September 27, 2019

Cepheid
Sudhakar Marla, Ph.D.
Senior Director, Regulatory Affairs
904 Caribbean Drive
Sunnyvale, California 94089

Re: K190076
Trade/Device Name: Xpert BCR-ABL Ultra, GeneXpert Dx System, GeneXpert Infinity-48s and GeneXpert Infinity-80 Systems
Regulation Number: 21 CFR 866.6060
Regulation Name: BCR-ABL quantitation test
Regulatory Class: Class II
Product Code: OYX, OOI
Dated: August 15, 2019
Received: August 19, 2019

Dear Sudhakar Marla:

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,

for Reena Philip, Ph.D.
Director
Division of Molecular Genetics and Pathology
OHT7: Office of In Vitro Diagnostics
and Radiological Health
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: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K190076

Device Name
Xpert® BCR-ABL Ultra

Indications for Use *(Describe)*

The Xpert BCR-ABL Ultra test is an in vitro diagnostic test for the quantitation of BCR-ABL1 and ABL1 mRNA transcripts in peripheral blood specimens of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR-ABLl fusion transcripts type e13a2 and/or e14a2. The test utilizes automated, quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR). The Xpert BCR-ABL Ultra test is intended to measure BCR-ABL1 to ABL1 percent ratios on the International Scale (IS), and also expressed as a log molecular reduction (MR value) from a baseline of 100% (IS), in t(9;22) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs).

The test does not differentiate between e13a2/b2a2 or e14a2/b3a2 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 Xpert BCR-ABL Ultra test is intended for use only on the Cepheid GeneXpert® Dx System and the GeneXpert Infinity System.

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

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 548-8946
Contact:	Sudhakar Marla, Ph.D.
Date of Preparation:	September 24, 2019
Device:	
Trade name:	Xpert® BCR-ABL Ultra
Common name:	Xpert BCR-ABL Ultra
Type of Test:	Reverse transcription, quantitative, polymerase chain reaction (RT-qPCR) based nucleic acid amplification
Regulation number, Classification name, Product code:	21 CFR 866.6060, BCR-ABL quantitation test, OYX 21 CFR 862.2570, Instrumentation for clinical multiplex test systems, OOI
Classification Advisory Panel	Pathology (88)
Prescription Use	Yes
Predicate Device	Asuragen QuantideX qPCR BCR-ABL IS Kit [DEN160003]

Device Intended Use:

The Xpert BCR-ABL Ultra test is an *in vitro* diagnostic test for the quantitation of BCR-ABL1 and ABL1 mRNA transcripts in peripheral blood specimens of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR-ABLl fusion transcripts type e13a2 and/or e14a2. The test utilizes automated, quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR). The Xpert BCR-ABL Ultra test is intended to measure BCR-ABL1 to ABL1 percent ratios on the International Scale (*IS*), and also expressed as a log molecular reduction (MR value) from a baseline of 100% (*IS*), in t(9;22) positive CML patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs).

The test does not differentiate between e13a2/b2a2 or e14a2/b3a2 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 Xpert BCR-ABL Ultra test is intended for use only on the Cepheid GeneXpert® Dx System and GeneXpert Infinity System.

Special conditions for use statement(s):

For *in vitro* diagnostic use only

For Prescription use only

Device Description:

The Xpert BCR-ABL Ultra test is an automated *in vitro* diagnostic test for quantifying the amount of *BCR-ABL1* (BCR-ABL, hereafter) mRNA transcript as a ratio of BCR-ABL/ABL per the International Scale (*IS*).

The test is performed on the Cepheid GeneXpert® Dx System and GeneXpert Infinity System (referred to as the GeneXpert systems). The GeneXpert systems require the use of single-use, disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized. The GeneXpert systems have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR and RT-PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), and a proprietary I-CORE® thermocycler for performing real-time PCR and RT-PCR and detection.

The Xpert BCR-ABL Ultra test includes reagents to detect BCR-ABL fusion genes resulting from two major breakpoints, translocation e13a2/b2a2 and e14a2/b3a2 and the ABL transcript as an endogenous control in peripheral blood specimens. The amount of BCR-ABL transcript in the patient sample is reported as a percent ratio of BCR-ABL/ABL on the International Scale (*IS*), and also expressed as a log molecular reduction (MR value) from a baseline of 100% (*IS*), using the GeneXpert software.

There are two controls included in each Xpert BCR-ABL Ultra test, which are the ABL Endogenous Control and the Probe Check Control (PCC). The ABL Endogenous Control normalizes the BCR-ABL target and ensures that sufficient sample is used in the test. The PCC verifies reagent rehydration, PCR tube filling, and that all reaction components, including probes and dyes, are present and functional in the cartridge.

