510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
ASSAY AND INSTRUMENT COMBINATION

I. Background Information

A. 510(k) Number

K252243

B. Applicant

Suzhou Precigenome Ltd, Co;

C. Proprietary and Established Names

Trade Name: FastPlex™ BCR-ABL %IS digital PCR Kit for use on the RainSure DropMDx digital PCR system

Common Name: BCR-ABL1 Digital PCR Test

D. Regulatory Information

Product Code:	OYX PHG
Device Class:	Class II
Classification Regulation:	21 CFR 866.6060 21 CFR 862.2570
Classification Panel:	Pathology

II. Submission/Device Overview

A. Purpose for Submission:

New device

B. Measurand:

BCR-ABL1 and ABL1 transcripts

C. Type of Test:

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

III. Intended Use/Indications for Use:

A. Intended Use(s):

The Fastplex™ BCR-ABL (p210) %IS digital PCR 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 Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is a reverse transcription-quantitative PCR performed on the RainSure DropMDX digital PCR 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 Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is intended for use only on the RainSure DropMDX digital PCR 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

B. Indications for Use

Same as above

C. Special conditions for use statement(s)

For *in vitro* diagnostic use
For prescription use only.

D. Special instrument requirements

RainSure DropMDx digital PCR System
DropMDx Software v2.1

IV. Device/System Characteristics

A. Device Description:

The Fastplex™ BCR-ABL (p210) %IS digital PCR Kit uses randomly primed reverse transcription product from the whole blood and is then detected by Digital PCR (dPCR). This kit is able to quantify copies of the BCR-ABL fusion transcripts (e13a2 and/or e14a2) in the FAM channel and the ABL transcript as an endogenous control in the HEX channel. A description of the reagents provided with the kit is described below in Table 1.

Table 1. Reagents Provided in the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit

Item	Description	Use
Reverse Transcription Reaction Buffer	Buffer for Reverse Transcriptase with salts, dNTPs, Reverse Transcriptase Primers, RNase Inhibitor	Reaction Buffer component of the RT reaction to generate cDNA from RNA template
Reverse Transcriptase	Reverse Transcriptase	Generate cDNA from RNA template
BCR-ABL 210 Digital PCR Reaction Buffer	Buffer for Deoxyoligonucleotide primers and dye - and quencher - conjugated probes, salt buffer, dNTPs	Provides dNTPs, primers and probes for PCR amplification and detection of target sequences.
Taq Enzyme	Taq DNA Polymerases	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.
BCR-ABL 210 10%IS Calibrator	K562 cell RNA, HL60 cell RNA mixture, and BCR-ABL and ABL RNA formulated to approximately 10%IS BCR-ABL/ABL	Per run calibrators to check against acceptance criteria for use of electronic WHO-IS CF factor and reporting of WHO-IS value results.
BCR-ABL 210 0.1%IS Calibrator	K562 cell RNA, HL60 cell RNA mixture, and BCR-ABL and ABL RNA formulated to approximately 0.10%IS BCR-ABL/ABL	Per run calibrators to check against acceptance criteria for use of electronic WHO-IS CF factor and reporting of WHO-IS value results.
BCR-ABL 210 Positive Control 1	K562 cell RNA, HL60 cell RNA mixture, and BCR-ABL and ABL RNA formulated to approximately 10%IS BCR-ABL/ABL	Control used to ensure that RT and ddPCR steps performed properly by generating expected MR value
BCR-ABL 210 Positive Control 2	K562 cell RNA, HL60 cell RNA mixture, and BCR-ABL and ABL RNA formulated to approximately 0.01%IS BCR-ABL/ABL	Control used to ensure that RT and ddPCR steps performed properly by generating expected MR value
BCR-ABL Negative Control	HL60 cell RNA	Control used to ensure that RT and ddPCR steps performed properly and identify falsely positive results due to contamination

B. Principles of Operation

The Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is a quantitative digital PCR-based in vitro diagnostics test, best performed on the RainSure DropMDx digital PCR system. Primers and probes are designed to detect BCR-ABL and ABL mRNA transcripts in peripheral blood specimens of diagnosed t(9;22)-positive chronic myeloid leukemia (CML) patients expressing BCR-ABLl fusion transcripts type e13a2 and/or e14a2.

The PCR mix containing target cDNA molecules is randomly partitioned into 20,000 water-in-oil droplets in nanoliter size. These droplets self-organize to form a monolayer in the reaction chamber located downstream of the droplet generation channels. The water-in-oil emulsions are then subjected to PCR reaction as the droplets are confined in the chamber. The droplet generation and digital PCR thermal cycling are both performed using the RainSure DropMDx Sample Prep Station.

The RNA sample and Reverse Transcription Mix are combined to produce complementary DNA (cDNA), which is then added to the dPCR Mix 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 20 microliters of the PCR-ready sample is loaded into a 4-well RainSure Digital PCR Cartridge. Place digital PCR cartridge on cartridge holder, as well as required consumables (Generation Oil for Probes and Cartridge sealing covers) are loaded into the RainSure DropMDx Sample Prep Station. The dPCR mix containing target cDNA molecules is randomly partitioned into 20,000 water-in-oil droplets in nanoliter size. These droplets self-organize to form a monolayer in the reaction chamber located downstream of the droplet generation channels. The water-in-oil emulsions are then subjected to PCR reaction as the droplets are confined in the chamber. The droplet generation and digital PCR thermal cycling are both performed using the RainSure DropMDx Sample Prep Station. Each test must contain 8-well run controls which are 2-well BCR-ABL 210 10%IS Positive Control, 2-well BCR-ABL 210 0.1%IS Positive Control and 4-well BCR-ABL Negative Control. The cartridge holder is then loaded into the RainSure DropMDx Dscanner4-1000.

The Dscanner4-1000 scans each individual droplet in both brightfield and two different fluorescence channels (FAM and HEX). The DScanner4-1000 measures the fluorescence intensity of the droplets to determine which contain the target (positive) and which do not (negative) for each of the targets identified with the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit. It also measures the diameter of each individual droplet based on the bright-field images of the droplets and counts the total number of droplets. The digital PCR is performed in duplicate on all samples, and controls (calibrator checks).

After the detection is completed, the copies of BCR-ABL1 and ABL1 genes are calculated, and the %IS and MR values of the sample are calculated according to the conversion factor (CF) of the kit. The Negative Control should be merged and BCR-ABL1 copy ≤2, the measured %IS values of BCR-ABL 210 10%IS Positive Control should be merged and between minimum and maximum values provided in the label of the kit (about between MR0.5 and MR1.5), the measured %IS values of BCR-ABL 210 0.1%IS Positive Control should be merged and between minimum and maximum values provided in the label of the kit (about between MR2.5 and MR3.5). The test results are issued after passing all these requirements above.

