510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I. Background Information

A. 510(k) Number:

K221869

B. Applicant:

Suzhou Sniper Medical Technologies Co., Ltd.

C. Proprietary and Established Names:

BCR-ABL1 (p210) %IS Kit (Digital PCR Method)

D. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OYX PHG	Class II	21 CFR 866.6060 21 CFR 862.2570	88-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. Indications for Use:

1. Indications for use:

The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) 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) adult patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2. The BCR-

ABL1 (p210) %IS Kit (Digital PCR Method) is a reverse transcription-quantitative PCR performed on the Sniper Digital PCR All-in-One 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 BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is intended for use only on the Sniper Digital PCR All-in-One 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:

   Sniper Digital PCR All-in-One System

IV. Device Description:

The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is designed for detection of the BCR-ABL1 fusion gene (p210) and ABL1 gene, with specific primers and specific fluorescence probes. The test is performed in three parts. The first part is to extract ribonucleic acid (RNA) from peripheral blood of CML patients. The second part is to detect the BCR-ABL1 fusion gene (p210) and ABL1 internal reference gene in RNA samples by Reverse Transcription-Droplet PCR (RT-dPCR) reaction solution using the Sniper Digital PCR All-in-One System (DQ24-Dx). The third part is to analyze the results.

A description of the reagents provided with the kit is described below in Table 1.

Table 1. Reagents in the BCR-ABL1 (p210) %IS Kit (Digital PCR Method)

Composition	Main components	Application
One-step RT-dPCR Master Mix	One-step RT-dPCR Buffer, dNTP/dUTP Mix, $MgCl_2$, FAM Reference Dye, RNase Inhibitor, etc.	Reaction mix component of the RT reaction to generate cDNA from RNA template.
BCR-ABL1 Primer Probe Mix	Primers, Probes	Provides primers and probes for ddPCR amplification and detection of target sequences.
BCR-ABL1 Enzyme Mix	Taq DNA Polymerases, Reverse Transcriptase, RNase Inhibitor, Uracil-	Catalyzes the amplification of Primers hybridize to templates from the cDNA. Enzyme exonuclease activity degrades

Composition	Main components	Application
	DNA Glycocasylase	hybridized probes to release fluorescence for the detection of amplicons in each PCR cycle.
Calibrator 10%IS	K562 cell RNA, HL60 cell RNA mixture	Per run calibrators to check against acceptance criteria for use of electronic WHO-IS CF[1] factor and reporting of WHO-IS value results.
Calibrator 0.1%IS	K562 cell RNA, HL60 cell RNA mixture	Per run calibrators to check against acceptance criteria for use of electronic WHO-IS CF factor and reporting of WHO-IS value results.
Positive Control 1 (%IS of 10)	K562 cell RNA, HL60 cell RNA mixture	Control used to ensure that ddPCR steps performed properly by generating expected MR value.
Positive Control 2 (%IS of 0.01)	K562 cell RNA, HL60 cell RNA mixture	Control used to ensure that ddPCR steps performed properly by generating expected MR value.
Negative Control	HL60 cell RNA	Negative control used to ensure that RT and ddPCR steps performed properly and identify false positive results due to contamination.
Nuclease free water	DNase/RNase-Free water	Adjust volume of RT & ddPCR reactions.

[1] WHO-IS CF: World Health Organization International Standard Conversion Factor

Instrument

The Sniper Digital PCR All-in-One System consists of one instrument, which can be used in conjunction with the associated consumables, and BCR-ABL1 (p210) %IS Kit (Digital PCR Method) to complete the detection of samples.

The Sniper Digital PCR All-in-One System is designed to partition the PCR reaction solution into a large number of independent micro-reaction units to carry out the PCR amplification reaction and read the number of positive and negative micro-reaction units through fluorescent signals, then calculate the concentration of PCR template quantitatively according to the volume of the micro-reaction units and the principle of Poisson Distribution.

Software:

DQ24-Dx-Sight Software (v1.0.2), is used to control the system and analyze test results. This software is embedded in the Sniper Digital PCR All-in-One System.

V. Substantial Equivalence Information:

1. Predicate device name(s):

QXDx BCR-ABL %IS Kit
QXDx Automated Droplet Generator
QXDx Droplet Reader
QXDx Software 1.2

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

   K181661

3. Comparison with predicate:

Similarities		
Item	**Subject Device**	**Predicate Device**
Indications	The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) 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) adult patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2. The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is a reverse transcription-quantitative PCR performed on the Sniper Digital PCR All-in-One 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 BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is intended for use only on the Sniper Digital PCR All-in-One 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.	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 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.

Similarities		
Item	**Subject Device**	**Predicate Device**
Measurement Type	Quantitative	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
Fundamental Technology	Digital PCR	Same
Calibrators	Two levels are formulated at 0.1%IS and 10%IS BCR-ABL1/ABL1.	Same

Differences		
Item	**Subject Device**	**Predicate Device**
Measuring Range	MR0.3 to MR4.5	MR0.3 to MR4.7
RNA Input	500 ng	1000 ng
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	Sniper DQ24-Dx	Bio-Rad QXDx™ AutoDG™ ddPCR System
Instrument Computer Operating System	Embedded software, Ubuntu18.04.5	Microsoft Windows 10
Degree of Automation	Automated control of amplification, detection, and data analysis.	Requires manual transfer of amplification mixture to amplification/detection instrument. Automated control of detection and data analysis, except amplification functionality.
Amplification Reaction Volume	22 µL in Sniper PCR plates.	20-25 µL in 96-well Bio-Rad PCR plates.

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

CLSI EP07-Ed3, Interference Testing in Clinical Chemistry
CSLI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.
CSLI EP15-A3, User Verification of Precision and Estimation of Bias - Third Edition.
CLSI EP06- 2nd Edition, Evaluation of the Linearity of Quantitative Measurement Procedures.
CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents.

VII. Test Principle:

The BCR-ABL1 (p210) %IS Kit quantitatively detects the RNA of fusion genes BCR-ABL1 (p210, b2a2 (e13a2) and b3a2 (e14a2)) and ABL1 in the peripheral blood of adult patients with Chronic Myeloid Leukemia (CML) by designed specific primers and probes combined with Sniper Digital PCR All-in-One System.

Total RNA is extracted from whole blood containing EDTA anticoagulant for detection. Sample RNA is mixed with One-step RT-dPCR Master Mix, BCR-ABL1 Primer Probe Mix, and BCR-ABL1 Enzyme Mix to prepare a 22 μL PCR reaction. The BCR-ABL1 primers and probes are designed to detect the breakpoint translocation of BCR-ABL1 p210 [b2a2 (e13a2) and b3a2 (e14a2)], and to detect the ABL1 sequence.

A total of 22 μL of PCR reaction is loaded into each of 8 consecutive tubes, which is placed on the sample rack of the Sniper Digital PCR All-in-One System. After the amplification procedure, the detection is conducted without separate reverse transcription. The supporting consumables required for the detection process include droplet generation oil, PCR four-well plate, droplet generation needle, 3 quality controls, and 2 calibrators for each run. After amplification, Sniper Digital PCR All-in-One System will photograph each sample, control, and calibrator, according to the fluorescence channels to distinguish negative and positive droplets, and the photos are stored.

