September 5, 2023

Suzhou Sniper Medical Technologies Co., Ltd
% Huifang Zhao
Consultant
Sinow Medical AS
Hoyteknologisenteret, Thormohlens gate 55
Bergen, 5006
Norway

Re: K221869
Trade/Device Name: BCR-ABL1 (p210) %IS Kit (Digital PCR Method)
Regulation Number: 21 CFR 866.6060, 21 CFR 862.2570
Regulation Name: BCR-ABL quantitation test
Regulatory Class: Class II
Product Code: OYX, PHG
Dated: April 3, 2023
Received: April 4, 2023

Dear Huifang Zhao:

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

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Pamela S. Ebrahimi -S

Digitally signed by Pamela S. Ebrahimi -S
Date: 2023.09.05 15:40:06 -04'00'

Pamela Gallagher Ebrahimi, Ph.D.
Deputy Branch Chief
Division of Molecular Genetics
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OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K221869

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

Indications for Use *(Describe)*
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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

A. Submitter:

Submitter: Suzhou Sniper Medical Technologies Co., Ltd.

Address: Unit 301, Building A6, Suzhou BioBAY, No. 218 Xinghu Street, Suzhou Industrial Park, Suzhou Area, China (Jiangsu) Pilot Free Trade Zone, Suzhou, China.

Email: zhangpeng@sniper-tech.com

Contact Person: Peng Zhang

B. Device

Trade Name:	BCR-ABL1 (p210) %IS Kit (Digital PCR Method)
Common Name:	BCR-ABL1 Digital PCR Test
Type of Test	Reverse transcription, quantitative, digital droplet polymerase chain reaction (ddPCR) based nucleic acid amplification
Regulation section:	21 CFR 866.6060 21 CFR 862.2570
Classification:	Class II
Product Code:	OYX PHG
Classification/Advisory Panel	Pathology (88)

C. Predicate device:

Product Name:	QXDx BCR-ABL %IS Kit for use on the QXDx AutoDG ddPCR System
510(k) Number:	K181661
Product Code:	OYX, PHG
Manufacture:	Bio-Rad Laboratories, Inc.

D. 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

E. 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 process includes three parts. The first part is to extract ribonucleic acid (RNA) from peripheral blood of CML patients. The second part is to detect BCR-ABL1 fusion gene (p210) and ABL1 internal reference gene in RNA samples by RT-dPCR (Reverse Transcription-Droplet PCR) 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.

Composition	Main components	Application
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-DNA Glycosylase	Catalyzes the amplification of primers hybridized to templates from the cDNA. Enzyme exonuclease activity degrades 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 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.

Instrument:

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

The Sniper Digital PCR All-in-One System divides the sample into about 20000 droplets and carries out PCR amplification, read the number of positive and negative droplets through fluorescent signals, and then calculate the concentration of nucleic acid quantitatively according to the volume of the droplets 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.

F. Substantial Equivalence Information:

1. Predicate device name(s):

The 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:

Table 2: Similarity comparison

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.
Measurement Type	Quantitative	Same
Specimen Type	RNA from whole blood (EDTA)	Same
Anti-coagulant	EDTA	Same

Similarities		
Item	**Subject Device**	**Predicate Device**
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

Table 3: Different comparison

Differences		
Item	**Subject Device**	**Predicate Device**
Measuring Range	MR 0.3 to MR 4.5	MR 0.3 to MR 4.7
RNA Input	500 ng	1000n g
Quality calibrators	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	Same. 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.

G. 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.

H. 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. The detection is conducted directly without separated reverse transcription. The supporting consumables required for the detection process include droplet generation oil, PCR four-well plate, droplet generation needle, 4 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, set the threshold line at 1/3 of the distance between negative and positive droplet clusters, and the ratio of BCR-ABL1 and ABL1 genes is calculated, and the %IS value and MR value of the sample are calculated according to the conversion factor (CF) of the kit. Secondly, the quality of the test results should be controlled, in which the blank control should satisfy BCR-ABL1 copy ≤1 and ABL1 copy ≤10, the negative control should satisfy 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. Finally the results are outputted.

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 4:

Table 4: %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. It 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.

$$\%IS = BCR\text{-}ABL1copy / ABL1copy \times 100 \times CF$$

The test results should be interpreted according to the following standards (table5):

Table 5: Interpretation of results

Test results	Report results	Explanation of test results
Copy number of BCR-ABL1 as 0.	Report: Negative	It 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 ≤ 10,000, the report is invalid. A retest should be performed with increased RNA input.