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 Molecular Reduction, or MR value. The MR value is traditionally written as $MR^{x.x}$. However, for simplicity and legibility, the Fastplex™ BCR-ABL (p210) %IS digital PCR 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 shown below:

Table 2. %IS and MR comparison table

IS (%)	MR
50	0.3
32	0.5
10	1.0
3.2	1.5
1	2.0
0.32	2.5
0.1	3.0 (MMR)
0.032	3.5
0.01	4.0
0.0032	4.5
0.002	4.7
0.001	5.0

The results are interpreted automatically by the RainSure DropMDx Software from measured droplet counts, fluorescent signals, and embedded calculation algorithms that report out BCR-ABL and ABL copies concentration. An indication of sample suitability is indicated as the ABL1 copies Sufficient to MRx.x column. International Scale Percent Ratio (%IS) is calculated as the copy number of BCR-ABL1 divided by the copy number of ABL1, then multiplied by 100 times the conversion factor (CF) of the kit, i.e.,

%IS = BCR-ABL1 copy / ABL1 copy x 100 x CF

The test results should be interpreted as identified below:

Table 3. Interpretation of results[1,2]

Test results	Report results	Explanation of test results
Positive droplet number of BCR-ABL1 ≤2.	Report: Negative	Indicates that there is no BCR-ABL1 fusion gene in the test sample.
MR>5.0 or %IS<0.001	Report: MR value> 5.0 detected or %IS value <0.001 detected	BCR-ABL1 fusion gene is detected, but the results are beyond the LoQ.
MR≤5.0 or %IS≥0.001	Report: %IS(MR) value	BCR-ABL1 fusion gene detected and %IS (MR) value measured.
Copy number of ABL1≤10000 per well[3]	Report: Invalid	The copy number of ABL1 gene is too low.

[1]If there are less than 3 BCR-ABL positive droplets in the 2-well merged test, a 4-well merged test is recommended.
[2]In the case of ABL1 copies > 3×105 Copies/well and the BCR-ABL positive droplet number > 2, the ABL1 copy number exceeds the linear range, which it will affect the quantitative accuracy. A retest should be performed with reduced RNA input.
[3]When the number of ABL1 copies is≤10000 per well, the report is invalid. A retest should be performed with increased RNA input.

C. Instrument

The RainSure DropMDx digital PCR System consists of DropMDx Sample Prep Station (SG32-3000) and Dscanner4-1000, and their associated consumables.

The DropMDx Sample Prep Station (SG32-3000) partitions samples into approximately 20,000 nanoliter-sized droplets and PCR; droplets (PCR-positive and PCR-negative) from each sample are counted and analyzed individually on the DropMDx Dscanner4-1000 to provide direct quantification of nucleic acid in digital form.

D. Software:

DropMDx Software is used to analyze all test results. This software is provided with the RainSure DropMDx digital PCR System.

E. Substantial Equivalence Information:

1. Predicate device name(s):

   QXDx BCR-ABL %IS Kit
   QXDx Automated Droplet Generator
   QXDx Droplet Reader
   QXDx Software 1.0

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

   K181661

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate
Intended Use	The Fastplex™ BCR-ABL (p210) %IS digital PCR 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 Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is a reverse transcription-quantitative PCR performed on the RainSure DropMDX digital PCR 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 Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is intended for use only on the RainSure DropMDX digital PCR 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.	Same
Measurement Type	Quantitative	Same
Specimen	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	Both %IS and Molecular Response(MR)	Same
Fundamental Technology	Digital PCR	Same

Calibrators	Two levels are formulated at 0.1%IS and 10%IS BCR-ABL/ABL.	Same
Reporting Units	Both %IS and Molecular Response (MR)	Same
Differences		
Item	New Device	Predicate
RNA Input	1250-2500ng	1000 ng
Measuring Range	MR0.3 to MR4.57 in 2-well test and to MR5.00 in 4-well test	MR0.3 to MR4.7
Quality Controls	3 levels of external control Positive control 1 (%IS of 10) Positive control 2 (%IS of 0.01) Negative Control	3 levels of external control RNA High (%IS of 18) RNA Low(%IS of 0.03) RNA Negative
Instrument	RainSure DropMDx digital PCR System	Bio-Rad QXDx™ AutoDG™ ddPCR System
Degree of Automation	Requires manual transfer of amplification mixture to amplification/detection instrument. Amplification functionality is included Automated control of amplification, detection and data analysis	Requires manual transfer of amplification mixture to amplification/detection instrument Amplification functionality is not included Automated control of amplification, detection and data analysis
Primary Operational Amplification and Detection Components	Integrated thermal cycler and nanoliter droplet detection for walk away PCR amplification and detection	Nanoliter droplet fluorimeter for walk away PCR detection.
Instrument Computer Operating System	Embedded software, Lubuntu22.04	Microsoft Windows 10
Amplification Reaction Volume	20 µL in RainSure Digital PCR cartridges	20-25µL in 96-well Bio-Rad PCR plates

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

The following FDA guidance documents and standards were consulted:

CLSI EP06 2nd Edition, Evaluation of the Linearity of Quantitative Measurement Procedures.
CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry
CLSI EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edtion.
CSLI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.
CLSI EP 25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.

G. Performance Characteristics (if/when applicable):

1. **Analytical performance:**

a. Precision/Reproducibility:

Precision and Reproducibility were assessed using 3 panels of positive pools at 6 MR levels. The 3 positive pools were prepared by mixing 2 BCR-ABL1 positive p210 (e13a2) RNA samples (pool 1), 2 BCR-ABL1 positive p210 (e14a2) RNA samples (pool 2), and 1 BCR-ABL1 positive RNA samples p210 (e13a2) mixed with 1 BCRABL1 positive RNA samples p210 (e14a2) (pool 3). A negative pool was used as a diluent and was prepared by mixing BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate 6 samples with different concentrations: MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0.

Samples were assayed in 5 replicates per run for 1 run per day for 5 non-consecutive days at 3 sites (2 Instruments at each site) with 3 reagent lot for a total of 225 replicates. Each run was performed by an independent operator (2 operators per site). The total precision (CV%) values needed to meet the acceptance criteria shown in Table 4:

Table 4. Precision acceptance criteria corresponding to different concentrations

MR	Precision (CV%) acceptance criteria
MR0.3-2.49	≤ 10%
MR2.5-3.49	≤ 20%
MR3.5-5.0	≤ 50%

A total of 4050 observations were included in a variance components analysis with random effects for site, day, reagent lot and run to assess repeatability, between-day precision, between-site precision, between-lot and reproducibility of measured MR level. Total MR and %IS precisions were calculated for the assay (Table 5 and Table 6, respectively). Results indicated very low variability, including sites, days and lots, and all acceptance criteria were satisfied. Total MR precision was calculated for in-kit calibrators and controls (Table 7).

