Technical Training
Xpert® BCR-ABL Ultra p190

GXBCRABLP190-CE-10

For CE-IVD Only

302-8412 Rev. C June 2023

Training Agenda

1. Overview
2. Kit Storage and Handling
3. Specimen Collection, Storage and Transport
4. Cartridge Preparation
5. Quality controls
6. Results Interpretation
7. Troubleshooting
8. Retest Procedures

Training Objectives

At the end of the training, users will be able to:

- Properly store and handle the Xpert® BCR-ABL Ultra p190 cartridge kit and sample collection
- Follow proper laboratory safety precautions
- Collect and transport appropriate specimen
- Prepare a cartridge and run the Xpert BCR-ABL Ultra p190 test
- Report the various software generated results
- Understand the Xpert BCR-ABL Ultra p190 control strategy

The Cepheid Solution

- Quantitative detection
  - BCR-ABL1 p190 and ABL1 mRNA transcripts in peripheral blood specimens
- On-board internal controls for each sample
  - Probe Check Control (PCC)
  - ABL1 Endogenous Control
- Time to Results approximately 2.5 hours (include hands-on time)
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

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.

Intended User/Environment

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

Targets

- The Xpert® BCR-ABL Ultra p190 is an automated test for quantifying the amount of BCR-ABL1 p190 transcript as a ratio of BCR-ABL1 p190/ABL1.
- BCR-ABL1 p190 and ABL1 mRNA transcripts in peripheral blood specimens of diagnosed [t(9;22)(q34;q11)] positive chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) patients expressing BCR-ABL1 fusion transcript type e1a2.

Xpert® BCR-ABL Ultra p190 Requirements

GeneXpert® Systems

- GeneXpert Dx software **v6.2** or higher

Test Kits

- GXBCRABLP190-CE-10

Sample Collection

- Whole blood

Materials Required but Not Provided

- EDTA Tubes
- Printer
- Vortex Mixer
- Microcentrifuge (1,000 x g minimum)
- Pipettes and aerosol filter pipettes
- 50 mL conical tubes
- Reagent grade absolute ethanol

Other Materials

- Personal Protective Equipment (PPE)
- 1:10 dilution bleach
- 70% ethanol or denatured ethanol

Good Laboratory Practice Review

Personnel Protective Equipment (PPE)

- Wear clean lab coats, wear safety glasses and gloves
- Change gloves between processing samples

Lab Bench Area

- Clean work surfaces routinely with:
  - ✓ 1:10 dilution of household bleach*
  - ✓ 70% ethanol solution
- After cleaning, ensure work surfaces are dry

Specimens, Samples, and Kits Storage

- Store specimens and samples away from kit to prevent contamination

Equipment

- Use filtered pipette tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment

* Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country.

Kit Storage and Handling

Xpert® BCR-ABL Ultra p190 Kit Contents

Catalog Number	GXBCRABLP190-CE-10
Cartridges* Per Kit	10
Reagent Vials (10 of each)	Proteinase K (PK) Lysis Reagent (LY) Wash Reagent (1)
Kit CD	Xpert® BCR-ABL Ultra p190 Assay Definition File (ADF) Xpert® BCR-ABL Ultra p190 Import Instructions Instructions for Use (PDF)
Storage	2-8°C

* *Cartridges contain chemically hazardous substances - please see Instructions for Use (IFU) and Safety Data Sheet for more detailed information.*

Warnings and Precautions

- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that:
  - appears wet, has leaked or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it
  - has a damaged reaction tube
  - has been used; each cartridge is single-use to process one test
  - has expired
- Do not reuse pipettes

Waste Disposal Warnings and Precautions

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of 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.
- Please Note: 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.

Xpert® BCR-ABL Ultra p190 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

Xpert® BCR-ABL Ultra p190 Limitations (Continued)

- 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 in Instruction For Use for additional information.

Xpert® BCR-ABL Ultra p190 Limitations (Continued)

- 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).

Specimen Collection, Storage and Transport

Specimen Transport and Storage

Whole blood specimens should be collected in EDTA tubes following your institution's guidelines.