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

Table 1. Reagents in the Xpert BCR-ABL Ultra Test Kit

Item	Description	Use
Proteinase K	Serine protease	Digests proteins and inactivates nucleases in EDTA whole blood specimen during the sample preparation and nucleic acid purification steps
Lysis Reagent	Guanidinium chloride buffered solution	Denaturant used to lyse cells, release of nucleic acids and decrease nuclease activity during the sample preparation step
Wash Reagent	Guanidinium thiocyanate and ethanol solution	Reagent used to remove cellular contaminants during nucleic acid binding step
Xpert BCR-ABL Ultra Cartridges with Integrated Reaction Tubes	Single-use test cartridges that house, buffered solutions (rinse and elution), lyophilized beads containing reverse transcriptase, DNA polymerase, primers and probes	Reagents used to perform the on-board nucleic acid isolation, purification and real-time RT-qPCR.
CD/Software	Compact disc	Contains assay definition file (ADF), instruction to import ADF into GeneXpert software and information for use

The peripheral blood specimens are collected in EDTA blood tubes following the user institution's standard procedures and can be stored for up to 72 hours prior to use when stored at 4°C. The test sample, from a peripheral blood specimen, is prepared in a sample preparation tube provided with the kit, with reagents that are provided with the kit (proteinase K and lysis reagent) plus user-supplied ethanol, following instructions in the package insert. After sample preparation, the prepared sample and one reagent provided with the kit (Wash Reagent) are pipetted to separate chambers of the Xpert BCR-ABL Ultra cartridge. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off additional sample preparation and nested, real-time, quantitative reverse transcription polymerase chain reaction (RT-qPCR) for detection of RNA. In this platform, the additional sample preparation, amplification, and real-time detection are all fully-automated and completely integrated. The time to result for the Xpert BCR-ABL Ultra test including offline sample preparation and real-time RT-qPCR is approximately 2.25 hours.

Substantial Equivalence:

The Xpert BCR-ABL Ultra is substantially equivalent to the Asuragen QuantideX qPCR BCR-ABL IS Kit [DEN160003]. The performance of the Xpert BCR-ABL Ultra test was evaluated in a multi-site clinical study using whole blood specimens and was compared to a FDA-cleared molecular assay. The results from the clinical study demonstrated that the performance of Xpert BCR-ABL Ultra is substantially equivalent to the predicate device.

Table 2 shows the similarities and differences between the Xpert BCR-ABL Ultra test and the predicate device.

Table 2: Comparison of Similarities and Differences of Xpert BCR-ABL Ultra with the Predicate Device

Similarities		
Item	Device	Predicate
Intended Use	For the quantitation of BCR-ABL1 and ABL mRNA transcripts in peripheral blood specimens of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR ABLl fusion transcripts type e13a2 and/or e14a2.	Same
Target Population/Indication	CMP-positive patients during monitoring of treatment with Tyrosine Kinase Inhibitors (TKIs)	Same
Test Limitation	The test does not differentiate between e13a2/b2a2 or e14a2/b3a2 fusion transcripts and does not monitor other rare fusion transcripts resulting from t(9;22). This test is not intended for the diagnosis of CML.	Same
Measurand	BCR-ABL1 fusion transcripts (e13a2/b2a2 and/or e14a2/b3a2) and the ABL1 endogenous control mRNA	Same
Measurement type	Quantitative	Same
Principle of Assay	Reverse transcription, quantitative, polymerase chain	Same

Similarities		
Item	Device	Predicate
	reaction (RT-qPCR) based nucleic acid amplification	
Traceability Standard	1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR	Same
Units	Both % (*IS*) and Molecular Response (MR)	Same
Specimen Type	Whole Blood (EDTA)	Same

Differences		
Item	Device	Predicate
Extraction and Assay Preparation	Single Use cartridge	Extraction steps may be manual followed by manual assay preparation
Instrument	Cepheid GeneXpert® Dx GeneXpert Infinity-48s, and GeneXpert Infinity-80	Applied Biosystems 7500 Fast Dx Real Time PCR Instrument
Input Range	RNA is isolated from white blood cell count	RNA input range of 1 to 5µg

Differences		
Item	Device	Predicate
	(WBCC) range 1.5×10^5 to 3.0×10^7 cells/mL	
Controls	Probe Check Control	Three controls RNA High (MR 1.5), mRNA Low (MR 3.5), and RNA Negative. Each provided in separate tubes
Calibrators	None provided. Each lot of Xpert BCR-ABL Ultra is calibrated to secondary standards that were calibrated to the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.	Four levels formulated to MR1.0, 2.0, 3.0, 4.0 traceable to the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.

The Xpert BCR-ABL Ultra test has the same general intended use as the predicate device and has the same technological characteristics as the predicate device. The differences between the Xpert BCR-ABL Ultra test and the predicate device do not raise different questions of safety and effectiveness. The clinical study demonstrates that the Xpert BCR-ABL Ultra test is acceptable for its intended use and is substantially equivalent to the predicate device described above.