After the detection is completed, the ratio of BCR-ABL1 and ABL1 genes is calculated, and the %IS and MR values of the sample are calculated according to the conversion factor (CF) of the kit. The blank control should be BCR-ABL1 copy ≤1 and ABL1 copy ≤10, the negative control should be BCR-ABL1 copy ≤1, the measured values of positive control 1 and calibrator 10%IS should be between MR0.5 and MR1.5, the measured value of positive control 2 should be between MR3.5 and MR4.5, and the measured value of calibrator 0.1%IS should be 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, repetitive detection of a sample is 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 MRx.x. However, for simplicity and legibility, the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) 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:

Table 2: %IS and MR comparison table

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

MMR: Major Molecular Response

The results are interpreted automatically by the embedded Software DQ24-Dx-Sight from measured droplet counts, fluorescent signals, and embedded calculation algorithms. The Software will report out BCR-ABL1 and ABL1 copies. An indication of sample suitability is that the ABL1 copies are sufficient for the 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.,

$$\%IS = \text{BCR-ABL1 copy} / \text{ABL1 copy} \times 100 \times CF$$

The test results should be interpreted according to the following standards in Table 3:

Table 3: Interpretation of results

Test results	Report results	Explanation of test results
Copy number of BCR-ABL1 as 0.	Report: Negative	Indicates that there is no BCR-ABL1 fusion gene in the test sample.
MR > 4.5 or %IS < 0.0032	Report: MR value > 4.5 detected or %IS value < 0.0032 detected	BCR-ABL1 fusion gene is detected, but the results are beyond the limit of quantitation.
MR ≤ 4.5 or %IS ≥ 0.0032	Report: %IS (MR) value	BCR-ABL1 fusion gene detected and %IS (MR) value measured.
Copy number of ABL1 ≤10000	Report: Invalid	The copy number of ABL1 gene is too low.

Note:

1) When the number of ABL1 copies is ≤ 10000, the report is invalid. A retest should be performed with increased RNA input.
2) In the case of 10000 < ABL1 copy number ≤ 32,000, and BCR-ABL1 copy number = 0, ABL1 copy number is too low, and a retest should be performed with increased RNA input.
3) In the case of ABL1 copy number > 140000 and the BCR-ABL1 copy number > 0, the ABL1 copy number exceeds the linear range, which it will affect the quantitative accuracy. A retest should be performed with reduced RNA input.

VIII. Performance Characteristics (if/when applicable):

1. Analytical Performance:

a. Precision/Reproducibility:

Precision and Reproducibility were assessed using 3 pools of positive samples at 5 MR levels. The 3 positive pools were prepared by mixing 5 BCR-ABL1 positive p210 (e13a2) RNA samples with an MR value of 0.3 (pool 1), 5 BCR-ABL1 positive p210 (e14a2) RNA samples with an MR value of 0.3 (pool 2), and 5 BCR-ABL1 positive RNA samples p210 (e13a2) and p210 (e14a2) with an MR value of 0.3 (pool 3). A negative pool was used as a diluent and was prepared by mixing 30-60 BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate 5 samples with different concentrations: MR1.0, MR2.0, MR3.0, MR4.0, and MR4.5.

Samples were assayed in 2 replicates per run for 2 runs per day for 3 non-consecutive days (1st, 3rd, and 5th) at 3 sites (1 instrument at each site) with 1 reagent lot for a total of 36 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 the following Table 4:

Table 4: Precision acceptance criteria corresponding to different concentrations

MR value	Precision (CV%) acceptance criteria
MR0.3 – MR2.0	≤10%
MR2.1 – MR3.49	≤15%
MR3.5 – MR4.0	≤20%
LoQ	≤20%

A total of 540 observations were included in a variance components analysis for site, day, and run (operator) to assess repeatability, within-day precision, within-site precision, and reproducibility of measured MR level. Total MR and %IS precision were calculated for the assay (Table 5 and Table 6, respectively) and in-kit calibrators and controls (Table 7). Results of MR level (CVs ≤10%) indicated low variability and the acceptance criteria (≤10%-20%) were satisfied.

Table 5: Precision analysis results (MR) of samples

Sample	Variant	N	MR expec-ted	MR mean	Within-run		Within-day		Between-day		Within-site		Between-site		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR1.0	e13a2	36	1.00	1.05	0.001	0.06%	0.006	0.58%	0.012	1.12%	0.012	1.12%	0.016	1.57%	0.019	1.85%
	e14a2	36	1.00	1.04	0.005	0.51%	0.013	1.22%	0.020	1.89%	0.020	1.96%	0.020	1.96%	0.024	2.31%
	mix	36	1.00	1.05	0.009	0.84%	0.016	1.56%	0.017	1.64%	0.019	1.84%	0.014	1.29%	0.025	2.38%
MR2.0	e13a2	36	2.00	1.99	0.008	0.40%	0.013	0.66%	0.023	1.13%	0.024	1.20%	0.023	1.14%	0.036	1.82%
	e14a2	36	2.00	1.98	0.016	0.80%	0.020	1.02%	0.009	0.48%	0.018	0.92%	0.021	1.04%	0.030	1.54%
	mix	36	2.00	1.98	0.008	0.41%	0.017	0.84%	0.017	0.88%	0.019	0.96%	0.017	0.87%	0.031	1.56%
MR3.0	e13a2	36	3.00	2.99	0.014	0.47%	0.032	1.06%	0.007	0.24%	0.016	0.52%	0.027	0.91%	0.071	2.37%
	e14a2	36	3.00	2.98	0.047	1.57%	0.010	0.34%	0.020	0.68%	0.050	1.69%	0.040	1.35%	0.093	3.11%
	mix	36	3.00	3.00	0.029	0.96%	0.035	1.16%	0.020	0.68%	0.035	1.16%	0.052	1.72%	0.077	2.55%
MR4.0	e13a2	36	4.00	3.92	0.057	1.45%	0.044	1.12%	0.023	0.60%	0.061	1.55%	0.041	1.05%	0.135	3.43%
	e14a2	36	4.00	3.94	0.036	0.90%	0.053	1.34%	0.026	0.65%	0.044	1.10%	0.036	0.90%	0.091	2.31%
	mix	36	4.00	3.91	0.025	0.64%	0.041	1.04%	0.025	0.64%	0.035	0.90%	0.063	1.62%	0.119	3.04%
MR4.5	e13a2	36	4.50	4.66	0.049	1.05%	0.055	1.19%	0.091	1.95%	0.103	2.21%	0.194	4.18%	0.255	5.48%
	e14a2	36	4.50	4.59	0.100	2.17%	0.099	2.14%	0.045	0.97%	0.108	2.35%	0.170	3.70%	0.261	5.68%
	mix	36	4.50	4.59	0.048	1.05%	0.121	2.64%	0.041	0.88%	0.062	1.36%	0.164	3.58%	0.227	4.95%

Mix: Represents a mixture of e13a2 and e14a2 transcripts.

