Cepheid

Instructions For Use

Xpert® BCR-ABL Ultra

For use with GeneXpert® System with Touchscreen

Catalog Numbers

REF GXBCRABL-US-10

303-0946 | Rev. B | 2024-06

℞only IVD *In Vitro* Diagnostic Medical Device

Trademark, Patents and Copyright Statements

See Revision History for a description of changes.

Table of Contents

Getting Started

Product Information

Proprietary Name

Xpert® BCR-ABL Ultra

Common or Usual Name

Xpert BCR-ABL Ultra

Intended Use, Summary, and Principle of Procedure

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 GeneXpert® Instrument Systems.

Summary and Explanation

Chronic myelogenous leukemia (CML) is one of the most common hematologic malignancies and accounts for 15-20% of all cases of leukemia.[1] The incidence of CML is approximately 1.8/100,000, meaning 1 out of every 55,555 men and women will be diagnosed with CML during their lifetime.[2] More than 95% of patients with CML have the distinctive Philadelphia chromosome (Ph1) that results from a reciprocal translocation

between the long arms of chromosomes 9 and 22.[2] The translocation involves the transfer of the Abelson or ABL1 (ABL hereafter) gene on chromosome 9 to the breakpoint cluster region (BCR) of chromosome 22, resulting in a fused BCR-ABL1 (BCR-ABL hereafter) gene. The fusion gene produces BCR- ABL, a tyrosine kinase with deregulated activity that plays a key role in the development of CML.[3] Xpert BCR-ABL Ultra detects the chromosomal translocation mRNA transcripts for the p210 form resulting from two major breakpoints, translocation e13a2/b2a2 and e14a2/b3a2.

The clinical utility of monitoring BCR-ABL mRNA levels by RT-PCR was established in the International Randomized Study of Interferon and STI571 (IRIS), in which patients received interferon therapy and/or tyrosine kinase inhibitor (TKI) treatment. BCR-ABL results were normalized under a standardized baseline common to the three laboratories participating in the trial.[4] Subsequently, it was proposed that BCR-ABL monitoring tests align to an international scale (*IS*) that is anchored to two values defined in the IRIS trial, thereby allowing results to be expressed on a common scale.[5] The first of these is the standardized baseline which represents 100% (*IS*). The second is Major Molecular Response (MMR) which is defined as a 3-log reduction from the standardized baseline which represents 0.10% (*IS*)/MR3. A 3-log reduction is associated with a favorable survival outcome.[6] In this fashion, *IS*-standardized molecular testing provides an essential aid for clinicians to manage their CML patients' disease.[6]

The Xpert BCR-ABL Ultra test quantifies BCR-ABL mRNA level as % (*IS*) via calibration of the test to the first World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL mRNA. According to the recommended protocol[7], Cepheid has developed and validated secondary quantitative standards which are aligned to the primary WHO reference panel. This allows for the determination of a lot-specific conversion factor, including test 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.

Principle of the Procedure

Xpert BCR-ABL Ultra is an automated test for quantifying the amount of BCR-ABL transcript as a ratio of BCR-ABL/ABL. The test utilizes automated, quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR).

The test is performed on Cepheid GeneXpert system with touchscreen. The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and target sequence detection in simple or complex samples using RT- qPCR and qPCR tests. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use, disposable GeneXpert cartridges that hold the RT-qPCR and qPCR reagents and host the reactions. For a full description of the systems, refer to the relevant system operator manual.

Xpert BCR-ABL Ultra includes reagents 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.[7,8,9,10,11] The amount of BCR-ABL transcript in the patient sample is reported as the percent ratio of BCR-ABL/ABL, and also expressed as a log molecular reduction (MR value) from a baseline of 100% on the International Scale (*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.

Reagents, Instruments, and Materials

Reagents

Material Provided

The Xpert BCR-ABL Ultra kit (GXBCRABL-US-10) contains sufficient reagents to process 10 specimens or quality control samples.The kit contains the following:

Xpert BCR-ABL Ultra Reagents	**10 of each per kit**
• Proteinase K (PK)	10 x 130 µL per vial
• Lysis Reagent (LY) (Guanidinium Chloride)	10 x 5.3 mL per vial

• Wash Reagent (1)
  ○ Ethanol
  ○ Guanidinium thiocyanate
	10 x 2.9 mL per ampoule

Xpert BCR-ABL Ultra Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, 2, 3 and 4 (freeze-dried)	1 of each per cartridge
• Rinse Reagent	2.0 mL per cartridge
• Elution Reagent	2.5 mL per cartridge

CD	**1 per kit**
• Assay Definition File (ADF) • Instruction to import ADF into GeneXpert software • Instructions for Use (Package Insert)	
Certificate of Analysis	1 per kit

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

Materials Required but Not Provided

- GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.0 or higher, and *GeneXpert System with Touchscreen Operator Manual*.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Microcentrifuge (1,000 X g minimum)
- Pipettes and aerosol filter pipette tips
- 50 mL conical tubes
- Reagent grade absolute ethanol

Materials Recommended but Not Provided

Xpert BCR-ABL IS Panel C130, catalog number C130 are quality controls from Maine Molecular Quality Controls, Inc.

Warnings and Precautions

General

- For *in vitro* diagnostic use. Rx only
- Treat all biological specimens, including used cartridges and reagents, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from U.S. Centers for Disease Control and Prevention[12] and Clinical and Laboratory Standards Institute.[13]
- Follow safety procedures set by your institution for working with chemicals and handling biological samples.
- Performance characteristics of this test have been established with blood collected in EDTA tubes only. The performance of this test with other specimen types or samples has not been evaluated.
- Reliable results are dependent on adequate specimen collection, transport, storage and processing. Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the target transcript in the specimen is below the limit of detection of the test. Careful compliance with the instructions for use and the respective operator manual are necessary to avoid erroneous results.
- Performing the Xpert BCR-ABL Ultra test outside the recommended kit or specimen storage temperature ranges and time may produce erroneous or invalid results.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges

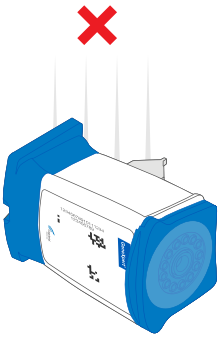
should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[14]

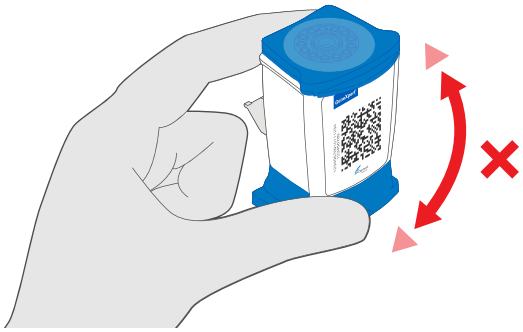
Specimen

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Specimen Collection, Transport and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Do not freeze whole blood specimens.
- Proper sample collection, storage, and transport are essential for correct results.