Specimen Type	Storage
Whole blood specimen	2-8 ºC for ≤ 72 Hours

Cartridge Preparation

Xpert® BCR-ABL Ultra p190 Cartridge Preparation

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

Lysate and Cartridge Preparation

- Xpert® BCR-ABL Ultra
- Xpert® BCR-ABL Ultra p190
- Xpert® NPM1 Mutation

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support
US office (888) 838-3222, Option 2
techsupport@cepheid.com
European office +33 563 82 53 19

20 minutes before starting the procedure, allow the following to come to room temperature (20°C – 30°C)
- blood specimen
- cartridge
- sample preparation reagents

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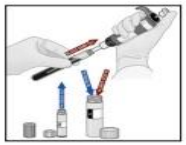
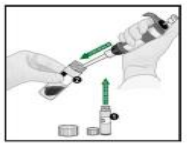
1 Remove EDTA whole blood and sample prep reagents from refrigerator. Place EDTA blood on rocker or invert 8 times prior to sampling.

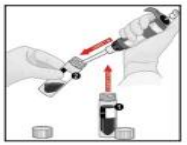
2 Briefly centrifuge PK reagent. To a 50mL conical tube, add 100uL of PK reagent. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.



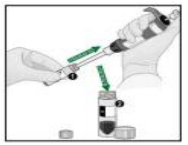
3 Add 2.5mL of lysis reagent (LY) to same tube, vortex 10 sec, and incubate 5 min at RT. Vortex again for 10 sec and incubate a 2nd time for 5 min. Mix by tapping tube 10x.



4 Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.



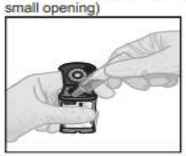
5 Add 1.5mL of lysis reagent (LY) to the new conical tube containing previously prepared lysate. Vortex for 10 sec and incubate for 10 min at RT.



6 To the same conical tube, add 2mL of reagent grade absolute EtOH. Vortex for 10 sec and set aside. Discard remaining PK or LY reagents.

7 Open the Xpert test cartridge lid.

8 Transfer entire contents of Wash Reagent ampoule into Wash Reagent Chamber (with small opening)



9 Pipette entire contents of final prepared lysate from conical tube.

10 Transfer entire contents (~4.5mL) of prepared sample into the sample chamber.

11 Close the Xpert cartridge lid.

12 Start the test within the timeframe specified in the package insert.

CE IVD *In Vitro* Diagnostic Use *In vitro* medical diagnostic device. May not be available in all countries. Not available in the United States.

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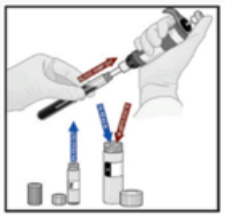
Xpert® BCR-ABL Ultra p190 Cartridge Preparation

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

1. Ensure the blood sample is well-mixed by inverting the EDTA blood collection tube 8 times immediately before pipetting.

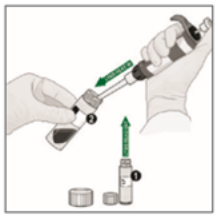
5. Open the cartridge lid.

2. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K). Add 50 µL of blood sample. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min.



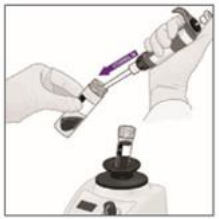
6. Transfer the entire contents of Wash Reagent ampoule into Chamber 1.

3. To the same tube, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Repeat vortex and incubation step for same times.



7. Pipette the entire contents of the prepared sample into the sample chamber (large opening)

4. To the same conical tube add 2 mL of reagent grade absolute ethanol. Mix the sample with vortex mixer at maximum setting continuously for 10 seconds. Set aside at room temperature. Discard any remaining PK or LY reagents.



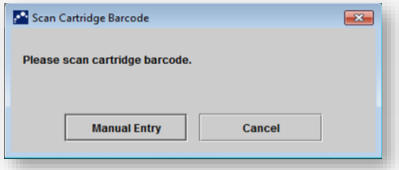
8. Close the cartridge lid and start the assay.

Run a Test on GeneXpert® Dx

1 Create a test.

Start the test within 1 hour minutes after adding the sample to the cartridge.

