determined to meet or exceed the criteria of 0.01%. The LoQ was determined to be 0.003% IS BCR-ABL/ABL or MR4.56 with a target criteria %CV <76%. The studies met all acceptance criteria and support an LoD of 4.7 and LoQ of 4.56 for the assay. (See footnote 1)

e. Analytical specificity:

Interference

A sample pool was prepared by mixing whole blood from a CML positive and CML negative patient. The potential interfering substance was added to the test pool and diluent was added to the control pool in concentrations recommended by CLSI EP7-A2. For both the control and test samples, 5 replicate extractions were performed and each extracted sample was tested in replicates of 2 for a total of 10 tests per sample type. For the MR values, the mean test MR value needed to fall within the 95% confidence interval plus or minus 0.5 log. In all cases, samples passed the acceptance criteria as shown in the table below. For the %IS data, the 95% confidence interval of the mean %IS for test samples needed to intersect the within run precision range for control samples. In all cases, not only did the test 95% confidence interval intersect the control precision range, but also the test mean %IS fell within the within run precision range, passing the acceptance criteria. The potential interfering substances evaluated were cholesterol, conjugated and inconjugated bilirubin, EDTA, hemoglobin, sodium heparin, triglycerides, guanidinium- containing lysis buffer, ethanol, phenol, final wash buffer, and genomic DNA. Results demonstrated that these endogenous and exogenous substances tested did not impact test results as compared to control.

Primer Specificity

Two (2) samples were prepared by blending in-vitro transcribed p190 or p230 with RNA extracted from normal, healthy, human blood from 2 donors. Four (4) dilutions of each sample were prepared by varying the amount of negative RNA used. The QXDx™ BCR-ABL %IS Kit performed as intended detecting only the major e13a2 and e14a2 (p210) variants as shown in the table below. Test specificity was 100% thus exceeding the required specification of ≥90%. Results in Table 7 support that the kit does not detect the minor e1a2 (p190) and micro e19a2 (p230) variants even when present in high concentrations.

Table 7. Primer Specificity Results

Variant Sample ID	Dilution Number	N	Mean Specific Assay Ratio %	% CV Specific Assay Ratio	Mean QXDx BCR-ABL %IS	% CV QXDx BCR-ABL %IS
p190	1	4	36.1329%	2.1%	0.000%	0.000%
	2	4	0.7148%	5.3%	0.000%	0.000%
	3	4	0.0749%	10.7%	0.000%	0.000%
	4	8	0.0080%	42.8%	0.000%	0.000%
p230	1	4	31.7429%	1.1%	0.000%	0.000%
	2	4	0.6207%	2.5%	0.000%	0.000%
	3	4	0.0630%	11.0%	0.000%	0.000%
	4	8	0.0056%	63.4%	0.000%	0.000%

Carryover Contamination

Two 96 well plates were set up with high positive at MR0.2 and negative wells in alternating rows. A total of 4 rows each containing 12 replicates or possible 48 carry over events were tested per plate. Two (2) plates were tested on each of 3 QXDx™ Droplet Reader instruments for a total test sample size of 288. Testing was conducted per the QXDxTM BCR-ABL %IS Kit IFU. Of the 286 replicates used in the analysis, signal was measured in only one negative well. One well contained 1 copy of BCR-ABL and 0 copies of ABL. No signal was measured in the remaining 285 negative wells. Acceptance criteria were met which demonstrates that the device does not generate significant carryover between wells.

RNA Input

A study was conducted to demonstrate the acceptable RNA input for the QXDx BCR-ABL %IS Kit. Four (4) RNA samples were created by mixing RNA extracted from1 negative donor blood with 4 RNA pools, consisting of 2 patients each, extracted from CML+ donor blood. Samples targeted MR levels from 1-4. Samples were diluted to 200 ng/µL and varying volumes were tested with the QXDx BCR-ABL %IS Kit targeting RNA inputs from 125ng to 1500ng according to the IFU. Samples were tested in

replicates of 4 or 8. Table 8 shows RNA inputs from 125ng to 1500ng do not significantly change the reported MR values for the QXDx BCR-ABL %IS kit.