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

Sample	Variant	N	%IS expec-ted	%IS mean	Within-run		Within-day		Between-day		Within-site		Between-site		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR1.0	e13a2	36	10.000	8.985	0.016	0.2%	0.123	1.4%	0.240	2.7%	0.241	2.7%	0.336	3.7%	0.397	4.4%
	e14a2	36	10.000	9.070	0.112	1.2%	0.270	3.0%	0.409	4.5%	0.424	4.7%	0.308	3.4%	0.503	5.5%
	mix	36	10.000	9.022	0.185	2.1%	0.327	3.6%	0.356	3.9%	0.400	4.4%	0.274	3.0%	0.507	5.6%
MR2.0	e13a2	36	1.000	1.017	0.019	1.9%	0.030	3.0%	0.053	5.2%	0.056	5.5%	0.054	5.3%	0.085	8.4%
	e14a2	36	1.000	1.051	0.038	3.6%	0.049	4.7%	0.022	2.1%	0.044	4.2%	0.050	4.8%	0.073	7.0%
	mix	36	1.000	1.048	0.020	1.9%	0.037	3.6%	0.042	4.0%	0.046	4.4%	0.040	3.8%	0.072	6.9%
MR3.0	e13a2	36	0.100	0.103	0.003	2.9%	0.008	7.4%	0.002	2.0%	0.004	3.5%	0.006	6.0%	0.017	16.3%
	e14a2	36	0.100	0.107	0.010	9.6%	0.002	2.3%	0.005	5.1%	0.012	10.8%	0.009	8.7%	0.022	20.9%
	mix	36	0.100	0.101	0.006	5.6%	0.007	7.0%	0.004	3.9%	0.007	6.8%	0.011	10.6%	0.016	15.8%
MR4.0	e13a2	36	0.010	0.013	0.002	12.1%	0.001	10.6%	0.001	5.2%	0.002	13.0%	0.001	9.2%	0.004	29.5%
	e14a2	36	0.010	0.012	0.001	7.6%	0.001	11.4%	0.001	5.6%	0.001	9.4%	0.001	8.4%	0.002	20.2%
	mix	36	0.010	0.013	0.001	5.4%	0.001	10.7%	0.001	6.1%	0.001	8.1%	0.002	16.1%	0.004	29.7%

Sample	Variant	N	%IS expec-ted	%IS mean	Within-run		Within-day		Between-day		Within-site		Between-site		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR4.5	e13a2	36	0.0032	0.0026	0.0001	5.8%	0.0004	16.3%	0.0004	16.5%	0.0004	17.5%	0.0010	40.3%	0.0015	56.6%
	e14a2	36	0.0032	0.0030	0.0004	14.7%	0.0008	25.3%	0.0004	13.8%	0.0006	20.0%	0.0009	31.2%	0.0015	51.3%
	mix	36	0.0032	0.0029	0.0003	9.9%	0.0007	23.1%	0.0002	5.3%	0.0003	11.1%	0.0010	33.6%	0.0013	44.8%

Mix: Represents a mixture of e13a2 and e14a2 transcripts.

Table 7: Calibrator and Control Precision Analysis Results

Sample	N	MR				%IS			
		target	mean	SD	CV	target	mean	SD	CV%
Calibrators 10%IS	54	1.00	1.05	0.022	2.10%	10.00	8.861	0.443	5.00%
Calibrators 0.1%IS	54	3.00	3.01	0.054	1.79%	0.10	0.098	0.012	12.20%
Positive control 1	54	1.00	1.06	0.023	2.14%	10.00	8.766	0.454	5.18%
Positive control 2	54	4.00	3.91	0.105	2.68%	0.01	0.013	0.003	23.86%
Negative control	54	--	NA	NA	NA	--	0.000	0.000	NA
Blank control	54	--	NA	NA	NA	--	NA	NA	NA

Lot-to-lot Precision

Lot-to-lot precision was assessed using 2 pools of positive samples at 4 MR levels. The positive pools were prepared by mixing 5 BCR-ABL1 positive RNA samples [p210 (e13a2) with an MR value of 0.3, pool 1], and 5 BCR-ABL1 positive RNA samples [p210 (e14a2) with an MR value of 0.3, pool 2]. A negative pool was used as a diluent and was prepared by mixing 60 BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate four samples with different concentrations: MR1.0, MR3.0, MR4.0, and MR4.5.

Samples were tested in 3 replicates per run for 2 runs per day for 3 non-consecutive days (1st, 3rd, and 5th) on 2 instruments with 3 reagent lots for a total of 108 replicates. Each run was performed by an independent operator (2 operators). The total precision (CV%) values needed to meet the acceptance criteria shown in Table 4 above.

A total of 864 observations were included in a variance components analysis with random effects for day, run, operator, instrument, and lot to assess precision of measured MR level. Results of MR level indicated low variability, including between lots, and the acceptance criteria were satisfied (CVs ≤10%). Total MR and %IS precision were calculated for the assay (Table 8 and Table 9) and in-kit calibrators and controls (Table 10).

Table 8: Lot-to-lot precision analysis results (MR) of samples

Sample	Variant	N	MR Expec-ted	MR mean	Within-run		Day		Operator		Instrument		Lot		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR1.0	e13a2	108	1.00	1.02	0.031	3.04%	0.006	0.62%	0.008	0.76%	0.013	1.31%	0.018	1.76%	0.031	3.06%
	e14a2	108	1.00	1.03	0.029	2.85%	0.006	0.55%	0.017	1.64%	0.008	0.76%	0.018	1.73%	0.030	2.86%
MR3.0	e13a2	108	3.00	2.98	0.092	3.07%	0.007	0.24%	0.025	0.85%	0.017	0.58%	0.063	2.10%	0.092	3.07%
	e14a2	108	3.00	2.99	0.085	2.84%	0.008	0.28%	0.030	1.01%	0.012	0.39%	0.059	1.96%	0.084	2.82%
MR4.0	e13a2	108	4.00	3.97	0.179	4.51%	0.017	0.42%	0.032	0.81%	0.024	0.60%	0.122	3.06%	0.179	4.52%
	e14a2	108	4.00	3.95	0.181	4.58%	0.004	0.10%	0.043	1.09%	0.066	1.67%	0.125	3.17%	0.180	4.55%

Sample	Variant	N	MR Expec-ted	MR mean	Within-run		Day		Operator		Instrument		Lot		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR4.5	e13a2	108	4.50	4.35	0.202	4.65%	0.103	2.37%	0.045	1.04%	0.084	1.92%	0.141	3.24%	0.218	5.01%
	e14a2	108	4.50	4.33	0.236	5.45%	0.007	0.17%	0.059	1.35%	0.039	0.90%	0.164	3.78%	0.235	5.41%

