510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190076

B. Purpose for Submission:

New Device

C. Measurand:

BCR-ABL1 and ABL1 transcripts

D. Type of Test:

Reverse transcription, quantitative, polymerase chain reaction (RT-qPCR) based nucleic acid amplification

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Trade name: Xpert® BCR-ABL Ultra
Common name: Xpert BCR-ABL Ultra

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.6060
   21 CFR 862.2570

2. Classification:

   Class II

3. Product code:

   OYX: BCR/ABL1 monitoring test
   OOI: Real Time Nucleic Acid Amplification System

4. Panel:

   88 - Pathology

H. Intended Use:

1. Indications for 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 the GeneXpert Infinity System.

2. Special conditions for use statement(s):

   For *in vitro* diagnostic use only
   For Prescription use only

3. Special instrument requirements:

   GeneXpert Dx System (I, II, IV, XVI) or the GeneXpert Infinity Systems (48s and -80). These instruments are collectively referred to as the GeneXpert Instrument System. The GeneXpert Dx system requires GeneXpert Dx software version 5.1 or higher. The GeneXpert Infinity Systems requires Xpertise software version 6.6 or higher. (Not intended for use on the GeneXpert Xpress)

I. 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) and is run on the GeneXpert Instrument Systems and the GeneXpert Infinity (48s and 80). The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. A single-use, disposable GeneXpert cartridge holds the RT-PCR and PCR reagents. A description of the reagents provided 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Asuragen QuantideX qPCR BCR-ABL IS Kit

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

   DEN160003

3. Comparison with predicate:

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	Same

Similarities		
Item	Device	Predicate
	Kinase Inhibitors (TKIs)	
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 reaction (RT-qPCR) based nucleic acid amplification	Same
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 (WBCC) range 1.5×10^5 to 3.0×10^7 cells/mL	RNA input range of 1 to 5µg
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	Four levels formulated to MR1.0, 2.0, 3.0, 4.0 traceable to the World Health Organization (WHO) international genetic

Differences		
Item	Device	Predicate
	Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.	reference panel for quantitation of BCR-ABL transcript.

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

- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2014.

- CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2005.

- CLSI EP09-A2, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2002.

- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2012.

- BS EN ISO 23640, In vitro diagnostic medical devices. Evaluation of Stability Testing of In Vitro Diagnostic Reagents, June 2015.

- General Principles of Software Validation; Final Guidance for Industry and FDA Staff, issued January 11, 2002.

- Guidance for Industry and FDA Staff – Content of Premarket Submissions for Management of Cybersecurity in Medical Devices, issued on October 2, 2014.

- Guidance for Industry - Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software, issued January 14, 2005.

- Guidance for Industry and FDA Staff – Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, issued May 11, 2005.

- Guidance for Industry, FDA Reviewers and Compliance on Guidance for Off-the-Shelf Software Use in Medical Devices; issued September 9, 1999.

L. Test Principle:

Xpert BCR-ABL Ultra is an automated quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR) test for quantifying the amount of BCR-ABL transcript as a ratio of BCR-ABL/ABL. Whole blood specimens are collected in EDTA. Four (4) ml of blood is manually processed with proteinase K, followed by lysis buffer and ethanol. The entire contents are transferred to the Xpert BCR-ABL Ultra cartridge. The system is configured for the use of single-use, disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the reactions needed to detect BCR-ABL fusion genes resulting from two major p210 breakpoints, translocation e13a2/b2a2 and e14a2/b3a2, and the ABL transcript as an endogenous control in peripheral blood specimens. 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.

The test is performed on Cepheid GeneXpert Instrument Systems (GeneXpert and GeneXpert Infinity). The GeneXpert Instrument Systems perform further sample purification, nucleic acid amplification, and target sequence detection. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The amount of BCR-ABL transcript in the patient sample is reported as the ratio of BCR-ABL/ABL, as well as a log molecular reduction (MR value) from a baseline of 100% on the International Scale (IS), using the GeneXpert software.

The Xpert BCR-ABL Ultra quantifies the BCR-ABL1 mRNA level on the International Scale (IS) using ABL1 as a housekeeping gene and is calibrated using lot specific parameters that are embedded within the test cartridge barcode for quantitation of BCR-ABL1 mRNA. The values of lot-specific calibrations are determined in quality control testing of each assay lot using secondary standards derived from and calibrated to the World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL transcript.

