February 13, 2019

Bio-Rad Laboratories, Inc.
Steve Lin
Director of Regulatory Affairs and Quality Assurance
5731 W. Las Positas Blvd
Pleasanton, California 94588

Re: K181661
Trade/Device Name: QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System
Regulation Number: 21 CFR 866.6060, 862.2570
Regulation Name: BCR-ABL quantitation test
Regulatory Class: Class II
Product Code: OYX, PHG
Dated: January 3, 2019
Received: January 11, 2019

Dear Steve Lin:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Yun-fu Hu -S

for Reena Philip, Ph.D.
Director
Division of Molecular Genetics and Pathology
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*

K181661

Device Name
QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

1. Submitter

Bio-Rad Laboratories, Inc.
Establishment Registration Number: N/A
5731 W. Las Positas Blvd
Pleasanton, CA 94566
Contact Name: Steve Lin
Phone Number: (925) 474-9018
Fax Number: (925) 474-8644
Email: steve_lin@bio-rad.com
Summary was prepared on February 10, 2019

2. Name of Device

Trade name:	QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System
Common name:	BCR-ABL1 Digital PCR Test
Classification Name:	Bcr/Abl1 Monitoring Test, OYX (per 21 CFR section 866.6060); Instrumentation For Clinical Multiplex Test Systems, PHG (per 21 CFR section 862.2570)

3. Predicate Devices

Device Name	**Premarket Notification**
QuantideX qPCR BCR-ABL IS Kit	De Novo DEN160003
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software	Class II K141220

4. Device Description

QXDx AutoDG ddPCR SYSTEM

The QXDx AutoDG 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 from each sample are analyzed individually on the QXDx Droplet Reader. PCR-positive and PCR-negative droplets are counted to provide direct quantification of nucleic acid in digital form. Results are analyzed on QXDx Software running on a Windows based computer.

The QXDx AutoDG ddPCR System contains:

- QXDx Automated Droplet Generator
- QXDx Droplet Reader
- Laptop Computer with QXDx Software
- Accessory components:
    - ddPCR Dx AutoDG Consumable Pack
        - Automated Droplet Generation Oil for Probes
        - DG32 Cartridges w/ Gaskets
        - ddPCR Pipet Tips
        - ddPCR 96 Well Plates
        - ddPCR Pierceable Foil Seals
    - ddPCR Dx AutoDG Droplet Reader Oil Pack

QXDx BCR-ABL %IS KIT

Components of the kit 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 PCR 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, and glycerol	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, glycerol, and surfactants	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	
QXDx™ BCR-ABL Neg-CTRL	ABL RNA	Control used to ensure that RT and PCR steps performed properly and protect against contamination and falsely positive samples 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	

5. 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.

6. Intended Use

Same as indications for use.

7. Substantial Equivalence Information:

The QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System is substantially equivalent to the following legally marketed devices:

Device Name	**Premarket Notification**
QuantideX qPCR BCR-ABL IS Kit	De Novo DEN160003
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software	Class II K141220

The table on the following pages compares the technical characteristics of the QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System. As can be seen from the table, the QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System is substantially equivalent in technological characteristics.

Comparison Table of Predicate and Subject Device

Features	QXDx BCR-ABL %IS Kit	QuantideX qPCR BCR-ABL IS Kit
Intended Use	Same	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.

Platform	QXDx AutoDG ddPCR System	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software
Measuring Range	Same	MR 0.3 to MR 4.7
Specimen Type	Same	Human whole blood
Assay Principle	Droplet Digital PCR	Real-Time PCR
Matrices	Same	EDTA
Standardization	Same	WHO-IS
Features	**QXDx AutoDG ddPCR System**	**Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software**
Fundamental Technology	**Digital PCR**	**Real-Time PCR**
Multiplex capable	Same	Able to measure and sort multiple signals generated by an assay from a clinical sample.

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 uL in 96-well Bio-Rad PCR plates	10-30 uL in 96-well Fast PCR plates

Conclusion:

Differences in technological characteristics do not raise any questions of safety and effectiveness. The subject device is substantially equivalent to the predicate device.

8. Principles of the Procedure

Testing begins with RNA purified from peripheral whole blood samples (see Section 9 for proposed sample preparation method). 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.