Non-Clinical Studies:

Linearity/Dynamic Range

Linearity was evaluated independently for each of the two major breakpoints, e13a2/b2a2 and e14a2/b3a2, using CML clinical specimens that were specific for a high level of either the e13a2/b2a2 or e14a2/b3a2 breakpoint. Lysate from each high level of BCR-ABL transcript CML specimen was diluted in a background lysate prepared from CML-negative clinical specimen to target ranges of ~50% (*IS*)/MR0.30 to 0.000625% (*IS*)/MR5.20. The panel members, including the negative level, were tested on two assay kit lots in replicates of 4 per kit lot.

Testing and statistical analyses were conducted in accordance with CLSI EP06-A. Linear regression analyses were performed for first, second and third order polynomials. The results for each breakpoint were considered linear if the polynomial regression coefficients were insignificant (p-values > 0.05). The linear regression curves for both transcripts are shown in Figure 1 and Figure 2 below.

Figure 1: Linear Regression Curves for Breakpoint Transcript e13a2/b2a2

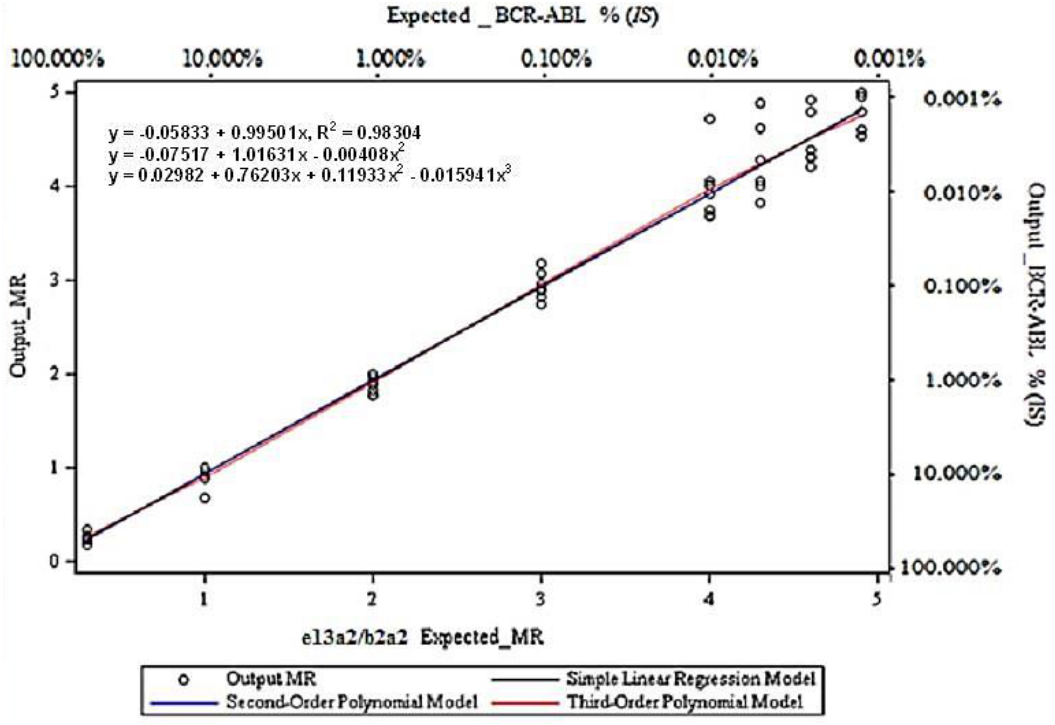
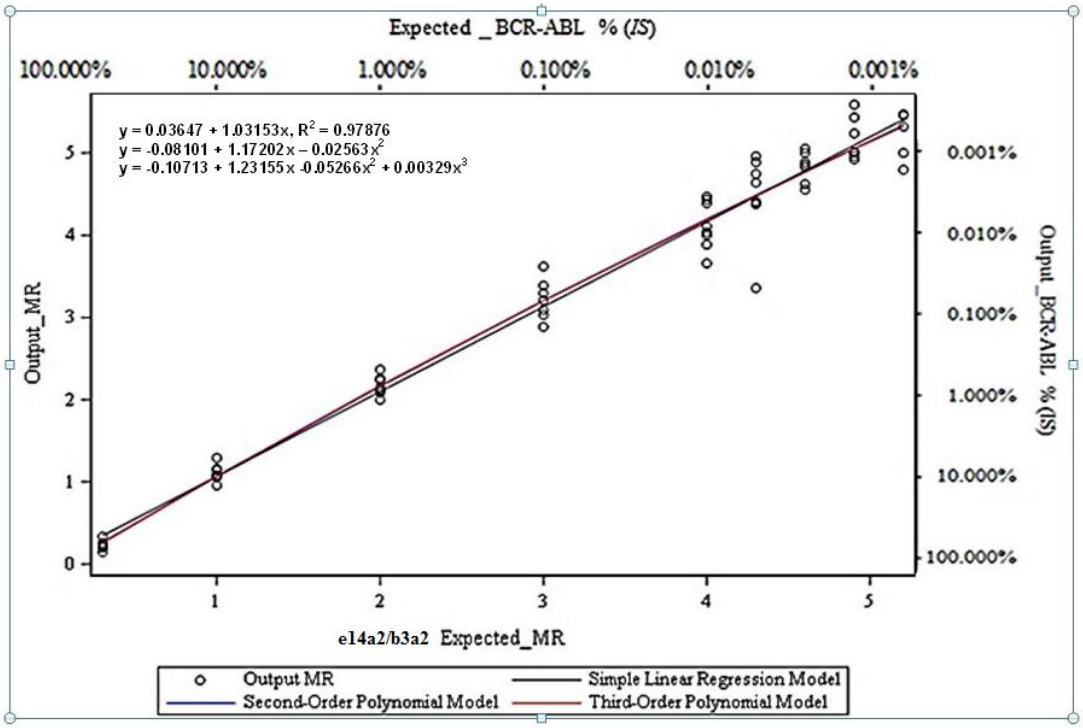