(2) In 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 > 140,000 and the BCR-ABL1 copy number > 0, the ABL1 copy number exceeds the linear range, which will affect the quantitative accuracy. A retest should be performed with reduced RNA input.

I. Performance Characteristics (if/when applicable):

1. Analytical Performance:

a. Precision/Reproducibility:

Precision and Reproducibility were assessed using 3 positive pools at 5 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 with a ratio value of 0%. The positive sample pools were diluted with the negative sample pool to generate five samples with different concentrations: MR1.0, MR2.0, MR3.0, MR4.0 and MR4.5. Each sample was stored separately at the temperature of -20℃±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 (one instrument at each site) with one reagent lot for a total of 36 replicates. Each run was performed by an independent operator (2 operators per site). The total precision (CV, %) values were required to meet the requirements shown in the following table 6.

Table 6: Precision requirements corresponding to different concentrations

MR value	Precision (CV, %) requirements
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 with random effects for site, day, and run (operator) to assess repeatability, within-day precision, within-site precision and reproducibility of measured MR level. Results of MR level indicated low variability, including between sites, and all acceptance criteria were satisfied (CVs ≤10%). Total MR and %IS precision were calculated for the assay (see table 7 and table 8) and in-kit calibrators and controls (see table 9). The acceptance criteria were met.

Table 7: Precision analysis results (MR) of samples

Sample	Variant	N	MR expected	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%

Sample	Variant	N	MR expected	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%
	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 8: Precision analysis results (%IS) of samples

Sample	Variant	N	%IS expected	%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%
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 9: 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

Precision between batches

Precision between batches were assessed using 2 positive pools at 4 levels. The 2 positive pools were prepared by mixing 5 BCR-ABL1 positive p210(e13a2) RNA samples with an MR value of 0.3 (pool 1), and 5 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 60 BCR-ABL1 negative RNA samples with a ratio value of 0%. 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. Each sample was stored separately at the temperature of -20℃±5℃.

Samples were tested in 3 replicates per run for 2 runs per day for 3 non-consecutive days (1st, 3rd and 5th) at 1 site (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 were required to meet the requirements shown in the following Table 10.

Table 10: Precision requirements corresponding to different concentrations

MR value	Precision (CV, %) requirements
MR0.3-MR2.0	≤10%
MR2.1-MR3.49	≤15%
MR3.5-MR4.0	≤20%
LOQ	≤20%

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 very low variability, including between lots, and all acceptance criteria were satisfied (CVs ≤10%). Total MR and %IS precision were calculated for the assay (see table 11 and table 12) and in-kit calibrators and controls (see table 13). The acceptance criteria were met.

Table 11: Precision analysis results (MR) of samples

Sample	Variant	N	MR expected	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%
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 12: Precision analysis results (%IS) of samples

Sample	Variant	N	%IS expected	%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%

Table 13: 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

The performance and sensitivity of the BCR-ABL 1 (p210) %IS Kit (Digital PCR Method) is dependent on the RNA quantity, purity and integrity. For the extraction of RNA in peripheral blood samples, Whole Blood Nucleic Acid

Extraction Reagent produced by Suzhou Sniper Medical Technologies Co., Ltd. is required.

This study was assessed using 3 positive pools at 5 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 with a ratio value of 0%. 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. The respective assigned peripheral blood samples were pretreated on Day 1 by two operators using Whole Blood Nucleic Acid Extraction Reagent, and the processed peripheral blood samples were stored at -70 ℃.

Samples were extracted in 2 times for 2 operators per day for 3 non-consecutive days (1st, 3rd and 5th) with 1 reagent lot (Whole Blood Nucleic Acid Extraction Reagent). A total of 180 results were included in RNA Extraction Method study. When following the requirements of the following table (see table 14) for RNA quality control, 5 samples of RNA quality control failed (2 samples did not meet the RNA concentration requirement and 3 samples did not meet the RNA purity requirement), and the proportion of extraction in line with the requirements was 97%, The quality control qualified RNA were tested, and the results showed that the CV% of all samples were less than 10% (see table 15).