<u>Interpretation of Results</u>

The numerical value of the World Health Organization (WHO) International Scale is %IS, the ratio expressed as a percentage of BCR-ABL1 expression to the expression of a control gene (ABL1 in this instance). The International Scale (%IS) is a geometric progression and therefore repeated measurements of a sample are non-normally distributed about the mean. %IS values require log-transformation prior to performing any statistical analyses that require normally-distributed data.

Another value commonly reported in the literature is the Molecular Reduction, or MR value. The MR value is traditionally written as MRx.x. The MR value is the log10 reduction from the internationally standardized baseline, defined as 100% IS. Therefore,

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

This test reports both %IS and MR values. MR values with corresponding IS values are shown in Table 1 below.

Table 1: MR Values and Corresponding %IS Values

MR	%IS
0.0	100
0.5	32
1.0	10
1.5	3.2
2.0	1
2.5	0.32
3.0	0.1
3.5	0.032
4.0	0.01
4.5	0.0032
5.0	0.001

M. Performance Characteristics:

1. **Analytical performance:**

a. Precision/Reproducibility:

i. The precision/reproducibility of the Xpert BCR-ABL Ultra test was in accordance with CLSI EP05-A3. A panel of 11 samples (described in Table 2) was prepared 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 and included the following: One sample negative for BCR-ABL, 2 samples near the limit of detection (LoD) and 8 samples at molecular response (MR) levels 1-4, using the 2 targets detected by the Xpert BCR-ABL Ultra test: e13a2/b2a2 and e14a2/b3a2.

Table 2: Specimen Representation for the Reproducibility Panel

Sample No.	Description	Target % (*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 11 panel members was tested in duplicate 2 times per day on 4 different days by each of 3 different operators at 3 different sites. Three (3) lots of Xpert BCR ABL Ultra kits were used and each operator performed testing with 1 lot (3 sites x 3 lots x 1 operator/lot x 4 days x 2 runs/operator x 2 replicates/run = 144 replicates per panel member). The observed total standard deviation for samples at MR1, MR2 and MR3 was ≤ 0.15. The maximum observed total standard deviation for samples at MR4 was 0.17. Below MR4 the maximum observed SD was 0.33. The results are summarized in Table 3 below.

Table 3. Reproducibility Study: Results from Analysis of Variance

Sample	N	Mean (MR)	Site/ Instrument SD	Operator/ Lot SD	Day SD	Within -run SD	Total SD[d]
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[a]	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 MR4.0 e13a2/b2a2	140[b]	4.36	0.04	0.04	0	0.33	**0.33**
Target near MR 4.0 e14a2/b3a2	143[c]	4.22	0.03	0.08	0	0.17	**0.19**

a. One replicate meeting the outlier requirements at the 99% level per CLSI EP15-A3 was removed from the analysis.
b. 4 samples out of the 144 test results yielded a NEGATIVE result
c. 1 sample out of the 144 test results yielded a NEGATIVE result
d. 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.

ii. Because the predicate assay precision study was performed using extracted RNA and the Xpert BCR ABL Ultra test, a second study head-to-head comparison was conducted to demonstrate the precision performance at MR3 was comparable to the predicate beginning with whole blood for the predicate. Precision was evaluated with 4 specimens at two levels (MR3 and MR4) for each breakpoint (e13a2/b2a2 and e14a2/b3a2). Each sample was run 3 times for each of 5 days (N = 15 extractions/replicates). The data demonstrated comparable precision performance when compared to the predicate. The results are shown in Table 4.

Table 4. Precision Comparison of the Xpert BCR-ABL Ultra to the Predicate

		Xpert BCR-ABL Ultra			QuantideX BCR-ABL		
		Median	Mean	SD	Median	Mean	SD
e13a2/b2a2	MR3	3.00	2.98	0.11	3.15	3.16	0.09
	MR4	3.85	3.82	0.16	3.98	3.93	0.17
e14a2/b3a2	MR3	3.00	3.04	0.11	3.04	3.02	0.12
	MR4	4.11	4.11	0.11	4.04	4.11	0.19

The study data support the conclusion that the assay has acceptable precision to MR4. Precision was not evaluated at MR = 4.5 and cannot be assured. The assay is not indicated for discontinuation from TKIs or for monitoring for discontinuation.

iii. Between-Lot Reproducibility: Between lot reproducibility was assessed using three lots in the 3 site reproducibility study described above. The imprecision ranged 0.02 SD to 0.08 SD across transcripts and levels.