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 (~40cycles) using a thermal cycler.

The thermal cycled plate is 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).

The QXDx Droplet Reader connects to a laptop computer running QXDx Software. The software provides measured levels of BCR-ABL and reference gene (BCR-ABL/ABL concentration ratio), quality values, World Health Organization International Scale (IS) calibrated results and quality associated with any controls or calibrators run.

9. 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,

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

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 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

10. Analytical Performance

Precision and Multisite Reproducibility (CLSI EP15-A3) – instrument to instrument and operator to operator reproducibility

Precision and Reproducibility was assessed using two panels of eight test samples each. Each panel consisted of six contrived samples and two controls. The contrived samples were prepared by mixing six independent pools of RNA isolated from BCR-ABL negative whole blood specimens with six independent pools of RNA isolated from BCR-ABL positive whole blood specimens, representing both e13a2 and e14a2 variants, in BCR-ABL:ABL ratios that spanned the assay range. 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). The reproducibility samples were prepared by Bio-Rad.

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 (2 reps x 2 runs x 3 days x 3 sites/instruments x 1 reagent lot = 36 replicates). Each run was performed by an independent operator (2 operators per site).

Acceptance Criteria:

When samples are 1ug +/- 25% RNA yielding >100,000 ABL copies and loads ranging from 640ng/test

- Total CV must be
  - ≤50% at LoQ

    - MR0.3-2.49 ≤ 10%
    - MR2.5-3.49 ≤ 20%
    - MR3.5-4 ≤ 50%
- Within run (CV <15% at MR3)
- Between instrument (CV<15% at MR3)
- Between day (CV <15% at MR3)
- Between operator (CV <15% at MR3)

A total of 576 observations were included in a variance components analysis with random effects for site, day, and run to assess repeatability, within-day precision, within-site precision, and reproducibility of measured MR level. Results shown on the following table indicated very low variability, including within sites, and all acceptance criteria were satisfied (all CVs were < 15%).

Criteria		Conclusion
-Total CV (Reproducibility)	≤ 50% at LoQ	Success (S012 %CV was 4.84)
	≤ 10% at MR 0.3 – 2.49	Success (maximum %CV was for S07 at 4.52)
	≤ 20% at MR 2.5 – 3.49	Success (maximum %CV was for S05 at 2.57)
	≤ 50% at MR 3.5 – 4	Success (maximum %CV was for S12 at 4.84)
Within run CV (Repeatability)-	< 15% at MR 3	Success – all CVs were less than 5.0%
Between instrument (site) CV / (Reproducibility)	< 15% at MR 3	Success – all CVs were less than 5.0%
Between day CV (Within-site Precision)-	< 15% at MR 3	Success – all CVs were less than 5.0%
Between operator (run) CV (Within day precision)	< 15% at MR 3	Success – all CVs were less than 5.0%

Lot to Lot Reproducibility (CLSI EP15-A3)

Sixteen (16) BCR-ABL1 negative whole blood and one-hundred (100) BCR-ABL1 positive RNA samples representing both the e13a2 and e14a2 variants were procured from commercial vendors. RNA was extracted from the whole blood samples and pooled to create six (6) negative patient RNA pools. Each negative pool used four (4) negative whole blood samples and each pool used at least 2 unique samples as compared to other pools. The positive RNA samples were pooled to create six (6) positive patient RNA pools. Each positive pool used minimally fifteen (15) samples each of which was unique to one (1) pool. The positive and negative RNA pools described above were blended with poly A RNA for stability. A high positive control sample (IHC 17%) was prepared by spiking Meg-01 (ATCC) BCR-ABL positive e13 variant cell line into a BCR-ABL negative HeLa cell line. A low positive control sample (MR4-5) was prepared by spiking BCR-ABL positive e14 variant cell line into a negative patient RNA pool and a BCR-ABL negative HeLa cell line.

Assay kit calibration checks and controls were tested in replicates of one (1) on each plate in order to qualify the run. Each sample, including control samples, was tested by 2 operators on 2 instruments and with 3 lots of reagents. All samples were tested in triplicate for 3 non-consecutive days. "Day 1" did not necessarily mean the same day. The mean, standard deviation (SD) and coefficient of variation (%CV) were calculated for each sample according to CLSI EP5-A2 prescribed methods for data analysis (3 replicates x 2 operators x 3 days x 3 lots x 2 instruments = 108 data points per sample).