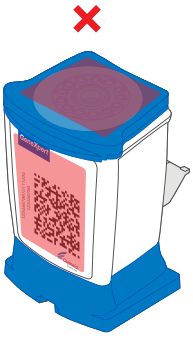
Test/Reagent

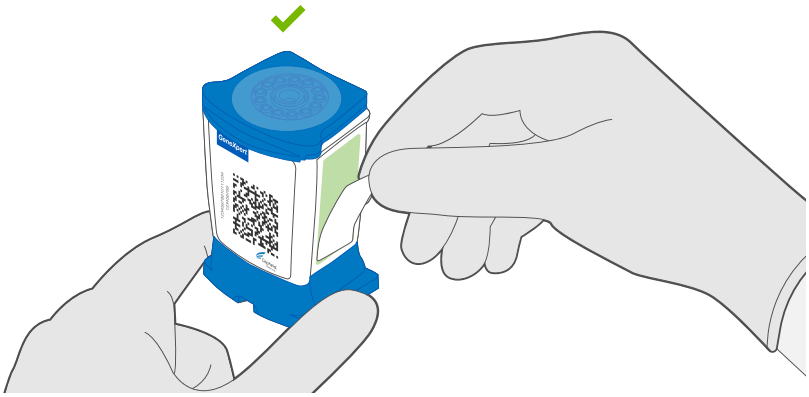
- Do not substitute Xpert BCR-ABL Ultra reagents with other reagents.
- Do not open the Xpert BCR-ABL Ultra cartridge lid except when adding specimen and Wash Reagent.
- Do not use a cartridge that has been dropped after removing it from the packaging.





- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge.





- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- It is recommended that the Xpert BCR-ABL Ultra cartridges be at room temperature (20°C – 30°C) when used for testing.
- Each single-use Xpert BCR-ABL Ultra cartridge is used to process one test. Do not reuse processed cartridges.

- Do not reuse pipette tips.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use the Xpert BCR-ABL Ultra cartridge if a reagent is added to the wrong opening.
- Do not open Xpert BCR-ABL Ultra cartridges after the test is completed.
- Excessively high white blood cell counts might cause pressure to build in the cartridge and lead to aborted runs.
- Dedicate a set of pipettes and reagents exclusively to sample preparation.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

Chemical Hazards, Storage and Handling

Chemical Hazards[15,16]

Note The information below applies to the entire product containing Proteinase K, Lysis, Wash, and Rinse Reagents.

- UN GHS Hazard Pictogram:

- Signal Word: DANGER
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Highly flammable liquid and vapour
  - Causes skin irritation
  - Causes serious eye irritation
  - May cause drowsiness or dizziness
  - Suspected of causing genetic defects.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Obtain special instructions before use.
    - Do not handle until all safety precautions have been read and understood.
    - Keep away from heat, sparks, open flames and/or hot surfaces. No smoking.
    - Keep container tightly closed.
    - Avoid breathing mist/vapours/spray.
    - Wash thoroughly after handling.
    - Use only outdoors or in a well-ventilated area.

- Wear protective gloves/protective clothing/eye protection/face protection.
- Use personal protective equipment as required.

- **Response**
  - In case of fire: Use appropriate media for extinction.
  - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - Call a POISON CENTER or doctor/physician if you feel unwell.
  - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
  - Specific treatment, see supplemental first aid information.
  - Take off contaminated clothing and wash before reuse.
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
  - If exposed or concerned: Get medical advice/attention.
- **Storage/Disposal**
  - Keep cool.
  - Store in a well-ventilated place. Keep container tightly closed.
  - Store locked up.
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

Storage and Handling

- Store the Xpert BCR-ABL Ultra kit contents at 2–8 °C until the expiration date provided on the label.
- Do not open the cartridge lid until you are ready to perform the test.
- Do not use cartridges that have passed the expiration date.
- The Wash Reagent is a clear, colorless liquid. Do not use the Wash Reagent if it has become cloudy or discolored.
- Twenty (20) minutes before starting the procedure, remove the blood specimen, cartridge and sample preparation reagents from storage to allow them to come to room temperature (20°C – 30°C).

Specimen Collection, Testing, and Results

Specimen Collection

Specimen Collection, Transport and Storage

- Whole blood specimens should be collected in EDTA tubes following your institution's guidelines. Samples should be stored at 4°C for no longer than 3 days (72 hours) prior to testing. Plasma from cells should not be separated.
- Proper specimen collection, storage, and transport are critical to the performance of this test. Specimen stability under shipping and storage conditions other than those listed below have not been evaluated with the Xpert BCR-ABL Ultra test.

Procedure

Before You Start

Twenty (20) minutes before starting the procedure, remove the blood specimen and Sample Preparation reagents (including the cartridges) from refrigerated storage to allow them to come to room temperature and briefly spin down the Proteinase K (PK) in a microcentrifuge.

Important **Start the test within one (1) hour of adding the Sample Reagent-treated sample to the cartridge.**

Important **Remove the cartridge from the cardboard packaging before preparing the sample. (See Preparing the Cartridge .)**

Preparing the Sample

1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K).
2. Ensure blood specimen is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing Proteinase K, add 4 mL of blood specimen.
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.

5. Incubate at room temperature for 1 minute.
6. To the same tube, add 2.5 mL of Lysis Reagent (LY).
   Note Retain the remaining lysis reagent to use again in step 13.
7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. Mix the sample by tapping the bottom of the tube 10 times.
12. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.
    Note Remaining lysate can be stored at 4 °C for up to 4 hours or stored at -20 °C or lower for up to 24 hours.
13. To the new conical tube containing lysate, add 1.5 mL of retained Lysis Reagent (LY) from step 6.
14. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
15. Incubate at room temperature for 10 minutes.
16. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
17. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
18. Discard any remaining PK or LY reagents.

Preparing the Cartridge

To add the sample to the Xpert BCR-ABL Ultra cartridge:

1. Remove the cartridge from the cardboard packaging.
2. Inspect the cartridge for damage. If damaged, do not use it.
3. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent Chamber (with small opening). See Figure 1.
4. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.

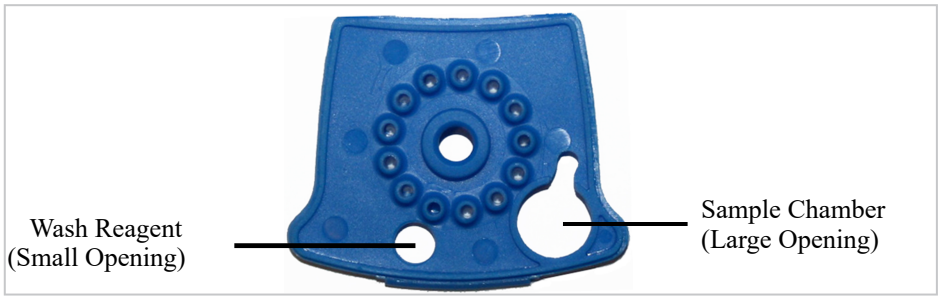


Figure 1 Xpert BCR-ABL Ultra Cartridge (Top View)

5. Close the cartridge lid. Ensure the lid snaps firmly into place. Initiate test (see Starting the Test: GeneXpert System with Touchscreen).