2 Scan barcode for Patient and/or Sample ID.



Do not click on Manual Entry or Cancel.

3 Scan the cartridge.

For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx Operator Manual.

Run a Test on GeneXpert® Dx (Continued)

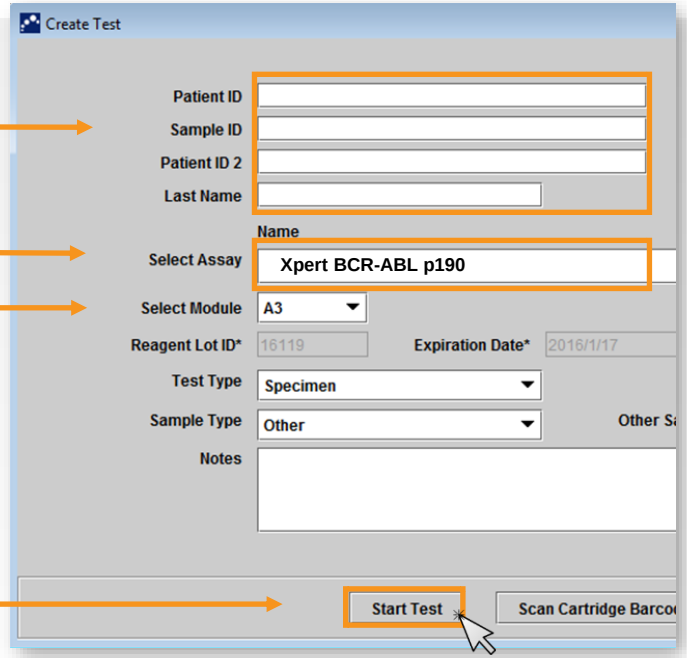
4 Complete the fields as required.

5 Xpert® BCR-ABL p190 test is selected automatically.

6 The module is selected automatically.

7 Click on Start Test.

8 A green light will flash on the module.
Load the cartridge into module and close the door.



Automated Xpert® BCR-ABL Ultra p190 Protocol

1. Sample is added to the cartridge
2. The cartridge is loaded into the System
3. Nucleic acids are purified
4. Purified nucleic acids mix with the PCR reagents
5. Simultaneous amplification and detection occurs
6. Results are ready to view

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Quality Controls

Xpert® BCR-ABL p190 Control Strategy

CONTROL

- Xpert® BCR-ABL p190 Quality Controls
  - Each Xpert cartridge is a self-contained test device
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge. On-board internal controls for each sample
    - Probe Check Control (PCC)
    - ABL1 Endogenous Control

Refer to 301-4868 GeneXpert® Quality Control Features for all Cepheid Xpert tests.