Table 9: Precision analysis results (%IS) of samples

Sample	Variant	N	%IS Expec-ted	%IS mean	Within-run		Day		Operator		Instrument		Lot		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
MR1.0	e13a2	108	10.000	9.4487	0.6866	7.3%	0.0836	0.9%	0.1678	1.8%	0.1905	2.0%	0.4607	4.9%	0.6892	7.3%
	e14a2	108	10.000	9.3064	0.6392	6.9%	0.0453	0.5%	0.3642	3.9%	0.1409	1.5%	0.4326	4.6%	0.6397	6.9%
MR3.0	e13a2	108	0.100	0.1058	0.0208	19.6%	0.0030	2.8%	0.0050	4.8%	0.0050	4.7%	0.0145	13.7%	0.0207	19.6%
	e14a2	108	0.100	0.1043	0.0200	19.2%	0.0009	0.9%	0.0068	6.5%	0.0054	5.1%	0.0140	13.4%	0.0199	19.0%
MR4.0	e13a2	108	0.010	0.0116	0.0045	39.2%	0.0004	3.3%	0.0004	3.8%	0.0007	6.1%	0.0032	27.6%	0.0045	39.0%
	e14a2	108	0.010	0.0122	0.0047	38.3%	0.0002	1.8%	0.0014	11.6%	0.0006	5.1%	0.0032	26.5%	0.0046	38.1%
MR4.5	e13a2	108	0.0032	0.0050	0.0020	39.6%	0.0010	19.6%	0.0002	4.5%	0.0004	8.1%	0.0014	27.6%	0.0021	42.5%
	e14a2	108	0.0032	0.0052	0.0024	45.1%	0.0001	1.8%	0.0005	9.1%	0.0005	9.3%	0.0016	31.4%	0.0023	44.8%

Based on the results of the lot-to-lot precision study, the results of the calibrators and controls were summarized and the precision was analyzed. The results showed that the precision (CV%) of the calibrators and controls were ≤10% (Table 10) and met the acceptance criteria for precision in Table 4.

Table 10: Calibrator and Control Precision Analysis Results

Sample	N	MR				%IS			
		Target	Mean	SD	CV	Target	Mean	SD	CV%
Calibrators 10%IS	96	1.00	1.04	0.027	2.59%	10.00	9.045	0.570	6.30%
Calibrators 0.1%IS	96	3.00	3.00	0.060	1.99%	0.10	0.101	0.014	13.90%
Positive control 1	96	1.00	1.05	0.029	2.74%	10.00	8.998	0.600	6.66%
Positive control 2	96	4.00	3.93	0.125	3.18%	0.01	0.012	0.003	28.07%
Negative control	96	--	NA	NA	NA	--	0.000	0.000	NA
Blank control	96	--	NA	NA	NA	--	NA	NA	NA

RNA Extraction Method

RNA is extracted from peripheral blood samples using the Whole Blood Nucleic Acid Extraction Reagent (Cat.# DT007048C) produced by Suzhou Sniper Medical Technologies Co., Ltd.

This study was assessed using 3 positive pools at 5 MR levels. The 3 positive pools were prepared by mixing 6 BCR-ABL1 positive p210 (e13a2) peripheral blood samples with an MR value of 0.3 (pool 1), 7 BCR-ABL1 positive p210 (e14a2) peripheral blood samples with an MR value of 0.3 (pool 2), and k562 cells (pool 3). A negative pool was used as a diluent and was prepared by mixing 80 BCR-ABL1 negative peripheral blood samples. The positive sample pools were diluted with the negative sample pool to generate 5 samples with different concentrations: MR0.5-1.0, MR1.5-2.0, MR2.5-3.0, MR3.5-4.0 and MR4.2-4.5, respectively.

Samples were extracted in duplicate by 2 operators per day for 3 non-consecutive

days (1st, 3rd, and 5th) with 1 lot of Whole Blood Nucleic Acid Extraction Reagent. The sample was considered to meet the acceptance criteria when the concentration of extracted RNA was equal or above 100 ng/μL and the purity OD_{260nm}/OD_{280nm} was above 1.6 based on the requirements listed in Table 11. A total of 180 results were included in RNA Extraction study, and 5 samples failed the quality requirements (2 samples did not meet the RNA concentration requirement and 3 samples did not meet the RNA purity requirement). The results showed that 97% of extracted RNA samples met the requirements and the precision (CV%) of all samples was less than 10% (Table 12).

Table 11: RNA quality

Category	**Requirements**
Peripheral blood volume	2-10 mL
RNA concentration	≥100 ng/μL
RNA purity	OD_{260}/OD_{280} ratio > 1.6

Table 12: Extraction of peripheral blood and analysis of test results

Variant	**MR value**	**Peripheral blood sample extraction**			**Sample detection**			
		Sample N	**Qualified N**	**Qualified proportion**	**Tests N**	**SD**	**AVG**	**CV%**
e13a2	MR0.5~1.0	12	12	97%	12	0.016	0.81	2.04%
	MR1.5~2.0	12	12		12	0.028	1.75	1.61%
	MR2.5~3.0	12	11*		11*	0.043	2.50	1.70%
	MR3.5~4.0	12	12		12	0.129	3.80	3.41%
	MR4.2~4.5	12	12		12	0.246	4.51	5.44%
e14a2	MR0.5~1.0	12	10*		10*	0.012	0.83	1.44%
	MR1.5~2.0	12	12		12	0.023	1.75	1.33%
	MR2.5~3.0	12	12		12	0.040	2.52	1.58%
	MR3.5~4.0	12	12		12	0.103	3.83	2.70%
	MR4.2~4.5	12	12		12	0.320	4.63	6.91%
K562	MR0.5~1.0	12	12		12	0.016	0.81	2.02%
	MR1.5~2.0	12	12		12	0.033	1.74	1.88%
	MR2.5~3.0	12	10*		10*	0.071	2.52	2.82%
	MR3.5~4.0	12	12		12	0.139	3.76	3.71%
	MR4.2~4.5	12	12		12	0.211	4.43	4.76%

* Indicates that RNA concentration or purity did not meet requirements during extraction and no subsequent testing

b. Linearity/assay reportable range:

Linearity/assay reportable range was assessed using 2 pools of positive samples at 10 MR levels. Two (2) positive BCR-ABL1 [p210 (e13a2)] peripheral blood samples with an MR value of approximately 0.3 were mixed to generate pool 1. Two (2) positive BCR-ABL1 [p210 (e14a2)] peripheral blood samples with an MR value of approximately 0.3 were mixed to generate pool 2. A negative pool was used as a diluent and was prepared by mixing 22 BCR-ABL1 negative RNA samples. The positive sample pools 1 and 2 were diluted with the negative pool to generate 10 samples with different concentrations: MR0.3, MR0.5, MR1.0, MR1.5, MR2.0,

MR2.5, MR3.0, MR4.0, MR4.5, and MR4.7.

Samples were tested in 4 replicates on 1 day with 1 reagent lot. The precision and deviation analyses were required to meet the acceptance criteria shown in Table 13, below.