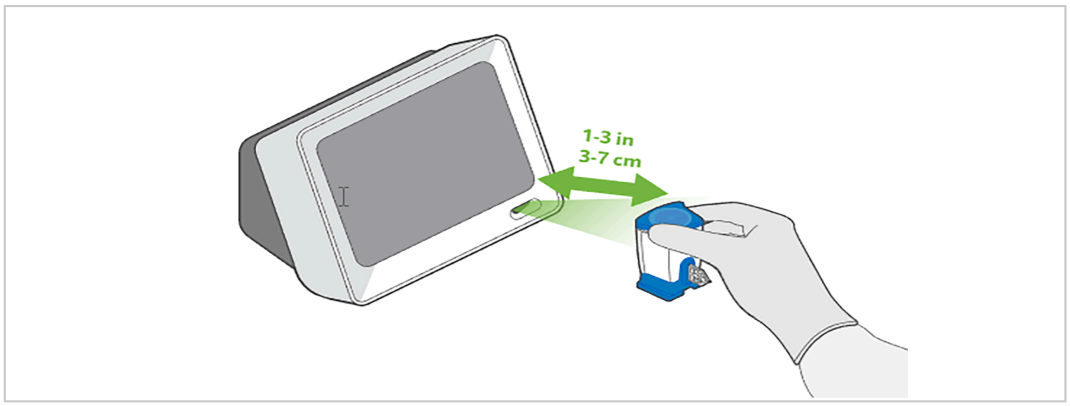
Starting the Test: GeneXpert System with Touchscreen

Important **Before you start the test, make sure that:**

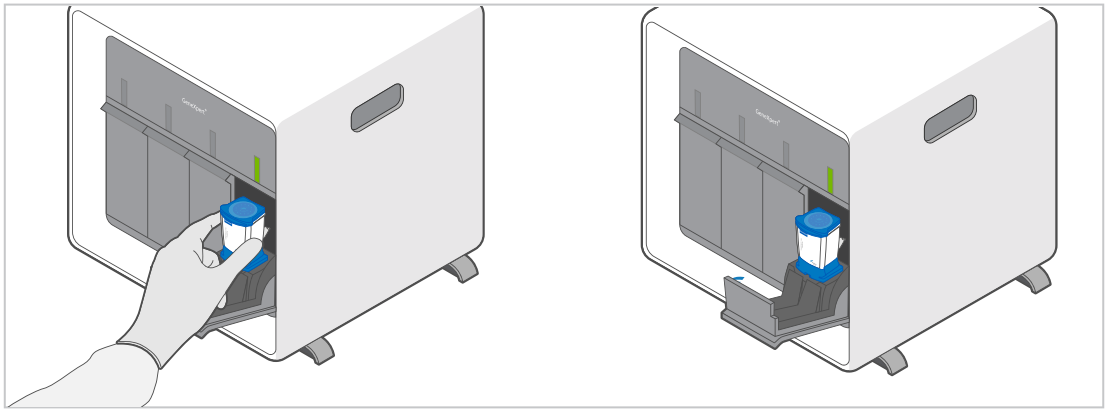
- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

Note The default workflow is shown. Your system administrator may alter the workflow.

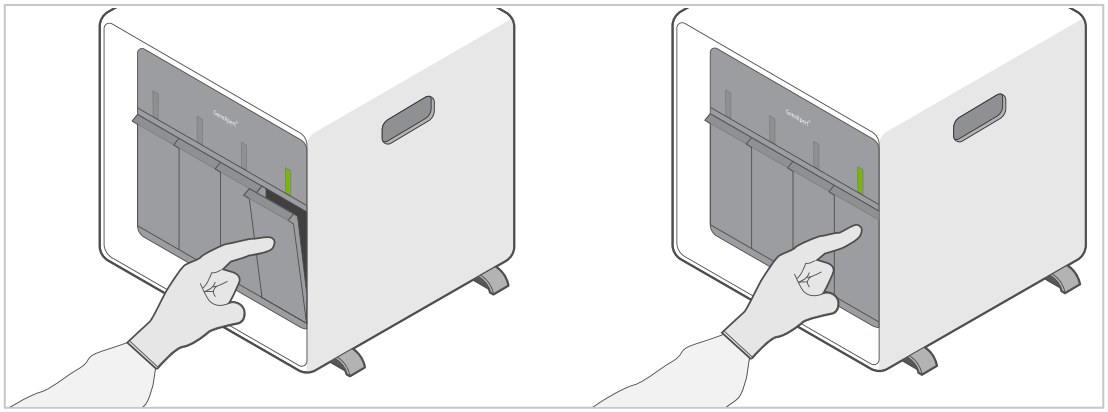
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue**.



6. Select the desired test and touch **Continue**.
7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm**.
9. Open the module door under flashing green light and insert the cartridge.



10. Close cartridge module door completely by pressing until it latches. The test starts.



11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution's standard practices.

Viewing Results: GeneXpert System with Touchscreen

The GeneXpert system with touchscreen results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**. More detailed instructions for viewing and uploading results are available in your system operator manual.

Quality Control

Each cartridge includes an ABL Endogenous Control and Probe Check Control (PCC).

ABL Endogenous Control — The ABL Endogenous Control verifies that sufficient sample is used with the test. Additionally, this control detects sample-associated inhibition of the real-time PCR test. The ABL passes if it meets the assigned acceptance criteria.

Probe Check Control (PCC) — Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, and if all reaction components are functional in the cartridge. The PCC passes if it meets the assigned acceptance criteria.

External Controls

External controls described in Materials Recommended but Not Provided are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert BCR-ABL Ultra, perform the following steps:

1. Allow the Xpert BCR-ABL IS Panel C130 controls to come to room temperature (20°C – 30°C), approximately 30 minutes.

2. Immediately before use, mix the external control tube by inverting the tube 5 to 10 times followed by briefly vortexing the tube for 10 to 15 seconds at medium speed.
3. To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).
4. To the tube already containing Proteinase K, add 4 mL of the external control.
5. Mix the external control with a vortex mixer at maximum setting continuously for 3 seconds.
6. Incubate at room temperature for 1 minute.
7. To the same tube, add 2.5 mL of Lysis Reagent (LY).
   Note Retain the remaining lysis reagent to use again in step 14.
8. Mix the external control with a vortex mixer at maximum setting continuously for 10 seconds.
9. Incubate at room temperature for 5 minutes.
10. Mix the external control with a vortex mixer at maximum setting continuously for 10 seconds.
11. Incubate at room temperature for 5 minutes.
12. Mix the external control by tapping the bottom of the tube 10 times.
13. Transfer 1 mL of the prepared external control sample into a new 50 mL conical tube.
14. To the new conical tube containing the external control sample, add 1.5 mL of retained Lysis Reagent (LY) from step 7.
15. Mix the external control with a vortex mixer at maximum setting continuously for 10 seconds.
16. Incubate at room temperature for 10 minutes.
17. To the same conical tube, add 2 mL of reagent grade absolute ethanol (not provided).
18. Mix the external control with a vortex mixer at maximum setting continuously for 10 seconds.
19. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
20. Pipette the entire contents of the prepared external control into the sample chamber (large opening). See Figure 1 .
21. Close cartridge lid. Initiate test (see Starting the Test: GeneXpert System with Touchscreen).

Results

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the **View Results** window. The possible results and interpretations are shown in Table 1.