Table 5. Precision analysis results (MR) of samples

MR Bin	Variant	Num	Mean MR	Within-run		between-lot		between-day		between-site		between-instrument		Total	
				SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1.0	e13a2	225	0.94	0.005	0.57%	0.000	0.00%	0.000	0.00%	0.008	0.89%	0.000	0.00%	0.010	1.06%
	e14a2	225	0.94	0.005	0.49%	0.000	0.00%	0.000	0.00%	0.008	0.86%	0.002	0.19%	0.010	1.01%
	e13a2+e14a2	225	0.94	0.006	0.64%	0.000	0.00%	0.000	0.00%	0.008	0.87%	0.000	0.00%	0.010	1.08%
2.0	e13a2	225	1.78	0.013	0.71%	0.000	0.00%	0.000	0.00%	0.004	0.24%	0.009	0.48%	0.016	0.89%
	e14a2	225	1.77	0.012	0.65%	0.000	0.00%	0.003	0.17%	0.008	0.44%	0.000	0.00%	0.014	0.81%
	e13a2+e14a2	225	1.78	0.013	0.73%	0.000	0.00%	0.000	0.00%	0.007	0.37%	0.000	0.02%	0.014	0.82%
3.0	e13a2	225	2.86	0.093	3.24%	0.001	0.02%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.093	3.24%
	e14a2	225	2.86	0.091	3.18%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.091	3.18%
	e13a2+e14a2	225	2.86	0.091	3.18%	0.000	0.01%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.091	3.18%
4.0	e13a2	225	3.83	0.194	5.08%	0.002	0.06%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.194	5.08%
	e14a2	225	3.82	0.196	5.12%	0.001	0.03%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.196	5.12%
	e13a2+e14a2	225	3.82	0.197	5.16%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.197	5.16%
4.5	e13a2	225	4.51	0.121	2.67%	0.000	0.00%	0.017	0.37%	0.000	0.00%	0.056	1.24%	0.134	2.97%
	e14a2	225	4.49	0.108	2.40%	0.000	0.00%	0.006	0.13%	0.000	0.00%	0.089	1.98%	0.140	3.11%
	e13a2+e14a2	225	4.50	0.105	2.34%	0.000	0.00%	0.020	0.45%	0.000	0.00%	0.074	1.66%	0.131	2.91%
5.0	e13a2	225	4.87	0.063	1.29%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.123	2.53%	0.138	2.84%
	e14a2	225	4.88	0.059	1.21%	0.000	0.00%	0.013	0.26%	0.000	0.00%	0.129	2.65%	0.143	2.93%
	e13a2+e14a2	225	4.88	0.056	1.14%	0.000	0.00%	0.013	0.27%	0.000	0.00%	0.132	2.71%	0.144	2.95%

Table 6. Precision analysis results (%IS) of samples

MR Bin	Variant	Num	Mean %IS	Within-run		between-lot		between-day		between-site		between-instrument		Total	
				SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1.0	e13a2	225	11.3922	0.142	1.25%	0.000	0.00%	0.000	0.00%	0.219	1.93%	0.000	0.00%	0.261	2.29%
	e14a2	225	11.3928	0.122	1.07%	0.000	0.00%	0.000	0.00%	0.213	1.86%	0.048	0.42%	0.250	2.19%
	e13a2+e14a2	225	11.4311	0.158	1.39%	0.000	0.00%	0.000	0.00%	0.215	1.88%	0.000	0.00%	0.266	2.34%
2.0	e13a2	225	1.6619	0.047	2.82%	0.000	0.00%	0.000	0.00%	0.015	0.92%	0.033	2.00%	0.059	3.58%
	e14a2	225	1.6883	0.044	2.64%	0.000	0.00%	0.011	0.68%	0.030	1.77%	0.000	0.00%	0.054	3.25%
	e13a2+e14a2	225	1.6719	0.049	2.90%	0.000	0.00%	0.000	0.00%	0.026	1.53%	0.004	0.21%	0.056	3.29%
3.0	e13a2	225	0.1408	0.028	19.80%	0.000	0.13%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.028	19.80%
	e14a2	225	0.1410	0.027	19.31%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.027	19.31%
	e13a2+e14a2	225	0.1395	0.027	19.40%	0.000	0.08%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.027	19.40%
4.0	e13a2	225	0.0159	0.007	42.77%	0.000	0.60%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.007	42.77%
	e14a2	225	0.0160	0.007	43.15%	0.000	0.24%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.007	43.15%
	e13a2+e14a2	225	0.0159	0.007	43.51%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.007	43.51%
4.5	e13a2	225	0.0032	0.001	27.24%	0.000	0.00%	0.000	3.59%	0.000	0.00%	0.000	13.88%	0.001	30.78%
	e14a2	225	0.0033	0.001	24.45%	0.000	0.00%	0.000	1.15%	0.000	0.00%	0.001	20.07%	0.001	31.65%
	e13a2+e14a2	225	0.0032	0.001	23.80%	0.000	0.00%	0.000	4.10%	0.000	0.00%	0.001	17.30%	0.001	29.71%
5.0	e13a2	225	0.0014	0.000	16.46%	0.000	0.00%	0.000	0.81%	0.000	0.00%	0.000	26.62%	0.000	31.30%
	e14a2	225	0.0014	0.000	15.24%	0.000	0.00%	0.000	2.91%	0.000	0.00%	0.000	27.97%	0.000	31.98%
	e13a2+e14a2	225	0.0014	0.000	14.68%	0.000	0.00%	0.000	3.53%	0.000	0.00%	0.000	28.71%	0.000	32.44%