Figure 2: Linear Regression Curves for Breakpoint Transcript e14a2/b3a2



The estimated regression intercepts, slopes and R^2 values from the linear model are shown in Table 3.

Table 3: Regression Coefficients from Linear Model

Breakpoint	Intercept	Slope	R^2
e13a2/b2a2	-0.05833	0.99501	0.98304
e14a2/b3a2	0.03647	1.03153	0.9788

Collectively, the data support an observation of linearity from at least 55% (*IS*)/MR 0.26 to ~0.0019% (*IS*)/MR4.75 with a maximum SD of 0.26. The reportable range spans from the limits of linearity at 55% (*IS*)/MR0.26 to the LoD/LOQ at 0.0030% (*IS*)/MR4.52.

Analytical Sensitivity

The limit of detection (LoD) was estimated for both e13a2/b2a2 and e14a2/b3a2 breakpoints by testing serial dilutions of High CML positive specimens [>10% (*IS*)/MR1] as well as testing Low CML positive specimens [<0.1% (*IS*)/MR3]. Data for each breakpoint across dilutions and specimens were separately compiled and the LoD was estimated by using probit regression analysis. The resulting analysis yielded an estimated LoD of 0.0035% (*IS*)/MR4.45 for the e13a2/b2a2 breakpoint and 0.0030% (*IS*)/MR4.52 for the e14a2/b3a2 breakpoint.

The LoD was verified by adapting the non-parametric method described in the CLSI guidance document, EP17-A2 (Table 4). Two unique CML positive specimens representing each breakpoint were diluted to a targeted 0.0030% (*IS*)/MR4.52 level. For e13a2/b2a2, 94 replicates were tested by 2 operators across 4 test kit lots over 4 days. For e14a2/b3a2, 101 replicates were tested by 2 operators across 4 test kit lots over 7 days.

Table 4: Verified Limit of Detection in % (*IS*)/MR

Breakpoint	Positives/ Replicates	% of Positives	Median % (*IS*)/MR
e13a2/b2a2	90/94	95.74%	0.0030% (*IS*)/MR4.52
e14a2/b3a2	97/101	96.04%	0.0029% (*IS*)/MR4.55

Since the Xpert BCR-ABL Ultra test does not distinguish between the two breakpoints, e13a2/b2a2 and e14a2/b3a2, the higher of the two is claimed as the assay LoD. Thus, the overall Xpert BCR-ABL Ultra LoD for both e13a2/b2a2 and e14a2/b3a2 is 0.0030% (*IS*)/MR4.52.

The limit of quantitation (LoQ) was estimated with the data obtained from the LoD studies. The mean and standard deviation for the % (*IS*) values and MR values were calculated for replicates at levels equal to the LoD, 0.0030% (*IS*)/MR4.52, or greater with positivity greater or equal to 95%. The LoQ of the test is constrained by the LoD of the test; therefore, the LoQ was determined to be equal to the LoD, 0.0030% (*IS*)/MR4.52. The results were also evaluated against the acceptance criteria for standard deviation (SD) ≤ 0.36. The MR standard deviation for both e13a2/b2a2 (observed SD range MR0.27-MR0.34) and e14a2/b3a2 (observed SD range MR0.29-MR0.31) were within the acceptance criteria.

The limit of blank (LoB) was determined with 50 presumptive non-CML, normal healthy

donor blood specimens, drawn into EDTA tubes. No measurable BCR-ABL values were observed for any of the tests. Thus, the overall LoB was determined to be 0.00% (*IS*).

Analytical Specificity

The analytical and clinical specificity of Xpert BCR-ABL Ultra was evaluated for exclusivity by analyzing EDTA whole blood specimens drawn from fifty (50) healthy donors (non-CML) and twenty (20) leukemic specimens (AML/ALL). Breakpoint specificity was determined by testing normal healthy donor EDTA blood spiked with five (5) different leukemia cell lines representing 3 different types of leukemia (CML, ALL and APL) and 5 disease-breakpoints: K562 (CML/e14a2/b3a2) and BV173 (CML/e13a2/b2a2) served as positive controls; SUP-B15 (ALL/e1a2), AR230 (CML/e19a2) and NB4 (APL/PML-RARA) were evaluated for specificity.