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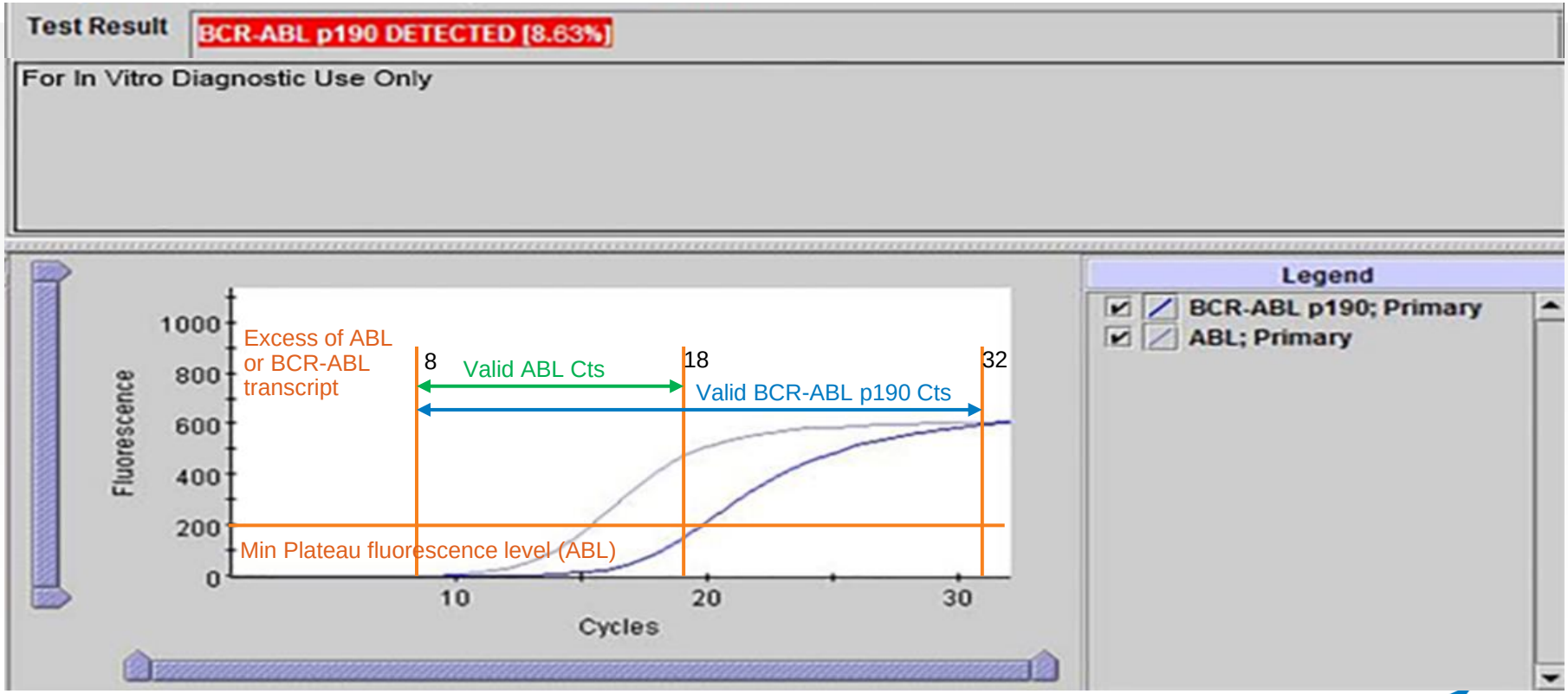
Results Interpretation

Quantitative Results

- 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.
  - Percent ratio = $\text{Efficiency}^{(\Delta Ct)}$ x Scaling Factor x 100
- Efficiency and Scaling factor values calibrate the quantitation of BCR-ABL p190 (e1a2) and ABL 1 transcripts to copy numbers of synthetic BCR-ABL p190 and ABL 1 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.

Valid Ct and Fluorescence Values



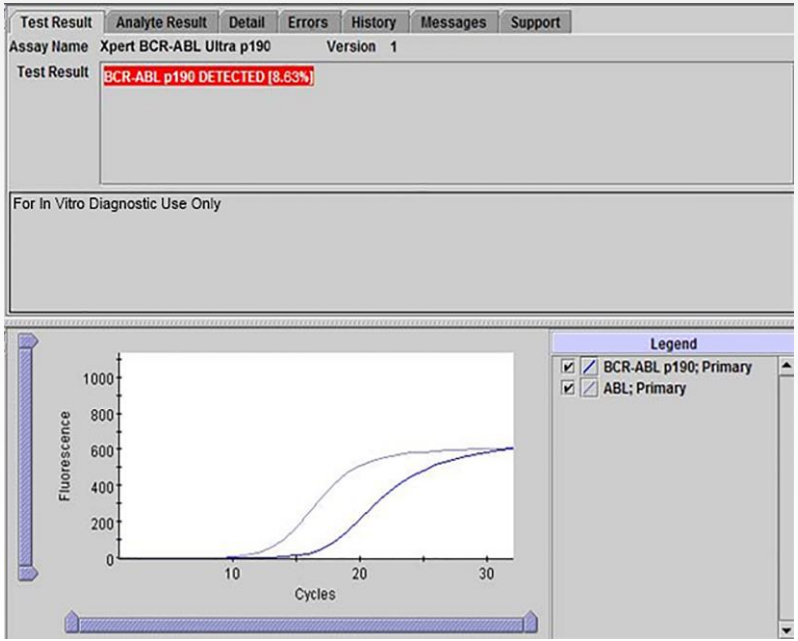
BCR-ABL p190 Detected Results

- Probe Check Control (PCC) must PASS
- ABL Endogenous Control must PASS:
  - Cycle threshold (Ct) within valid range 8≤Ct≤18,
  - and Endpoint above threshold setting
- BCR-ABL p190 must be Detected:
  - Cycle threshold (Ct) within valid range 8≤Ct≤32
  - And Endpoint above threshold