Total MR and %IS precision was calculated for patient samples, cell line control samples and in-kit calibrators/controls. While the specifications as outlined were described for only MR, we evaluated total precision results for both measurement types across all samples. Cell line control 2 (MR4-5) led to an extremely low %IS (below LoQ) preventing a reliable calculation for %CV, thus, the data was not used. Similarly the in kit negative control also yielded a value too low for a realistic evaluation and was marked as "N/A – too low". All samples included in the evaluation met the acceptance criteria.

Additional precision metrics for both MR and %IS, including within-run, between run, between operator, between lot and between instruments are displayed for patient and cell line control samples only. As in-kit calibrator checks and controls were run in replicates of one (1) per plate, these metrics could not be calculated for these samples and total precision must represent overall performance. While the specification described a 15% or better CV across all metrics for samples close to MR3.0, we included all MR sample data in the evaluation. All samples met the specification as written for MR.

Cross Reactivity

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 measured in all samples was 0.000% demonstrating the kit detects neither the minor e1a2 (p190) nor micro e19a2 (p230) variants. All calibrators and controls met acceptance criteria.

Variant Sample ID	Dilution Number	N	Mean Specific Assay Ratio %	% CV Specific Assay Ratio	Mean QXDx BCR-ABL %IS	% CV QXDx BCR-ABL %IS	% Specificity	Pass/Fail
p190	1	4	36.1329%	2.1%	0.000%	0.000%	100.000%	PASS
	2	4	0.7148%	5.3%	0.000%	0.000%	100.000%	PASS
	3	4	0.0749%	10.7%	0.000%	0.000%	100.000%	PASS
	4	8	0.0080%	42.8%	0.000%	0.000%	100.000%	PASS
p230	1	4	31.7429%	1.1%	0.000%	0.000%	100.000%	PASS
	2	4	0.6207%	2.5%	0.000%	0.000%	100.000%	PASS
	3	4	0.0630%	11.0%	0.000%	0.000%	100.000%	PASS
	4	8	0.0056%	63.4%	0.000%	0.000%	100.000%	PASS

Interference (CLSI EP07-A2)

For each test, a sample pool was prepared by mixing whole blood from a CML positive and CML negative patient. Samples were mixed on ice and stored less than 1 hour prior to extraction to prevent blood type incompatability hemolysis. For each interfering substance tested, the sample pool used was split into two (2) parts, a test and control. 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.

Interferent	Positive Patient Sample ID	Base % BCR-ABL/ABL Result	Negative Patient Sample ID	Concentration Tested	Control Diluent Used	Extraction Date
Cholesterol	CMLBR017	0.16%	M5366	6.47 mmol/L	3.8mmol/L methyl-β-cyclodextrin	1/24/17
Conjugated Bilirubin	CMLBR017	0.16%	M5366	86 μM	PBS	1/24/17

Interferent	Positive Patient Sample ID	Base % BCR-ABL/ABL Result	Negative Patient Sample ID	Concentration Tested	Control Diluent Used	Extraction Date
EDTA	CMLBR017	0.16%	M5366	7 mg/ml	PBS + 0.1N NaOH	1/25/17
Hemoglobin	CMLBR020	1.30%	85962	200 g/L	PBS	2/14/17
Sodium Heparin	CMLBR017	0.16%	M5366	3000 U/L	PBS	1/25/17
Triglycerides	CMLBR017	0.16%	M5366	5.6 mmol/L	2.5% glycerol in nuclease-free water	1/24/17
Unconjugated Bilirubin	CMLBR017	0.16%	M5366	257μM	PBS + 0.1N NaOH	1/24/17

For both the control and test samples, five (5) replicate extractions were performed using the Promega Maxwell CSC RNA Blood Kit. Following extraction, absorbance at 260nm was measured and replicates normalized to 100ng/μL by adding DNA suspension buffer as needed. Each extracted sample was tested in replicates of two (2) for a total of ten (10) tests per sample type.