Table 14: RNA quality control

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

Table15: RNA extraction and detection

Variant	MR value	Peripheral blood sample extraction			Sample detection			
		Sample N	Qualified N	Qualified proportion	Tests N	MR SD	MR Mean	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 does not meet requirements during extraction and no subsequent testing.

b. Linearity/Assay reportable range:

Linearity/Assay reportable range were assessed using 2 positive pools at 10 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 22 BCR-ABL1 negative RNA samples with a ratio value of 0%. The positive sample pools were diluted with the negative sample pool to generate ten 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. Each sample was stored separately at the temperature of 2-8℃.

Samples were assayed in 4 replicates for one day at 1 instrument with 1 reagent lot. The precision analysis and deviation analysis were required to meet the requirements shown in the following Table 16.

Table 16：Precision and deviation requirements

Category	Requirements
Precision	⩽10%
% Deviation	⩽±15%

Samples with concentrations from 50%IS (MR0.3) to 0.002%IS (MR 4.7) were determined for the e13a2 and e14a2 variants. Precision analysis showed that the precision 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 meet the requirement of ≤±15% (see table 17).

Table 17: Precision analysis results of different samples

Variant	Sample	MR values									
		Precision					Deviation				
		Mean Y	Expected E	SD	CV %	Acceptable range	Predicted y=AE	Deviation	%Deviation	Acceptable range	Pass
e13a2	1	0.26	0.30	0.009	3.26%	CV⩽10%	0.30	-0.03	-10.91%	≤±15%	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

Variant	Sample	MR values									
		Precision					Deviation				
		Mean Y	Expected E	SD	CV %	Acceptable range	Predicted y=AE	Deviation	%Deviation	Acceptable range	Pass
	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 e13a2 and e14a2 types showed that R^2 and slope met the requirements (see table 18).

Table 18: Linear range regression analysis for variants e13A2 and e14a2

Variant	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, considering that the quantitation limit of the kit is MR4.5, therefore the assay reportable range for both e13a2 and e14a2 of the BCR-ABL 1 (p210) %IS Kit is 50%IS(MR0.3) -0.0032%IS(MR4.5).

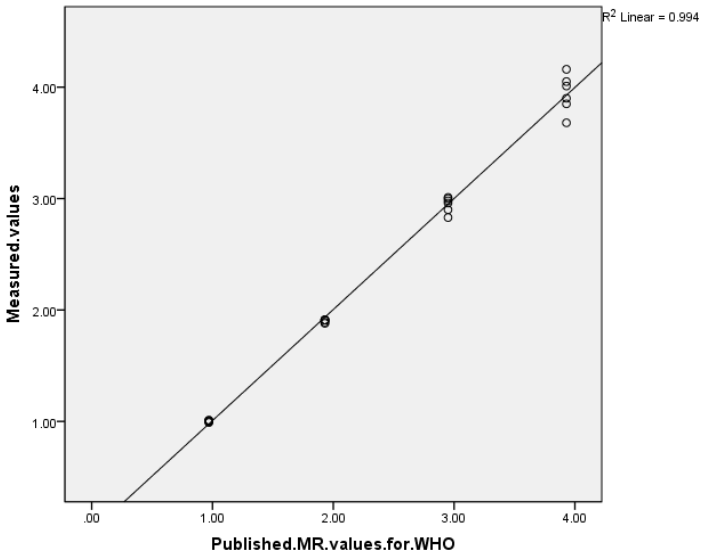
c. Traceability, stability, expected values (kit, calibrators, reference or methods):

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 was tested in 6 replicates across 9 runs (1 run per lot for 9 lots). The measured MR values for each level of the WHO Reference Panel were adjusted by 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 one kit lot is shown in Figure 1.

Figure 1: Measured MR and published MR regression analysis



d. Detection limit:

Limit of Blank

The limit of blank was determined by testing independent BCR-ABL negative samples by 1 operator using 2 kit lots. Out of 144 test results, 138 had no detectable BCR-ABL values. Six 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-ABL 1 (p210) %IS Kit (Digital PCR Method) kit is 0 copy.

Limit of Detection/ Limit of Quantitation

Limit of Detection/ Limit of Quantitation were assessed using 2 positive pools at 3 levels. The 2 positive pools were prepared by marking 1 BCR-ABL1 positive p210(e13a2) RNA sample 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 with a ratio value of 0%. The positive sample pools were diluted with the negative sample pool to generate three samples with different concentrations: MR4.5, MR4.7 and MR4.9. Each sample was stored separately at the temperature of -20℃±5℃.

Samples were assayed in 20 replicates per day for 3 days with 2 reagent lots for a total of 120 replicates. Based on Probit method and Precision analysis, the LoD and LoQ of the kit can be obtained. The hit rate and precision were required to meet the requirements shown in the following Table 19.