Table 13: Precision and deviation acceptance criteria

Category	Acceptance criteria
Precision (CV%)	⩽10%
% Deviation	⩽±15%

Samples with concentrations from 50%IS (MR 0.3) to 0.002%IS (MR4.7) were determined for the e13a2 and e14a2 variants. Precision analysis showed that CV% of all samples met the requirement of ≤10%. Regression analysis showed that the appropriate type of regression analysis was a weighted least squares (WLS) linear regression analysis with no intercept (Y=AE). Based on WLS linear regression analysis, deviation analysis showed that the % deviations of all samples met the requirement of ≤±15% (Table 14).

Table 14: Precision analysis results of different samples

Variant	Sample	MR values							
		Precision				Deviation			
		Mean Y	Expected E	SD	CV%	predicted Y=AE	Deviation	% Deviation	Pass
e13a2	1	0.26	0.30	0.009	3.26%	0.30	-0.03	-10.91%	YES
	2	0.46	0.50	0.013	2.76%	0.49	-0.03	-6.43%	YES
	3	1.03	1.00	0.012	1.15%	0.98	0.05	4.72%	YES
	4	1.45	1.50	0.014	0.94%	1.48	-0.02	-1.55%	YES
	5	1.97	2.00	0.010	0.49%	1.97	0.00	0.08%	YES
	6	2.53	2.50	0.070	2.77%	2.46	0.07	2.94%	YES
	7	2.98	3.00	0.058	1.93%	2.95	0.03	1.00%	YES
	8	3.87	4.00	0.107	2.76%	3.93	-0.07	-1.71%	YES
	9	4.40	4.50	0.080	1.82%	4.43	-0.02	-0.51%	YES
	10	4.81	4.70	0.207	4.30%	4.62	0.19	4.11%	YES
e14a2	1	0.28	0.30	0.011	4.08%	0.30	-0.02	-6.50%	YES
	2	0.47	0.50	0.022	4.53%	0.50	-0.02	-4.65%	YES
	3	1.05	1.00	0.016	1.53%	0.99	0.05	5.24%	YES
	4	1.47	1.50	0.013	0.89%	1.49	-0.02	-1.65%	YES
	5	1.97	2.00	0.017	0.84%	1.99	-0.02	-0.76%	YES
	6	2.54	2.50	0.043	1.70%	2.49	0.05	2.17%	YES
	7	3.03	3.00	0.035	1.16%	2.98	0.05	1.54%	YES
	8	3.92	4.00	0.275	7.01%	3.98	-0.06	-1.61%	YES
	9	4.45	4.50	0.222	4.98%	4.48	-0.03	-0.62%	YES
	10	4.80	4.70	0.181	3.76%	4.68	0.13	2.74%	YES

In addition, the linear range regression analysis of variants e13a2 and e14a2 showed that the results for R^2 and slope met the study acceptance criteria (Table 15).

Table 15: Linear range regression analysis for variants e13a2 and e14a2

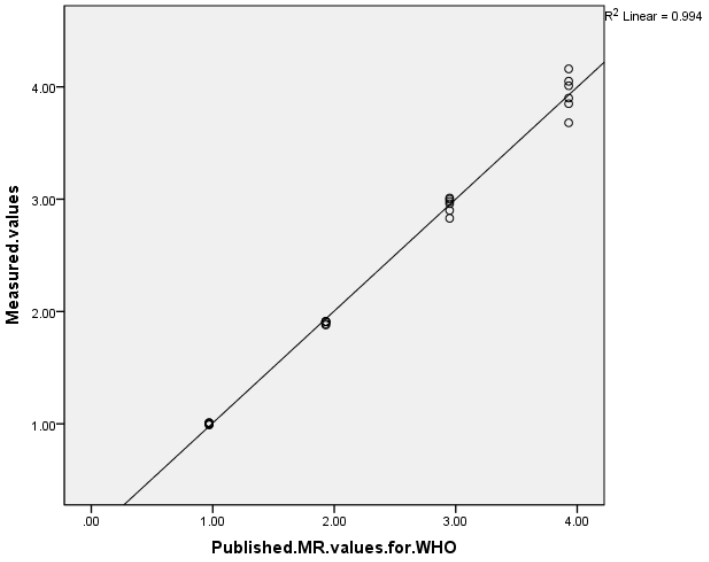
Type	Linear range (%IS, MR)	Slope	95% confidence interval for slope	R^2	Intercept	Acceptable range		Pass
						R^2	95% confidence interval for slope	
e13a2	50%IS-0.002%IS MR0.3-MR4.7	1.000	0.98-1.02	0.996	-0.023	≥0.98	0.83-1.20	YES
e14a2	50%IS-0.002%IS MR0.3-MR4.7	1.004	0.98-1.03	0.994	-0.011			YES
e13a2 and e14a2 together	50%IS-0.002%IS MR0.3-MR4.7	1.002	0.99-1.02	0.995	-0.017			YES

Based on the linear range, the deviation between the measured value and theoretical value for samples with different concentrations for variant e13a2 was -0.13–0.11, and that for different concentrations for variant e14a2 was -0.08–0.10 (the results of deviation analysis for samples with different variants and concentrations are shown in Table 14). Considering that the lower quantitation limit of the kit is MR4.5, the reportable range for both e13a2 and e14a2 of the BCR-ABL1 (p210) %IS Kit is 50%IS (MR0.3) - 0.0032%IS (MR4.5).

c. *Traceability, Expected values (controls, calibrators, or methods):*
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 9 independent BCR-ABL1 (p210) %IS Kit (Digital PCR Method) 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 (08/192, 08/194, 08/196 and 08/198) with a different concentration was tested in 6 replicates across 9 lots. The measured MR values for each level of the WHO Reference Panel were adjusted by the correction factor, CF (0.62-0.83). 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 R^2 values of 0.989-0.997. The slope of the regression lines varied between 0.889 and 0.997, and the intercepts were between 0.011 and 0.222. An example of 1 kit lot is shown in Figure 1.

Figure 1: Measured MR vs. published MR regression analysis



d. Detection limit:

Limit of Blank

The limit of blank (LoB) was determined by testing 84 independent BCR-ABL negative samples by 1 operator using 2 kit lots on 3 non-consecutive days. Out of 144 test results, 138 had no detectable BCR-ABL values. Six (6) had measurements below the LoD of the test and were reported as "MR value ＞4.5". Based on nonparametric analysis method, the LoB of the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) kit is 0 copies.

Limit of Detection/Limit of Quantitation

Limit of Detection (LoD)/Limit of Quantitation (LoQ) were assessed using 2 pools of positive samples. The 2 positive pools were prepared by mixing 1 BCR-ABL1 positive p210 (e13a2) RNA samples with an MR value of 0.3 (pool 1), and 3 BCR-ABL1 positive p210 (e14a2) RNA samples with an MR value of 0.3 (pool 2). A negative pool was used as a diluent and was prepared by mixing 39 BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate 3 samples with different concentrations: MR4.5, MR4.7, and MR4.9.