All in-kit calibrator check and control results met the run acceptance criteria. All samples were under MR3. 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.

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.

MR test results, acceptance criteria and status.

Test	Measured Mean MR		MR 95% Confidence Interval		MR Acceptable Range (Control CI ± 0.5 Log)	Result (Does the Test Sample MR CI fall within the Control Sample CI ± 0.5 Log?)
	Control	Test	Control	Test		
Cholesterol	4.38	4.38	4.04 - Inf*	3.97 - Inf*	3.54 - Inf*	PASS
Conjugated	4.30	4.15	4.00 - Inf*	3.84 - Inf*	3.50 - Inf*	PASS

Bilirubin						
EDTA	3.00	3.03	2.85 - 3.25	2.83 - 3.38	2.35 - 3.75	PASS
Hemoglobin	3.34	3.35	3.21 - 3.54	3.26 - 3.46	2.71 - 4.04	PASS
Heparin	3.04	3.00	2.85 - 3.38	2.88 - 3.17	2.35 - 3.88	PASS
Triglycerides	4.21	4.41	3.89 - Inf*	4.02 - Inf*	3.39 - Inf*	PASS
Unconjugated Bilirubin	4.29	4.32	3.92 - Inf*	3.95 - Inf*	3.42 - Inf*	PASS

%IS test results, acceptance criteria and status.

Test	N		Mean %IS		%IS Control Precision %CV	%IS Control Precision Range	Does the Test %IS fall in the Control Precision Range?	Test %IS 95% Confidence interval	Does the Test %IS 95% CI intersect the Control Precision Range?
	Control	Test	Control	Test					
Cholesterol	10	10	0.0042%	0.0042%	60.88%	0.0016% - 0.0067%	Yes	0.0000% - 0.0108%	Yes - Pass
Conjugated Bilirubin	10	10	0.0050%	0.0071%	51.42%	0.0024% - 0.0075%	Yes	0.0000% - 0.0144%	Yes - Pass
EDTA	10	10	0.0989%	0.0943%	22.20%	0.0769% - 0.1208%	Yes	0.0420% - 0.1466%	Yes - Pass
Hemoglobin	10	9*	0.0453%	0.0447%	18.24%	0.0370% - 0.0536%	Yes	0.0349% - 0.0546%	Yes - Pass
Heparin	10	10	0.0912%	0.1002%	27.43%	0.0662% - 0.1162%	Yes	0.0683% - 0.1321%	Yes - Pass
Triglycerides	10	10	0.0061%	0.0039%	56.99%	0.0026% - 0.0096%	Yes	0.0000% - 0.0095%	Yes - Pass
Unconjugated Bilirubin	10	10	0.0052%	0.0048%	67.75%	0.0017% - 0.0087%	Yes	0.0000% - 0.0113%	Yes - Pass

**One failing sample due to operator error*

Assay Linearity (CLSI EP06-A)

Two (2) positive BCR-ABL RNA patient pools were prepared by mixing RNA extracted from BCR-ABL positive whole blood. Pool 1 contained RNA from five (5) patients positive for the E13a2 variant and Pool 2 contained RNA from five (5) patients positive for the E14a2 variant. The RNA concentration of each pool was measured by absorbance at 260nm and was brought to a final concentration of 100ng/µL with 3T3 mouse BCR-ABL1 negative RNA. One (1) negative BCR-ABL RNA sample pool was prepared by mixing RNA from fifteen (15) BCR-ABL negative whole blood samples with RNA extracted from a HeLa cell line to bring the final concentration to 100ng/µL. The negative RNA pool was used in the dilution of the positive patient sample pools.

Results from Linearity Study:

Variant	Regression line slope (m)	Acceptable Range, m	Result	R^2	Acceptable Range, R^2	Result	Range %IS	Acceptable Range, %IS	Result
E13a2	1.04	0.8-1.2	PASS	0.996	0.97-1.0	PASS	50%-0.002%	10%-0.1%	PASS
E14a2	1.01		PASS	0.992		PASS	50%-0.002%	10%-0.1%	PASS

Evaluation of Detection Capability (CLSI EP17-A2)

Two (2) contrived positive BCR-ABL RNA samples were prepared to achieve MR values at approximately MR4.7, as determined a priori in LoD by Probit. 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.