Table 1. Xpert BCR-ABL Ultra Results and Interpretation

Result	Interpretation

POSITIVE	BCR-ABL transcript was detected.
	• BCR-ABL POSITIVE – BCR-ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
	• Possible positive results:
	  ○ POSITIVE [#.##% (*IS*) and MR#.##]; .
	  ○ POSITIVE [Above upper LoQ]; .
	  ○ POSITIVE [Below LoD; >MR4.52/<0.003% (*IS*)]; .
	• ABL PASS; ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
	• When ABL Ct value is below 18, a minimum of 32,000 ABL copy number was present in the reaction.[17,18]
	• Probe Check PASS; all probe check results passed.

NEGATIVE	BCR-ABL transcript was not detected. • BCR-ABL NEGATIVE [Sufficient ABL transcript] – BCR-ABL transcript was not detected and has a cycle threshold (Ct) above the valid cycle threshold. • ABL PASS; ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting • When ABL Ct value is below 18, a minimum of 32,000 ABL copy number was present in the reaction.[17,18] • Probe Check PASS; all probe check results passed.
INVALID	BCR-ABL transcript level cannot be determined. • INVALID – BCR-ABL transcript level cannot be determined due to sample containing excess BCR-ABL and/or ABL transcripts. See Troubleshooting Guide for additional instructions for retesting the specimen. • ABL FAIL – ABL cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting. See Troubleshooting Guide for additional instructions for retesting the specimen. • Probe Check PASS; all probe check results passed.
ERROR .	BCR-ABL transcript level cannot be determined. See Troubleshooting Guide for additional instructions for retesting the specimen. • BCR-ABL – NO RESULT • ABL – NO RESULT • Probe Check FAIL – all or one of the probe check results failed. • Probe Check PASS or NA (not applicable) and Pressure Abort. If the probe check passed or shows N/A, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	BCR-ABL transcript level cannot be determined. Insufficient data were collected to produce a test result. For example, this can occur if the operator stopped a test that was in progress. See Troubleshooting Guide for additional instructions for retesting the specimen. • BCR-ABL NO RESULT • ABL NO RESULT • Probe Check NA (not applicable)

Quantitative Results

A certificate of analysis is supplied with each Xpert BCR-ABL Ultra Test kit and contains a lot-specific standard curve for the Xpert BCR-ABL Ultra kit and an Efficiency Value ($E_{\Delta Ct}$). The Efficiency Value is embedded in the barcode of the Xpert BCR-ABL Ultra cartridge. See the certificate of analysis for detailed calculations of the Efficiency Value. Each kit lot also contains a lot specific scaling factor (*SF*) embedded in the barcode that ties the quantitative test output to the International Scale (*IS*).[7] Test results are provided with quantitative test output in both % (*IS*) and molecular response (MR) scales (see Table 2and Table 3). These quantitative values

should be interpreted in the context of the precision of the Xpert BCR-ABL test (see Precision and Reproducibility).

Table 2. Log Reduction, International Scale (IS), and Molecular Response (MR) Correlation

Log Reduction in % BCR-ABL/ABL (*IS*)	MR	% BCR-ABL/ABL (*IS*)[a]
0	0	100
1	1	10
2	2	1
3	3	0.1
4	4	0.01
4.5	4.5	0.0032
5	5	0.001

a. **% BCR-ABL/ABL (*IS*) = % (*IS*).**

MRxx.x = $\log_{10}$[100/Determined % (*IS*)]=$\log_{10}$(100)−$\log_{10}$[Determined % (*IS*)]=2−$\log_{10}$[Determined % (*IS*)]

Table 3. Examples of Xpert BCR-ABL Ultra Test Results

Test	BCR-ABL		ABL		Xpert BCR-ABL Ultra Test Results	Notes
	Ct	Result	Ct	Result		
1	7.1	INVALID	7.3	FAIL	INVALID [Too high BCR-ABL and ABL transcripts]	Calculated % value: 149.92%
2	8.1	INVALID	7.9	FAIL	INVALID [Too high ABL transcript]	Calculated % value:121.05%
3	7.9	INVALID	8.1	PASS	INVALID [Too high BCR-ABL transcript]	Calculated % value:149.92%
4	11.4	POS	10.9	PASS	POSITIVE [Above upper LoQ]	Calculated % value:78.92%
5	18.2	POS	13.5	PASS	POSITIVE [33.93% (*IS*) and MR0.47]	Calculated % value:33.93%
6	21.4	POS	13.4	PASS	POSITIVE [4.68% (*IS*) and MR1.33]	Calculated % value:4.68%
7	28.6	POS	15.2	PASS	POSITIVE [0.012% (*IS*) and MR3.92]	Calculated % value:0.012%
8	30.0	POS	12.7	PASS	POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)]	Calculated % value:0.0008%
9	0	NEG	13.3	PASS	NEGATIVE [Sufficient ABL transcript]	0%
10	31.6	INVALID	18.2	FAIL	INVALID [Insufficient ABL transcript]	NA
11	0	INVALID	18.6	FAIL	INVALID [Insufficient ABL transcript]	NA
12	0	INVALID	0	FAIL	INVALID [No ABL transcript]	NA
13	0	NO RESULT	0	NO RESULT	ERROR	For example, Error5017: [ABL] probe check failed

POSITIVE [#.##% (*IS*) and MR#.##]

BCR-ABL has been detected at a level of #.##% (*IS*) and MR#.##.

For a **POSITIVE [#.##% (*IS*) and MR#.##]** result, BCR-ABL is detectable with a BCR-ABL Ct greater than or equal to “8” and less than or equal to the cut-off of “32” and an ABL Ct greater than or equal to “8” and less than or equal to “18”. The GeneXpert software calculates the % (*IS*) using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL Ct:

% (*IS*) = E $_{\Delta Ct}^{(\Delta Ct)}$ x 100 x Scaling Factor (*SF*)

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the test cartridge barcode. The value of this factor and the lot-specific test Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each test lot using secondary standards calibrated to the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.[7] Together, the secondary standards and the lot-specific $E_{\Delta Ct}$ and *SF* values, align the quantitative output of the test to the *IS*. The $E_{\Delta Ct}$ is set for 1.92 and *SF* value is set for 1.22 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 1.92; *SF* = 1.22

Test's ABL Ct = 11.3; BCR-ABL Ct = 18.0 ; ΔCt = -6.7

%(*IS*)= 1.92 $^{(-6.7)}$ x 100 x 1.22 = 1.54% (*IS*)

MRx.xx=log_{10}[100/Determined % (*IS*)]=log_{10}(100)−log_{10}(1.54)=2−log_{10}(1.54)=MR1.81

Result: **POSITIVE [1.54% (*IS*) and MR1.81]**.

POSITIVE [Above upper LoQ]

BCR-ABL has been detected at a level of >55% (*IS*) and <MR0.26.