BCR-ABL p190 Detected Results

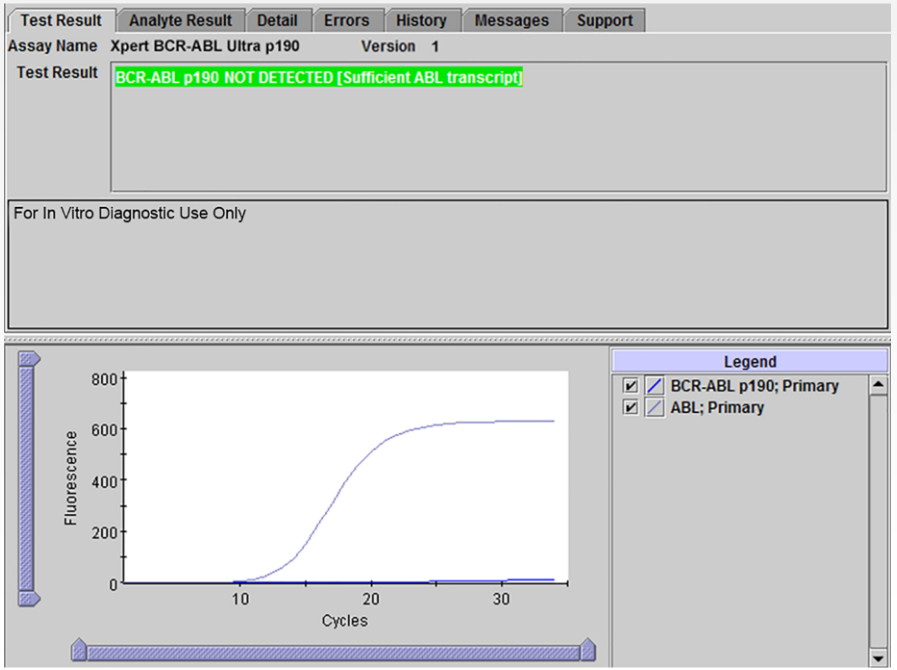
- Assay Result:
  - BCR-ABL p190 DETECTED [#,##%]
  - BCR-ABL p190 DETECTED [Below LoD, <0,0065%]
  - BCR-ABL p190 DETECTED [Above upper LoQ]

- Refer to IFU for additional examples of Detected results



BCR-ABL p190 NOT DETECTED

- Probe Check Control (PCC) must PASS
- ABL Endogenous Control must PASS:
  - Cycle threshold (Ct) within valid range 8≤Ct≤18,
  - and Endpoint above threshold setting
- BCR-ABL p190 must be NOT Detected:
  - No Cycle threshold (Ct) (Ct=0), or
  - Endpoint lower than threshold setting
- Assay Result:
- BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]



