GeneXpert
Powered By CEPHEID INNOVATION

Xpert® BCR-ABL Ultra p190

REF **GXBCRABLP190-CE-10**

Instructions for Use

IVD

Cepheid

In Vitro Diagnostic Medical Device

302-6673, Rev. C
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Trademark, Patents and Copyright Statements

See Section 26, Revision History for a description of changes.

Xpert® BCR-ABL Ultra p190

For *in vitro* diagnostic use.

1 Proprietary Name

Xpert® BCR-ABL Ultra p190

2 Common or Usual Name

Xpert BCR-ABL Ultra p190

3 Intended Purpose

3.1 Intended Use

The Xpert® BCR-ABL Ultra p190 test is an *in vitro* diagnostic test for use on the Cepheid GeneXpert® Dx System for the quantitation of the BCR-ABL1 p190 and ABL1 mRNA transcripts in peripheral blood specimens of diagnosed Philadelphia positive (Ph+) [t(9;22)(q34;q11)] chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) patients expressing BCR-ABL1 fusion transcript type e1a2. The test utilizes automated, quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR) and is intended to measure the percent ratio of BCR-ABL1 p190 mRNA versus ABL1 mRNA in t(9;22) positive CML or ALL patients during monitoring of treatment.

The test does not monitor other fusion transcripts resulting from t(9;22) and is not intended for the diagnosis of CML or ALL.

3.2 Intended User/Environment

The Xpert BCR-ABL Ultra p190 test is intended for use by trained users in a laboratory setting.

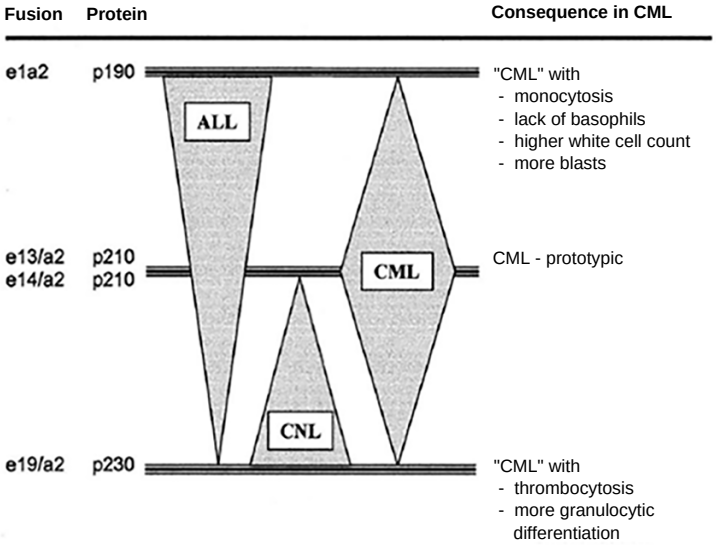
4 Summary and Explanation

Philadelphia chromosome (Ph) is a shortened chromosome that results from the translocation of the 3’ part of the ABL gene on chromosome 9 to the 5’ part of the BCR gene on chromosome 22. The breakpoint on the ABL gene is fairly constant occurring at the 5’ end of exon a2 whereas the breakpoints of the BCR gene is variable but is mainly clustered in 3 different regions (breakpoint cluster regions or bcr). Depending on the breakpoint on chromosome 22, different size segments are joined with the 3’ sequences of the ABL gene. There are major (M-bcr), minor (m-bcr) and micro-breakpoints each of which result in different size mRNA fusion transcripts.[1]

The Ph chromosome is observed in greater than 95% of patients with chronic myeloid leukemia (CML) and up to 20-30% of adults with acute lymphoblastic leukemia (ALL), 5% of children with ALL and in 1-2% of patients with acute myeloid leukemia (AML).[1]

In CML the BCR-ABL p210 is present in greater than 95% of patients and is also found in approximately 30% of Ph-positive (Ph+) ALL patients. In the remaining patients with Ph+ ALL and in rare cases of CML (1-3%), the BCR-ABL p190 is present. In CML, the BCR-ABL p210 and p190 can co-exist. Both the p210 and p190 fusion proteins demonstrate increased tyrosine phosphokinase activity compared to the normal p145 c-abl protein.[1,2]

In Ph+ ALL patients, the p190 form is detected in approximately 80% of Ph+ childhood ALL and 20-40% of Ph+ adult ALL.[1] In addition, the frequency of the Ph chromosome increases with age, being present in 10% in ages 15-30, 25% in ages 40-49 and 20-40% in ALL patients older than 50 years of age.[3-5]



Acute lymphoblastic leukemia (ALL) is a hematologic malignancy in which there is an accumulation of immature poorly differentiated white blood cells (WBC); lymphoblasts, in bone marrow, blood and other tissues. ALL is classified as a rare cancer (orphan disease number ORPHA:513; GARD 522) with a prevalence of 1.7/100,000. In the United States ALL is the most common cancer in children from birth to 15 years of age accounting for 75% of all cases childhood leukemia.[6, 7]

The presence of the Ph chromosome in ALL patients after consolidation is a significant predictor of relapse and monitoring is recommended. However, there are currently no established guidelines defining the monitoring frequency of ALL patients using measurements of BCR-ABL p190 transcript for detection of minimal residual disease (MRD). The NCCN guidelines have definitive timepoints for monitoring BCR-ABL p210 in CML patients, so measuring BCR-ABL p190 to monitor ALL is done in similar frequencies.[5]

Chronic myeloid leukemia (CML) is characterized by the presence of the Ph chromosome with >95% of cases being associated with BCR-ABL p210 and only 1-3% of cases associated with BCR-ABL p190.[2,3]

Unlike the BCR-ABL World Health Organization international standard (WHO *IS*) for the p210 transcript, currently there is no internationally recognized reference which can be used to standardize the p190 fusion transcript. Therefore, current molecular assays for p190 typically detect the fusion transcript and report it as a percent relative to the expression of an internal control gene (e.g. ABL).

5 Principle of the Procedure

The Xpert BCR-ABL Ultra p190 is an automated test for quantifying the amount of BCR-ABL1 p190 transcript as a ratio of BCR-ABL p190/ABL1. The test is performed on Cepheid GeneXpert Dx System, which automates and integrates specimen purification, nucleic acid amplification, and target sequence detection in simple or complex specimens using real-time RT-PCR and nested PCR tests. The system consists of an instrument, computer, and pre-loaded software for running tests and viewing the results. The system requires the use of single-use, disposable GeneXpert cartridges that hold the RT-PCR and nested PCR reagents and host the RT-PCR and nested PCR processes. For a full description of the system, refer to the appropriate *GeneXpert Dx System Operator Manual*.

The Xpert BCR-ABL Ultra p190 cartridge includes reagents to detect BCR-ABL1 p190 fusion genes resulting from a minor breakpoint, translocation e1a2, and the ABL1 transcript as an endogenous control in peripheral blood specimens. The amount of BCR-ABL1 p190 transcript is quantified as the percent ratio of BCR-ABL1 p190/ABL1. There are two controls included in the Xpert BCR-ABL Ultra p190 test – the Endogenous Control (ABL1) and a Probe Check Control (PCC). The ABL1 endogenous control normalizes the BCR-ABL1 p190 target and ensures that sufficient specimen 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.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert BCR-ABL Ultra p190 kit (GXBCRABLP190-CE-10) contains sufficient reagents to process 10 test specimens or quality control specimens. The kit contains the following:

Xpert BCR-ABL Ultra Reagents **10 of each per kit**

Proteinase K (PK)	**10 x 130 µL per vial**
Component	**Reagent Ingredient**
Proteinase K	< 5%

Lysis Reagent (LY) (Guanidinium Chloride)	**10 x 5.3 mL per vial**
Component	**Reagent Ingredient**
Guanidinium chloride	25 - 50%
Urea	25 - 50%
Sodium dodecyl sulphate	< 2%

Wash Reagent	**10 x 2.9 mL per ampoule**
Component	**Reagent Ingredient**
Ethanol	< 50%
Guanidinium thiocyanate	< 50%

Xpert BCR-ABL Ultra p190 Cartridges with Integrated Reaction Tubes		**10 per kit**
Component	**Reagent Ingredient**	**Amount**
Bead 1 (freeze-dried)	Enzyme: Taq DNA polymerase < 50U/bead	1 per cartridge
	dNTPs < 0.05%	
Bead 2 (freeze-dried)	Primers and probes < 0.005%	1 per cartridge
Bead 3 (freeze-dried)	Primers and probes < 0.005%	1 per cartridge
Bead 4 (freeze-dried)	Enzyme: Taq DNA polymerase < 50U/bead	1 per cartridge
	dNTPs < 0.05%	
Rinse Reagent	Potassium chloride < 4%	2 mL per cartridge
	Sodium azide < 0.1%	
	Polyethylene glycol < 15%	
	Tween 20 < 0.2%	
Elution Reagent	Trizma base < 0.3%	2.5 mL per cartridge
	Trizma hydrochloride < 0.1%	
	Sodium azide < 0.05%	

CD	**1 per kit**

- Assay Definition File (ADF)
- Instruction to import ADF into GeneXpert Dx software
- Instructions for Use (Package Insert)

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.

Note Certificates of Analysis and Lot Specifications Data Sheets are available through Cepheid Technical Support.

6.2 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and Operator Manual.
- For GeneXpert Dx System: GeneXpert Dx software version 6.2 or higher
- 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

7 Storage and Handling

- Store the Xpert BCR-ABL Ultra p190 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 specimen preparation reagents from storage to allow them to come to room temperature (20 – 30 °C).