For a **POSITIVE [Above upper LoQ]** result, BCR-ABL is detectable with a BCR-ABL Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and an ABL Ct greater than or equal to "8" and less than or equal to "18". The GeneXpert software calculates the % (*IS*) using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL Ct:

% (*IS*) = E $_{\Delta Ct}^{(\Delta Ct)}$ x 100 x Scaling Factor (*SF*)

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the test cartridge barcode. The value of this factor and the lot-specific test Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each test lot using secondary standards calibrated to the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.[7] Together, the secondary standards and the lot-specific $E_{\Delta Ct}$ and *SF* values, align the quantitative output of the test to the *IS*. The $E_{\Delta Ct}$ is set for 1.92 and *SF* value is set for 1.10 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 1.92; *SF* = 1.10

Test's ABL Ct = 13.4; BCR-ABL Ct = 14.2 ; ΔCt = -0.8

%(*IS*)= 1.92 $^{(-0.8)}$ x 100 x 1.10 = 65% is greater than the defined test upper LoQ at 55% (*IS*)

MRx.xx=log_{10}[100/Determined % (*IS*)]=log_{10}(100)−log_{10}(65)=2−log_{10}(65)=MR0.19 is less than the defined test upper LoQ at MR0.26.

Result: **POSITIVE [Above upper LoQ]**.

POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)]

BCR-ABL has been detected at a level of <0.0030% (*IS*) and >MR4.52.

For a **POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)]** result, BCR-ABL is detectable with a BCR-ABL Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and an ABL Ct greater than or equal to "8" and less than or equal to "18". The GeneXpert software calculates the % (*IS*) using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL Ct

% (*IS*) = E $_{\Delta Ct}^{(\Delta Ct)}$ x 100 x Scaling Factor (*SF*)

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the test cartridge barcode. The value of this factor and the lot-specific test Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each test lot using secondary standards calibrated to the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.[7] Together, the secondary standards and the lot-specific $E_{\Delta Ct}$ and *SF* values, align the quantitative output of the test to the *IS*. The $E_{\Delta Ct}$ is set for 1.91 and *SF* value is set for 1.14 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 1.91; *SF* = 1.14

Test's ABL Ct = 12.5; BCR-ABL Ct = 29 ; ΔCt = -16.6

%(*IS*)= $1.91^{(-16.6)}$ x 100 x 1.14 = 0.0025% is less than the defined test LoD at 0.0030% (*IS*)

MRx.xx=log_{10}[100/Determined % (*IS*)]=log_{10}(100)−log_{10}(0.0025)=2−log_{10}(0.0025)=MR4.60 is greater than the defined test LoD at MR4.52.

Result: **POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)]**.

NEGATIVE [Sufficient ABL transcript]

BCR-ABL was not detected with BCR-ABL Ct equal to "0" or greater than the cut-off of "32" and ABL Ct greater than "8" and less than or equal to "18".

When BCR-ABL is undetectable with BCR-ABL Ct equal to "0" or greater than the cut-off of "32", the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is greater than or equal to "8" and less than or equal to "18" to ensure having "Sufficient ABL transcript". See Table 1.

Example:

Test's BCR-ABL Ct = 0; ABL Ct = 11.3 is less than "18".

Result: **NEGATIVE [Sufficient ABL transcript]**.

INVALID [Insufficient ABL transcript]

BCR-ABL was detected or not detected with ABL Ct greater than "18".

When BCR-ABL is either detected or not detected, the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is less than or equal to "18" to ensure having "Sufficient ABL transcript". Refer to Troubleshooting Guide.

Example:

Test's BCR-ABL Ct = 0 ; ABL Ct = 24 is greater than "18".

Result: **INVALID [Insufficient ABL transcript]**.

Retest Procedure for ERROR or INVALID (Type 1)

Retest samples with **ERROR** or **INVALID** results due to the ABL cycle threshold (Ct) exceeding the maximum valid Ct cut-off (Ct >18) or the endpoint is below the threshold setting (<200). Also refer to Troubleshooting Guide.

1. If *sufficient* blood specimen volume is available, re-test from original blood specimen collection tube following the procedure in Preparing the Sample.
   -OR-

   If blood specimen volume is *insufficient*, re-test can be performed with the retained lysate from Preparing the Sample, step 12.

   a. If retained lysate from Preparing the Sample, step 12 is stored frozen, thaw to room temperature before use.

   b. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle. Go to step 2.

2. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.
3. To the new conical tube containing lysate, add 1.5 mL of Lysis Reagent (LY).
4. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
5. Incubate at room temperature for 10 minutes.
6. To the same conical tube, add 2 mL of reagent grade absolute ethanol (not provided).
7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
9. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
10. Close cartridge lid. Initiate test (see Starting the Test: GeneXpert System with Touchscreen).

Retest Procedure for ERROR (Code 2008) or INVALID (Type 2)

Retest samples with BCR-ABL and/or ABL transcript levels below the valid minimum Ct cut-off (Ct<8) and/or when pressure limit is exceeded. Also refer to Troubleshooting Guide.

1. To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).
2. If *sufficient* blood specimen volume is available, re-test from original blood specimen collection tube. Ensure blood specimen is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. Go to step 4.
   -OR-

   If blood specimen volume is *insufficient*, re-test can be performed from the retained lysate from Preparing the Sample, step 12.

   a. If retained lysate from Preparing the Sample, step 12 is stored frozen, thaw to room temperature before use. If refrigerated lysate is used, allow to come to equilibrate to room temperature before use.

   b. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle. Go to step 4.

3. To the tube already containing Proteinase K, add 50 µL of blood specimen, if available, or 80 µL of left-over lysate from Preparing the Sample.

   a. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.

   b. Incubate at room temperature for 1 minute.

4. To the new conical tube containing lysate, add 2.5 mL of Lysis Reagent (LY).
5. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
6. Incubate at room temperature for 10 minutes.
7. To the same conical tube, add 2 mL of reagent grade absolute ethanol (not provided).
8. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
9. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.

10. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.

11. Close cartridge lid. Initiate test (see Starting the Test: GeneXpert System with Touchscreen).

Limitations

Limitations of the Test

- The product is intended for *in vitro* diagnostic use only.
- The test is not intended to be used with external calibrators.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- The precision of the test is not demonstrated or assured below MR4.5.
- The test is not indicated for determining discontinuation from TKI treatment nor for monitoring after discontinuation.
- The performance of the Xpert BCR-ABL Ultra test was evaluated using the procedures provided in these instructions for use only. Modifications to these procedures may alter the performance of the test.
- This product has been validated for blood collected in EDTA tubes.
- Do not use heparin as the anticoagulant because it can inhibit the PCR reaction.
- Sodium citrate (NaCitrate), buffy-coat and bone marrow sample types have not been validated.
- Erroneous test results might occur from improper specimen collection, handling or storage or sample mix-up. Careful compliance to the instructions in these instructions for use is necessary to avoid erroneous results.
- The Xpert BCR-ABL Ultra test is only designed to detect, but not distinguish between the p210 BCR-ABL fusion transcripts e13a2/b2a2 and e14a2/b3a2. The ability to detect other fusion transcripts has not been evaluated beyond those described in these instructions for use. The test does not detect minor or micro breakpoints, microdeletions, or mutations.
- The Xpert BCR-ABL Ultra is not intended to detect the e1a2 (p190), e19a2 (p230) or other minor translocations that may be present in a peripheral blood sample from a patient with leukemia.
- The Xpert BCR-ABL Ultra will not detect aberrant e13a2/b2a2 fusion transcripts in which parts of the sequence adjacent to the breakpoint are deleted.
- For some specimens with very high white blood cell counts (higher than 30 million cells/mL), Xpert BCR-ABL Ultra may report **INVALID** (Type 2) results due to excess BCR-ABL or ABL levels in the sample. See for additional information.
- Some specimens with very low levels of ABL transcript or with white blood cells lower than 150,000 cells/mL may be reported as **INVALID** (Type 1). A non-determinate result does not preclude the presence of very low levels of leukemic cells in the patient.
- CML p230 transcript with e19a2 micro breakpoint may report a BCR-ABL positive result below the test LoD (0.0030% (*IS*)/MR4.52) when tested at high target levels (>3.52 logs above LoD).
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.
- Some patients with very low levels of BCR-ABL1 transcript (i.e., below LoD 0.0030% (*IS*) or higher than MR4.52) may be reported as **NEGATIVE [Sufficient ABL transcript]**. Hence, an undetected result does not preclude the presence of low levels of leukemic cells in the patient.
- The test is validated for use on the GeneXpert system with touchscreen.