iv. Between Instrument Model Reproducibility: The Xpert BCR-ABL test is intended for use on the GeneXpert 1, II, IV, and XVI and GeneXpert Infinity 48s and GeneXpert Infinity 80. Each GeneXpert model is similar in that all contain the same modules but different number of modules. The GeneXpert Infinity models are higher-throughput models. To demonstrate the uniformity of results across all models, each site in the 3 site reproducibility study used each of 3 different GeneXpert Instrument Systems including the highest throughput the Infinity-80. The results demonstrated that performance is the same across all systems.

v. Precision of External Controls: The precision performance of external control materials recommended for use with the Xpert BCR-ABL Ultra was demonstrated. The Xpert BCR-ABL IS Panel C130 is a set of reference materials intended for use in monitoring the performance of the in vitro quantitative detection of the BCR-ABL translocation transcripts. The 6-member panel is traceable to the 1st World Health Organization (WHO) International Genetic Reference Panel and composed of different ratios of synthetic BCR-ABL1(e14a2/b3a2) fusion transcript to ABL1 control transcript suspended in a stabilizing matrix to yield approximately 0.0%, 0.0032%, 0.01%, 0.1%, 1% and 10% BCR-ABL1/ABL1 (IS) when analyzed using the Xpert BCR-ABL Ultra assay. They are manufactured by and available through Maine Molecular Quality Controls, Inc. (MMQCI; Scarborough, Maine, USA). Four

sites using the three lots MMQCI Xpert BCR-ABL IS Panel C130 external control and one lot of Xpert BCRABL Ultra assay tested each vial with 3 replicates. The results demonstrated acceptable precision of the controls. Precision is shown for the 3 lots in the Table 5.

Table 5. Mean % BCR-ABL/ABL (*IS*) and SD Measured by Xpert BCR-ABL Ultra at Clinical Sites for 3 MMQCI External Control Lots

C130 Panel Lot 1		**All Sites**	
		Average %IS/MR[a] Measured	**SD**
%*IS*/MR Assigned	0.0062%/MR 4.24	0.007%/MR4.20	0.17
	0.017%/MR 3.76	0.020%/MR3.71	0.1
	0.14%/MR2.85	0.13%/MR2.89	0.072
	1.07%/MR1.97	1.15%/MR1.94	0.027
	8.12%/MR1.09	7.75%/MR1.11	0.035
C130 Panel Lot 2		**All Sites**	
		Average %IS/MR[a] Measured	**SD**
%*IS*/MR Assigned	0.0051%/MR4.30	0.0058%/MR4.28	0.22
	0.013%/MR3.88	0.015%/MR3.89	0.27
	0.13%/MR2.90	0.13%/MR2.91	0.11
	1.08%/MR1.96	1.14%/MR1.95	0.06
	7.64%/MR1.11	7.28%/MR1.14	0.05

C130 Panel Lot 3		**All Sites**	
		Average %IS/MR[a] Measured	**SD**
%*IS*/MR Assigned	0.005%/MR4.31	0.0050%/MR4.34	0.16
	0.015%/MR3.82	0.013%/MR3.88	0.09
	0.13%/MR2.90	0.12%/MR2.94	0.083
	1.97%/MR1.70	2.17%/MR1.67	0.059
	9.6%/MR0.98	11.79%/MR0.93	0.059

[a.] MR values were calculated [MR value = -Log(% *(IS)*/100)] from the individual value for % (*IS*) then averaged for the final mean MR value.

b. Linearity/assay reportable 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. The estimated regression intercepts, slopes and R2 values from the linear model are shown in Table 6. The reportable range of the assay is MR0.26 to MR 4.52.