No BCR-ABL signal was detected by Xpert BCR-ABL Ultra in any of the healthy non-CML specimens or AML/ALL leukemic specimens evaluated in this study.

Among the leukemia cell lines tested, CML cell lines (K562 and BV173) with p210 major breakpoints yielded the expected positive results. The CML cell line (AR230) with the p230 e19a2 breakpoint reported POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)] for 1 of 4 replicates tested at the targeted 10% (IS)/MR1.00 level based on the number of K562 cell. The positive result for the AR230 cell line was for a target level 3.52 logs above assay LoD and was not observed at the lower levels of 1% (*IS*)/MR2.00 and 0.1% (*IS*)/MR3.00.

Xpert BCR-ABL Ultra is specific to the p210 BCR-ABL fusion transcript associated with CML and has an analytical specificity of 100% for non-CML EDTA blood specimens.

Potentially Interfering Substances Study

This study evaluated five substances that may be present in EDTA whole blood specimens with the potential to interfere with the performance of the Xpert BCR-ABL Ultra test. The compounds and levels tested (Table 5) were based on guidance from the CLSI document EP07-A2. Interferents were tested in the background of CML clinical EDTA whole blood specimens representing three levels with five specimens per level: >1% (*IS*)/<MR2, 0.1-1% (*IS*)/MR3-MR2, and <0.1% (*IS*)/>MR3). Test controls consisted of CML clinical specimens in EDTA whole blood at the respective BCR-ABL transcript level without the interfering substance. Each CML specimen was tested in the absence and presence of the five individual interferents at 4 replicates per condition.

A substance was considered non-interfering if in its presence the % mean (*IS*)/MR ratio observed was within 3-fold difference when compared to the control.

No clinically significant inhibitory effects on the Xpert BCR-ABL Ultra test were observed with any of the interfering substances evaluated in this study. Although some variability and statistically significant differences (p-value <0.05) in some tested

conditions were observed, the reported % (*IS*)/MR ratios for test and control conditions were within the acceptable 3-fold range.

Table 5: Potentially Interfering Substances Tested Using the Xpert BCR-ABL Ultra

Interfering Substances	Concentration Tested
Unconjugated Bilirubin	20 mg/dL
Cholesterol, Total	500 mg/dL
Triglycerides, Total (Lipids)	1800 mg/dL
Heparin	3500 U/L
EDTA (short draw)	750 mg/dL (5X)

Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination from cartridges run sequentially in the same module. To demonstrate this, negative samples were run following very high positive samples in the same GeneXpert module. This study consisted of processing a **NEGATIVE** EDTA normal sample (CML-negative blood) in the same GeneXpert module immediately following a high **POSITIVE** sample (simulated CML positive blood) with 4.5 x 10^5 cells/mL of K562 cells spiked into CML-negative blood to yield ~10% (*IS*)/MR1.00. This testing sequence was repeated five times on each of the four GeneXpert modules. All twenty BCR-ABL positive samples were correctly reported as **POSITIVE [#.##% (*IS*) and MR#.##]**, while all twenty BCR-ABL negative samples were correctly reported as **NEGATIVE [Sufficient ABL transcript]**.

Traceability to WHO Panel

Traceability to the 1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR (NIBSC code: 09/138) was demonstrated by measuring the WHO Reference Panel with 3 lots of the Xpert BCR-ABL Ultra test and comparing the measured values to the values published in the Reference Panel's Instructions for Use. Each of the 4 Reference Panel members was tested with a minimum of 10 replicates per assay kit lot. The measured MR values for each level of the WHO Primary panel were calculated by regression to each lot of the Xpert BCR-ABL Ultra test (i.e., the WHO panel members were treated as clinical samples and fit to the linear regression model of the assay's standard curve). Furthermore, the measured MR values were compared to the published MR values through an additional regression analysis to determine slope and intercept values (Figure 3). The slope of the line was close to unity (0.96 to 1.1) and the intercept was calculated to be close to 0 (-0.03 to -0.06).