Table 8. QXDx™ BCR-ABL %IS Kit RNA Input Study

MRgroup	input (ng)	n_tests	positive_tests	Mean MR	MR SD
MR1	125.0	4	4	0.75	0
MR1	250.0	4	4	0.74	0.01
MR1	500.0	4	4	0.73	0
MR1	1000.0	4	4	0.77	0
MR1	1500.0	4	4	0.83	0.01
MR2	125.0	4	4	2.06	0.04
MR2	250.0	4	4	2.01	0.03
MR2	500.0	4	4	1.99	0
MR2	1000.0	4	4	2.01	0.02
MR2	1500.0	4	4	2.02	0.01
MR3	125.0	8	8	3.06	0.12
MR3	250.0	8	8	3.07	0.05
MR3	500.0	8	8	3.02	0.04
MR3	1000.0	8	8	3.04	0.06
MR3	1500.0	8	8	3.08	0.07
MR4	125.0	8	8	3.77	0.28
MR4	250.0	8	8	3.92	0.12
MR4	500.0	8	8	3.74	0.15
MR4	1000.0	8	8	3.81	0.17
MR4	1500.0	8	8	3.87	0.09

f. Stability Studies

Kit Real-Time Stability

Reagent shelf life stability studies were conducted using 3 lots with testing ongoing at T0, T1, T2.5, T5, T11, T12, T19 and T25 months. Three samples at MR2.0, 3.0, and 4.0 along with calibrators and controls were tested by thawing reagents stored at -20°C at each time point.

The kit performance met the following acceptance criteria:
1. Kit shelf life stability: 12 months at -20°C.
2. Stability performance Indicator: Controls (0.1%IS, 10.0%IS, H-CTRL, L-CTRL, Neg-CTRL) and Sample values must be within pre-established ranges:
   a) Controls must meet within run precision %CV at the defined %BCR-ABL/ABL level

b) The upper or lower confidence interval of the mean must be ≤0.5 log between Time Zero (T0) and Time Final (Tf) readings for controls, calibrators and samples
c) Must have two consecutive failures to be considered failed product/time point

These results support the conclusion that reagents are stable under the storage conditions for 12 months.

Kit Freeze-thaw Stability

A study was conducted to determine the allowable number of freeze-thaw cycles for the components of the QXDx BCR-ABL IS Kit. The study was conducted by cycling kit contents from -20°C to ambient temperature multiple times and assessing the performance of the kit in response to the freeze-thaw cycling. Four QXDx™ BCR-ABL %IS kits from one lot stored at -20°C were used in this study. With the exception of the QXDx™ iScript Advanced reverse transcriptase, all components of one kit were fully thawed at ambient temperature for 15 minutes, all kit component caps were removed, components were held uncapped for 2 minutes, components were capped and materials were returned to -20°C for a minimum of 8 hours prior to the next temperature cycle. The QXDx™ iScript Advanced reverse transcriptase was thawed on an ice block. This cycle was repeated 1, 4 or 8 times. One kit (reference) remained frozen throughout the study duration.

The acceptance criteria were that after each freeze-thaw cycle, controls and calibrators must be within %CV for precision at the defined %BCR-ABL/ABL level and the upper or lower 95% confidence interval of the mean must be ≤0.5 log between control and freeze-thawed samples. Results support that all components of the QXDx BCR-ABL IS kit demonstrated stable performance for at least 5 freeze-thaw cycles.

Specimen Stability

One BCR-ABL positive patient sample (< MR1) and three (3) BCR-ABL negative patient samples were collected in EDTA tubes and shipped to Bio-Rad within 24 hours of the draw date. Each sample was divided into 4 aliquots which was processed immediately or stored at 2-8°C for 1 or 2 additional days. The positive blood sample was serially diluted with a negative blood sample creating dilutions spanning the clinical decision point at each day/timepoint to prevent hemolysis. Each dilution was divided into multiple aliquots and the RNA was extracted. Four replicate extractions were conducted for dilutions 0 and 1 and 11 replicate extractions were conducted for dilutions 2 and 3. Each extracted RNA sample replicate was tested using one (1) lot of the QXDx™ BCR-ABL %IS Ki, by one operator and on one QXDx™ AutoDG ddPCR system as per the kit IFU. For each sample, the allowable range was calculated as the mean MR value obtained on the reference time point (Day 1) plus or minus 0.5 log. For the remaining time points, the mean MR value and the 95% CI were calculated for each dilution. Each time point and sample type was considered to pass if the confidence interval (CI) for each test sample fell entirely within the allowable range.