8 Warnings and Precautions

8.1 General

- For *in vitro* diagnostic use.
- 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[9] and Clinical and Laboratory Standards Institute.[10]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- 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 (LoD) of the test. Careful compliance with the Package Insert instructions and the *GeneXpert Dx System Operator Manual* is necessary to avoid erroneous results.
- Performing the Xpert BCR-ABL Ultra p190 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.[11]

8.2 Specimen

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 10). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Do not freeze whole blood specimens.
- Proper specimen collection, storage, and transport are essential for correct results.

8.3 Test/Reagent

- Do not substitute Xpert BCR-ABL Ultra p190 reagents with other reagents.
- Do not open the Xpert BCR-ABL Ultra p190 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.
- Xpert BCR-ABL Ultra p190 cartridges should be at room temperature (20 °C – 30 °C) when used for testing.
- Each single-use Xpert BCR-ABL Ultra p190 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 p190 cartridge if a reagent is added to the wrong opening. Do not open Xpert BCR-ABL Ultra p190 cartridges after the test is completed.
- Dedicate a set of pipettes and reagents exclusively to specimen preparation.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a specimen or control spill, wear gloves and absorb the spill with paper towels. Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Final active chlorine concentration should be 0.5%. After the work area is dry, follow by wiping the surface with 70% ethanol. For equipment, follow the manufacturer's recommendations for decontamination of equipment. Alternately, follow your institution's standard procedures for a contamination or spill event.
- Used cartridges may contain potentially infectious materials, as well as highly amplified PCR target(s). Do not open or attempt to alter any part of the cartridge for disposal.

9 Chemical Hazards[12,13]

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

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

- UN GHS Hazard Pictogram: 
- Signal Word: DANGER
- **UN GHS Hazard Statements**
  - Harmful if swallowed H302
  - Highly flammable liquid and vapor H225
  - Causes skin irritation H315
  - Causes serious eye irritation H319
  - May cause drowsiness or dizziness H336
  - Suspected of causing genetic defects H341
- **UN GHS Precautionary Statements**
  - **Prevention**

- Refer to Safety Data Sheet for special instructions before use.
- Do not handle until all safety precautions have been read and understood.
- Use personal protective equipment: gloves, eyewear, face shield and clothing.
- Use only in well-ventilated areas.
- Keep away from heat, sparks, open flames and/or hot surfaces.
- Avoid breathing mist, vapors, or spray.
- Wash hands thoroughly after handling.

- **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 victim feels unwell.
  - If SPILLED: Immediately remove contaminated clothing. If on skin or hair, rinse with water/shower.
  - If SKIN IRRITATION occurs: Get medical advice/attention.
  - If IN EYES: Remove contact lenses, if present. Rinse eyes thoroughly with water for several minutes. If eye irritation persists: Get medical advice/attention.
  - Specific treatment: see supplemental first aid measures in Safety Data Sheet.
  - If exposed or concerned: Get medical advice/attention.
- **Storage/Disposal**
  - Store under refrigerated conditions.
  - Keep containers tightly closed.
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

10 Specimen Collection, Transport and Storage

- The test requires whole blood specimens collected in EDTA vacuum tubes. Specimens may be held for up to 72 hours at 2-8 °C prior to use. Plasma should not be separated from cells.
- Proper specimen collection, storage, and transport are critical to the test function.

11 Procedure

11.1 Before You Start

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

Important **Remove the cartridge from the cardboard packaging before preparing the specimen. (See Section 11.2, Preparing the Specimen.)**

Important **Start the test on the GeneXpert Dx instrument within 1 hour of adding the prepared specimen to the cartridge.**

11.2 Preparing the Specimen

11.2.1 Preparing a Specimen with Unknown White Blood Cell (WBC) Count or Specimens with Less than 30 Million WBC/mL

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 specimen 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 specimen with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the specimen with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. Mix the specimen 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 used for retest. Store remaining lysate at 2–8 ºC for up to 4 hours or stored at -20 ºC or lower for up to 24 weeks.

13. To the new conical tube containing lysate, add 1.5 mL of retained Lysis Reagent (LY) from Step 6.
14. Mix the specimen 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 specimen with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
18. Discard any remaining PK or LY reagents.

11.2.2 Preparing a Specimen with WBC Count Greater than 30 Million cells/mL

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 50 µL of blood specimen.
4. Mix the specimen 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).
7. Mix the specimen with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the specimen with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
12. Mix the specimen with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
13. Discard any remaining PK or LY reagents.

11.3 Preparing the Cartridge

To add the specimen to the Xpert BCR-ABL Ultra p190 cartridge:

1. Remove the cartridge from the cardboard packaging.
2. Inspect the cartridge for damage. Do not use if damaged.
3. Lift the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent Chamber (small opening). See Figure 1.
4. Pipette the entire contents of the prepared specimen into the Sample Chamber (large opening). See Figure 1.

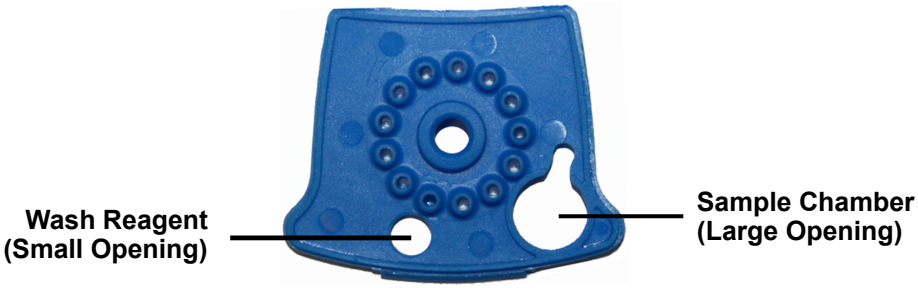


Figure 1. Xpert BCR-ABL Ultra p190 Cartridge (Top View)

5. Close the cartridge lid. Ensure the lid snaps firmly into place. Initiate test (see Section 11.4, Starting the Test).

11.4 Starting the Test

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

Important **Before initiating a test, ensure that the instrument is running GeneXpert Dx software version 6.2 or higher and that the correct Assay Definition File (ADF) is imported into the software.**

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert instrument:

   If using the GeneXpert Dx instrument, first turn on the GeneXpert Dx instrument, and then turn on the computer. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your username and password.
3. In the **GeneXpert System** window, click **Create Test** (GeneXpert Dx). The **Create Test** window opens. The **Scan Patient ID barcode** dialog box opens.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box opens.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box opens.
6. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the Assay Definition File (ADF) is not available, a screen will appear indicating the Assay Definition File (ADF) is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that appears, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door. Then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

12 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

13 Quality Control

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

ABL Endogenous Control — The ABL Endogenous Control verifies that sufficient specimen is used with the test. Additionally, this control detects specimen-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 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.

14 Interpretation of Results

Xpert BCR-ABL Ultra p190 quantitative outputs are provided as a percent ratio of BCR-ABL1 p190/ABL1. Examples of possible results and interpretations are presented in Table 1.

Table 1. Xpert BCR-ABL Ultra p190 Possible Results and Interpretation

Probe Check*	ABL Ct*	e1a2 Ct*	Xpert BCR-ABL Ultra p190 Test Result	Notes
PASS	PASS	POS	**BCR-ABL p190 DETECTED [#.##%]**	Calculated% ratio value is reported. See Figure 2.
			BCR-ABL p190 DETECTED [Below LoD;<0.0065%]	Calculated% ratio is below the limit of detection and is not reported. See Figure 3.
			BCR-ABL p190 DETECTED [Above upper LoQ]	Calculated% value is above the limit of quantitation and is not reported. See Figure 4.
		NEG	**BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]**	e1a2 Ct is zero or above the acceptance threshold. See Figure 5.
		INVALID	**INVALID [Too high BCR-ABL p190 transcript]**	e1a2 Ct is below the acceptance threshold.
	FAIL	POS, NEG, or INVALID	**INVALID [No ABL transcript]**	ABL Ct value is zero. No ABL detected. See Figure 6.
			INVALID [Insufficient ABL transcript]	ABL Ct is above the acceptance threshold. See Figure 7.
			INVALID [Too high ABL transcript]	ABL Ct is below the acceptance threshold.
		INVALID	**INVALID [Too high BCR-ABL p190 and ABL transcripts]**	Both e1a2 and ABL Ct values are below the acceptance thresholds. See Figure 8.
FAIL	PASS or FAIL	POS, NEG or INVALID	**ERROR**	Probe Check Control did not meet acceptance criteria. See Figure 9.
* See the Analyte Results tab in the GeneXpert Dx System Software for details				

Note GeneXpert systems calculate results automatically based upon *cycle threshold* (Ct) values generated by the test, and lot-specific parameters assigned during manufacturing. The software applies the following algorithm, wherein the ΔCt (Delta Ct) value is obtained from ABL Ct minus BCR-ABL p190 Ct, and Efficiency (*E*) and Scaling Factor (*SF*) are lot specific values:

$$\text{Percent ratio} = \text{Efficiency}^{(\Delta Ct)} \times \text{Scaling Factor} \times 100$$

Note Efficiency and Scaling Factor values calibrate the quantitation of BCR-ABL1 p190 (e1a2) and ABL1 transcripts to copy numbers of synthetic BCR-ABL p190 and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) primary standards. Efficiency and Scaling Factor values are embedded within each cartridge barcode. Lot Specifications Data Sheets are available through Cepheid Technical Support.