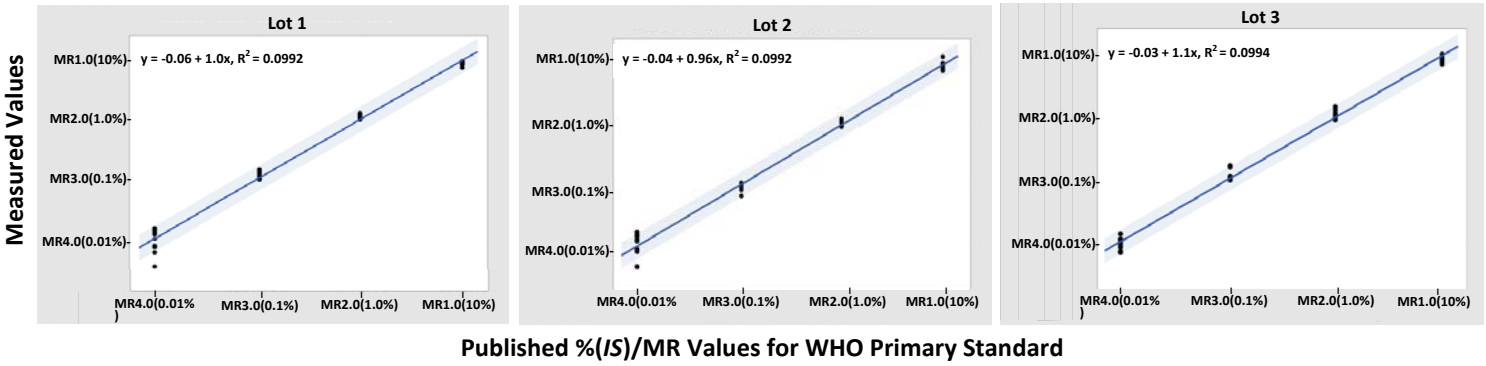


Figure 3. Measured vs Published Values for WHO Primary Reference Panel, Lot-to-Lot.

Xpert BCR-ABL Ultra test kit-generated MR values (y-axis) are plotted against the MR values published in the WHO Primary Reference Panel's Instruction for Use (x-axis). The three lots are represented by (black) data points. Regression analyses and confidence intervals are based upon data for each lot separately.

Clinical Studies

Clinical Performance

The clinical performance of the Xpert BCR-ABL Ultra test was evaluated at four institutions in the U.S as part of a multi-site clinical study. Three additional institutions served as specimen collection only sites. The study was conducted using fresh, prospectively collected EDTA whole blood specimens from patients with CML at any stage of disease, following initial diagnosis, with or without prior exposure to Tyrosine Kinase Inhibitor therapy or other CML treatment. In addition, the study included left over specimens stored as frozen lysates which were prepared from EDTA whole blood from the same patient population. The Xpert BCR-ABL Ultra test performance was compared to a FDA-cleared molecular assay which detects and quantifies the mRNA transcripts for the p210 translocation types (e13a2/b2a2 or e14a2/b3a2) and uses the ABL as the endogenous control mRNA transcript.

A total of 266 eligible specimens were initially enrolled in the study, from which 57 were excluded due to use of an obsoleted procedure for extraction method (27), subject did not complete blood draw (8), shipping or testing delay (6), insufficient volume for testing (6), comparator test failed (6) or testing with incorrect Xpert BCR-ABL Ultra assay definition file (4) leaving 209 specimens which were tested.

Of 209 specimens, 97.1% (203/209) of the Xpert BCR-ABL Ultra results were successful on the first attempt giving an initial non-determinate rate of 2.9% (6/209) and 99.5%

(208/209) were successful upon retesting giving a final non-determinate rate of 0.5% (1/209).

Of the 208 specimens available for analysis, 150 (72.1%) were frozen specimens and 58 (27.9%) were fresh, prospectively collected specimens, for which demographic information was available. Among the fresh specimens, 24 (41.4%) were collected from female subjects and 34 (58.6%) from male subjects. The mean subject age for those providing fresh specimens was 60.5 years (range 28-85 years).

Of the 208 results that were available for analysis, 147 had results that were within the quantitative reportable range for both assays [0.0030% - 55% (*IS*)/MR4.52 – MR0.26 for Xpert BCR-ABL Ultra and 0.002% - 50% (*IS*)/MR4.72 – MR0.30 for the Comparator Assay]: 117 of which were from frozen left-over lysates and 30 of which were fresh prospectively collected specimens. The performance of the Xpert BCR-ABL Ultra test versus the Comparator Assay was evaluated using a Deming regression to determine the slope and intercept. Figure 4 shows the Deming regression and linear regression analysis of the 147 assay results (MR values).