Table 19: Hit rate and precision requirements

Category	Hit rate	CV%
LoD	⩾95%	None
LoQ	100%	⩽10%

The hit rate and precision of different samples were as follows. The hit rates of MR4.5 samples were 100%, the precision was between 3.47% and 4.03%. The hit rates of MR4.7 samples were between 97% and 98%, the precision was between 4.09% and 4.79%. The hit rates of MR4.9 samples were between 78% and 90%, the precision was between 4.09% and 4.64%. When the samples concentration was MR4.7, the hit rates could meet the requirement of ≥95%. When the samples concentration was MR4.5, the hit rates were 100% and the precisions were ≤10%. The studies support an LoD of 4.7 and LoQ of 4.5 for the assay.

e. Analytical specificity:

Interference

A sample pool was prepared by mixing peripheral blood from CML positive patients, with an MR value around 3.0. The potential interfering substance was added to the test group and diluent was added to the control group in concentrations recommended by CLSI EP7-A2. For both the control and test samples, 2 replicate extractions were performed and each extracted sample was

tested in replicates of 3 for a total of 6 tests per sample type.

For the MR values, the mean test MR value and 95% confidence interval needed to fall within the 95% confidence interval ±0.5Log of the corresponding control group. In all cases, samples passed the acceptance criteria (see table 20).

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. In all cases, samples passed the acceptance criteria (see table 21). The potential interfering substances evaluated were cholesterol (6.47mmoL/L), conjugated bilirubin (86μM), EDTA (7mg/mL), hemoglobin (200g/L), sodium heparin (3000U/L), triglycerides (5.6mmoL/L), unconjugated bilirubin (257μM),10x red blood cell lysis buffer, phenol, residual ethanol, 10x PBS, and genomic DNA. Results demonstrated that these endogenous and exogenous substances tested did not impact test results as compared to control. 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 20: Statistical of test results (MR values) of different interfering substances

Interfering substances	Mean value of MR		95% confidence interval		The acceptable range of the AVG value of the test and its 95%CI (95% CI ±0.5 log of control group)	Result (if within acceptable range)
	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
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

Interfering substances	Mean value of MR		95% confidence interval		The acceptable range of the AVG value of the test and its 95%CI (95% CI ±0.5 log of control group)	Result (if within acceptable range)
	Control group	Test group	Control group	Test group		
Genomic DNA	2.99	3.05	2.95-3.03	2.95-3.15	2.45-3.53	YES

Table 21: Statistical of test results (%IS values) of different interfering substances

Interfering substances	Mean value of %IS		%IS		Does the detected range of control group intersect with the 95%CI of the detection
	Control group	Test group	Detected range of control group	%95 confidence interval 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 levels. 1 BCR-ABL1 positive p190(e1a2) RNA sample with a ratio of approximately 50%, 1 BCR-ABL1 positive p230(e19a2) RNA sample with a ratio of approximately 50%, 1 BCR-ABL1 positive p210(e13a2) RNA sample with an MR value of about 0.3, and 1 BCR-ABL1 positive p210(e14a2) RNA sample with an MR

value of about 0.3. A negative pool was used as a diluent and was prepared by mixing 5 BCR-ABL1 negative RNA samples with a ratio value of 0%. The p190(e1a2) and p230(e19a2) samples were diluted with the negative sample pool to generate four samples with different concentrations: 50%, 10%, 0.1%, and 0.005%. The p210 samples were diluted with the negative sample pool to generate four samples with different concentrations: MR0.3, MR1.0, MR3.0 and MR4.5. Each sample was stored separately at the temperature of 2-8 ℃.

Samples were assayed in 4 replicates per run with 1 reagent lot. For p190 and p230 samples, the negative specificity should be ≥95%. For p210 samples, the positive specificity should be 100% and the CV% should be ⩽10%.

The test results showed that for p190 and p230 samples, the negative specificity was 100%, for p210 samples, positive specificity and precision could meet the requirements. The results are shown in the Table 22 and Table 23 below.