Troubleshooting

Factors That Negatively Affect Results

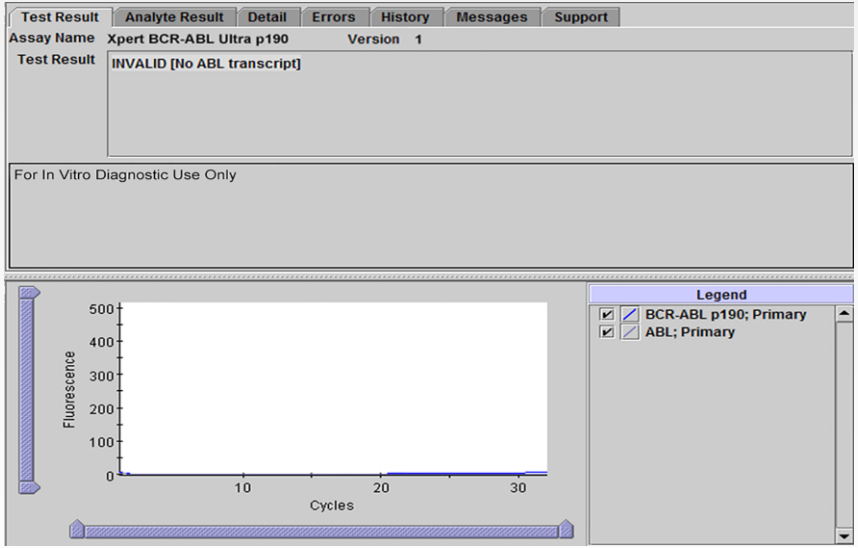
- Improper specimen collection.
  - The performance of this assay with other specimen types or samples has not been evaluated.
- Inadequate numbers of organisms are present in the specimen.
- Improper transport or storage of collected specimen.
  - Storage and transport conditions are specimen specific.
  - Refer to the Instructions For Use for the appropriate handling instructions.
- Improper testing procedure.
  - Modification to the testing procedures may alter the performance of the test.
  - Careful compliance with the Instructions For Use is necessary to avoid erroneous results.

BCR-ABL p190 INVALID Result

- Probe Check Control (PCC) must PASS
- ABL Endogenous Control FAIL/PASS
  - Cycle threshold (Ct) not within valid range 8≤Ct≤18, or
  - Endpoint below threshold setting
- BCR-ABL p190 INVALID
  - Cycle threshold (Ct) not within valid range 8≤Ct≤32

BCR-ABL p190 INVALID Result

- Assay Results
  - INVALID [No ABL transcript]
  - INVALID [Insufficient ABL transcript]
  - INVALID [Too high BCR-ABL p190 and ABL transcript]
  - INVALID [Too high BCR-ABL p190 transcript]
  - INVALID [Too high ABL transcript]

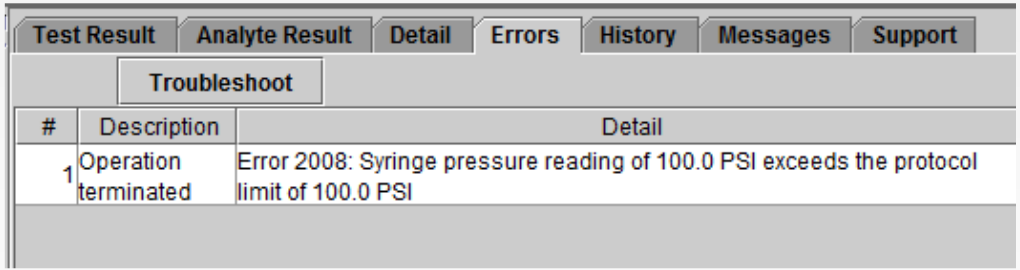


- Refer to IFU for additional examples of INVALID results

Error Result

BCR-ABL transcript level cannot be determined

- BCR-ABL No Result
- ABL No Result
- Probe Check Control FAIL



Possible Causes/Solution

- **Error 2008** – Pressure exceeding limit
- Check the Sample Quality
- Check for grossly elevated WBC count
- Retest Procedure Type 2

- Error 5006, 5007, 5008 and 5009 – Probe Check Failure

- Retest Procedure Type 1

*Refer to Table 2 Troubleshooting Guide in Instructions For Use

NO RESULT

- BCR-ABL transcript level cannot be determined
  - Data collection failure
  - For example, the operators stopped a test that was in progress, or a power failure occurred

*Refer to Table 2 Troubleshooting Guide in Instructions For Use

Reasons to Retest

- INVALID (Type 1) or ERROR
  - 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).
- INVALID (Type 2) or ERROR (Code 2008):
  - Retest samples with BCR-AL p190 and/or ABL transcript levels below the valid minimum Ct cut-off (Ct<8) and/or when pressure limit is exceeded.
- NO RESULT:
  - Due to data collection failure.