Figure 1. Linearity for breakpoint transcript e13a2/b2a2

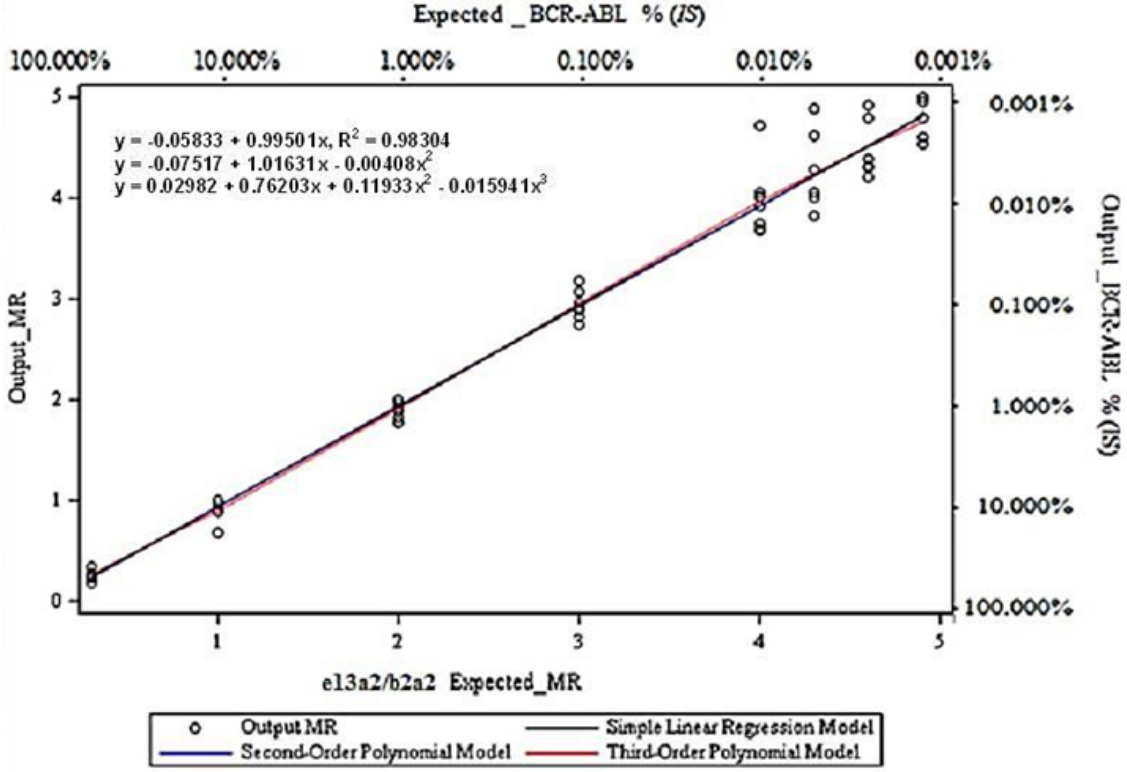


Figure 2. Linearity for breakpoint transcript e14a2/b3a2

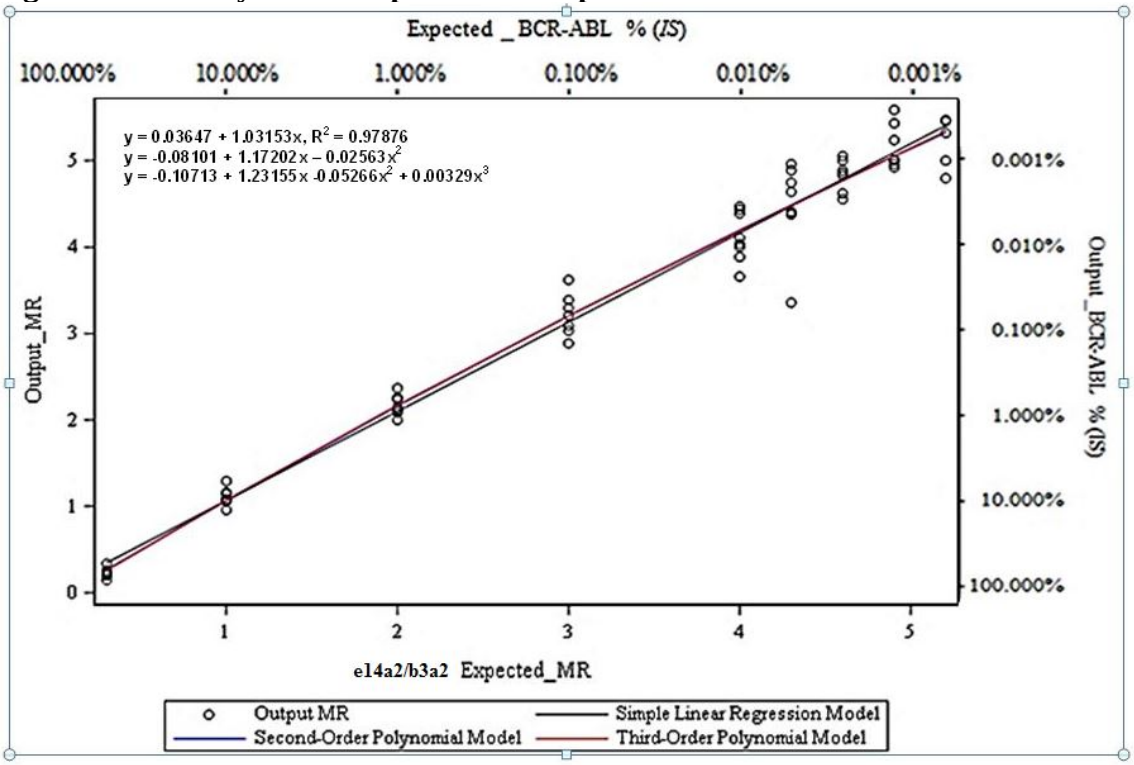


Table 6. Regression Coefficients from Linear Model

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

These results support the linearity from 55% IS (MR 0.3) to 0.002 IS% (MR 4.7) with a maximum SD of 0.26.