Samples were diluted to an Expected value of 4.7 and anchored to the WHO-IS with Expected values of MR3.0 (0.1%IS) and MR4.0 (0.01%IS), and a College of American Pathologists (CAP) Proficiency sample, MRD-01(2018) at MR4.4, nearer to the LoD.

All samples were tested according to the kit IFU. Samples were tested in replicates of twenty (20) per day for four (4) days by one (1) operator on one (1) QXDX™ AutoDG ddPCR system with two (2) independent kit lots (20 replicates. x 4 days x 2 lots x 1 instrument = 160 replicates per sample), Calibrator checks and controls met product acceptance criteria.

A nonparametric approach was used to identify the limit of detection (LoD). For each sample and lot combination the percentage of tests falling at or below the Limit of Blank (0 copies of BCR-ABL; 0.0000% IS) was determined. If the percentage of tests results at or below the LoB was ≤ 5%, then the LoD was the concentration of the test sample. The concentration of each sample was the median MR level of all results within each kit lot. The LoD was determined for each kit lot and sample combination. The LoD for the QXDx™ BCR-ABL %IS Kit was determined as the worst (least MR value) of the four median MR levels.

For the e13a2 sample, 98.1% (157/160) of tests were above the LoB, with a median MR level of 4.7. For the e14a2 sample, 99.4% (159/160) of tests were above the LoB, with a median MR level of 4.7. In all combinations, the worst (least) median MR value was MR4.7.

The calculated limit of detection by this model for two (2) lots and 2 samples was found to be 0.002% IS BCR-ABL or MR 4.7.

Kit, Calibrator and Control Stability (CLSI EP25-A)

a. 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 lots of the QXDx™ BCR-ABL %IS Kit, Lot D, Lot H and Lot I, which were stored at -15°C , the outer limit of a -20°C freezer. The Droplet Reader Oil and the Automated Droplet Generation Oil for Probes were stored at 30°C. At each specified time point, the kits were removed from the storage and tested per IFU to quantify the %BCR-ABL/ABL in the controls and contrived samples. The temperature of the freezers and incubators were monitored and recorded throughout the study.

To date, the BCR-ABL %IS kit lots along with the droplet generation and reader oils have met the acceptance criteria and are stable under the storage conditions at 12 months for Lot D and 5 months for Lot H and Lot I.

b. Freeze-thaw Stability

Three (3) samples were prepared for use in this study at approximately MR2, MR3 and MR4. The MR2 and MR4 samples were prepared by blending Meg01 cell line RNA (containing the e13a2 variant) and the K562 cell line (containing the e14a2 variant), with RNA extracted from HeLa cells (containing ABL only) in water. Poly-A RNA was added to each sample for stability and to adjust the MR2 and MR4 sample RNA concentration to approximately 100 ng/ µL. The MR3 sample was prepared by blending RNA extracted from twelve (12) BCR-ABL positive patient blood samples (containing both the e13a2 and the e14a2 variants) with RNA extracted from one (1) BCR-ABL negative patient sample and diluted in water to achieve an RNA concentration of approximately 100 ng/µL.

Four QXDx™ BCR-ABL %IS kits from one lot stored at -20oC were used in this study. With the exception of the QXDx™ iScript Advanced reverse transcriptase, all components of one (1) 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 -20oC 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.

All components of the QXDx BCR-ABL IS kit demonstrated stable performance for at least 5 freeze-thaw cycles.

c. Specimen Stability (Whole Blood Stability)

One (1) 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 2-8oC 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 using the Promega Maxwell CSC RNA blood kit and instrument as per the manufacturer's instructions. Four (4) replicate extractions were conducted for dilutions 0 and 1. To increase power at the lower end, eleven (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. All samples tested met the specification:

Dilution	Day	Use	n	Mean %IS Value	Mean MR Value	Lower 95% CI limit	Upper 95% CI limit	± 0.5 log Allowable Range	Pass/Fail
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		Pass
	3	Test	4	37.2260	0.43	0.39	0.47		Pass
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		Pass
	3	Test	4	6.5420	1.18	1.15	1.22		Pass
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		Pass
	3	Test	11	0.6583	2.18	2.16	2.21		Pass
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		Pass

	3	Test	11	0.0658	3.20	3.12	3.28		Pass

Verification of WHO Standard Quantification

WHO-IS primary reference standards were run as patient samples on seven kit lots. The %IS master calibration CF, 0.93, was applied when the calibrator check values on each plate fell within allowable manufacturing specifications.