Table 22: Primer specificity results for p190 and p230

Variant	N	Ratio%				Acceptable range	Pass
		Targeted value	Detected mean value	CV% of Detected mean value	Negative specificity	Negative specificity	
p190 (e1a2)	4	50%	0.00%	0.00%	100	≥95%	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 23: Primer specificity results for p210(e13a2) and p210(e14a2)

Variant	N	MR					Acceptable range		Pass
		Targeted value	Mean value	SD	CV, %	Positive specificity	Positive specificity	CV, %	
p210 (e13a2)	4	0.3	0.32	0.003	0.81%	100%	100%	≤10%	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

Variant	N	MR					Acceptable range		Pass
		Targeted value	Mean value	SD	CV, %	Positive specificity	Positive specificity	CV, %	
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 K562 cells RNA and HL60 cells RNA with an MR value of 0.3 (pool 1). The negative pool was prepared by mixing BCR-ABL1 negative RNA samples with a ratio value of 0% (pool 1). Each sample was stored separately at the temperature of 2-8℃.

Eight 8-well PCR plates were set up with high positive and negative wells in alternating rows. A total of 2 rows each containing possible 4 carryover contamination wells were tested per plate. Two plates were tested on each of 2 instruments (Sniper Digital PCR All-in-One System) for a total test sample size of 64. Of the 64 replicates used in the analysis, no signal was measured in the 32 negative wells. Acceptance criteria were met which demonstrates that the device does not generate significant carryover between wells.

RNA Input

RNA input was assessed using 2 positive pools at 4 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 with a ratio value of 0%. The positive sample pools were diluted with the negative sample pool to generate four samples with different concentrations: MR1.0, MR2.0, MR3.0 and MR3.5. Each sample was stored separately at the

temperature of 2-8 ℃.

By adjusting the samples volume, the amount of RNA input was controlled at 30 ng, 150 ng, 300 ng, 500 ng, 800 ng and 1000 ng, respectively. Samples were assayed in 3-5 replicates per run with 1 reagent lot. When the RNA input of the samples was 500ng, the positive detection rate was 100%, the deviation between the measured values and the theoretical values were within ±0.5, and the precision were ≤10%. Therefore, the RNA input of the kit is set to 500ng.

f. Stability Studies:

Real-Time Stability (kit, calibrators)

Real-time stability was assessed using 1 positive pool at 3 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 with a ratio value of 0%. The positive sample pool was diluted with the negative sample pool to generate three samples with different concentrations: MR2.0, MR3.0 and MR4.0. Each sample was stored separately at the temperature of -20℃±5℃.

Reagent real-rime stability studies were conducted using 3 lots with testing ongoing at T0 (baseline), T3 (3 months), T6 (6 months), T9 (9 months), T11 (11 months), T12 (12 months), and T13 (13 months). Three samples along with calibrators and controls were tested by thawing reagents stored at -20℃±5°C at each time point.

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.

The results showed that when the storage time of the kits reached the T3, T6, T9, T11, T12 and T13, the MR measured value of samples, controls, and calibrators and their 95% confidence interval were all within ±0.5 log of the result of T0 (baseline). Besides, the precision of the measured MR values was between 0.56% and 5.95%, acceptance criteria were met. Therefore, these results support the conclusion that reagents are stable under the storage conditions for 12 months at the temperature of -20℃±5℃.

Freeze-thaw Stability Kit (kit, calibrators)

A study was conducted to determine the allowable number of freeze-thaw cycles for the components of the BCR-ABL 1 (p210) %IS Kit. In this study, the same samples as real-time stability were used. 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 freeze-thaw cycling. 1 lot of kit was used in this study. The kits were fully thawed at ambient temperature for 15 minutes, all kits component caps were removed, components were held uncapped for 2 minutes, components were capped and materials were 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, the controls, calibrators, and samples were tested by kit.

The acceptance criteria were that after each freeze-thaw cycle, 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)and the CV% of samples must meet the requirement (CV≤10%) and the mean value of MR detected by the sample, 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 the 0 time.

The results showed that when the freeze-thaw cycle of the kits reached the 3,

5 and 6 times, the measured value of MR and 95% confidence interval were all within ±0.5 log of the result of 0 time. Besides, the precision of the measured MR values was between 0.83% and 5.95%, acceptance criteria were met. Therefore, these 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), approximately 10mL which 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 equally into 3 portions. Each portion should not be less than 2mL. The peripheral blood samples should be stored at 2-8℃. RNA was extracted from blood samples on the 0, 1, and 2 days after collection. Each extracted RNA sample was tested 6-8 replicates by using one lot of the kit.