c. Detection limit:

i. Limit of Blank

The limit of blank (LoB) was determined by testing 50 normal (non-CML) human EDTA whole blood specimens that were presumed to be negative for BCR-ABL. Testing was performed across 5 kit lots by 4 operators across multiple days. No measurable BCR-ABL values were observed for any of the tests and the overall LoB was determined to be 0.00% (IS).

ii. Limit of Detection

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)/MR4.56 for the e13a2/b2a2 breakpoint and 0.0030% (IS)/MR4.52 for the e14a2/b2a2 breakpoint.

The LoD was verified by adapting the non-parametric method described in the CLSI guidance document, EP17-A2. 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. Results are shown below in Table 7.

Table 7: LoD Verification for e13a2/b2a2 and e14a2/b3a2

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 assay is not indicated for discontinuation from TKIs or for monitoring for discontinuation.

iii. Limit of Quantitation

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 assay is constrained by the LoD of the assay; 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 LoQ is the same as the LoD MR4.52.

d. Traceability and Stability (Reagents and Specimen):

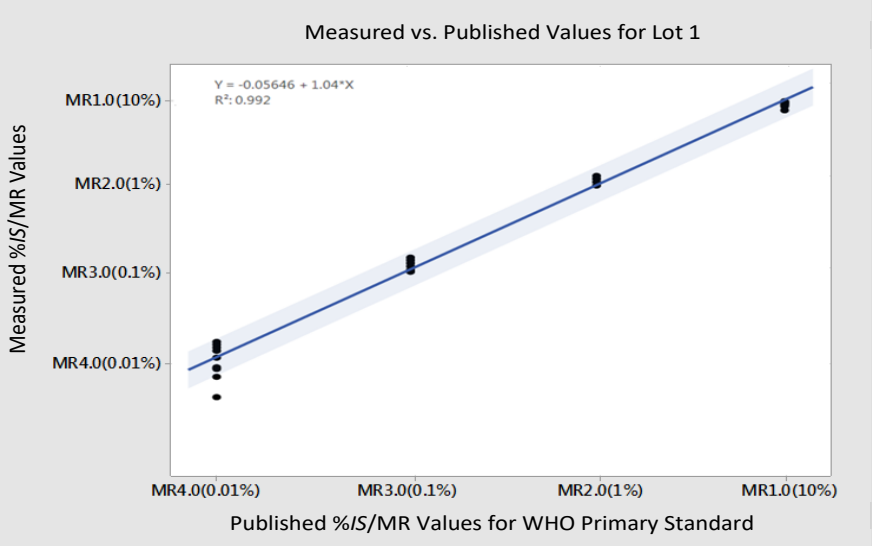
i. Traceability

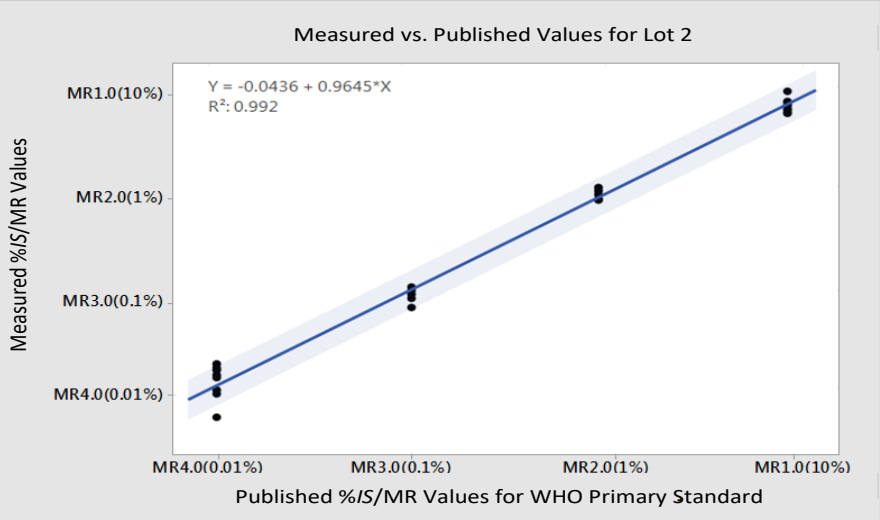
The Xpert BCR-ABL Ultra quantifies BCR-ABL mRNA level as % (IS) via calibration of the assay to validated secondary quantitative standards which are aligned to the primary (i.e., the first World Health Organization (WHO)) international genetic reference panel for quantitation of BCR-ABL mRNA.