Retest Procedures

Retest Procedures
(Refer to Table 2. Troubleshooting Guide in the Instructions For Use)

- INVALID
  - Endogenous control ABL failure due to either poor sample quality, RT-PCR inhibition, if ABL Ct>18, and/or endpoint < 200 => Retest procedure **Type 1**
  - BCR-ABL transcript level cannot be determined due to sample containing excess BCR-ABL and/or ABL transcripts (Ct < 8) => Retest procedure **Type 2**

- ERROR
  - ERROR (Code 2008) Pressure exceeding limit => **Type 2**
  - ERROR (Code 5006, 5007, 5008, and 5009) Probe check failure => **Type 1**

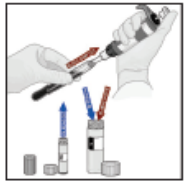
- NO RESULT
  - Data collection failure. For example, the operator stopped an assay that was in progress, or a power failure occurred => **Type 1**

Retest from Blood Collection Tube *(If sufficient blood sample volume is available)*
(Retest Procedure Type 1)

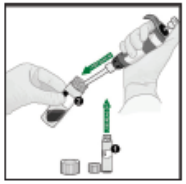
Start Here

1 Remove EDTA whole blood and sample prep reagents from refrigerator. Place EDTA blood on rocker or invert 8 times prior to sampling.

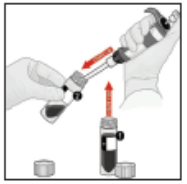
2 Briefly centrifuge PK reagent. To a 50mL conical tube, add 100uL of PK reagent. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.



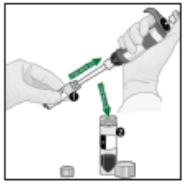
3 Add 2.5mL of lysis reagent (LY) to same tube, vortex 10 sec, and incubate 5 min at RT. Vortex again for 10 sec and incubate a 2nd time for 5 min. Mix by tapping tube 10x.



4 Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.



5 Add 1.5mL of lysis reagent (LY) to the new conical tube containing previously prepared lysate. Vortex for 10 sec and incubate for 10 min at RT.



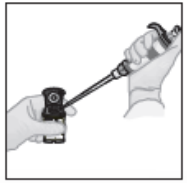
6 To the same conical tube, add 2mL of reagent grade absolute EtOH. Vortex for 10 sec and set aside. Discard remaining PK or LY reagents.