Four (4) levels of WHO primary standards were prepared and tested as patient samples as described in the QXDx™ BCR-ABL %IS kit IFU. The WHO primary standard cells were reconstituted in 600ul Qiagen RLT buffer, lysed using repeated pipette aspiration and RNA was extracted using the Qiagen RNeasy Mini Kit per NIBSC IFU. Each level was tested in four (4) replicates per the QXDx™ BCR-ABL %IS Kit IFU.

In all cases, the calibrator checks fell within their allowable ranges validating each of the runs. The WHO primary standard measured values as based on the master calibration curve and associated %IS correction factor (CF) were compared against the WHO assigned values via regression analyses. In all cases, the WHO standards measured within the allowable range of their actual assigned values from WHO:

	Slope (m)			Correlation (r2)			Intercept (b)		
Bio-Rad Kit Lot	Allowable Range	Measured	Status	Allowable Range	Measured	Status	Allowable Range	Measured	Status
Lot A	0.95 – 1.05	1.025	Pass	0.98 – 1.00	0.997	Pass	-0.2 – 0.2	-0.045	Pass
Lot B		1.025	Pass		0.996	Pass		-0.045	Pass
Lot C		0.972	Pass		0.997	Pass		0.053	Pass
Lot F		1.026	Pass		0.992	Pass		-0.001	Pass
Lot G		0.967	Pass		0.995	Pass		0.090	Pass
Lot H		1.02	Pass		0.998	Pass		-0.005	Pass
Lot I		1.002	Pass		0.997	Pass		0.029	Pass
Overall		1.014	Pass		0.995	Pass		-0.006	Pass

Carryover Contamination

A high positive sample pool was prepared by diluting RNA extracted from the MEG-01 cell line, which expresses the e13a2 variant at a BCR-ABL/ABL ratio

measuring MR0.2, diluted in commercially available DNA suspension buffer. A negative sample consisting of nuclease-free water was also tested.

The QXDx™ DR reads droplets column wise from the top to bottom. To examine for possible carryover, two (2), ninety-six (96) well plates were set up with high positive (Hi Pos) and negative (Neg) wells in alternating rows. A total of four (4) rows each containing twelve (12) replicates or possible 48 carry over events were tested per plate. Two plates were tested on each of 3 QXDx™ DR instruments for a total test sample size of 288. Testing was conducted per the QXDxTM BCR-ABL %IS Kit IFU.

Of the 288 replicate carryover tests, two-hundred and eighty-six (286) wells met inclusion criteria. Two (2) wells were excluded due to unacceptable droplet event counts. Instrument operation was verified by % droplet occupancy which demonstrates positive sample was distributed amongst droplets. Occupancy rate was >99%. Of the two-hundred and eighty-six (286) replicates used in the analysis, signal was measured in only one (1) 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.

11. Clinical Performance (Method Comparison)

A clinical evaluation method comparison study was designed to evaluate the performance of the QXDx BCR-ABL %IS Assay compared to the Asuragen Quantidex qPCR BCR-ABL IS Kit (IVD) in RNA derived from human blood in accordance with this protocol.

139 samples representing the intended use population and spanning the common dynamic range of the two methods were evaluated at a single testing lab.

Samples were acquired from at least two geographically distinct regions. 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 participating site tested de-identified leftover RNA samples that have been previously collected from a minimum of two (2) sites. The samples were extracted RNA from peripheral whole blood. Sample testing occurred at one clinical site.

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 excellent correlation with the Comparator Kit using a weighted Deming regression with a Pearson R correlation coefficient of 0.99, slope 1.037 and intercept was 0.1084.

12. Conclusion from the Clinical and Nonclinical Tests

The device is as safe, as effective, and performs as well as or better than the predicate device. The device is substantially equivalent to the predicate device.