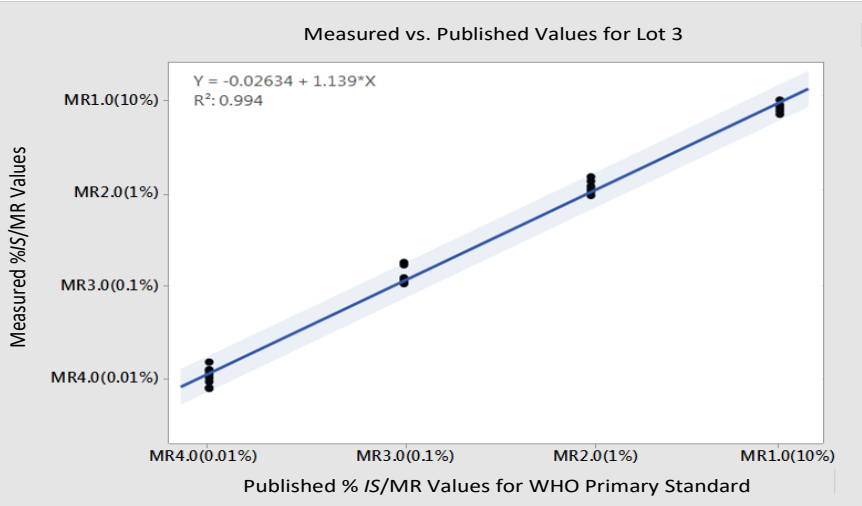
This allows for the determination of a lot-specific conversion factor, including assay efficiency (E) and scaling factor (SF) for each lot of Xpert BCR-ABL Ultra kits. The efficacy of calibration relative to the secondary standards is monitored on an ongoing basis.

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. 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 below).

Figure 3. Measured vs. Published %(*IS*)/MR Values for WHO Primary Standard on Xpert BCR-ABL Ultra by Lot for three lots.







Xpert BCR-ABL Ultra 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.

ii. Reagent Stability

a. Real Time Stability

Reagent shelf life stability studies were conducted with 3 lots, testing 2° C and 8° C at 0 month, 0.5 months, 1 month, 3 months, 6 months, 9 months, 12 months 25 months 31 months and 37 months. Three levels (0.1% IS, 0.01% IS and negative) were tested. At each timepoint, 8 replicates were tested for each lot. The results of the study support a 24 month stability claim.

b. Shipping Stability

Summer and winter shipping conditions were tested on a single lot. Summer shipping conditions were as follows:

- 43^0 C, 70% humidity, 4 hrs then
- 24^0 C, 50% humidity, 2 hrs then
- 43^0 C, 70% humidity, 2 hrs then
- 38^0 C, 95% humidity, 2 hrs then
- 15^0 C, 20 % humidity, 6 hrs then
- 41^0 C, 85% humidity, 2 hrs then
- 48^0 C, 90% humidity, 6 hrs then
- 43^0 C, 75% humidity, 24 hrs then
- 50^0 C, 75% humidity, 5 days

Winter shipping conditions were as follows:

- -2^0 C, uncontrolled humidity, 4 hrs then
- 24^0 C, 50% humidity, 2 hrs then
- -2^0 C, uncontrolled humidity, 2 hrs then
- -5^0 C, uncontrolled humidity, 2 hrs then
- 15^0 C, 10% humidity, 6 hrs then
- -14^0 C, uncontrolled humidity, 2 hrs then
- -18^0 C, uncontrolled humidity, 6 hrs then
- -24^0 C, uncontrolled humidity, 24 hrs then
- -18^0 C, uncontrolled humidity, 5 days