Table 7. Precision analysis results (MR) of controls and calibrators

Lot	Sample	Target MR	Num	Sepcification	Mean MR	MR Total Precision			Target %IS
						SD	CV	Status	
Lot1	BCR-ABL 210 Positive Control 1	0.972	165	CV≤ 10%	0.972	0.016	1.60%	YES	10
	BCR-ABL 210 Positive Control 2	3.826	165	CV≤ 10%	3.826	0.141	3.68%	YES	0.01
	BCR-ABL 210 10%IS Calibrator	0.978	165	CV≤ 10%	0.978	0.021	2.15%	YES	10
	BCR-ABL 210 0.1%IS Calibrator	2.842	165	CV≤ 10%	2.842	0.059	2.07%	YES	0.1
	BCR-ABL Negative Control	BCR-ABL-positive droplets< 3	165	N/A	Cannot log transform 0 value				0
Lot2	BCR-ABL 210 Positive Control 1	0.971	165	CV≤ 10%	0.971	0.02	2.10%	YES	10
	BCR-ABL 210 Positive Control 2	3.827	165	CV≤ 10%	3.827	0.11	2.87%	YES	0.01
	BCR-ABL 210 10%IS Calibrator	0.981	165	CV≤ 10%	0.981	0.021	2.18%	YES	10
	BCR-ABL 210 0.1%IS Calibrator	2.857	165	CV≤ 10%	2.857	0.047	1.65%	YES	0.1
	BCR-ABL Negative Control	BCR-ABL-positive droplets< 3	165	N/A	Cannot log transform 0 value				0
Lot3	BCR-ABL 210 Positive Control 1	0.885	165	CV≤ 10%	0.885	0.017	1.87%	YES	10
	BCR-ABL 210 Positive Control 2	3.813	165	CV≤ 10%	3.813	0.104	2.72%	YES	0.01
	BCR-ABL 210 10%IS Calibrator	0.987	165	CV≤ 10%	0.987	0.026	2.59%	YES	10
	BCR-ABL 210 0.1%IS Calibrator	2.856	165	CV≤ 10%	2.856	0.063	2.19%	YES	0.1
	BCR-ABL Negative Control	BCR-ABL-positive droplets< 3	165	N/A	Cannot log transform 0 value				0

RNA Extraction Method

This study was assessed using 4 BCR-ABL1 p210-positive blood samples which were pooled to prepare positive control sample P1. 1 BCR-ABL1 p210 positive blood sample was combined with 4 BCR-ABL1 p210 negative blood samples to prepare positive control sample P2. 4 BCR-ABL1 p210-negative blood samples were combined to prepare negative control sample NC.

Two (2) different BCR-ABL1 p210-positive blood concentration blood samples and 1 negative blood sample which were collected from homogenized EDTA anticoagulation tubes were extracted using 3 batches of RainSure RNA extraction Kit, and each blood sample was extracted in duplicate by 2 operators over 3 days. The RNAs obtained after the extractions were individually analyzed using the Nano-300 for concentration and purity, and after completion of the assay, an equal amount of 1.25μg of RNA was used for 1 batch of Fastplex™ BCR-ABL (p210) %IS digital

PCR Kit.

Acceptance criteria for this study were as follows: 1) the quantity of extracted RNA should be ≥ 2500ng; 2) CV% of positive samples must be ≤ 10%; 3) NC samples remain negative.

A total of 108 results were included in RNA Extraction study. 100% of the samples met the concentration requirements, and the OD260/OD280 ratio ranged from 1.61 to 2.10. All NC samples tested negative, and the CV for both P1 and P2 samples was below 10% (see Table 8).

Table 8. CV% for Positive Sample

Sample	P1	P2
N	36	36
Mean	0.862	4.225
$CV_{Within\text{-}run}$	5.48%	4.29%
$CV_{Between\text{-}extraction\ Lot}$	0.00%	0.71%
$CV_{Between\text{-}Day}$	0.00%	0.00%
$CV_{Between\text{-}Operator}$	3.74%	0.00%
CV_{Total}	6.64%	4.35%

b. Linearity/assay reportable range:

Three (3) 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 50 negative specimens to create 9 levels ranging from MR0.3 to MR5.0 with 3 replicates each. Pool 1 contained RNA from 2 BCR-ABL1 positive RNA for the E14a2 variant, Pool 2 contained RNA from 2 BCR-ABL1 positive RNA for the E13a2 variant, Pool3 contained RNA from 1 BCR-ABL1 positive RNA for the E13a2 variant and 1 BCR-ABL1 positive RNA for the E14a2 variant. The precision of each sample was first calculated based on the measurement results, and its detection rate should meet 100% and CV should be ≤10%. Second, according to the standard deviation (SD) of the measured results of each sample, the most suitable regression analysis type was determined, and the regression equation was obtained. From this equation, the predicted values, deviations, and deviations from linearity expressed as percentage (% deviations) were calculated for each sample, where the linear deviation should be ≤ ±15%. The slope was required to be 0.83-1.20 and the correlation coefficient had to be 0.98 to 1.0.

Results show the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is linear throughout the measuring range of 50%-0.001% in %IS ratio, and MR0.3-MR5.0 in log-space. Linearity of MR results was demonstrated from at least MR 0.3 (50%IS) to MR 5.0 (0.001%IS). Results are shown in Table 8.

Table 8. Linear range regression analysis for BCR-ABL (p210)

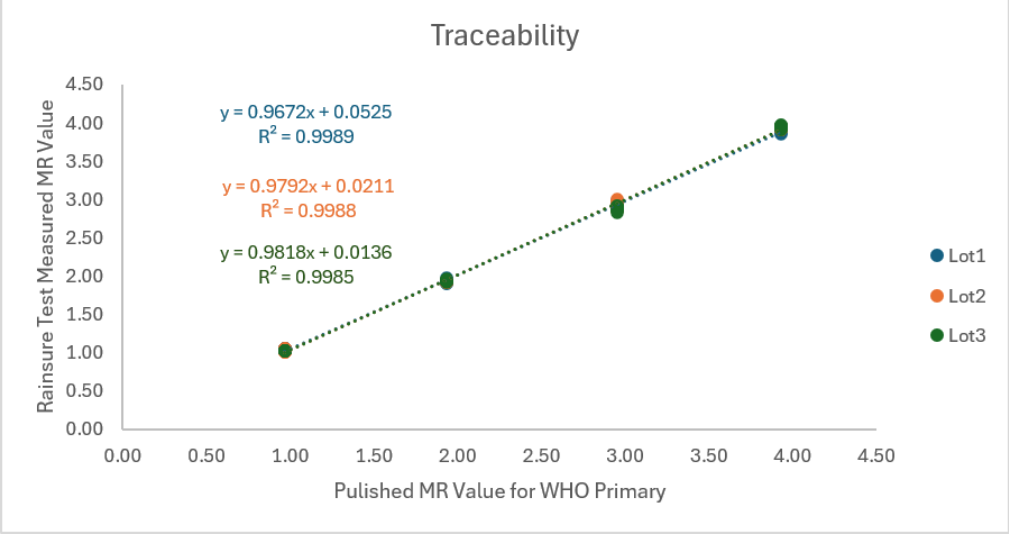
Breakpoint	**Linearity/ Dynamic Range**	**Linearity/ Dynamic Range**	**Slope (b1)**			**Intercept**			**R**		
			Lot1	Lot2	Lot3	Lot1	Lot2	Lot3	Lot1	Lot2	Lot3
e13a2	50% IS to 0.001% IS	MR 0.3 – MR 5.0	1.002	0.989	0.987	-0.02	0.00	0.01	0.9996	0.9998	0.9992
e14a2	50% IS to 0.001% IS	MR 0.3 – MR 5.0	1.019	1.020	1.038	-0.06	-0.07	-0.06	0.9990	0.9991	0.9992
e13a2 & e14a2	50% IS to 0.001% IS	MR 0.3 – MR 5.0	0.981	0.976	0.970	-0.01	0.00	0.02	0.9990	0.9981	0.9961