14.1 BCR-ABL p190 DETECTED [#.##]%

For a "**BCR-ABL p190 DETECTED [#.##%]**" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18".

Example: ABL Ct = 11.4; BCR-ABL p190 Ct = 15.6 ; ΔCt = -4.2
Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
% ratio = $2.05^{(-4.2)}$ x 100 x 1.76 = 8.63%

Result: **BCR-ABL p190 DETECTED [8.63%]**. See Figure 2.

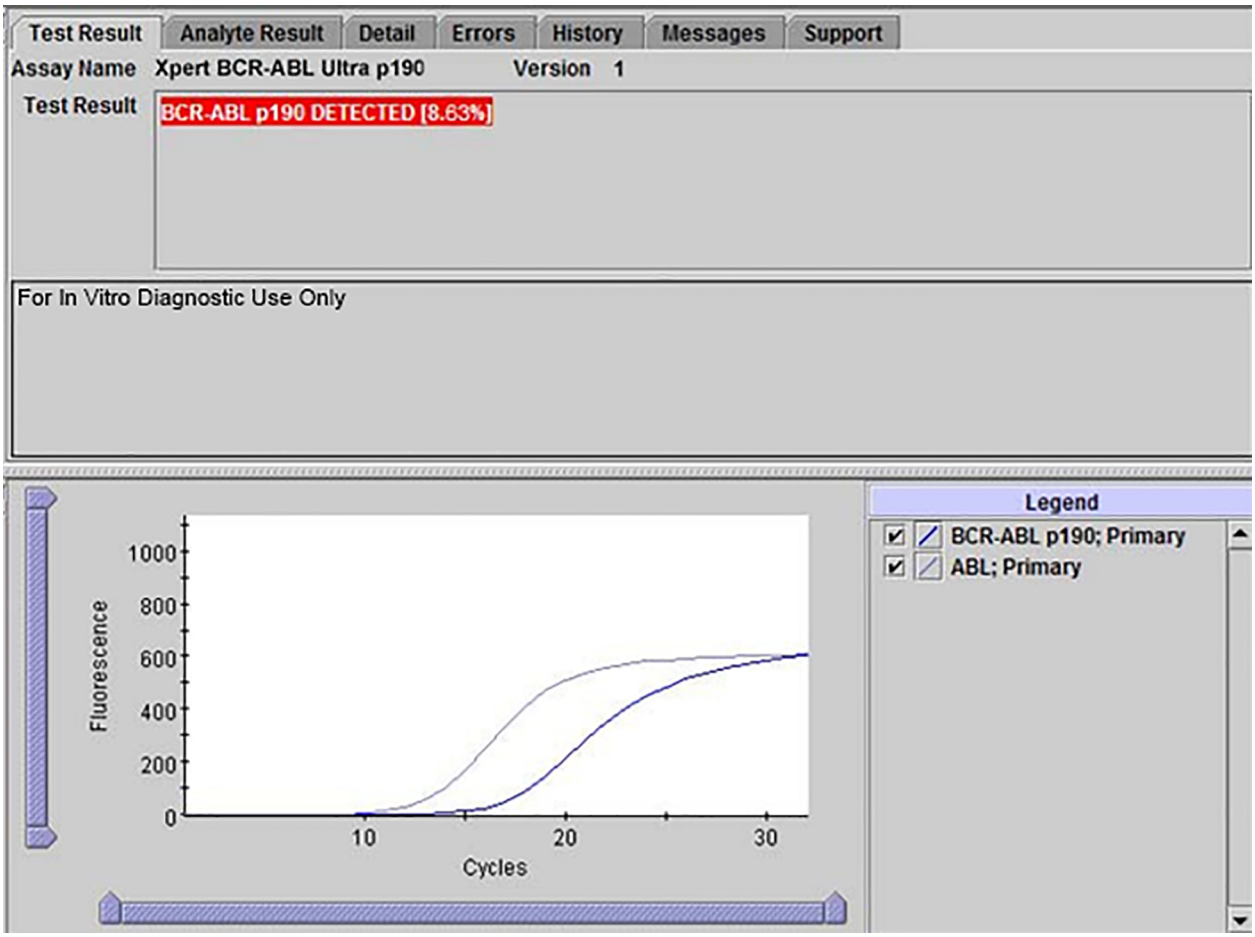


Figure 2. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [8.63%]

14.2 BCR-ABL p190 DETECTED [Below LoD; <0.0065%]

BCR-ABL p190 has been detected at a level < 0.0065%.

For a "**BCR-ABL p190 DETECTED [Below LoD; <0.0065%]**" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18".

Example: ABL Ct = 10.1; BCR-ABL p190 Ct = 24.8; ΔCt = -14.8
Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
% ratio = $2.05^{(-14.8)}$ x 100 x 1.76 = 0.0044% is less than the defined test LoD at 0.0065%

Result: **BCR-ABL p190 DETECTED [Below LoD; <0.0065%]**. See Figure 3.

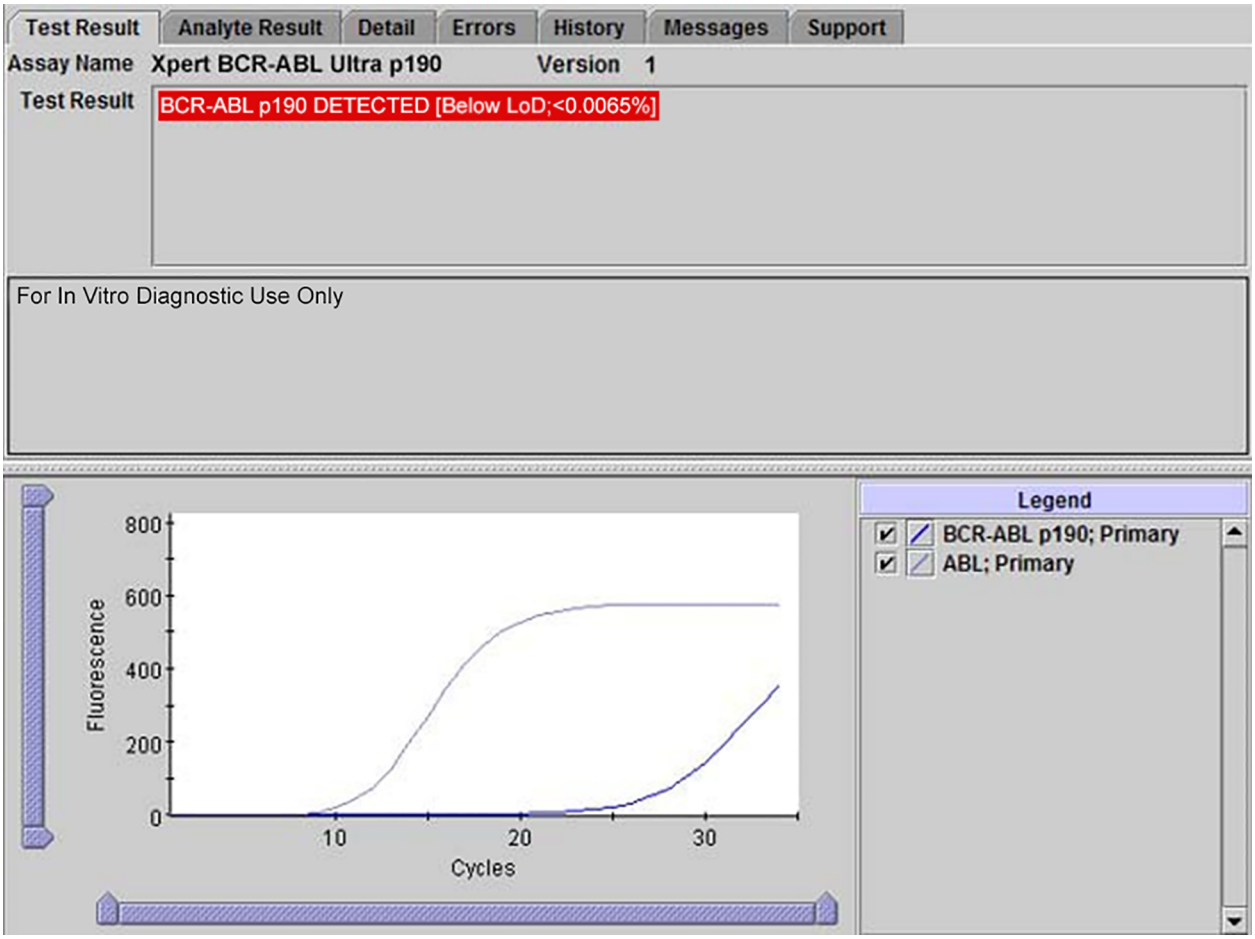


Figure 3. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [Below LoD; <0.0065%]

14.3 BCR-ABL p190 DETECTED [Above upper LoQ]

BCR-ABL p190 has been detected at a level > 25%.

For a "**BCR-ABL p190 DETECTED [Above upper LoQ]**" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18".

Example: ABL Ct = 17.2; BCR-ABL p190 Ct = 18.7; ΔCt = -1.6
Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
% ratio = $2.05^{(-1.6)}$ x 100 x 1.76 = 56.6% is greater than the defined test upper LoQ at 25%

Result: **BCR-ABL p190 DETECTED [Above upper LoQ]**. See Figure 4.