Upon completion of each condition, packages were further subjected to a series of drop testing conditions including shock and vibration tests, vehicle stacking simulation, loose load vibration simulation, altitude test simulation, and vehicle vibration simulation. Samples were tested at 0 month, 0.5 months, 1 month, 3 months, 6 months, 9 months, 12 months 25 months 31 months and 37 months. Three levels (0.1% IS, 0.01% IS and negative) were tested. At each timepoint, 8 replicates were tested for each lot. Acceptance criteria were met and these results support the conclusion that the assay is stable following a 7 day shipment.

iii. <u>Specimen Stability</u>

a. Blood stability

Testing was performed on CML EDTA blood specimens targeting 10% IS, 0.1% IS and 0.01% IS. The clinical specimens were stored at 4^0 C for 0 hours, 24 hours, 48 hours, 72 hours, and 96 hours. The results demonstrate that the test can be used on whole blood collected in EDTA tubes and stored 4^0 C for 72 hours.

b. Lysate stability

Whole blood is mixed with proteinase K and lysis reagent to create a lysate that is loaded on the Xpert BCR-ABL Ultra Cartridge. Users are instructed to store remaining lysate at 4 ºC for up to 4 hours or store at -20 ºC or lower for up to 24 hours in samples need to be retested. The stability of lysate at 4^0 C was tested at 0, 1, 3, 4, 6, 24 and 48 hours and the stability lysate at -20^0 C was tested for 1, 7 and 14 days. Each test used samples at 1% IS, 0.1% IS and 0.01% IS. The results support that lysates are stable up to 4 hours at 4º C and up to 24 hours at -20º C.

e. Analytical specificity:

i. Interfering Substances

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 were evaluated. The compounds and levels tested were based on 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)/MR2-MR3, and 0.001%-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 listed in Table 8.

Table 8: List of Potentially Interfering Substances Tested

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)

The data demonstrated no discernable interference and supported the conclusion that none of the tested agents interfere with the assay at the concentrations listed.

ii. Primer Specificity

The analytical and clinical specificity of Xpert BCR-ABL Ultra was evaluated for exclusivity by analyzing EDTA whole blood specimens drawn from 50 healthy donors (non-CML) and 20 leukemic specimens (AML/ALL). Breakpoint specificity was determined by testing normal healthy donor EDTA blood spiked with 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/<0.003% (IS)]" for 1 of 4 replicates tested at the targeted 10% (IS)/MR1.00 level based on the number of K562 cells. 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.

iii. Specimen Carryover

Each cartridge is single-use, and self-contained GeneXpert cartridges and therefore carry-over contamination from cartridges is not expected. To confirm, negative samples were run following very high positive samples in the same GeneXpert module. This study consisted of processing a negative sample in the same GeneXpert module immediately following a high positive sample (simulated CML positive blood) with 4.5×10^5 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. No contamination was observed as expected.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was designed to evaluate the performance of the Xpert BCR-ABL Ultra compared to the predicate device [Asuragen Quantidex qPCR BCR-ABL IS Kit (IVD)] in RNA derived from human blood samples obtained from individuals previously diagnosed with t(9;22) positive CML, in accordance with the assay protocol. The samples were tested at four institutions in the U.S. and these sites also served as specimen collection sites. 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 exposure to Tyrosine Kinase Inhibitor therapy or other CML treatments. In addition, the study included leftover specimens stored as frozen lysates which were prepared from EDTA whole blood from the same patient population. Study participants were required to be at least 18 years of age.