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

The results showed that when the peripheral blood samples were stored in 2-8°C for 1 and 2 days, the measured value of MR and 95% confidence interval were all within the range of ±0.5 log of the result of 0 day. Besides, the precision of the measured MR values was between 0.92% and 5.75%, acceptance criteria were met. Therefore, peripheral blood samples stored for 1 day at 2-8°C are stable and produce reliable results 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) (Sniper kit) compared to the predicate device QXDx BCR-ABL %IS Kit (Bio-Rad Laboratories, registration number K181661) (Bio-Rad kit) in RNA derived from peripheral blood samples obtained from individuals previously diagnosed with t(9;22) positive chronic myeloid leukemia (CML).

Clinical samples of 127 were collected from 2 hospitals for analysis retrospectively. 127 samples were screened, and 15 samples were excluded since they did not meet the sample screening conditions. Finally, 112 samples (MR value were distributed between 0.32 and 4.47) could be included in the statistics. The 112 samples cover the intended use and linear range of both kits.

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 size requirements:

A single sample could meet the requirement of detection by the Bio-Rad kit and the Sniper kit (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.

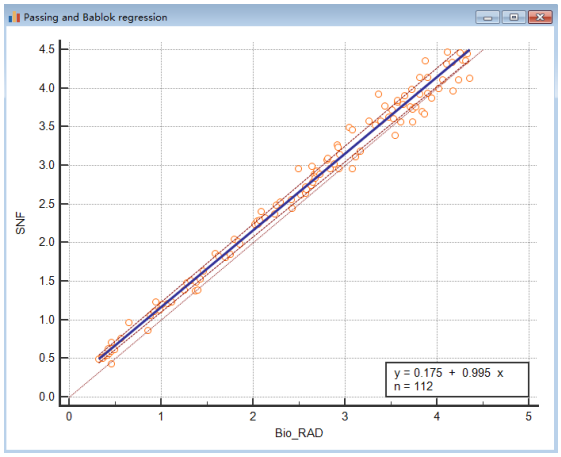
The clinical samples in the method comparison study were selected to span the testing as shown in the table below in Table 24:

Table 24: Sample MR value distribution

Bin	Bio-rad	Sniper
Lowest- < 1.5	29	26
1.5- < 2.5	19	19
2.5- < 3.5	31	29
3.5-4.5	33	38

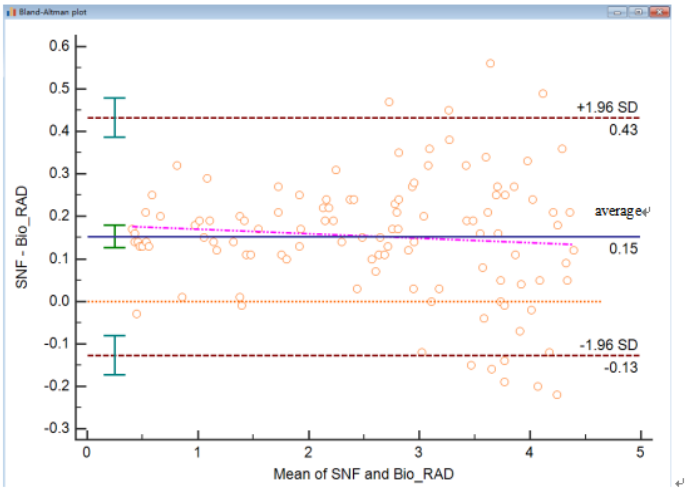
The outlier test by the ESD method showed that 112 test results had no outliers. Passing-Bablok regression (Figure 2) showed that the intercept A (95% CI): 0.17 (0.13-0.22), the slope B (95% CI): 0.99 (0.97-1.01), and the Spearman correlation coefficient: 0.988(P<0.0001) were greater than 0.95.

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) represent 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 demonstrate that over the assay measurement range of MR0.3-MR4.5 and the predicted biases and their 95% confidence intervals from regression analysis as blew (see table 23). The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is substantially equivalent to the predicate.

Table 25: Predicted biases and their 95% confidence intervals of different transcripts

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

J. Instrument Name:

Sniper Digital PCR All-in-One System

K. 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.

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

Not applicable

M. Proposed Labeling:

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

N. Conclusion:

The data presented in the analytical and clinical sections support the safety and effectiveness of the Sniper Digital PCR All-in-One System. A comparison of the Intended Use, device features, non-clinical and clinical data support that the Sniper Digital PCR All-in-One System is substantially equivalent to the predicate QXDx BCR-ABL %IS Kit.