Samples were tested in 20 replicates per day for 3 days with 2 reagent lots for a total of 120 replicates. The hit rate and CV% were required to meet the acceptance criteria shown in the following Table 16.

Table 16: Hit rate and CV% acceptance criteria

Category	Hit rate	CV%
LoD	≥95%	None
LoQ	100%	≤10%

Based on Probit method and precision analysis, the LoD and LoQ of the kit were

analyzed. The hit rates of MR4.5 samples were 100%, and the CV% was between 3.47% and 4.03%. The hit rates of MR4.7 samples were between 97% and 98%, and the CV% was between 4.09% and 4.79%. The hit rates of MR4.9 samples were between 78% and 90%, and the CV% was between 4.09% and 4.64%. When the sample concentration was MR4.7, the hit rates were ≥95%. When the sample concentration was MR4.5, the hit rates were 100% and the CV% were ≤10%. Therefore, the LoD was determined as 4.7 (0.002%IS) and the LoQ was determined as 4.5 (0.0032%IS) for the BCR-ABL1 (p210) %IS Kit.

e. *Analytical specificity:*

Interference

A sample pool was prepared by mixing 20 peripheral blood samples of positive BCR-ABL1 [p210 (e14a2)] with an MR value around 3.0. The potential interfering substance was added to the test group and the diluent was added to the control group in concentrations recommended by CLSI EP7-A2. The potential interfering substances evaluated were cholesterol (6.47 mmoL/L), conjugated bilirubin (86 μM), EDTA (7 mg/mL), hemoglobin (200 g/L), sodium heparin (3000 U/L), triglycerides (5.6 mmoL/L), unconjugated bilirubin (257 μM), 10x Red blood cell lysis buffer, phenol, residual ethanol, 10x PBS, and genomic DNA. For both the control and test samples, 2 replicate extractions were conducted and each extracted sample was tested in 3 replicates for a total of 6 tests per sample type. The %IS value and MR value were measured for each group individually.

For the MR values, the mean test MR value and 95% confidence interval needed to fall within the 95% confidence interval ±0.5 log of the corresponding control group. For the %IS data, the 95% confidence interval of the mean %IS for test samples needed to intersect the detected range of the corresponding control group. All the samples based on both the MR value and %IS passed the acceptance criteria (Table 17 and Table 18). Results demonstrated that these endogenous and exogenous substances tested did not impact test results compared to the controls. However, considering that ethanol is an organic solvent that causes fusion of the droplets during amplification testing, residual ethanol in RNA samples should be minimized.

Table 17: Different interfering substances results analysis (MR)

Interfering substances	AVG value of MR		95% CI		Acceptable range of the AVG value of the test and its 95%CI (95% CI ±0.5 log of control group)	Pass
	Control group	Test group	Control group	Test group		
Cholesterol	3.04	2.98	2.94-3.13	2.94-3.01	2.44-3.63	YES
Conjugated bilirubin	3.01	2.99	2.97-3.06	2.94-3.03	2.47-3.56	YES
EDTA	3.00	3.00	2.97-3.04	2.93-3.08	2.47-3.54	YES
Hemoglobin	3.03	3.06	3.00-3.05	3.01-3.11	2.50-3.55	YES
Heparin sodium	3.06	3.02	2.99-3.14	2.98-3.06	2.49-3.64	YES
Triglyceride	2.98	3.07	2.93-3.04	3.05-3.08	2.43-3.54	YES
Unconjugated bilirubin	2.98	3.02	2.96-3.00	2.97-3.08	2.46-3.50	YES

Interfering substances	AVG value of MR		95% CI		Acceptable range of the AVG value of the test and its 95%CI (95% CI ±0.5 log of control group)	Pass
	Control group	Test group	Control group	Test group		
10x Red blood cell lysis buffer	2.96	2.97	2.92-2.99	2.93-3.01	2.42-3.49	YES
Phenol	2.95	2.97	2.92-2.99	2.93-3.00	2.42-3.49	YES
Ethanol	2.95	2.95	2.91-2.99	2.92-2.97	2.41-3.49	YES
10x PBS	2.97	3.03	2.92-3.03	2.90-3.16	2.42-3.53	YES
Genomic DNA	2.99	3.05	2.95-3.03	2.95-3.15	2.45-3.53	YES

Table 18: Different interfering substances results analysis (%IS)

Interfering substances	AVG value of %IS		%IS		Pass
	Control group	Test group	Detected range of control group	95% CI of the detection	
Cholesterol	0.094%	0.106%	0.055%-0.112%	0.098%-0.114%	YES
Conjugated bilirubin	0.098%	0.104%	0.088%-0.116%	0.093%-0.114%	YES
EDTA	0.100%	0.101%	0.089%-0.113%	0.083%-0.119%	YES
Hemoglobin	0.094%	0.088%	0.084%-0.102%	0.078%-0.098%	YES
Heparin sodium	0.089%	0.096%	0.060%-0.114%	0.086%-0.105%	YES
Triglyceride	0.105%	0.086%	0.087%-0.125%	0.083%-0.088%	YES
Unconjugated bilirubin	0.106%	0.096%	0.098%-0.115%	0.083%-0.108%	YES
10x Red blood cell lysis buffer	0.111%	0.108%	0.098%-0.121%	0.098%-0.119%	YES
Phenol	0.112%	0.109%	0.097%-0.125%	0.100%-0.117%	YES
Ethanol	0.112%	0.114%	0.098%-0.134%	0.106%-0.121%	YES
10x PBS	0.108%	0.098%	0.080%-0.125%	0.074%-0.122%	YES
Genomic DNA	0.103%	0.091%	0.089%-0.115%	0.073%-0.110%	YES

Primer Specificity

Primer specificity was assessed using 4 positive samples at 4 MR levels. The 4 positive samples were 1 BCR-ABL1 positive RNA sample (p190(e1a2) positive with a ratio of approximately 50%, 1 BCR-ABL1 positive RNA sample (p230(e19a2) positive with a ratio of approximately 50%, 1 BCR-ABL1 positive RNA sample [p210 (e13a2)] positive with an MR value of about 0.3, and 1 BCR-ABL1 positive RNA sample [p210 (e14a2)] positive with an MR value of approximately 0.3. A negative pool was used as a diluent and was prepared by mixing 5 BCR-ABL1 negative RNA samples. p190 and p230 samples were diluted with the negative sample pool to generate 4 samples with different concentrations of around 50%, 10%, 0.1%, and 0.005%. The 2 p210 samples (e13a2 and e14a2) were diluted with the negative sample pool to generate four samples with different concentrations of approximately MR0.3, MR1.0, MR3.0 and MR4.5.

Samples were tested in 4 replicates per run with 1 reagent lot. For p190 and p230 samples, the negative specificity acceptance criterion was ≥95%. For p210 samples, the acceptance criteria were positive specificity of 100% and the CV% ≤10%. The test results showed that no positive results were detected from either p190 (e1a2) or p230 (e19a2) samples with different concentrations, and the negative specificity was 100%. The positive specificity of p210 (e13a2) and p210 (e14a2) samples at different

concentrations was 100% and the CV% met the acceptance criterion of ≤10% (Table 19 and Table 20). Therefore, the results demonstrated that the primers are specific for p210 detection.