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 3 independent Fastplex™ BCR-ABL (p210) %IS digital PCR 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 10 replicates. The measured MR values for each level of the WHO Reference Panel were adjusted by a common derived correction factor, CF (1.05-1.09). The measured values were compared to the published values through a regression analysis to determine slope and intercept values. The analysis showed correlation with R2 values of 0.9985-0.9989. The slope of the regression lines varied between 0.967 and 0.982, and the intercepts were between -0.0544136 and -0.0295525. The results are shown in Figure 1.

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



d. *Detection limit:*

Limit of Blank

Using three different reagent kit lots, six negative samples were tested twice daily (morning and evening) over five days (5 days × 6 samples × 2 replicates). Each reagent lot generated 60 data points for the 4-well merged analysis or 120 data points for the 2-well merged analysis.

In addition, three separate reagent kit lots were evaluated over 3 days, with 24 negative samples tested each day (3 days × 24 samples). Each lot yielded 72 data points for the 4-well merged analysis and 144 data points for the 2-well merged analysis. All results were ranked according to the total number of BCR-ABL p210-positive droplets detected.

The Fastplex™ BCR-ABL (p210) %IS Digital PCR Kit demonstrated a lower limit of blank (LoB) of 1 BCR-ABL p210-positive droplet at the 95% confidence level when a 2-well merged analysis was used for blank determination. When a 4-well merged analysis was used, the LoB was 2 BCR-ABL p210-positive droplets. Accordingly, the established LoB for the kit was 2 BCR-ABL p210-positive droplets, representing the maximum LoB observed across the analysis approaches evaluated.

Limit of Detection/Limit of Quantification

Limit of Detection (LoD)/Limit of Quantitation (LoQ) were assessed using 3 pools of positive samples. The 3 positive pools were prepared by mixing 2 BCR-ABL1 positive p210 (e13a2) RNA samples (pool 1), 2 BCR-ABL1 positive p210 (e14a2) RNA samples (pool 2) and 1 BCR-ABL1 positive p210 (e13a2) RNA samples mix with 1 BCR-ABL1 positive p210 (e14a2) RNA samples (pool 3). A negative pool was used as a diluent and was prepared by mixing 73 BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate 5 samples with different concentrations: MR4.49, MR4.92, MR5.05, MR5.15 and MR5.30.

Samples were tested in 24 wells per day for 4 days with 3 reagent lots in 3 instruments for a total of 96 wells. Each lot uses probit method to determine LOD in 2-well or 4-well measurements, as shown in Table 9.

Table 9. Determining LoD for the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit.

Mergerd	Breakpoint	Expected MR	Tests	Lot1		Lot2		Lot3	
				Positive test ratio	Probit hit	Positive test ratio	Probit hit	Positive test ratio	Probit hit
2-well	e14a2	MR5.30	48	27.08%	MR4.61	12.50%	MR4.67	6.25%	MR4.73
		MR5.15	48	29.17%		35.42%		25.00%	
		MR5.05	48	45.83%		41.67%		50.00%	
		MR4.92	48	70.83%		72.92%		75.00%	
		MR4.49	48	100.00%		100.00%		100.00%	
	e13a2	MR5.30	48	43.75%	MR4.57	14.58%	MR4.61	6.25%	MR4.67
		MR5.15	48	27.08%		22.92%		25.00%	
		MR5.05	48	43.75%		47.92%		52.08%	
		MR4.92	48	75.00%		75.00%		75.00%	
		MR4.49	48	100.00%		100.00%		100.00%	
	e14a2+e13a2	MR5.30	48	12.50%	MR4.64	22.92%	MR4.66	8.33%	MR4.74
		MR5.15	48	22.92%		20.83%		27.08%	
		MR5.05	48	41.67%		43.75%		52.08%	
		MR4.92	48	64.58%		72.92%		75.00%	
		MR4.49	48	100.00%		100.00%		100.00%	
4-well	e14a2	MR5.30	24	45.83%	MR5.03	54.17%	MR5.04	41.67%	MR5.07
		MR5.15	24	75.00%		75.00%		79.17%	
		MR5.05	24	87.50%		91.67%		91.67%	
		MR4.92	24	100.00%		100.00%		100.00%	
		MR4.49	24	100.00%		100.00%		100.00%	
	e13a2	MR5.30	24	70.83%	MR5.00	54.17%	MR5.07	33.33%	MR5.03
		MR5.15	24	83.33%		91.67%		79.17%	
		MR5.05	24	87.50%		91.67%		87.50%	
		MR4.92	24	100.00%		100.00%		100.00%	
		MR4.49	24	100.00%		100.00%		100.00%	

Mergerd	Breakpoint	Expected MR	Tests	Lot1		Lot2		Lot3	
				Positive test ratio	Probit hit	Positive test ratio	Probit hit	Positive test ratio	Probit hit
	e14a2+e13a2	MR5.30	24	70.83%	MR5.04	45.83%	MR5.03	41.67%	MR5.05
		MR5.15	24	91.67%		79.17%		87.50%	
		MR5.05	24	87.50%		87.50%		87.50%	
		MR4.92	24	100.00%		100.00%		100.00%	
		MR4.49	24	100.00%		100.00%		100.00%	

The calculated limit of detection (LoD) was determined to be MR4.57 using the 2-well merged analysis and MR5.00 using the 4-well merged analysis. The LoD was subsequently verified using three reagent kit lots and three BCR-ABL1 transcript variants (e13a2, e14a2, and a mixture of e13a2 and e14a2). Samples at MR4.57 and MR5.00 concentrations (Table 10) were tested. The MR4.57 samples were evaluated using the 2-well merged analysis, while the MR5.00 samples were evaluated using the 4-well merged analysis. Each sample was tested twice daily over a period of 3 days.