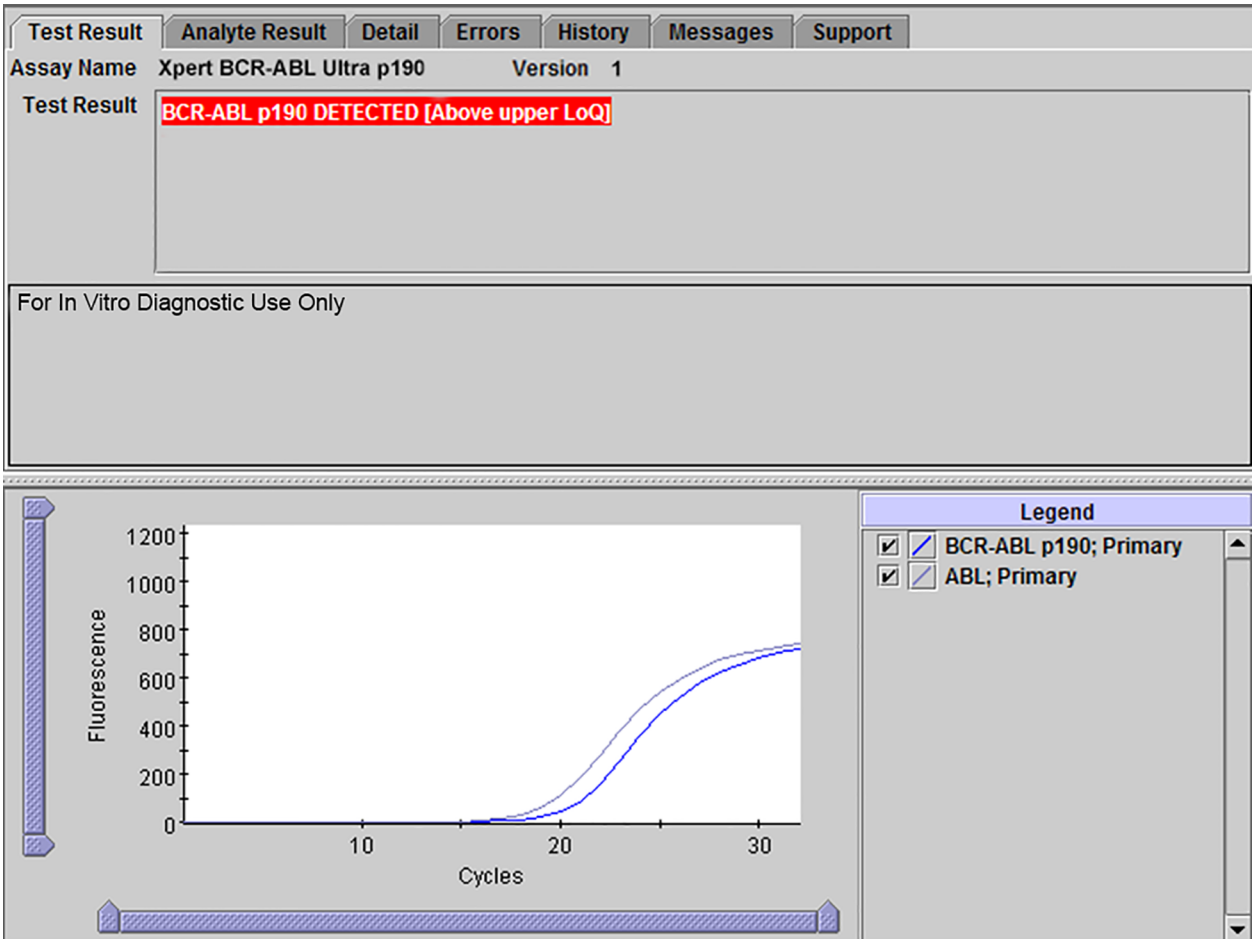


Figure 4. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [Above upper LoQ]

14.4 BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]

BCR-ABL p190 was not detected with BCR-ABL p190 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 p190 is undetectable with BCR-ABL p190 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 2.

Example: BCR-ABL p190 Ct = 0; ABL Ct = 11.6 is less than “18”.

Result: **BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]**. See Figure 5.

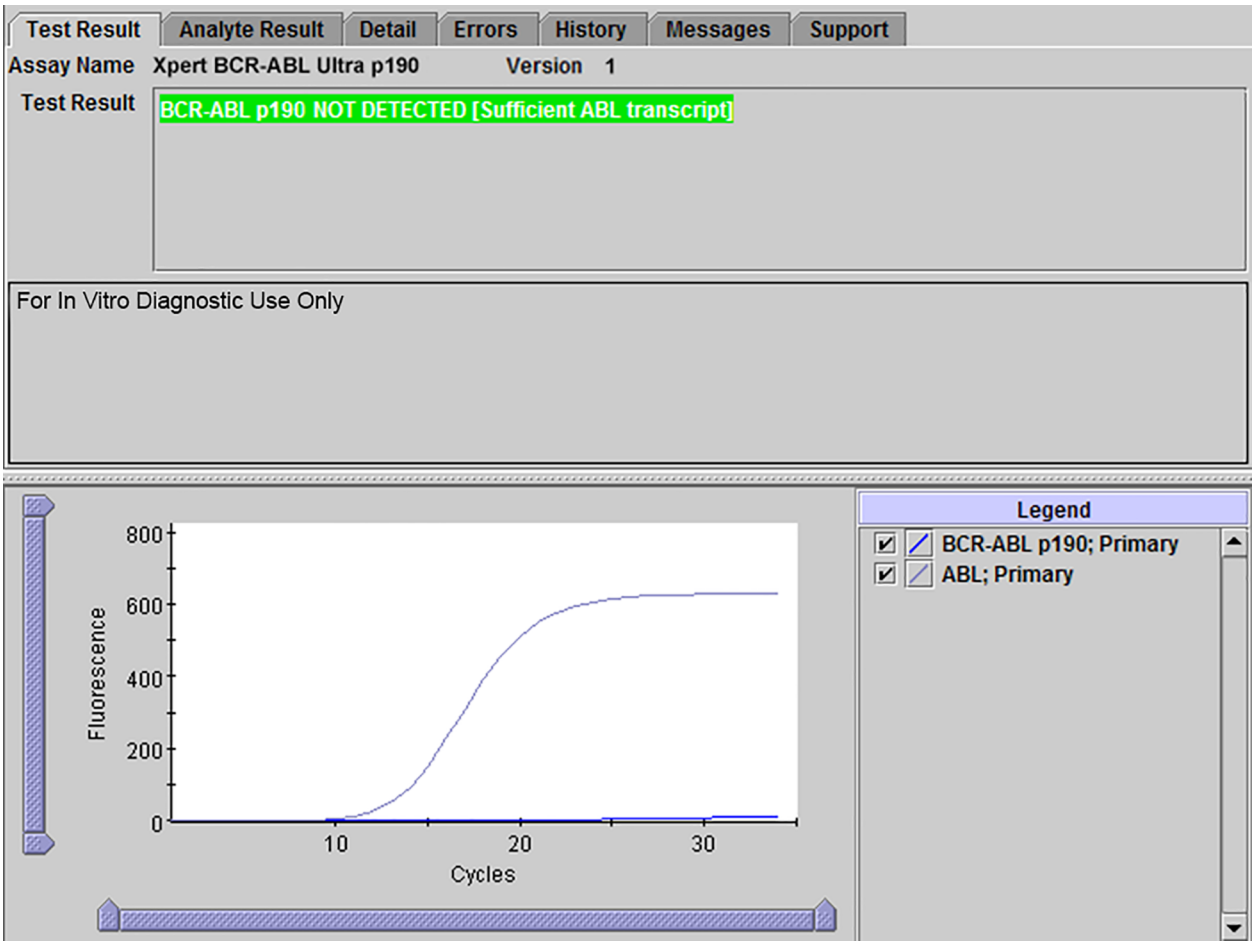


Figure 5. GeneXpert Dx View Results Window: BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]

14.5 INVALID [No ABL transcript]

BCR-ABL p190 was not detected with ABL Ct equal to “0”.

When BCR-ABL p190 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 Section 16, Troubleshooting Guide.

Example: BCR-ABL p190 Ct = 0; ABL Ct = 0.

Result: **INVALID [No ABL transcript]**. See Figure 6.