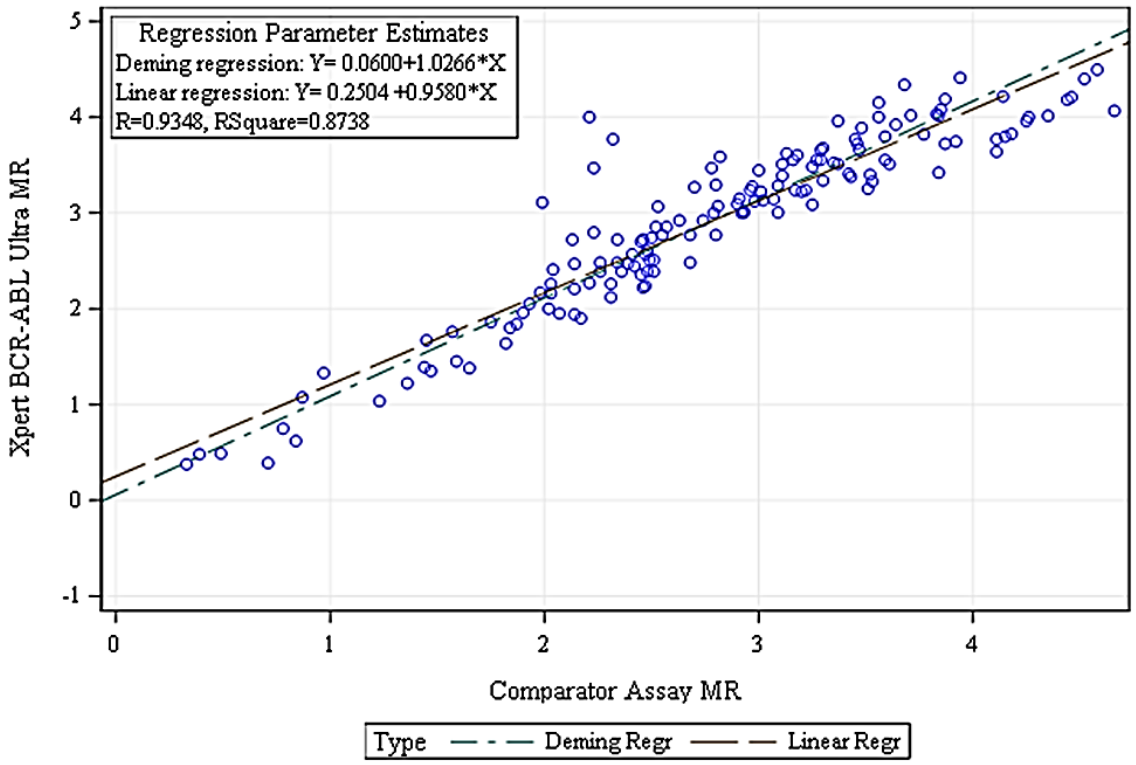


Figure 4: Deming and Linear Regression Analyses

The slope and intercept from the Deming regression were 1.0266 and 0.0600 respectively. From these results, the predicted bias at the MMR (MR3) was calculated to be MR0.1244 (95% confidence interval of 0.0969 – 0.1519).

A Bland-Altman difference analysis was also performed using the 147 quantitative results that were within the reportable range for both the Xpert BCR-ABL Ultra test and Comparator Assay. The Bland-Altman graph (Figure 5) shows the upper and lower 2SD of the mean difference that was observed. The trend line of the bias across the MR range is also shown.

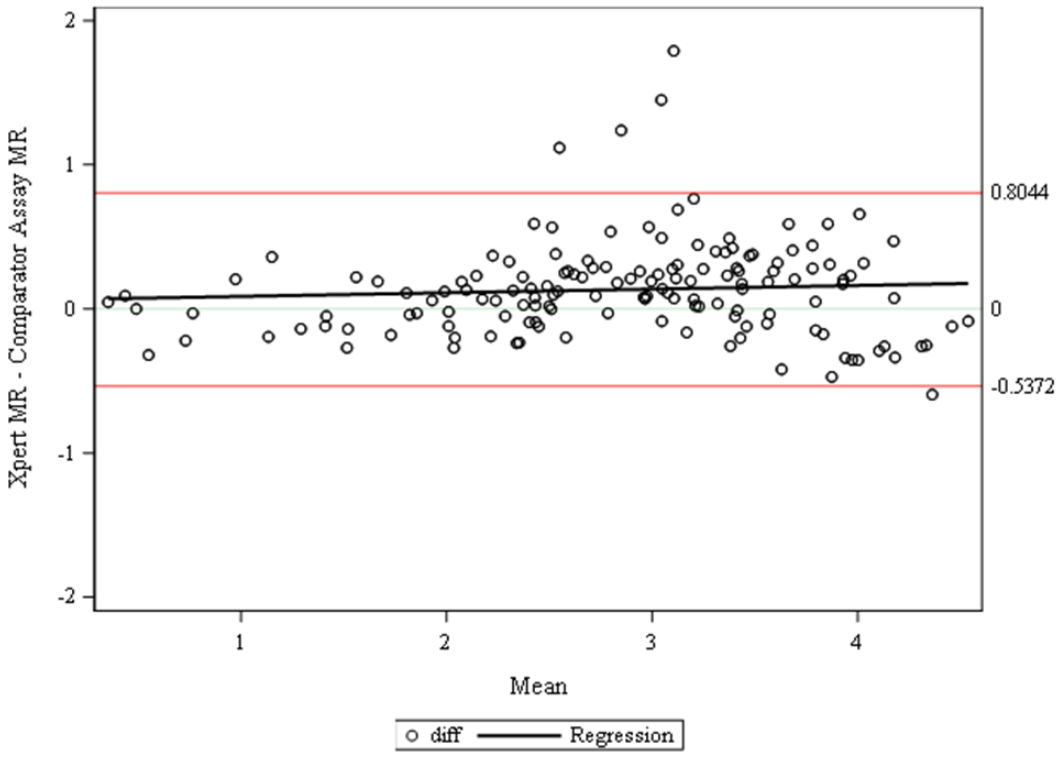


Figure 5: Xpert BCR-ABL Ultra Test MR vs Comparator Assay BCR-ABL MR Bland-Altman Difference Analysis

The mean difference (bias) was calculated to be 0.1336 with a SD of 0.3354. The majority (96.6%, 142/147) of the results were within the 2SD range (between -0.5372 and 0.8044).