For the MR4.57 samples, a total of 30 test results were generated for each reagent lot. Similarly, for the MR5.00 samples, a total of 30 test results were generated for each reagent lot. The calculated LoD was found to be MR 4.57 in 2-well test and MR 5.00 in 4-well test. The estimated limit of quantification (LoQ) under the 2-well merged analysis was MR4.57. Under the 4-well merged analysis, the estimated limit of quantification (LoQ) was MR5.00.

Table 10. Verifying LoD for the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit

Variant	Expected MR	Observed MR	2-well Test			4-well Test		
			Lot1	Lot2	Lot3	Lot1	Lot2	Lot3
e14a2	4.57	4.47	100%	100%	100%	/	/	/
e14a2	4.57	4.50						
e13a2	4.57	4.40						
e13a2	4.57	4.53						
e14a2 &e13a2	4.57	4.49						
e14a2	5.00	4.80	/	/	/	100%	100%	100%
e14a2	5.00	4.85						
e13a2	5.00	4.76						
e13a2	5.00	4.92						
e14a2 &e13a2	5.00	5.01						

e. Analytical specificity:

Interference

Seven (7) BCR-ABL1 p210-positive clinical samples (with MR values of approximately 0.3) and 95 BCR-ABL1-negative clinical samples were prepared. The positive samples were diluted with a negative sample pool to generate samples at approximately MR1.0, MR3.0, and MR4.5 concentrations.

Potential interfering substances were added to the test group, while diluent was added to the

corresponding control group at the concentrations recommended in CLSI EP7-A2. The potential interfering substances evaluated included hemoglobin (1000 mg/dL), triglycerides (1500 mg/dL), EDTA (7.5 mg/mL), genomic DNA (10 µg/mL), unconjugated bilirubin (40 mg/dL), cholesterol (400 mg/dL), 10× red blood cell lysis buffer (5%), RNA lysis buffer (5%), chloroform (5%), isopropanol (5%), and ethanol (10%).

For both the control and test samples, five replicate extractions were performed, and each extracted sample was tested in duplicate, resulting in a total of 10 test results per sample type. The %IS and MR values were measured independently for each group.

For MR values, the mean MR value of the test group and its 95% confidence interval were required to fall within the 95% confidence interval ±0.5 log units of the corresponding control group. For %IS values, the 95% confidence interval of the mean %IS for the test group was required to overlap the observed range of the corresponding control group.

All samples met the predefined acceptance criteria based on both MR and %IS values. The results demonstrated that the endogenous and exogenous substances evaluated did not interfere with assay performance and did not affect test results compared with the control samples.

Primer Specificity

Two (2) samples were prepared by p190 RNA extracted from SUP-B15 cells or p230 RNA extracted from AR230-r cells. Three dilutions of each sample were prepared by varying the amount of negative RNA used. The Fastplex™ BCR-ABL (p210) %IS digital PCR Kit performed as intended detecting only the major e13a2 and e14a2 (p210) variants as shown. Test specificity was 100% thus exceeding the required specification of ≥90%. Results in Table 11 support that the kit does not detect the minor e1a2 (p190) and micro e19a2 (p230) variants even when present in high concentrations.

Table 11. Primer Specificity Results

Variant	Target Ratio	Observed Ratio	Fastplex™ BCR-ABL (p210) %IS digital PCR Kit					
			Lot 1		Lot 2		Lot 3	
			Test Num.	Tested positive	Test Num.	Tested positive	Test Num.	Tested positive
e1a2 （P190）	50%	42.07%	4	0	4	0	4	0
	10%	9.02%	4	0	4	0	4	0
	1%	1.40%	4	0	4	0	4	0
e19a2 （P230）	50%	44.46%	4	0	4	0	4	0
	10%	9.94%	4	0	4	0	4	0
	1%	1.54%	4	0	4	0	4	0

Carryover Contamination

Carryover contamination was assessed using one high-positive sample pool t MR1.0 and one negative sample pool. For the 2-well merged analysis, eight cartridges were configured with high-positive and negative wells arranged in alternating rows, resulting in a total of 16 sample replicates per test. Each test included two rows, with each row containing four potential carryover events. Testing was performed across 3 runs, 3 instruments, and 3 reagent lots, yielding a total of 144 replicates (72 positive and 72 negative samples).

One positive well failed to meet the droplet count acceptance criterion and was

excluded from the analysis. Consequently, 71 positive and 72 negative replicates were included in the final analysis. Of the 72 negative replicates, only one well contained a single BCR-ABL1-positive droplet, which was below the established limit of blank (LoB). No BCR-ABL1-positive droplets were detected in the remaining 71 negative wells.

For the 4-well merged analysis, eight cartridges were configured with high-positive and negative wells arranged in alternating rows, resulting in a total of eight sample replicates per test. Each test included two rows, with each row containing two potential carryover events. Testing was performed across 3 runs, 3 instruments, and 3 reagent lots, yielding a total of 72 replicates (36 positive and 36 negative samples).

No BCR-ABL1-positive droplets were detected in any of the 36 negative replicates evaluated in the 4-well merged analysis. The results demonstrated that the device did not generate significant carryover contamination between samples.

RNA Input

A study was conducted to demonstrate the acceptable RNA input for the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit. A negative pool was used as a diluent and was prepared by mixing 5 BCR-ABL1 negative RNA samples. Two positive samples were diluted with the negative sample pool to generate 4 samples with different concentrations: MR1.0, MR2.0, MR3.0 and MR4.0.

Samples were diluted to 250 ng/μL and varying volumes were tested with the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit targeting RNA inputs from 312.5ng to 2500ng according to the IFU. Samples were tested in 4 replicates. When the RNA input of the samples was 1250 ng, the positive detection rate was 100%, the deviation between the measured values and the theoretical values were within ±0.5, and the CV% was ≤10% (Table 12). Therefore, the RNA input of the kit was determined to be 1250 ng to 2500 ng.