Expected Values

The Xpert BCR-ABL Ultra range covers key clinical decision points for monitoring of CML (spanning MR 1 to 4.5)[5] with the quantitative detection of BCR-ABL mRNA (transcripts e13a2/b2a2 or e14a2/b3a2) and the ABL endogenous control mRNA. Expected values are within the Xpert BCR-ABL Ultra range of 0.0030 to 55% (*IS*) (MR4.52 to MR0.26).

Troubleshooting Guide

Table 4. Troubleshooting Guide

Test Result	Possible Causes	Suggestions
INVALID	Type 1: Endogenous control ABL failure: • Poor sample quality • RT-PCR inhibition • If ABL Ct > 18, and/or endpoint <200	• Check the specimen quality (e.g., exceeded specimen storage requirement including time and temperature). • Repeat the test with original specimen (if available) or from retained lysate and a new cartridge following the procedure as described in Retest Procedure for ERROR or INVALID (Type 1).
	Type 2: BCR-ABL transcript level cannot be determined due to sample containing excess BCR-ABL and/or ABL transcripts (Ct < 8)	Repeat the test with original specimen (if available) or from retained lysate and a new cartridge following the procedure as described in Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 2008)	Pressure exceeding limit (error message 2008)	• Check the specimen quality. • Check for grossly elevated WBC count. • Repeat the test with original specimen (if available) or from retained lysate and a new cartridge following the procedure as described in Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 5006, 5007, 5008, and 5009[a])	Probe check failure	Repeat the test with original specimen (if available) or from retained lysate and with a new cartridge following the procedure as described in Retest Procedure for ERROR or INVALID (Type 1).
NO RESULT	Data collection failure. For example, the operator stopped a test that was in progress or a power failure occurred.	Repeat the test with original specimen (if available) or from retained lysate and with a new cartridge following the procedure as described in Retest Procedure for ERROR or INVALID (Type 1).

a. This is not an exhaustive list of ERROR codes.

Specific Performance Characteristics

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 test which detects and quantifies the mRNA transcripts for the p210 translocation types (e13a2/b2a2 or e14a2/b3a2) and uses 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 tests [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 Test]: 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 Test was evaluated using a Deming regression to determine the slope and intercept. Figure 2 shows the Deming regression and linear regression analysis of the 147 test results (MR values).

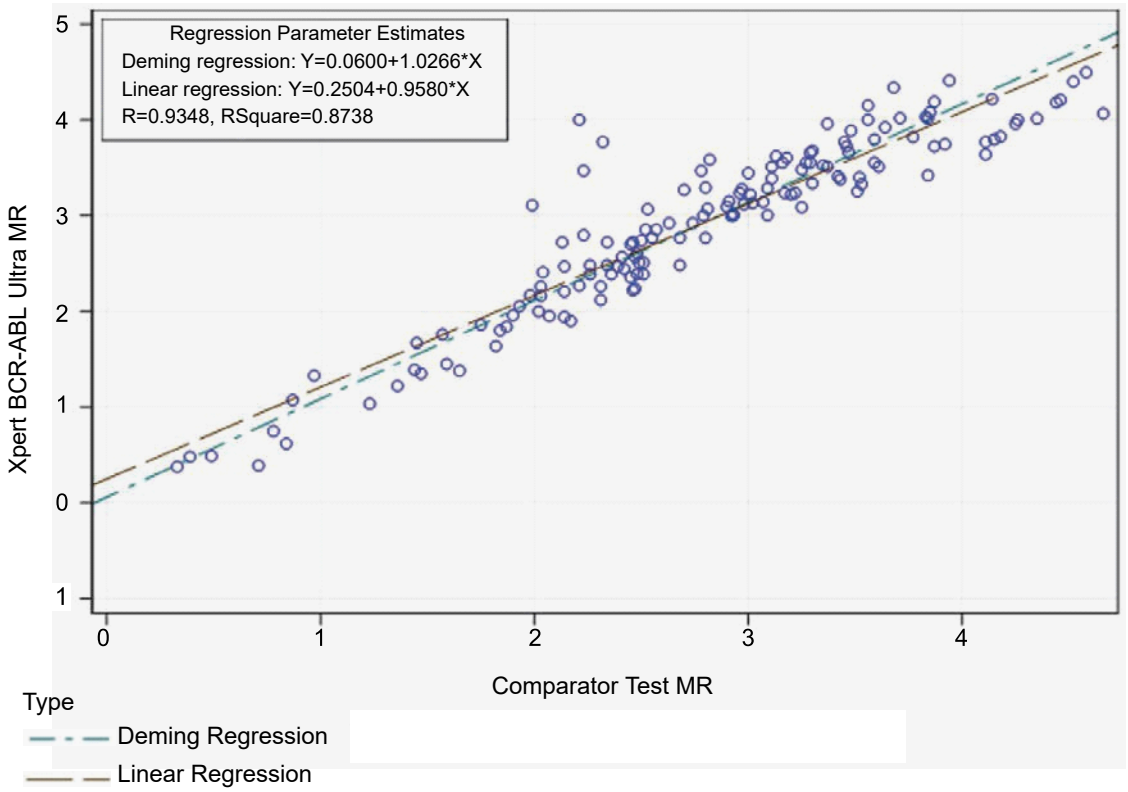


Figure 2 Deming and Linear Regression Analyses

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 Test. The Bland-Altman graph (see Figure 3) 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.