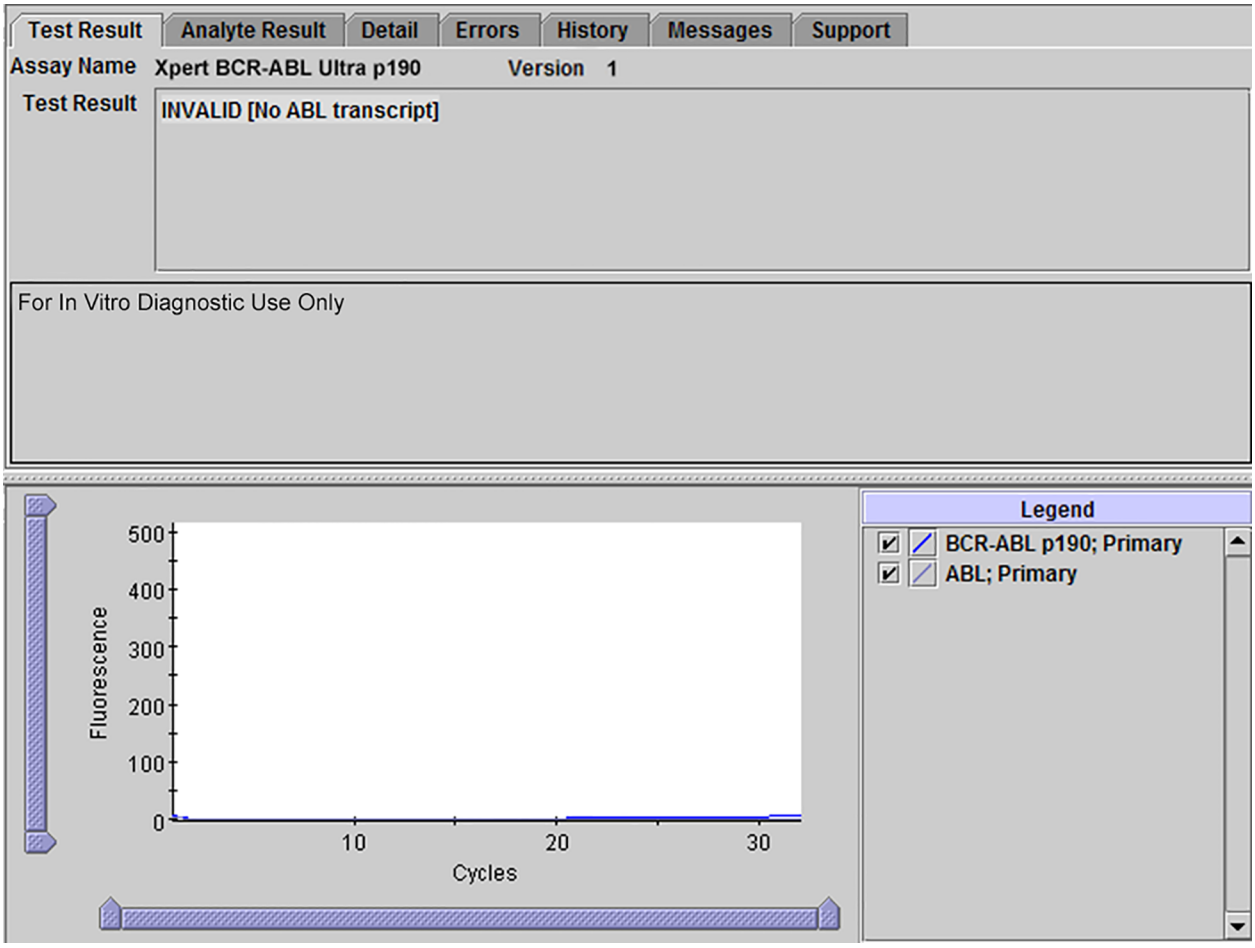


Figure 6. GeneXpert Dx View Results Window: INVALID [No ABL transcript]

14.6 INVALID [Insufficient ABL transcript]

BCR-ABL p190 was not detected with ABL Ct greater than "18".

When BCR-ABL p190 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 Section 16, Troubleshooting Guide.

Example: BCR-ABL p190 Ct = 31.2; ABL Ct = 28 is greater than "18".

Result: **INVALID [Insufficient ABL transcript]**. See Figure 7.

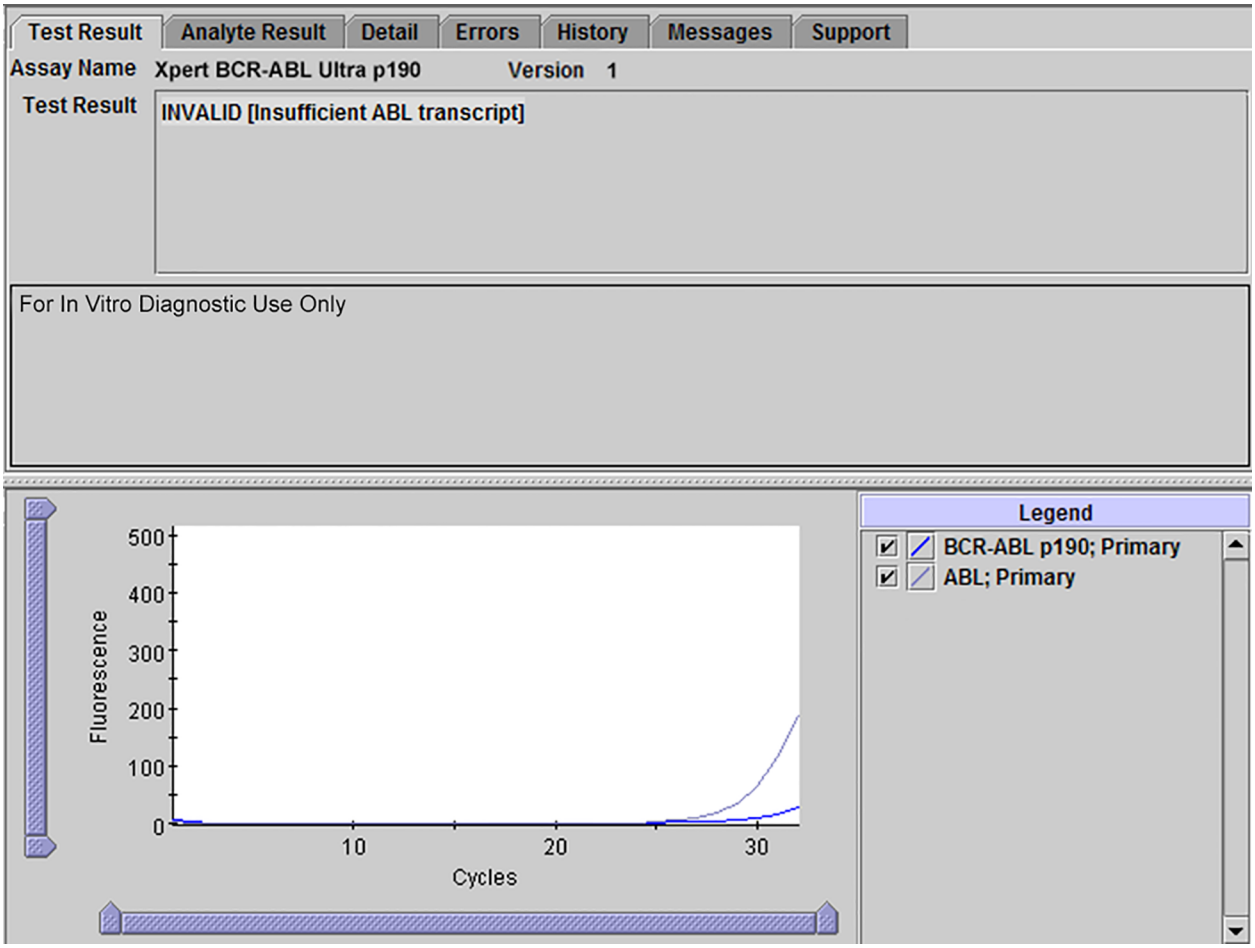


Figure 7. GeneXpert Dx View Results Window: INVALID [Insufficient ABL transcript]

14.7 INVALID [Too high BCR-ABL p190 and ABL transcripts]

BCR-ABL p190 was detected with both BCR-ABL p190 and ABL Cts less than "8".

When BCR-ABL p190 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 Section 16, Troubleshooting Guide.

Example: BCR-ABL p190 Ct = 7.9; ABL Ct = 7.6 is less than "8".

Result: **INVALID [Too high BCR-ABL p190 and ABL transcripts]**. See Figure 8.