Table 12. RNA Input Study

LOT			Lot1			Lot2			Lot3		
Sample	Target	input(ng)	Positive ratio	MR Mean	MR CV	Positive ratio	MR Mean	MR CV	Positive ratio	MR Mean	MR CV
MR 1.0	0.91	2500	100%	0.86	1.28%	100%	1.86	0.61%	100%	0.86	0.51%
		1250	100%	0.86	1.21%	100%	1.87	0.74%	100%	0.87	1.16%
		625	100%	0.85	1.20%	100%	0.86	0.95%	100%	0.87	0.99%
		312.5	100%	0.87	1.38%	100%	0.87	1.15%	100%	0.88	1.24%
MR 2.0	1.93	2500	100%	1.84	0.81%	100%	1.86	0.61%	100%	1.87	0.65%
		1250	100%	1.86	0.58%	100%	1.87	0.74%	100%	1.88	0.67%
		625	100%	1.87	1.34%	100%	1.88	1.79%	100%	1.89	1.83%
		312.5	100%	1.89	1.02%	100%	1.89	1.30%	100%	1.90	1.11%
MR 3.0	3.02	2500	100%	2.85	1.44%	100%	2.88	1.07%	100%	2.85	1.29%
		1250	100%	2.89	1.05%	100%	2.90	1.47%	100%	2.89	1.47%
		625	100%	2.90	3.50%	100%	2.90	3.80%	100%	2.92	3.92%
		312.5	100%	2.88	2.52%	100%	2.88	3.02%	100%	2.90	2.51%
MR 4.0	4.07	2500	100%	4.25	4.19%	100%	4.35	4.10%	100%	4.30	2.99%
		1250	100%	3.97	1.51%	100%	4.01	1.95%	100%	4.02	1.30%
		625	75%	4.17	4.87%	75%	4.21	4.19%	75%	4.16	5.30%
		312.5	25%	4.23	9.50%	25%	4.23	9.49%	25%	4.10	11.65%

f. Stability Studies

Kit Real-Time Stability

Real-time stability was assessed using 3 pools of positive samples and 3 pools of negative samples. The 3 positive pools were prepared by BCR-ABL1 positive p210 (e14a2) RNA from K562 cells (pool 1), BCR-ABL1 positive p210 (e13a2) RNA from BV-173 cells (pool 2) and 4 BCR-ABL1 positive p210 RNA samples (pool 3). The 3 negative pools were prepared by BCR-ABL1 p190 (e1a2) positive RNA from SUP-B15 cells (pool 1), BCR-ABL1 p230 (e19a2) positive RNA from AR230-r cells (pool 2) and BCR-ABL1 negative RNA samples from HL60 cells (pool 3). The positive sample pool 1 was diluted with the negative sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, the positive sample pool 2 was diluted with the negative sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, and the positive sample pool 3 was diluted with the negative sample pool 3 to generate 3 concentration samples: MR3.0, MR4.0 and MR5.0. The negative sample pool 1 was diluted with the negative sample pool 3 to generate about 10% ela2 sample and the negative sample pool 2 was diluted with the negative sample pool 3 to generate about 10% el9a2 sample.

Real-time reagent stability studies were conducted using three reagent lots at the following time points: T0 (baseline), 3 months (T3), 6 months (T6), 9 months (T9), 12 months (T12), and 13 months (T13). At each time point, 17 samples and the appropriate controls were tested using reagents that had been stored at temperatures below -15°C and then thawed for analysis. The sample panel included both e13a2 and e14a2 transcript variants at target concentrations of MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5, and MR5.0.

Samples were considered acceptable if the deviation between the measured MR value at the evaluated time point and the corresponding baseline (T0) MR value was within ±0.5 log units of the target MR value.

All samples met the predefined acceptance criteria throughout the study period, demonstrating the real-time stability of the reagents when stored below -15°C for up to 12 months.

Kit Transportation Stability

A study was conducted to determine, based on the actual transportation conditions of the reagents whether storage under transportation conditions affects the performance of the product and thus evaluates the stability of the product.

The study was conducted using three batches of Fastplex™ BCR-ABL (p210) %IS digital PCR Kit were transported from Suzhou to Guangzhou with dry ice (below -15℃) and then returned to Suzhou after transportation from Guangzhou (20220711-20220715, with a total time of 5 days). After transportation, these kits were stored below -20℃± 5℃, and the samples were tests in 3, 6, 9 and 10 months respectively.

The 3 positive pools were prepared by mixing BCR-ABL1 positive p210 (e14a2) RNA from K562 cells (pool 1), BCR-ABL1 positive p210 (e13a2) RNA from BV-173 cells (pool 2) and 4 BCR-ABL1 positive p210 RNA samples (pool 3). The 3 negative pools were prepared by mixing BCR-ABL1 p190 (e1a2) positive RNA from SUP-B15 cells (pool 1), BCR-ABL1 p230 (e19a2) positive RNA from AR230-r cells (pool 2) and BCR-ABL1 negative RNA samples from HL60 cells (pool 3). The positive sample pool 1 was diluted with the negative

sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, the positive sample pool 2 was diluted with the negative sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, and the positive sample pool 3 was diluted with the negative sample pool 3 to generate 3 concentration samples: MR3.0, MR4.0 and MR5.0. The negative sample pool 1 was diluted with the negative sample pool 3 to generate about 10% ela2 sample and the negative sample pool 2 was diluted with the negative sample pool 3 to generate about 10% el9a2 sample.

All samples met the predefined acceptance criteria throughout the study period, demonstrating the conditions for transportation of the kit can be set to be below -15℃.

Kit Freeze-thaw Stability

A study was conducted to determine the allowable number of freeze-thaw cycles for the components of the Fastplex™ BCR-ABL (p210) %IS digital PCR 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. 12 Fastplex™ BCR-ABL (p210) %IS digital PCR Kits from 3 lot3 stored at -20°C were used in this study. All components of one kit were fully thawed at ambient temperature for 30 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 overnight (a minimum of 8 hours) prior to the next temperature cycle. The Reverse Transcriptase and Taq Enzyme were thawed on an ice block. This cycle was repeated 2, 4 ,6 or 8 times.

The 3 positive pools were prepared by mixing BCR-ABL1 positive p210 (e14a2) RNA from K562 cells (pool 1), BCR-ABL1 positive p210 (e13a2) RNA from BV-173 cells (pool 2) and 4 BCR-ABL1 positive p210 RNA samples (pool 3). The 3 negative pools were prepared by BCR-ABL1 p190 (e1a2) positive RNA from SUP-B15 cells (pool 1), BCR-ABL1 p230 (e19a2) positive RNA from AR230-r cells (pool 2) and BCR-ABL1 negative RNA samples from HL60 cells (pool 3). The positive sample pool 1 was diluted with the negative sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, the positive sample pool 2 was diluted with the negative sample pool 3 to generate 7 concentration samples: MR0.3, MR1.0, MR2.0, MR3.0, MR4.0, MR4.5 and MR5.0, and the positive sample pool 3 was diluted with the negative sample pool 3 to generate 3 concentration samples: MR3.0, MR4.0 and MR5.0. The negative sample pool 1 was diluted with the negative sample pool 3 to generate about 10% ela2 sample and the negative sample pool 2 was diluted with the negative sample pool 3 to generate about 10% el9a2 sample.

