Assay Training: GeneXpert® BCR-ABL V2

Technical Training for Research Use Only (RUO) product only

301-7314, Rev. A March 2017

Training Agenda

- GeneXpert® BCR-ABL V2 Training
  - Reagents
  - Specimen transport and storage
  - Kit storage and handling
  - Preparing cartridge
  - Quality control
  - Results analysis
  - Discussion and Q&A

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Training Objectives

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

- Properly store and handle the GeneXpert BCR-ABL V2 cartridge kit.
- Follow proper laboratory safety precautions.
- Identify appropriate specimen types and transport specimen.
- Prepare a cartridge and run the assay.
- Report and understand the various software generated-results.
- Understand the assay control strategy.

Breakpoint Cluster Region-Abelson (BCR-ABL)

GeneXpert BCR-ABL V2 Overview

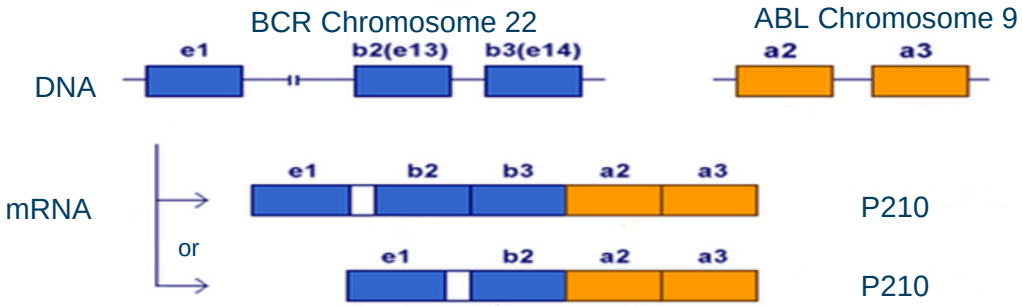
Xpert BCR-ABL V2 :
Xpert BCR-ABL V2 is a real-time RT-PCR (Reverse Transcription Polymerase Chain Reaction) offering unique features not available in current testing methods, including minimal hands-on time while delivering results in less than 2 hours.

The GeneXpert System:
The GeneXpert is the only system to combine sample preparation with real-time PCR amplification and detection for fully integrated and automated nucleic acid analysis. The system purifies, amplifies, detects, and identifies targeted nucleic acid sequences in less than 2 hours. The GeneXpert System requires minimal hands on time. For BCR-ABL, after a short sample preparation step, users simply add selected reagents and the prepared sample to the cartridge and the GeneXpert System does the rest.

GeneXpert BCR-ABL/ABL V2 Test (*IS*)
Xpert BCR-ABL/ABL V2 test reports results to the International Scale (*IS*) by using an assay-specific conversion factor determined by comparison to an IS reference assay.

GeneXpert BCR-ABL V2 Overview continued

- Quantitative, Multiplex, real-time RT-PCR assay
  - Peripheral Blood Specimen (EDTA or PAXgene Blood RNA collection tube)
- Detected with TaqMan® probes
  - BCR-ABL fusion genes resulting from two major breakpoints, translocations e13a2 (b2a2) and e14a2 (b3a2)
  - ABL transcript: Endogenous control



GeneXpert® BCR-ABL V2

The Cepheid Solution

- Simultaneous detection
  - Detects p210 major breakpoint translocations
  - e13a2 (b2a2) and e14a2 (b3a2) translocation
- Two controls for each sample
  - Endogenous Control (ABL)
  - Probe Check Control (PCC)
- High sensitivity and specificity
- Simple and easy to use
  - Closed cartridge system
- On-demand results
- Random access

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Summary

The GeneXpert BCR-ABL V2 assay, performed on the GeneXpert Instrument Systems, is a real-time RT-PCR (reverse transcription-polymerase chain reaction) test for the quantitative detection of the BCR-ABL1 chromosomal translocation mRNA transcripts (types e13a2/b2a2 or e14a2/b3a2) and the ABL1 endogenous control mRNA transcript in peripheral blood specimens.

The amount of BCR-ABL1 transcript is quantified as the ratio of BCR-ABL1/ABL1.

System and Reagent Requirements

GeneXpert Systems

- 6-color modules
- GXDX software v4.4a or higher
- Xpertise software v6.1 or higher

Test Kits (US-RUO)

- RBCRABL-10

Materials Required but not Provided

- Vortex mixer
- Microcentrifuge (1000 × g minimum)
- Pipettes and aerosol filter pipette tips
- 50mL conical tubes
- Reagent grade absolute ethanol
- 1N NaOH (if processing PAXgene samples)

GeneXpert BCR-ABL V2 Kit Components

GeneXpert BCR- ABL V2	
Catalog Number	RBCRABL-10
Tests per kit	10
Reagents (10 of each)	Proteinase K (PK)
	Lysis reagent (LY)
	Wash reagent (1)
Kit CD	Assay Definition File (ADF)
	Instructions to import ADF
	Package insert
Storage	2-8ºC

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Good Laboratory Practice

PCR laboratory setup	• Cartridge/reagent preparation → Sample addition → Detection
Specimen and reagent storage	• Store specimens separately from reagents to prevent reagent contamination.
Equipment	• Use filtered pipette tips, when needed. • Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment.