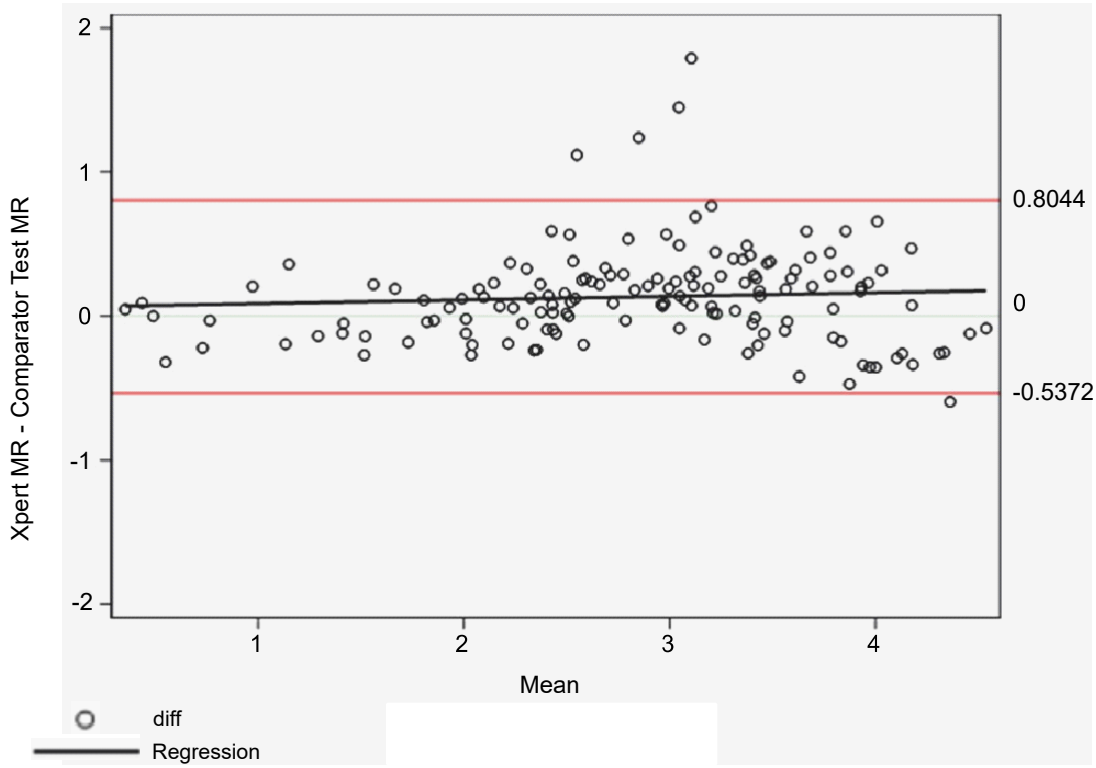


Figure 3 Xpert BCR-ABL Ultra Test MR vs Comparator Test BCR-ABL MR Bland-Altman Difference Analysis

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).

Analytical Performance

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.[19] Each of the 4 Reference Panel members was tested with a minimum of 10 replicates per test 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 test'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).

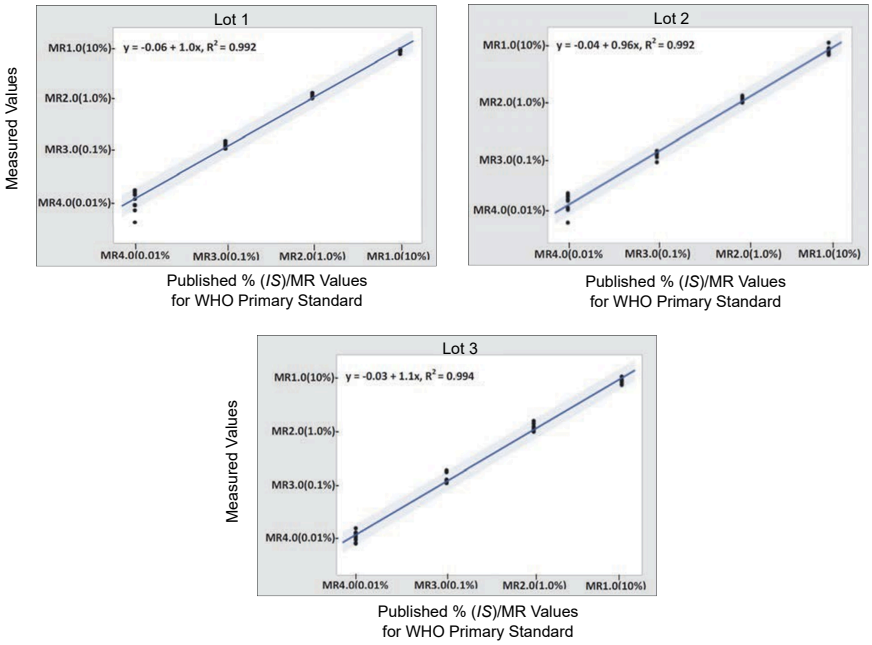


Figure 4 Measured vs Published Values for WHO Primary Reference Panel, Lot-to-Lot.

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.

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 test 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 5 and Figure 6 below.