The deviation between the baseline MR value and each freeze thaw cycle were within ±0.5 log of the MR value, thus meeting the acceptance criteria. Results support that all components of the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit demonstrated stable performance for at least 6 freeze-thaw cycles.

Specimen Stability

One (1) fresh peripheral blood sample from a BCR-ABL1-positive patient and five fresh peripheral blood samples from BCR-ABL1-negative patients were collected. All blood samples were collected within 24 hours of each patient and were stored in vacuum tubes containing K2-EDTA. Three (3) BCR-ABL1 p210-negative blood samples were pooled to prepare a negative sample pool. The positive sample was mixed with the negative sample

pool and diluted to prepare three BCR-ABL1 levels: MR1.0, MR3.0, and MR4.5.

Each blood sample was divided into five equal portions of approximately 3 mL each. RNA was extracted from the blood samples on Days 0, 1, 2, 3, and 4. Each extracted RNA sample underwent eight replicate tests using a single batch of the kit.

Positive samples were considered acceptable if the coefficient of variation (CV%) was ≤10% and the mean MR value fell within the 95% confidence interval of the Day 0 MR value ±0.5 log units. Negative samples were required to yield 100% negative test results. Results indicate whole blood samples should be stored at 2-8°C for no longer than 3 days.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was designed to evaluate the performance of the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit compared to QXDx BCR-ABL %IS Kit (Bio-Rad Laboratories, K181661) in RNA derived from peripheral blood samples obtained from individuals previously diagnosed with t(9;22) positive chronic myeloid leukemia (CML).

Clinical samples from 159 CML patients were collected from 2 hospitals for retrospective analysis. Of the 159 samples screened, 16 samples were excluded since they did not meet the sample screening criteria. A total of 143 samples (MR values were distributed between 0.32 and 4.7) were included in the statistics.

Subject inclusion criteria:

- 18 years of age or older
- Previously diagnosed as t(9;22) positive CML p210 or Major variant type

Subject exclusion criteria:

- Samples that did not meet the inclusion criteria
- Specimen type other than peripheral blood
- Extracted RNA concentration is less than 125 ng/µL
- Insufficient Samples for testing

Sample inclusion criteria

- Peripheral blood stored in blood collection tube containing EDTA anticoagulant and stored at 4°C for no more than 72 hours.

Sample extraction requirements

- RNA from the patient's peripheral blood samples were extracted by the RainSure RNA extraction Kit and stored at -80°C after the RNA extractions were completed.
- Extracted RNA ≥ 2.5µg

The clinical samples in the method comparison study were selected to span the testing range as shown below:

Table 13. Range of clinical specimens in the method comparison study.

Bin	Predicate	BCR-ABL (p210) %IS digital PCR Kit
0.3-<1.5	33	33
1.5-<2.5	23	22
2.5-<3.5	36	33
3.5-<4.7	51	55

The mean bias (95%CI) between RainSure Fastplex™ BCR-ABL (p210) %IS digital PCR Kit and QXDx BCR-ABL %IS Kit calculated using a Bland-Altman analysis was -0.01 (-0.33 to 0.31), indicating that the limits of agreement (LOA) between the two methods should lie between -0.33 and 0.31 for 95% of the time (Figure 2). Passing-Bablok regression analysis (Figure 3) was also performed and resulted in intercept A (95% CI): -0.06768 (-0.1214, -0.02208), and the slope B (95% CI): 1.0194 (0.9984, 1.0416). Both results from the method comparison study demonstrate that the Fastplex™ BCR-ABL (p210) %IS digital PCR Kit is substantially equivalent to the predicate.

Figure 2. Bland-Altman result

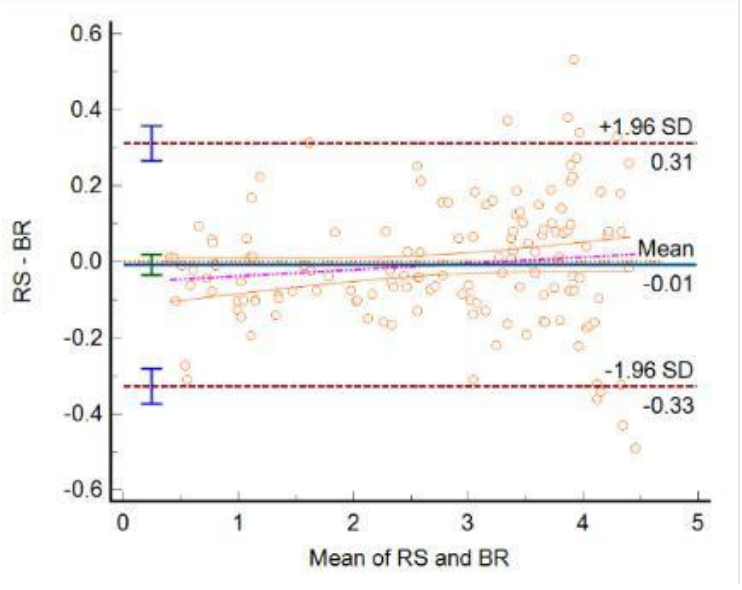
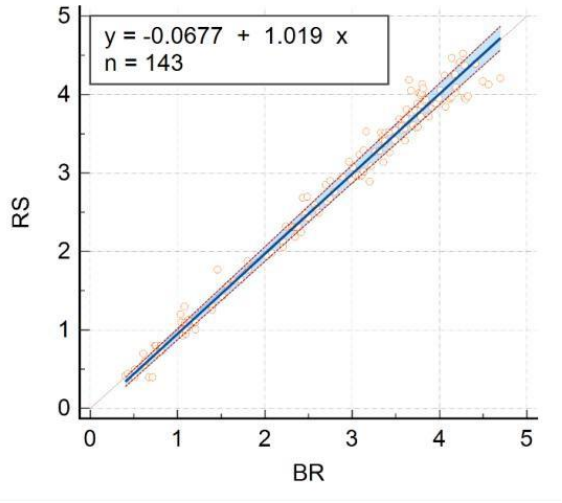


Figure 3. Passing-Bablok Regression Result



i. Matrix comparison: Not applicable

b. Clinical studies:

i. Clinical Sensitivity: Not applicable

ii. Clinical specificity: Not applicable

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

c. Clinical cut-off**:** Not applicable

3. Instrument Name:

RainSure DropMDx digital PCR System

4. System Descriptions**:**

a. 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__

b. 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 controls 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.

H. Other Supportive Instrument Performance Characteristics Data Not Covered in The "Performance Characteristics" Section above:

Not applicable

I. Proposed Labeling:

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

J. Conclusion:

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