7 Open the Xpert cartridge lid.

8 Transfer entire contents of Wash Reagent ampoule into Chamber 1.

9 Pipette entire contents of final prepared lysate from conical tube.

10 Transfer entire contents (~4.5mL) of prepared sample into the sample chamber.

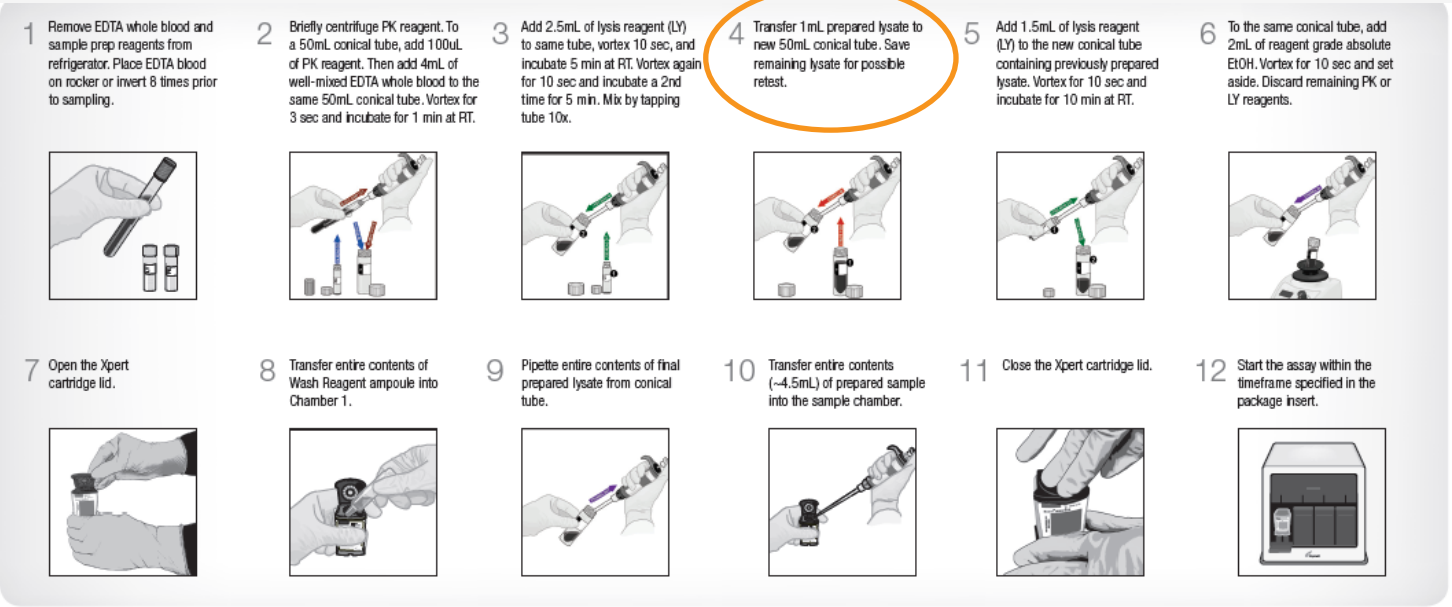


11 Close the Xpert cartridge lid.

12 Start the assay within the timeframe specified in the package insert.

Retest from Lysate (If blood sample volume is *insufficient)*
(Retest Procedure Type 1)

- Thaw lysate to room temperature before use
- 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. Then start here

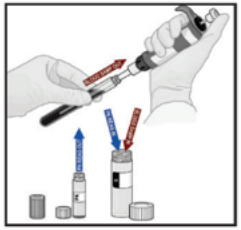


Retest from Blood
(Retest Procedure Type 2)

1. Ensure the blood sample is well-mixed by inverting the EDTA blood collection tube 8 times immediately before pipetting

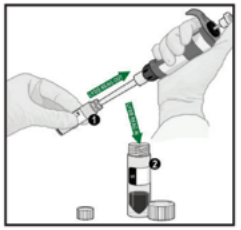
5. Open the cartridge Lid.

2. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K). Add 50 µL of blood sample. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min.



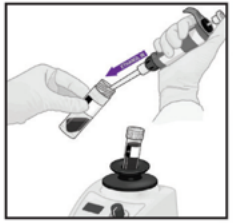
6. Transfer the entire contents of Wash Reagent ampoule into Chamber 1.

3. To the same tube, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Repeat vortex and incubation step for same times.



7. Pipette the entire contents of the prepared sample into the sample chamber (large opening)

4. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside at room temperature.



8. Close the cartridge lid and start the assay.

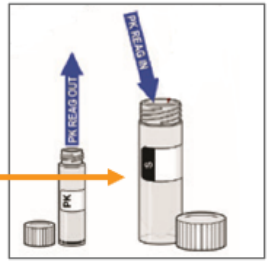
Retest from Lysate
(Retest Procedure Type 2)

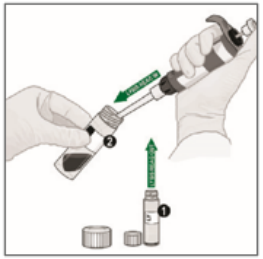
1. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle.

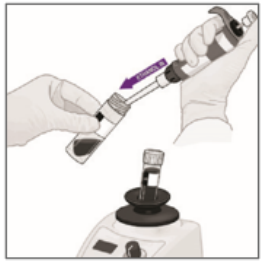
2. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K). Add 80 µL of lysate. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min.

3. To the same tube, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Repeat vortex and incubation step for same times.

4. To the same conical tube, add 2 mL of reagent grade absolute ethanol. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside at room temperature.







5. Open the cartridge Lid. Transfer the entire contents of Wash Reagent ampoule into Chamber 1.

6. Pipette the entire contents of the prepared sample

7. Transfer it into the sample chamber (large opening) Close the cartridge lid and start the assay.

8. Close the Xpert® cartridge lid

9. Start the assay in the timeframe specified in the Package Insert

Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the System
  - Error messages (if any)
  - Software version

- Log your complaint online using the following link ***http://www.cepheid.com/en/support:*** *Create a Support Case*

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