The results of the study (Table 9) show that blood samples stored for up to 2 days at 2-8°C are stable and produce reliable results with the BCR-ABL %IS Kit. Acceptance

criteria were met.

Table 9. Specimen Stability.

Dilution	Day	Use	n	Mean %IS Value	Mean MR Value	Lower 95% CI	Upper 95% CI	± 0.5 log Allowable Range
0	1	Reference	4	44.9645	0.35	0.31	0.39	0-0.85
	2	Test	4	19.8590	0.71	0.57	0.85	
	3	Test	4	37.2260	0.43	0.39	0.47	
1	1	Reference	4	7.6568	1.12	1.07	1.16	0.62-1.62
	2	Test	4	3.7152	1.44	1.27	1.62	
	3	Test	4	6.5420	1.18	1.15	1.22	
2	1	Reference	11	0.7302	2.14	2.11	2.17	1.64-2.64
	2	Test	11	0.6424	2.19	2.17	2.22	
	3	Test	11	0.6583	2.18	2.16	2.21	
3	1	Reference	11	0.0511	3.32	3.20	3.42	2.82-3.82
	2	Test	11	0.0617	3.24	3.11	3.38	
	3	Test	11	0.0658	3.20	3.12	3.28	

Thermal Cyler Study

The purpose of this study was to verify the performance of the QXDx BCR-ABL IS kit on different PCR devices for both reverse transcription (RT) and droplet digital polymerase chain reaction (ddPCR). It is intended to check the performance on at least 3 instruments, across the assay range. The three Reaction thermal cyclers must yield mean BCR-ABL ratios (MR values) within 95% CI of ≤ 0.5 log difference. Samples were cell line RNAs blended to the desired levels containing both the e13a2 (Meg01) and e14a2 (K562) variants with ABL1 copies (Hela) ranging from ranging from 10% to 0.03% IS and tested in replicates of either 6 or 12. The data emonstrated that the 3 thermal cyclers produced mean BCR-ABL ratios (MR values) within 95% CI of ≤ 0.5 log difference of the Bio-Rad C1000 Touch™ Deep Well demonstrating equivalent performance of the instruments for use in the QXDx™ BCRABL %IS Kit supporting that any thermal cycler is acceptable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was designed to evaluate the performance of the QXDx BCR-ABL %IS Assay compared to the predicate device [Asuragen Quantidex qPCR BCR-ABL IS Kit (IVD)] in RNA derived from human blood samples obtained from individuals previously diagnosed with t(9;22) positive CML, in accordance with the assay

protocol. A total of 155 deidentified samples were retrospectively collected for analysis. Samples were obtained from 2 geographically distinct sites. Of the 155 patient specimens, 16 were either not paired or excluded due to protocol deviations. The remaining 139 samples representing the intended use population and spanning the common dynamic range of the two comparison methods were analyzed at a single testing lab. The samples were collected and stored by the sites using a pre-specified protocol with eligibility criteria that fit this test including a system compatible RNA extraction method. The samples were extracted RNA from peripheral whole blood.

Subject inclusion criteria:

- 18 years of age or older
- Previously diagnosed as t(9;22) positive CML p210 or Major variant type
- No more than one sample per subject

Subject exclusion criteria:

• Other clinically significant leukemias or cancers

Sample inclusion criteria

• Whole blood samples must have been collected and processed to meet the following criteria:

- Processed within 72 hours of collection
- Collection tube type –EDTA
- Storage – not frozen and stored at 2-8^{0}C

• RNA samples must have been extracted and stored to meet the following criteria:

- Stored at –80^{0}C
- No greater than 1 freeze/thaw cycle
- Sample matrix is RNA derived from white blood cells collected from whole blood in EDTA collection tubes