Precision and Reproducibility Study

The precision and reproducibility of the Xpert BCR-ABL Ultra test was evaluated in a multisite study in accordance with CLSI EP05-A3, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline" and CLSI EP15-A3, "User

Verification of Performance for Precision and Trueness, Approved Guideline".

A panel of eleven samples was prepared which included the following: One sample negative for BCR-ABL, two samples near the limit of detection (LoD) and eight samples at molecular response (MR) levels 1-4, using the two targets detected by the Xpert BCR-ABL Ultra test: e13a2/b2a2 and e14a2/b3a2. The sample panel was made by diluting a bulk lysate of high %BCR-ABL/ABL specimens from patients with CML into pooled whole blood collected from healthy donors to obtain the desired level.

Table 6 shows the eleven samples included in this study.

Table 6: Reproducibility Panel for Xpert BCR-ABL Ultra

Sample No.	Description	% (*IS*)
1	MR1.0 e13a2/b2a2	BCR-ABL at ~ 10% (*IS*)
2	MR1.0 e14a2/b3a2	BCR-ABL at ~ 10% (*IS*)
3	MR2.0 e13a2/b2a2	BCR-ABL at ~ 1% (*IS*)
4	MR2.0 e14a2/b3a2	BCR-ABL at ~1% (IS)
5	MR3.0 e13a2/b2a2	BCR-ABL at ~ 0.1% (*IS*)
6	MR3.0 e14a2/b3a2	BCR-ABL at ~0.1% (*IS*)
7	MR4.0 e13a2/b2a2	BCR-ABL at ~ 0.01% (*IS*)
8	MR4.0 e14a2/b3a2	BCR-ABL at ~0.01% (*IS*)
9	Near LoD e13a2/b2a2	BCR-ABL at ~ 0.005% (*IS*)
10	Near LoD e14a2/b3a2	BCR-ABL at ~ 0.005% (*IS*)
11	Negative	BCR-ABL Not Detected

Each of the eleven panel members was tested in duplicate two times per day on four different days by each of three different operators at three different sites. Three lots of Xpert BCR ABL kits were used and each operator performed testing with one lot (3 sites x 3 lots x 1 operator/lot x 4 days x 2 runs/operator x 2 replicates/run = 144 replicates/panel member).

The quantitative results were analyzed by Analysis of Variance (ANOVA) and the major components of variance were identified.

The ANOVA analysis for each panel member are shown in Table 7.

Table 7: Reproducibility Study: Results from Analysis of Variance

Sample	N	Mean (MR)	Site/Instrument SD	Operator/Lot SD	Day SD	Within-run SD	Total SD[a]
Target MR1.0 e13a2/b2a2	144	0.96	0	0.05	0.01	0.06	0.08
Target MR1.0 e14a2/b3a2	144	0.99	0	0.06	0	0.08	0.1
Target MR2.0 e13a2/b2a2	143	2.04	0	0.06	0.02	0.10	0.11
Target MR2.0 e14a2/b3a2	144	2.09	0.03	0.07	0.02	0.10	0.13
Target MR3.0 e13a2/b2a2	144	2.89	0.06	0.04	0.03	0.10	0.12
Target MR3.0 e14a2/b3a2	144	3.12	0.06	0.08	0	0.11	0.15
Target MR4.0 e13a2/b2a2	143[b]	3.67	0.03	0.02	0	0.15	0.15
Target MR4.0 e14a2/b3a2	144	3.91	0.05	0.08	0.04	0.14	0.17
Target near MR 4.0 e13a2/b2a2	140[c]	4.36	0.04	0.04	0	0.33	0.33
Target near MR 4.0 e14a2/b3a2	143[d]	4.22	0.03	0.08	0	0.17	0.19

a. The Xpert BCR-ABL Ultra test performed on the GeneXpert Instrument Systems integrates sample purification and nucleic acid amplification. The overall variability of the test observed in this study (expressed as Total SD) includes variability contributed by both the on-board sample preparation and RT-qPCR steps.
b. One replicate meeting the outlier requirements at the 99% level per CLSI EP15-A3 was removed from the analysis.
c. 4 samples out of the 144 test results yielded a NEGATIVE result
d. 1 sample out of the 144 test results yielded a NEGATIVE result

The observed total standard deviation for samples at MR1, MR2 and MR3 was ≤ 0.15. The maximum observed total standard deviation for samples near the LoD and MR4 was 0.33.

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert BCR-ABL Ultra is substantially equivalent to the predicate device.