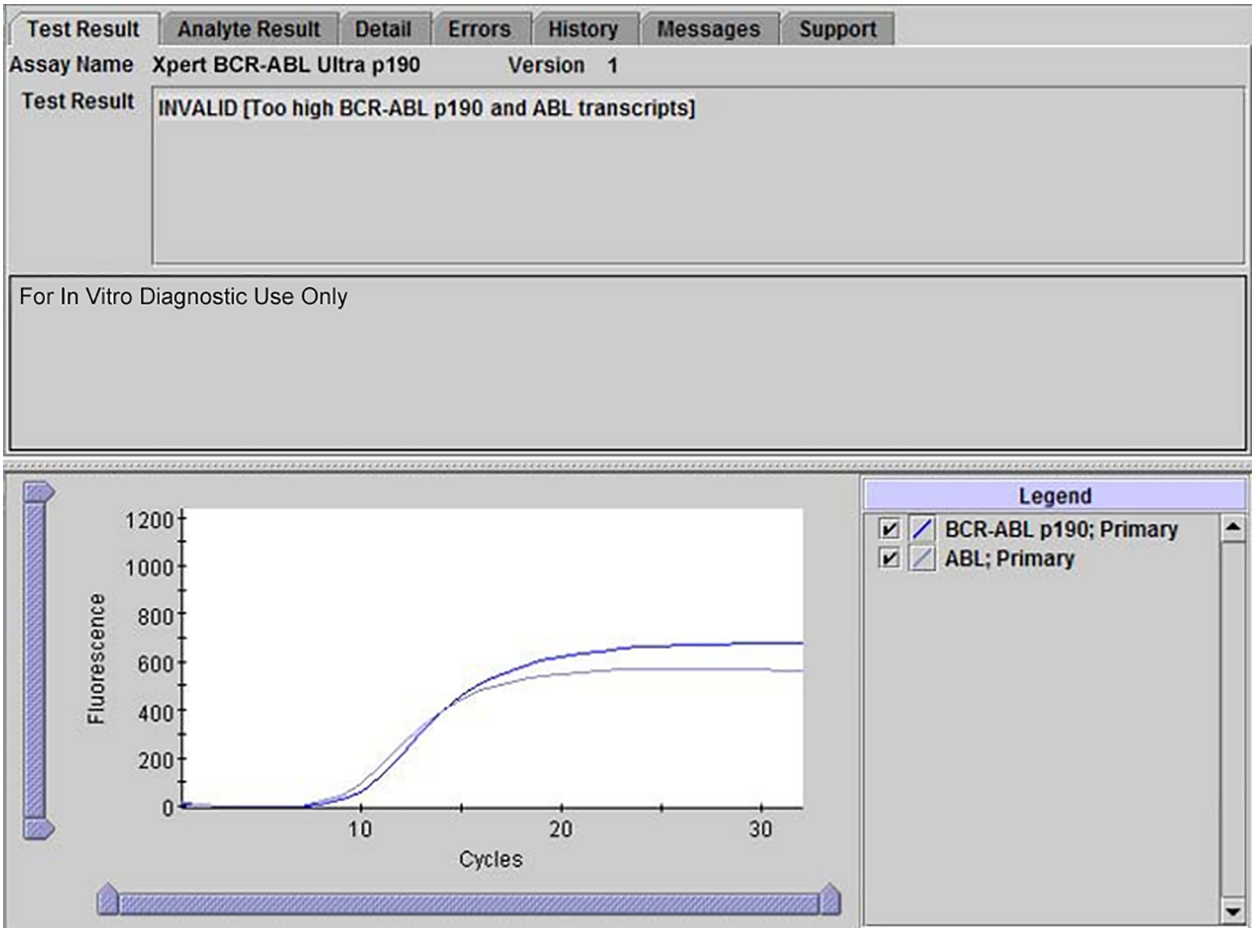


Figure 8. GeneXpert Dx View Results Window: INVALID [Too high BCR-ABL p190 and ABL transcripts]

14.8 ERROR

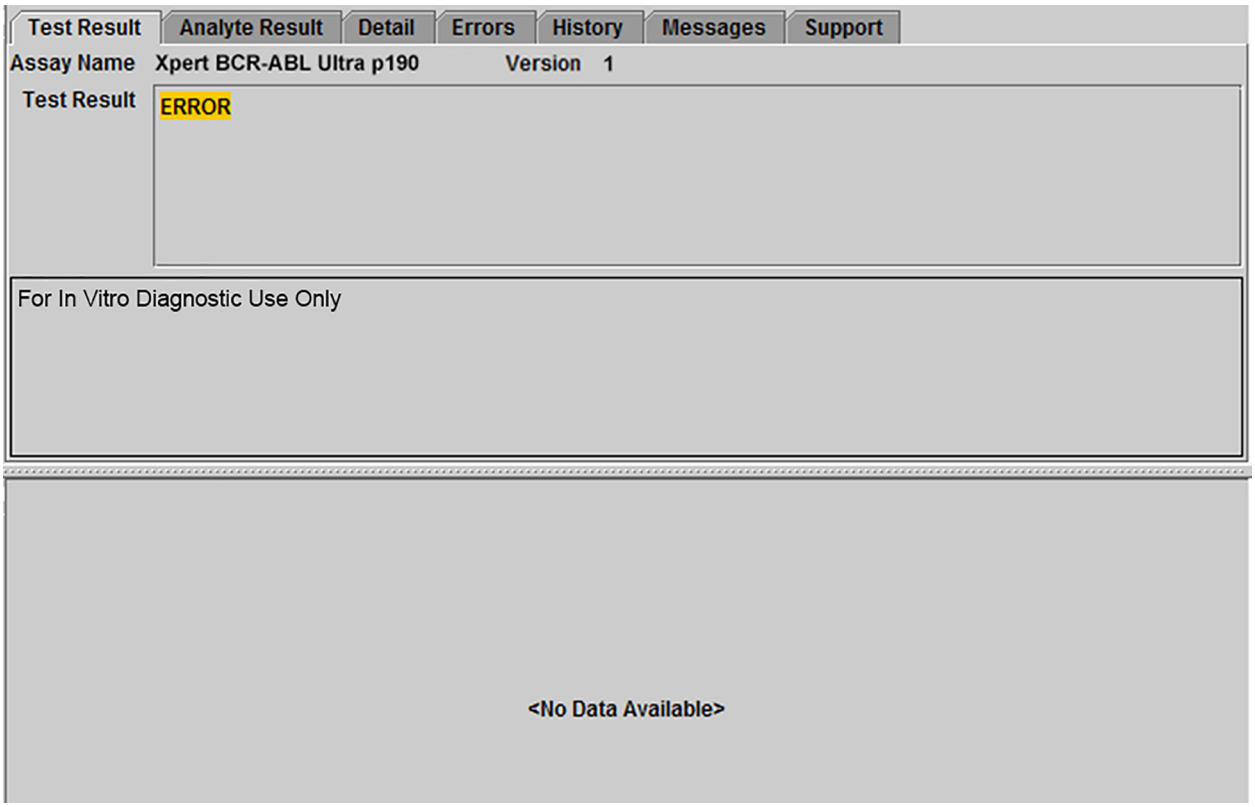


Figure 9. GeneXpert Dx View Results Window: ERROR

15 Limitations

- The product is intended for *in vitro* diagnostic use only.
- The test is not intended to be used with external calibrators.
- The test is not indicated for determining discontinuation from TKI treatment nor for monitoring after discontinuation.
- The performance of the Xpert BCR-ABL Ultra p190 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 (Na Citrate), buffy-coat and bone marrow specimen types have not been validated.
- Erroneous test results might occur from improper specimen collection, handling, storage, or specimen mix-up. Strict adherence to the Instructions for Use is necessary to avoid erroneous results.
- The Xpert BCR-ABL Ultra p190 test is only designed to detect the p190 BCR-ABL fusion transcript e1a2. The ability to detect other fusion transcripts has not been evaluated beyond those described in these instructions for use. The test does not detect major or micro breakpoints, microdeletions, or mutations.
- The Xpert BCR-ABL Ultra p190 is not intended to detect the e13a2/b2a2 and e14a2/b3a2 (p210), e19a2 (p230) or other minor translocations that may be present in a peripheral blood specimen from a patient with leukemia.
- For some specimens with very high white blood cell counts (higher than 30 million cells/mL), Xpert BCR-ABL Ultra p190 may report **INVALID** (Type 2) results due to excess BCR-ABL p190 or ABL levels in the specimen. See Table 2 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 (0.0065%) 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 0.0065%) may be reported as **BCR-ABL p190 NOT DETECTED [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 Dx System (GX-I, GX-II, GX-IV, GX-XVI).

16 Troubleshooting Guide

Table 2. Troubleshooting Guide

Test Result	Possible Causes	Suggestions
INVALID	Type 1: Endogenous control ABL failure: ● Poor specimen 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 Section 17.1, Retest Procedure for ERROR or INVALID (Type 1).
	Type 2: BCR-ABL transcript level cannot be determined due to specimen containing excess BCR-ABL p190 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 Section 17.2, 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 Section 17.2, 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 Section 17.1, 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 Section 17.1, Retest Procedure for ERROR or INVALID (Type 1).

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

17 Retests

17.1 Retest Procedure for ERROR or INVALID (Type 1)

Retest specimens 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 Table 2.

1. Measure blood specimen volume:
   - If *sufficient* blood specimen volume is available, re-test from original blood specimen collection tube following the procedure in Section 11.2.1.

     -OR-
   - If blood specimen volume is *insufficient*, re-test can be performed with the retained lysate from Section 11.2.1 step 12.

     a. If retained lysate from Section 11.2.1 step 12 is stored frozen, thaw to room temperature before use.
     b. Ensure lysate is well-mixed by mixing the specimen 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 retained 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. Follow steps 14-17 in Section 11.2.1 to make the final lysate.
5. 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.
6. Pipette the entire contents of the prepared specimen into the Sample Chamber (large opening) See Figure 1.
7. Close the cartridge lid. Initiate test (see Section 11.4).

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

Retest specimens 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 Table 2.

1. To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).
2. Measure blood specimen volume:
   - 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 3.

     -OR-
   - If blood specimen volume is *insufficient*, re-test can be performed from the retained lysate from Section 11.2.1 Step 12.

     a. If retained lysate from Section 11.2.1 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 specimen 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 3.
3. To the tube already containing Proteinase K, add 50 µL of original blood specimen, if available, or 80 µL of retained lysate from Section 11.2.1 Step 12.
4. Mix the specimen with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. Follow the Steps 6-13 in Section 11.2.2 to make the final lysate.
7. 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.
8. Pipette the entire contents of the prepared specimen into the Sample Chamber (large opening). See Figure 1.
9. Close cartridge lid. Initiate test (see Section 11.4).

18 Expected Values

The Xpert BCR-ABL Ultra p190 range covers key clinical decision points for monitoring of CML and ALL. Expected values are expressed as percent ratio of BCR-ABL p190 mRNA (e1a2) to the ABL mRNA and range between 0.0065% and 25%. Measurements below this range are reported as undetected or below the limit of detection (LoD). Measurements above this range are reported as above the limit of quantitation (LoQ). Refer to Section 14 for details.