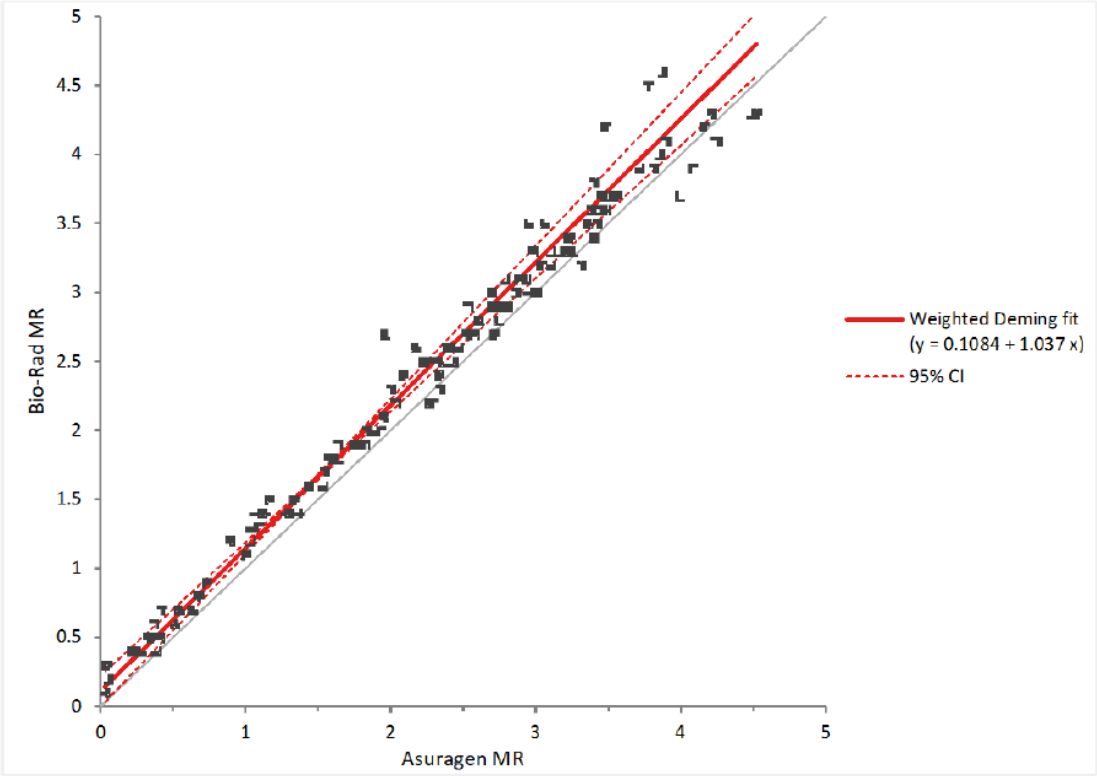
The 139 samples were selected to span the testing range as shown in Table 10 below:

Table 10. Range of clinical specimens in the method comparison study.

Bin	Asuragen	BioRad
Lowest - <1.5	35	31
1.5 - <2.5	34	25
2.5 - <3.5	52	54
3.5 - 4.5	18	29

The mean bias (95%CI) between Bio-Rad and Asuragen using a Bland-Altman was 0.16 (0.14 to 0.19) indicating that the limits of agreement (LOA) between the two methods should lie between 0.14 and 0.19 for 95% of the time. The Bio-Rad QXDx BCR-ABL %IS assay showed correlation with the predicate using a weighted Deming regression with a Pearson R correlation coefficient of 0.99, slope 1.037 (95% CI, 0.96 to 1.11) and intercept was 0.1084 (95% CI, -0.005 to 0.22) as shown in Figure 2 below.

Figure 2. Weighted Deming Regression



Results from the method comparision study demonstrate that the QXDx BCR-ABL %IS assay is substantially equivalent to the predicate.

b. Matrix comparison:
Not applicable

3. Clinical studies:

a. Clinical Sensitivity:
Not applicable

b. Clinical specificity:
Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable

4. Clinical cut-off:
Not applicable

N. Instrument Name:

QXDx AutoDG ddPCR System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:

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

   Yes ____X____ or No _____

5. Calibration & Quality Controls:
   The assay uses calibrators by which the BCR-ABL/ABL is calculated. The instrument and assay employ both in-process QC Checks and physical controls. See description in traceability section for calibrator value assignments.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.