Table 19: Primer specificity results for p190 and p230

Variant	N	Ratio%				Pass
		Targeted value	Detected mean value	CV% of Detected mean value	Negative specificity	
p190 (e1a2)	4	50%	0.00%	0.00%	100%	YES
	4	10%	0.00%	0.00%	100%	YES
	4	0.1%	0.00%	0.00%	100%	YES
	4	0.005%	0.00%	0.00%	100%	YES
p230 (e19a2)	4	50%	0.00%	0.00%	100%	YES
	4	10%	0.00%	0.00%	100%	YES
	4	0.1%	0.00%	0.00%	100%	YES
	4	0.005%	0.00%	0.00%	100%	YES

Table 20: Primer specificity results for p210 (e13a2) and p210 (e14a2)

Sample type	N	MR					Pass
		Targeted value (MR)	Detected mean value	SD	CV%	Positive specificity	
p210 (e13a2)	4	0.3	0.32	0.003	0.81%	100%	YES
	4	1.0	1.02	0.006	0.60%	100%	YES
	4	3.0	2.97	0.045	1.51%	100%	YES
	4	4.5	4.46	0.100	2.24%	100%	YES
p210 (e14a2)	4	0.3	0.33	0.009	2.85%	100%	YES
	4	1.0	1.02	0.011	1.07%	100%	YES
	4	3.0	3.00	0.039	1.31%	100%	YES
	4	4.5	4.58	0.269	5.89%	100%	YES

Carryover Contamination

Carryover contamination was assessed using 1 pool of high positive samples and 1 pool of negative samples. The positive pool was prepared by mixing RNA obtained from K562 cells and RNA obtained from HL60 cells with an MR value of 0.3. The negative pool was prepared by mixing BCR-ABL1 negative RNA samples.

Eight (8)-well PCR plates were set up with high positive and negative wells in alternating rows. A total of 2 rows each containing 4 possible carryover events were tested per plate. The samples were tested duplicate using 2 instruments for a total of 64 sample replicates (32 positive and 32 negative samples, respectively).

The results showed that no signal was measured in the 32 negative wells, which demonstrated that the device does not generate significant carryover between wells.

RNA Input

RNA input was assessed using 2 pools of positive samples at 4 MR levels. The 2 positive pools were prepared by mixing 2 BCR-ABL1 positive p210 (e13a2) RNA

samples with an MR value of 0.3 (pool 1), and 2 BCR-ABL1 positive p210 (e14a2) RNA samples with an MR value of 0.3 (pool 2). A negative pool was used as a diluent and was prepared by mixing 35 BCR-ABL1 negative RNA samples. The positive sample pools were diluted with the negative sample pool to generate 4 samples with different concentrations: MR1.0, MR2.0, MR3.0 and MR3.5.

The RNA input of each sample (different variants and concentrations) was 30 ng, 150 ng, 300 ng, 500 ng, 800 ng, and 1000 ng, respectively. Samples were tested in 3-5 replicates per run with 1 reagent lot. When the RNA input of the samples was 500 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%. Therefore, the RNA input of the kit was determined to be 500 ng.

f. Stability Studies

Kit Real-Time Stability

Real-time stability was assessed using 1 pool of positive samples at 3 MR levels. The positive pool was prepared by mixing 5 BCR-ABL1 positive p210 (e14a2) RNA samples with an MR value of 0.3. A negative pool was used as a diluent and was prepared by mixing 10 BCR-ABL1 negative RNA samples. The positive sample pool was diluted with the negative sample pool to generate 3 samples with different concentrations: MR2.0, MR3.0, and MR4.0.

Reagent real-time stability studies were conducted using 3 reagent lots at the following time points: T0 (baseline), 3 months (T3), 6 months (T6), 9 months (T9), 11 months (T11), 12 months (T12), and 13 months (T13).

The kit performance met the following acceptance criteria:

1. Controls , calibrators, and samples values must be within pre-established ranges (the deviation between the measured value and the expected value(controls (MR1.0/4.0), calibrators (MR1.0/3.0) and samples (MR2.0/3.0/4.0))were within ±0.5 log).
2. The CV% of samples must meet the requirement (CV≤10%).
3. The mean value of MR detected by the samples, controls, and calibrators and their 95% confidence intervals were all within the range of ±0.5Log of the MR value detected by the kit on T0.

Three (3) concentrations and 8-12 replicates per sample along with calibrators and controls were tested at each time point. The test results showed that the MR values detected by the kit, the mean value of MR detected by the sample and calibrators and their 95% confidence intervals were all within the range of ±0.5 log of the MR value detected at baseline (T0), and the CV% of the MR values detected by the samples of different concentrations were between 0.56% and 5.95% (≤10%). Therefore, the reagents are stable under the storage conditions for 12 months at the temperature of -20℃±5℃.

Kit Freeze-thaw Stability

Freeze-thaw stability was conducted by cycling kit contents from -20°C to ambient temperature multiple times and assessing the performance of the kit in response to freeze-thaw cycling. In this study, the same samples as kit real-time stability above were used.

One (1) lot of kit was used, which was fully thawed at ambient temperature for 15 minutes. All kit component caps were removed, held uncapped for 2 minutes, and then components were capped and returned to -20℃ ± 5°C for a minimum of 8 hours prior to the next temperature cycle. This cycle was repeated 3, 5, and 6 times, and the controls, calibrators, and 8-12 replicates per sample were tested by the kit.

The acceptance criteria were that after each freeze-thaw cycle, controls, calibrators, and sample values must be within pre-established ranges (the deviation between the measured value and the expected value was within ±0.5 log of the MR value detected by the kit at T0).

The results showed that the measured value of MR and 95% confidence interval were within ±0.5 log of the MR value detected by the kit at T0. Besides, the precision of the measured MR values was between 0.83% and 5.95% (≤10%). Therefore, the results support that all components of the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) demonstrated stable performance for at least 5 freeze-thaw cycles.

Specimen Stability (Peripheral blood)

Specimen stability was assessed using 3 fresh peripheral blood samples of BCR-ABL1 positive [p210 (e14a2)] patients. Approximately 10mL of blood were collected within 24 hours and stored in a vacuum blood collection tube containing EDTA. MR values of the samples were 0.3, 3.0, and 4.0, respectively. Each blood sample was divided into 3 aliquots, and each aliquot was ≥ 2 mL. The peripheral blood samples were stored at 2-8℃. RNA was extracted from blood samples on 0, 1, and 2 days after collection. Each extracted RNA sample was tested in 6-8 replicates using 1 kit lot.

The acceptance criteria were that the CV% of samples must be ≤ 10% and the mean value of MR detected by the sample and their 95% confidence intervals must be within the range of ±0.5 log of the MR value detected by the kit on day 0.