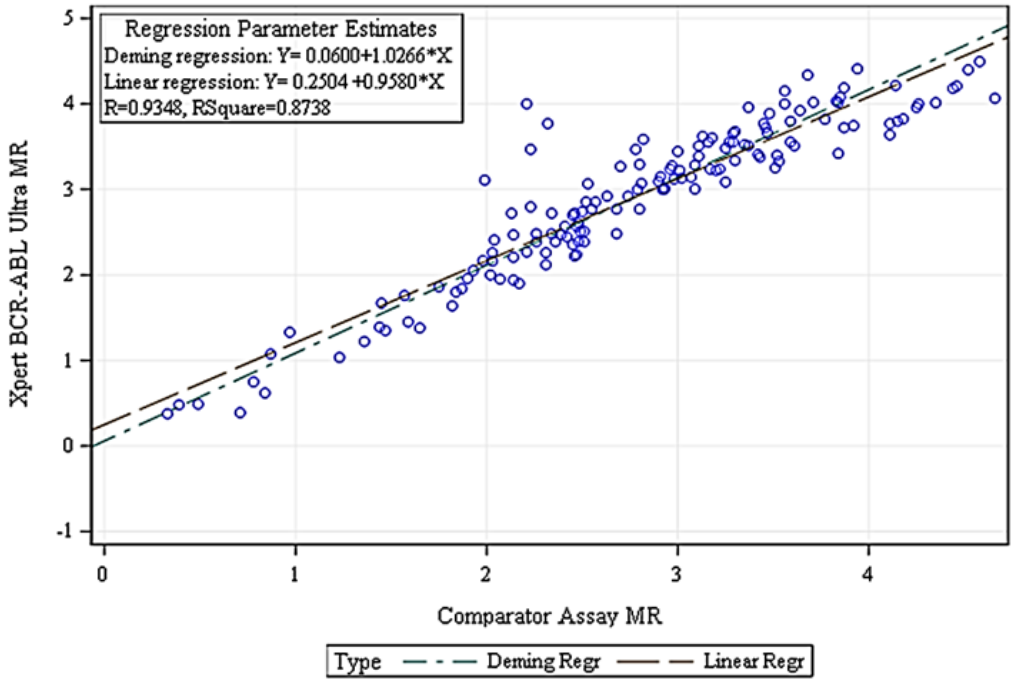
A total of 266 eligible specimens were initially enrolled in the study, from which 57 were excluded. The reasons for exclusions included obsolete extraction methods (n = 27), subjects who did not complete blood draw (n = 8), shipping or testing delays (n = 6), insufficient volumes for testing (n = 6), absence of comparator result (n = 6) or testing with incorrect Xpert BCR-ABL Ultra assay definition file (n = 4).

Of the remaining 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 0.0020% - 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).

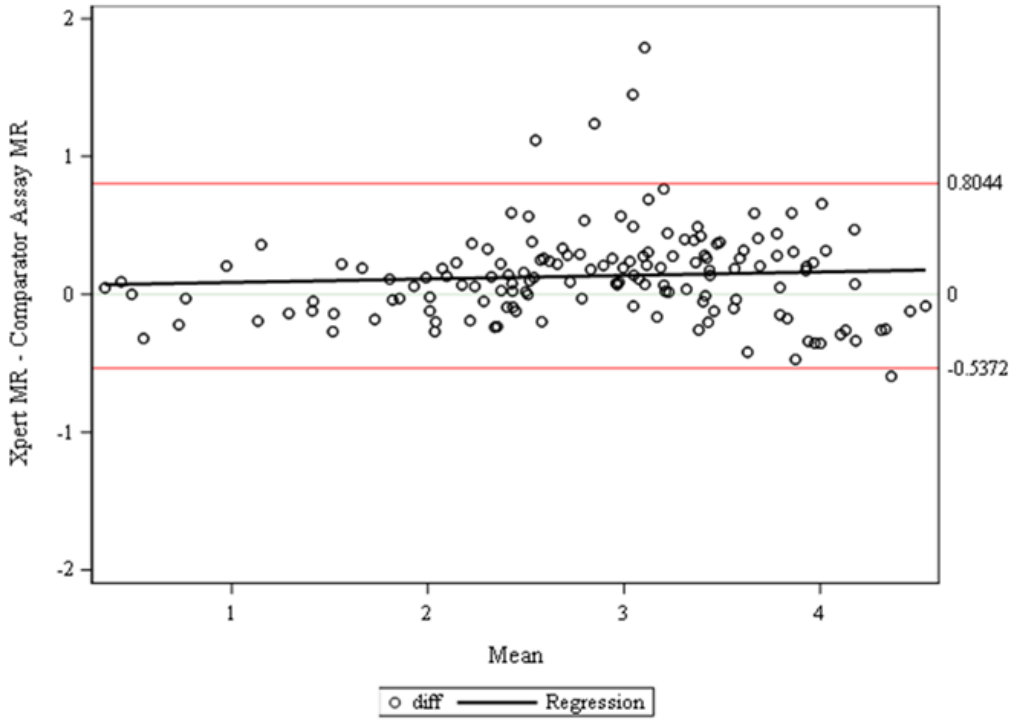
Figure 4. Deming and Linear Regression Analysis



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 MR 0.1244 (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 (see Figure 5) shows the upper and lower 2 SD of the mean difference that was observed. The trend line of the bias across the MR range is also shown.

Figure 5. 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).

Results from the method comparison study demonstrate that the Xpert BCR-ABL Ultra assay is substantially equivalent to the predicate.

b. *Matrix comparison:*
Not applicable

3. Clinical studies:
   a. *Clinical Sensitivity:*
   Not applicable

*b. Clinical specificity**:***
Not applicable

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

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

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type under 21 CFR 6060.

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