19 Clinical Performance

The clinical performance of the Xpert BCR-ABL Ultra p190 test was evaluated at three institutions in the U.S. as part of a multi-site clinical study. The study was conducted using prospectively collected EDTA peripheral blood (PB) specimens from acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) patients during monitoring of treatment. In addition, the study included left over specimens stored as frozen clinical lysates which were prepared from EDTA PB from the same patient population. The Xpert BCR-ABL Ultra p190 test performance was compared to a molecular test which detects and quantifies the mRNA transcripts for the p190 [t(9;22)(q34;q11)] positive CML and ALL patients expressing BCR-ABL1 fusion transcript type e1a2 and uses ABL as the endogenous control mRNA transcript.

A total of 47 specimens were enrolled into this study. Of these 47 specimens, 9 had RNA yield of < 100 ng/ml and were excluded from the analyses. A total of 9 specimens were excluded leaving 38 specimens included in the final dataset. It is important to note that all 9 specimens that were excluded yielded valid Xpert BCR-ABL Ultra p190 test results.

Age and gender were collected for the 38 specimens enrolled into this study. Specimens were collected from 25 males (65.8%) and 13 females (34.2%). All specimens were from patients between 20 and 88 years of age with mean 54.5 years. Twenty-three (61%) specimens were collected from patients diagnosed with ALL and 15 (39%) specimens were collected from patients diagnosed with CML.

Of the 38 eligible specimens, seven (7) specimens were excluded from the Deming regression since they were negative for at least one of the tests. Thirty-one specimens within the quantitative ranges of both tests were included in the Deming regression analysis.

The Deming regression analysis for percent ratio (PR) results show good correlation between the Xpert BCR-ABL Ultra p190 and comparator method measurements in terms of PR measurement. The intercept was 0.01 and slope was 1.08; both met the acceptance criteria. The Pearson's r was 0.814. Log Reduction (LR) was conducted to normalize the PR data distribution. Deming regression analysis using LR measurements were performed and presented in Figure 10 below.

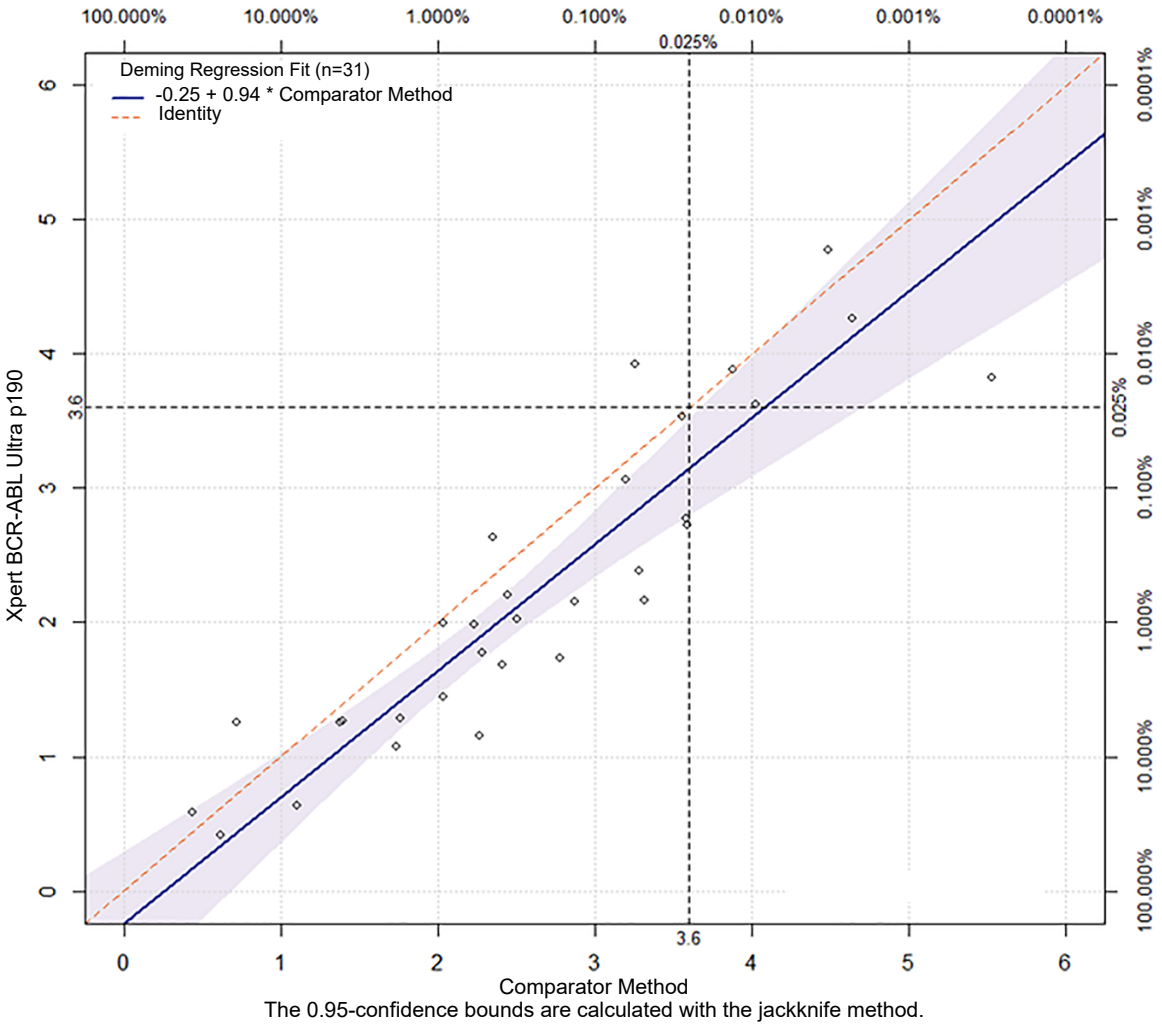


Figure 10. Deming Regression for LR

Figure 10 shows high correlation between Xpert BCR-ABL Ultra p190 and comparator method tests for LR measurements. The Deming regression has a slope of 0.94 and an intercept of -0.25. Deming Regression results for the LR values also met the acceptance criteria for the intercept and slope. The overall correlation (Pearson) r=0.904 was high.

The positive predicted bias of 0.01 in percent reporting (LR: -0.39) as well as the distribution indicates that for most specimens the Xpert test measures higher concentration of the p190 transcript compared to the comparator. The Xpert BCR-ABL Ultra p190 test showed high correlation of 0.904 with the comparator and had a low bias using LR measurements. The non-determinate rate observed in this study was 0% and the acceptance criteria of non-determinate ≤ 5% was also met. The Xpert BCR-ABL Ultra p190 test showed acceptable concordance with the comparator as demonstrated by the slope and intercept in a Deming regression analysis.

20 Analytical Performance

20.1 Linearity/Dynamic Range

Linearity was evaluated for the minor breakpoint, e1a2, using total RNA from ALL SUP-B15 cell line. Total RNA from BCR-ABL p190 transcript was diluted in a background lysate prepared from ALL-negative clinical specimen to target ranges of ~25% to 0.001% (LR [log reduction] 0.60 to LR5). 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 result for e1a2 breakpoint was considered linear if the polynomial regression coefficients were insignificant (p-values > 0.05). The linear regression curve is shown in Figure 11 below.

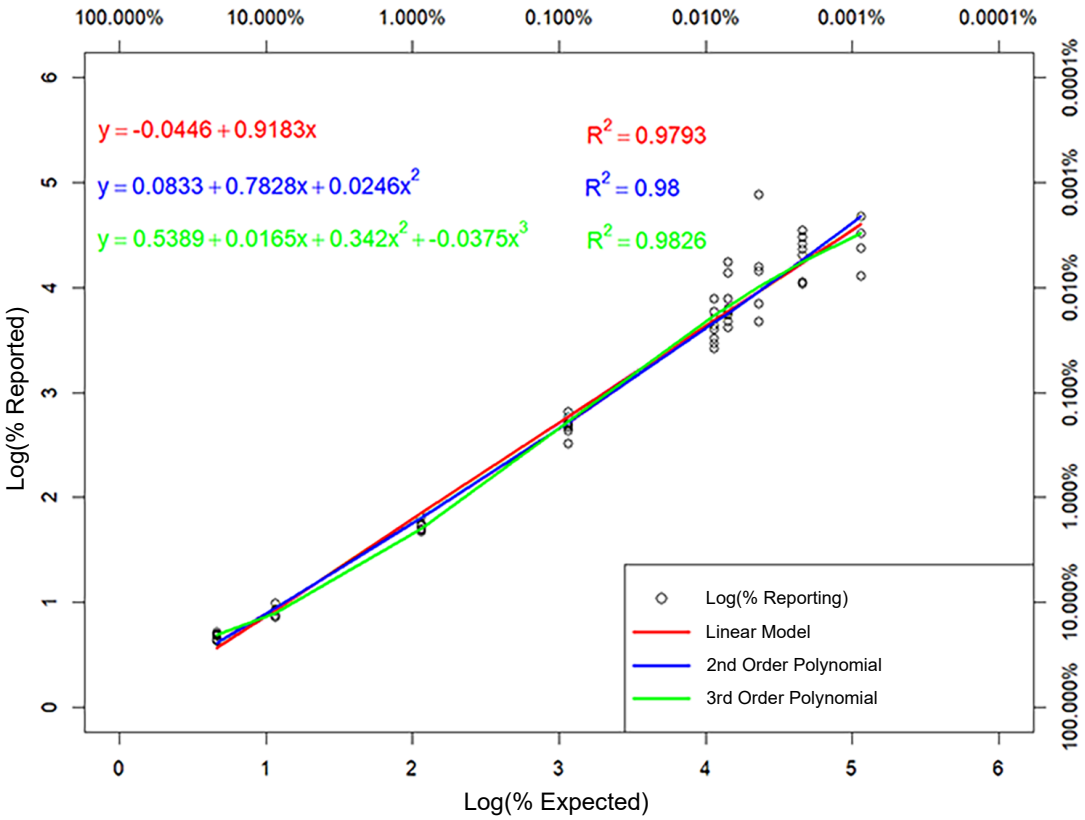


Figure 11. Linear Regression Curves for Breakpoint Transcript e1a2

The estimated regression intercepts, slopes and R2 values from the linear model are shown in Table 3.