The test results showed that when peripheral blood samples with MR0.3, MR3.0, and MR4.0 were stored at 2-8℃ for 1 and 2 days, the measured mean value of MR and its 95% confidence interval were within the range of ±0.5Log of the initial (day 0) measured mean value of MR of the corresponding samples. Besides, the precision of the measured MR values was between 0.92% and 5.75% (≤ 10%). Therefore, the peripheral blood sample is stable for 24 hours at 2-8℃ with the BCR-ABL1 (p210) %IS Kit (Digital PCR Method).

2. Comparison studies:

a. Method comparison with predicate device:
A method comparison study was designed to evaluate the performance of the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) compared to the predicate device 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 127 CML patients were collected from 2 hospitals for retrospective analysis. Of the 127 samples screened, 15 samples were excluded since they did not meet the sample screening criteria. A total of 112 samples (MR values were distributed between 0.32 and 4.47) were included in the statistics.

The study included the following inclusion/exclusion criteria:

1) Subject inclusion criteria:
- Over 18 years of age.
- Previously diagnosed with t(9;22) positive CML (p210).

2) Sample requirements:
- Peripheral blood stored in blood collection tube containing EDTA anticoagulant and stored at 4℃ for no more than 24 hours.

3) Sample extraction requirements:
- RNA from the patient's peripheral blood samples were extracted by the Whole Blood Nucleic Acid Extraction Reagent and stored at -80℃ after the RNA extraction were completed.

4) Sample mass requirements:
- A single sample can meet the requirement of detection by the Bio-Rad kit and the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) (RNA ≥3μg).

5) Sample exclusion criteria:
- Samples that did not meet inclusion criteria.
- Specimen type other than peripheral blood.
- Extracted RNA concentration and purity not meeting the assay requirements.
- Insufficient sample for testing.

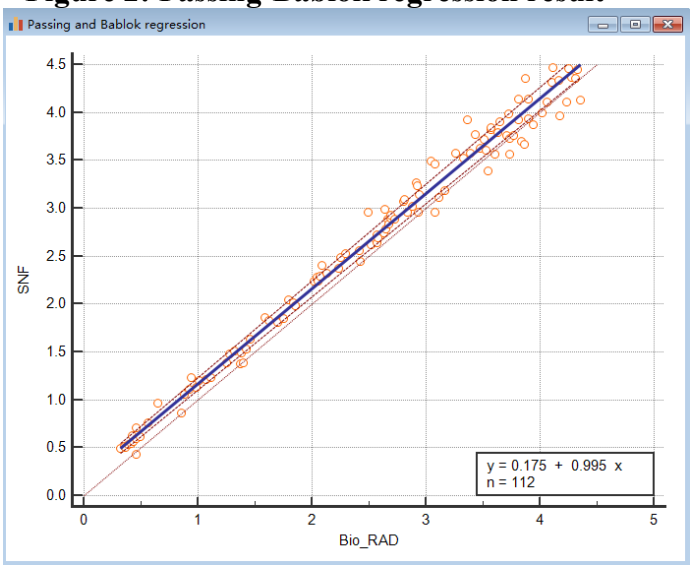
The clinical samples in the method comparison study were selected to span the testing range as shown below in Table 21:

Table 21: Sample MR value distribution

Bin	Bio-Rad	BCR-ABL1 (p210) %IS Kit
Lowest-<1.5	29	26
1.5-<2.5	19	19
2.5-<3.5	31	29
3.5-4.5	33	38

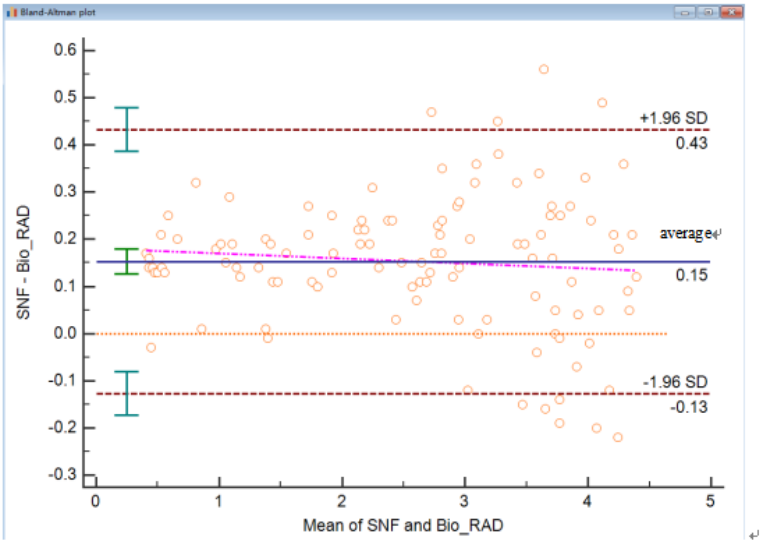
The outlier test by the ESD (extreme Studentized deviate) method showed that 112 test results had no outliers. Passing-Bablok regression (Figure 2) showed that the intercept A (95% CI): 0.17 (0.13-0.22), the slope B (95% CI): 0.99 (0.97-1.01), and the Spearman correlation coefficient: 0.988 ($p<0.0001$) were greater than 0.95.

Figure 2: Passing-Bablok regression result



The mean bias using Bland-Altman (Figure 3) was 0.15 (95% CI: 0.13–0.18, $p<0.0001$). The limits of agreement (0.43 to -0.13) represented the interval that was expected to contain 95% of the data from an approximately normal distribution. The slope of the regression line was -0.01 with a 95% CI of -0.032, 0.012 ($p = 0.3426$, not significant) and the intercept was 0.1806 (95% CI: 0.117–0.244, $p<0.0001$).

Figure 3: Bland-Altman result



Results from the method comparison study over the assay measurement range of MR0.3-MR4.5 and the predicted biases and their 95% confidence intervals from regression analysis are shown below (Table 22). The results demonstrate that the BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is substantially equivalent to the predicate.

Table 22: Predicted biases and their 95% confidence intervals of different transcripts

	Predicted Bias (95% CI)					
Variant	**MR0.3**	**MR1.0**	**MR2.0**	**MR3.0**	**MR4.0**	**MR4.5**
e13a2	-0.023 (-0.08, 0.03)	-0.023 (-0.07, 0.02)	-0.023 (-0.06, 0.01)	-0.023 (-0.06, 0.01)	-0.023 (-0.07, 0.02)	-0.023 (-0.08, 0.03)
e14a2	-0.010 (-0.08, 0.06)	-0.007 (-0.06, 0.05)	-0.003 (-0.04, 0.04)	0.001 (-0.04, 0.04)	0.005 (-0.05, 0.06)	0.007 (-0.06, 0.07)
e13a2 & e14a2 together	-0.016 (-0.09, 0.05)	-0.015 (-0.08, 0.05)	-0.013 (-0.06, 0.04)	-0.011 (-0.06,0.04)	-0.009 (-0.07, 0.05)	-0.008 (-0.08,0.06)

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:

Sniper Digital PCR All-in-One 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 _____

3. Calibration & Quality Controls:
   The assay uses calibrators by which the BCR-ABL1/ABL1 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.