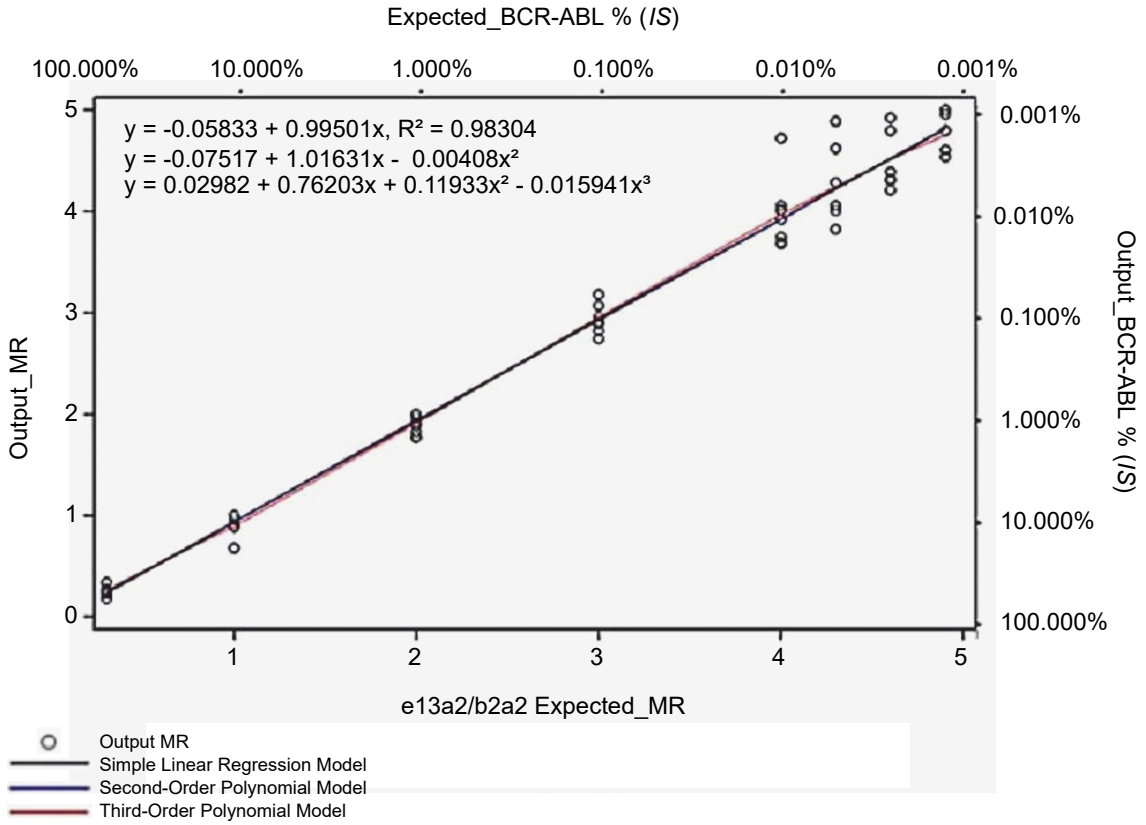


Figure 5 Linear Regression Curves for Breakpoint Transcript e13a2/b2a2

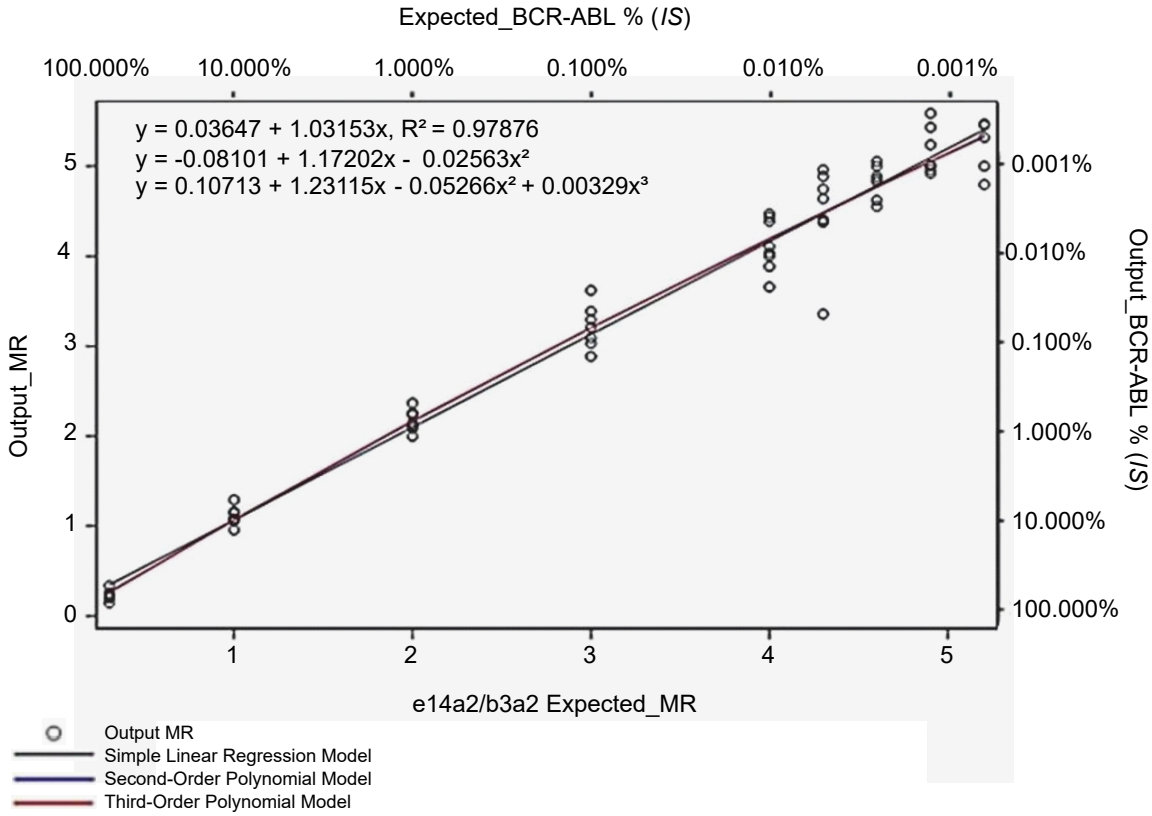


Figure 6 Linear Regression Curves for Breakpoint Transcript e14a2/b3a2

The estimated regression intercepts, slopes and R^2 values from the linear model are shown in Table 5.

Table 5. 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 LOQ at 0.0030% (*IS*)/ MR4.52.

Analytical Sensitivity (Limit of Detection, Limit of Quantitation, Limit of Blank)

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 6). 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 6. 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 test 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*)/MR 4.52. 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 test 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.

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]**.

Potentially Interfering Substances

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 (see Table 7) 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 7. Potentially Interfering Substances Tested Using 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)

Precision and Reproducibility

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 at 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 8 shows the eleven samples included in this study.

Table 8. 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 Ultra 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 9.

Table 9. 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 MR>4.0 e13a2/ b2a2	140[c]	4.36	0.04	0.04	0	0.33	0.33
Target 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 Dx and GeneXpert Infinity 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 onboard 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.

Appendix

Bibliography

1. Jemal A, Siegel R, Ward E, et al. Cancer Statistics 2008; CA Cancer J Clin. 2008;58:71-96.
2. NIH/NCI – Surveillance, Epidemiology, and End Results Program (SEER). Cancer Stat Facts: Chronic Myeloid Leukemia (CML). Accessed December 21, 2018. https://seer.cancer.gov/statfacts/html/cmyl.html
3. Thompson PA, Kantarjian HM, Cortes JE. Diagnosis and Treatment of Chronic Myeloid Leukemia in 2015. Mayo Clin Proc. 2015;90(10):1440-1454.
4. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005;105(7):2640-2653.
5. Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28-37.
6. NCCN. Clinical Practice Guidelines in Oncology; Chronic Myelogenous Leukemia (Access Version 1, 2019).
7. White H, Matejtschuk P, Rigsby P, et al. Establishment of the first World Organization International Generic Reference Panel for quantitation of BCR-ABL mRNA.Blood. 2010; 116:e111-e117.
8. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer Program. Leukemia. 2003;17:2318-2357.
9. Beillard E, Pallisgaard N, van der Velden VH, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) - a Europe Against Cancer Program. Leukemia. 2003;17:2474-2486.
10. van der Velden VH, Boeckx N, Gonzalez M, et al. Differential stability of control gene and fusion gene transcripts over time may hamper accurate quantification of minimal residual disease—a study within the Europe Against Cancer Program. Leukemia. 2004;18:884-886.
11. van der Velden VH, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17:1013-1034.
12. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
13. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).

14. World Health Organization. Safe management of wastes from health-care activities. Bulletin of the World Health Organization (refer to latest edition).

15. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).

16. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

17. Baccarani M. et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia. Blood. 2013 Jun;122(6):872-884.

18. Hochhaus A. et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow up. Annals of Oncology. 2017 May; 28(4):iv41-iv51.

19. WHO International Standard 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation NIBSC code: 09/138. Instructions for use. (Version 4.0., Dated 13/12/2012).

Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

Technical Assistance

Before Contacting Us

Collect the following information before contacting Cepheid Technical Support:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

United States

Telephone: + 1 888 838 3222
Email:
techsupport@cepheid.com

France

Telephone:+ 33 563 825 319:
Email:
support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website:www.cepheid.com/en/support/contact-us

Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
LOT	Batch code
	Do not reuse
	Expiration date
	Warning
	Consult instructions for use
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Temperature limitation
	Biological risks
	Flammable liquids
	Reproductive and organ toxicity
Rx only	For prescription use only

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

IVD

Revision History

Description of Changes: 303-0946 Rev. A to B

Section	Description of Change
Principle of the Procedure	Corrected errors.
Material Provided	Corrected catalog number.
Specimen Collection, Transport and Storage	Corrected errors.
Preparing the Sample	Corrected errors.
Limitations of the Test	Added federal law restriction. Corrected errors.
Clinical Performance	Corrected error.