Table 3. Regression Coefficients from Linear Model

Breakpoint	Intercept	Slope	R^2
e1a2	-0.0561	0.9248	0.9811

Collectively,the data support an observation of linearity from ~25%/LR 0.60 to 0.001%/LR5 with a maximum SD of 0.26. The reportable range spans from the limits of linearity at 25%/LR0.6 to the LOQ at 0.0065%/LR4.19.

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

The limit of detection (LoD) was estimated for e1a2 breakpoint by testing serial dilutions of ALL positive clinical specimen [>10%]. Data across dilutions were compiled and the LoD was estimated by using probit regression analysis. The resulting analysis yielded an estimated LoD of 0.0070% for the e1a2 breakpoint.

The LoD was verified by adapting the non-parametric method described in the CLSI guidance document, EP17-A2 (Table 4). Three unique ALL positive specimens representing e1a2 breakpoint were diluted to a targeted 0.0065% level. Two hundred and fifteen replicates were tested by 4 operators across 3 test kit lots over 3 days.

Table 4. Verified Limit of Detection in %

Breakpoint	Positives/Replicates	% of Positives	Average % Ratio
e1a2	206/215	96.0%	0.0065%

Xpert BCR-ABL Ultra p190 LoD for e1a2 is 0.0065%.

The limit of quantitation (LoQ) was estimated with the data obtained from the LoD and linearity studies. The mean and standard deviation for the % BCR-ABL p190/ABL values were calculated for the replicates at levels equal to the LoD or greater with positivity greater or equal to 95%. The LoQ is reported as the minimal % BCR-ABL p190/ABL reporting that can be reliably quantitated, meeting the precision goal of detecting the e1a2 transcript with a positivity greater than or equal to 95%, with a log reduction (LR) standard deviation of ≤ 0.36 LR. The LoQ of the test is constrained by the LoD of the test; therefore, the LoQ was determined to be equal to the LoD, 0.0065%. The results were also evaluated against the acceptance criteria for standard deviation (SD) ≤ 0.36 LR and were within the acceptance criteria.

The Limit of Blank (LoB) study was conducted to estimate the highest % BCR-ABL p190/ABL ratio that is likely to be detected in ≥ 95% of p190-negative whole EDTA blood specimens. The test LoB was determined from 387 valid data points in an uncensored non-parametric analysis, as described in CLSI EP17-A2, to estimate a LoB of 0.00032% BCR-ABL p190/ABL.

20.3 Analytical Specificity

The analytical specificity of Xpert BCR-ABL Ultra p190 was evaluated by testing EDTA whole blood specimens from twenty (20) healthy donors (non-CML and non-ALL). Each sample was tested in quadruplicate.

BCR-ABL p190 signal was detected in one of the 80 replicates, demonstrating that the Xpert BCR-ABL Ultra p190 test had an analytical specificity for the BCR-ABL p190 transcript of 98.8%.

20.4 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 specimens were run following very high positive specimens in the same GeneXpert module. This study consisted of processing a **NEGATIVE** EDTA normal specimen (ALL-negative blood) in the same GeneXpert module immediately following a high **POSITIVE** specimen (simulated ALL positive blood) with SUP-B15 cells spiked into ALL- negative blood to yield ≥10%. Testing scheme was repeated 10 times for each specimen, starting and ending with negative, on two GeneXpert modules, resulting in 21 negatives and 20 positives per module. All twenty BCR- ABL p190 positive specimens were correctly reported as **BCR-ABL p190 DETECTED [#.##%]**, while all twenty-one BCR-ABL p190 negative specimens were correctly reported as **BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]**.

20.5 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 p190 test. The compounds and levels tested (see Table 5) were based on guidance from the CLSI document EP07-A2. Interferents were tested in the background of ALL EDTA whole blood specimens, contrived with ALL SUP-B15 cells, representing three levels with five specimens per level: >1%, 0.1-0.02%, and Negative. Test controls consisted of SUP-B15 cells in EDTA whole blood at the respective BCR-ABL p190 transcript level without the interfering substance. Each ALL 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 % ratio observed was within 3-fold difference when compared to the control.

No clinically significant inhibitory effects on the Xpert BCR-ABL Ultra p190 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 % ratios for test and control conditions were within the acceptable 3-fold range.

Table 5. Potentially Interfering Substances Tested Using Xpert BCR-ABL Ultra p190

Interfering Substances	Concentration Tested
Unconjugated Bilirubin	20 mg/dL
Cholesterol, Total	500 mg/dL
Triglycerides, Total (Lipids)	3000 mg/dL
Heparin	3500 U/L
EDTA (short draw)	900 mg/dL

21 Reproducibility and Precision

The reproducibility and precision of the Xpert BCR-ABL Ultra p190 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.”

Table 6 shows the panel of five specimens that were prepared and included in this study.

Table 6. Reproducibility Panel for Xpert BCR-ABL Ultra p190

Specimen No.	Description of the Panel	BCR-ABL p190/ABL Level Detected (percent ratio)
1	LR1: e1a2	~10%
2	LR2: e1a2	~1%
3	LR3: e1a2	~0.1%
4	LR3.7: e1a2	~0.02%
5	Negative	Not Detected

Each of the five panel members was tested in duplicate two times per day on six different days by two different operators at three different sites. Three lots of Xpert BCR-ABL Ultra p190 kits were used and each operator performed testing with one lot (3 sites x 2 operators x 3 lots x 2 days (2 days of testing per cartridge lot) x 2 runs x 2 replicates = 144 replicates/panel member).

Table 7. Standard Deviation and Coefficient of Variation (CV) with Percent Ratio (PR)

Panel Member	N	Mean	Site		Op		Lot		Day		Run		Within Test		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LR1: e1a2 (~10% ratio)	144	14.04	0.20	1.44	0.00	0.00	3.14	22.35	0.55	3.94	0.00	0.00	1.63	11.60	3.58	25.53
LR2: e1a2 (~1% ratio)	144	1.65	0.14	8.58	0.00	0.00	0.61	36.80	0.00	0.00	0.00	0.00	0.32	19.35	0.70	42.45
LR3: e1a2 (~0.1% ratio)	144	0.16	0.01	6.15	0.00	0.00	0.08	50.18	0.01	5.26	0.00	0.00	0.04	24.42	0.09	56.39
LR3.7: e1a2 (~0.02% ratio)	143[a]	0.03	0.00	6.60	0.00	0.00	0.02	62.48	0.00	11.43	0.00	0.00	0.01	43.56	0.02	77.30

[a] One sample gave a non-determinate on both test and re-test result.

The total coefficient of variation (CV%) of the percent ratio reporting quantitative values ranged from 25.53 to 77.30 for the positive samples. The component of variance for the PR reporting values did not exceed 50% of the total test variance for the following factors: Site-to-site, Operator-to-Operator, Day-to-Day, and Run-to-Run. Analysis of variance over the Mean PR quantitative value gave similar results.

Table 8. Standard Deviation and Coefficient of Variation (CV) of Log Reduction (LR)

Panel Member	N	Mean	Site		Op		Lot		Day		Run		Within Test		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LR1: e1a2 (~10% ratio)	144	0.86	0.01	1.47	0.00	0.00	0.10	11.17	0.02	2.53	0.00	0.00	0.05	5.87	0.11	26.17
LR 2: e1a2 (~1% ratio)	144	1.81	0.03	1.93	0.00	0.00	0.15	8.48	0.00	0.00	0.00	0.00	0.07	3.64	0.17	40.75
LR 3: e1a2 (~0.1% ratio)	144	2.84	0.03	1.06	0.00	0.00	0.22	7.60	0.01	0.51	0.00	0.00	0.09	3.34	0.24	59.16
LR 3.7: e1a2 (~0.02% ratio)	143[a]	3.66	0.04	1.19	0.00	0.00	0.27	7.26	0.04	1.12	0.03	0.86	0.19	5.06	0.33	88.68

[a] One specimen gave a non-determinate on both test and re-test result.

The total coefficient of variation (CV) percent of the LR reporting quantitative values ranged from 26.17 to 88.68 for the positive samples.

22 References

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23 Cepheid Headquarters Locations

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24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- 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

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

25 Table of Symbols

Symbol	Meaning
REF	Catalog number
CE	CE marking – European Conformity
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
EC REP	Authorized Representative in the European Community
CH REP	Authorized Representative in Switzerland
	Importer

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26 Revision History

Description of Changes: 302-6673, Rev. B to Rev. C

Purpose: Updates to the Instructions for Use

Section	Description of Change
8.3	Added a warning statement to not open or alter cartridges for disposal.
11.2.1	Updated note regarding remaining lysate.
17	Updated Retest instructions and corrected section references.
19	Updated diagram labels in Figure 10.
21	Updated Reproducibility and Precision content.
25	Added CH REP and Importer symbols and definitions to Table of Symbols. Added CH REP and Importer information with Switzerland address.
26	Updated Revision History table.