Good Laboratory Practice, continued

Housekeeping	• Clean work surfaces with a 1:10 dilution of household bleach* in water and then a 70% ethanol solution. Wipe work surfaces dry.
Personnel	• Wear clean lab coats and gloves. • Change gloves between processing samples.
Lab bench area	• Clean the lab bench area routinely. • Keep the back of the instrument dust free.

** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*

GeneXpert BCR-ABL V2 Kit Storage and Handling

- Store the GeneXpert BCR-ABL V2 kit contents at 2–8ºC.
- Do not open the cartridge lid until you are ready to perform the assay.
  - Cartridge should be placed on the instrument within 60 minutes of adding the sample and reagents into the cartridge.
- Except for the Lysis Reagent (LY), do not use reagents that have become cloudy or discolored.
- Do not use expired cartridges.
- Do not use cartridge if a reagent is added to the wrong opening.
- Do not shake the cartridge.
- Do not use a cartridge that has leaked.
- Each single-use cartridge is used to process one test only. Do not reuse processed cartridges.

Specimen Collection

GeneXpert BCR-ABL V2
Specimen Transport and Storage

Specimen	Transport and Storage Temperature (˚C)	Storage Time
Whole Blood EDTA tubes	2-8 ºC	72 hours
PAXgene Blood RNA tube (PreAnalytiX)	2-8 ºC	120 hours

- ⚠ Do not use heparin as the anticoagulant because it can inhibit the PCR reaction.
- ⚠ Do not separate plasma from cells.

GeneXpert BCR-ABL V2 Testing Protocol

1. Twenty minutes prior to starting procedure, remove blood specimen and Sample Prep reagents from storage.
2. Briefly microcentrifuge the Proteinase K (PK) reagent prior to use
3. Ensure that blood sample is well-mixed by inverting the collection tube 8 times immediately before pipetting.

GeneXpert BCR-ABL V2- Lysate Preparation

GeneXpert® BCR-ABL V2 Lysate Preparation

• GeneXpert BCR-ABL V2

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

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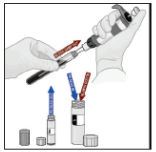
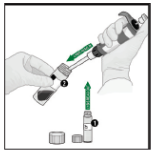
A better way.

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

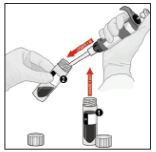
2 Briefly centrifuge PK reagent. Add 100uL of PK reagent to a 50mL conical tube. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.



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.

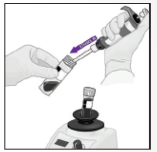


4 Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.



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.

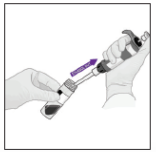
6 To the same conical tube, add 2mL of reagent grade absolute EtOH. Vortex for 10 sec and set aside. Discard remaining PK or LY reagents.



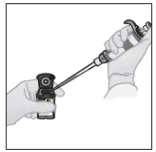
7 Open the Xpert cartridge lid.

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.

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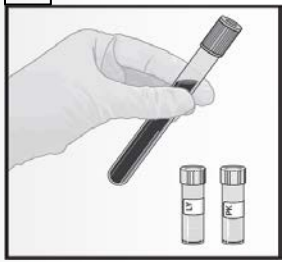
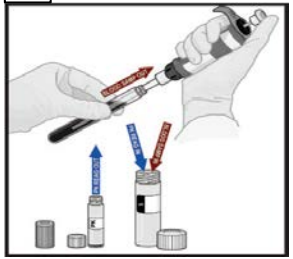
GeneXpert BCR-ABL V2 Lysate Preparation

1



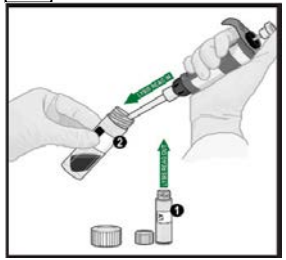
Remove EDTA whole blood and sample prep reagents from refrigerator. Place EDTA blood on rocker or invert 8 times prior to sampling.

2



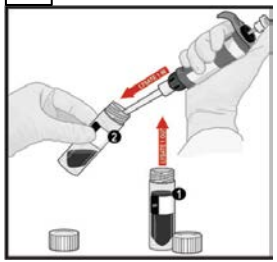
Briefly centrifuge PK reagent. Add 100uL of PK reagent to a 50mL conical tube. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.

3



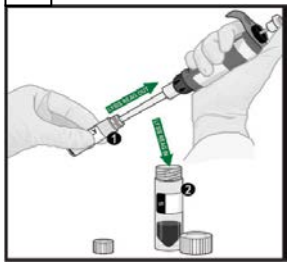
Add 2.5mL of lysis reagent (LY) to same tube, vortex 10 sec, and incubate 5 min at RT. Vortex again for 10 sec and incubate a 2nd time for 5 min. Mix by tapping tube 10x.

4



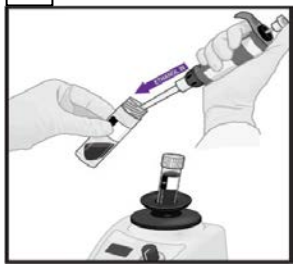
Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.

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.

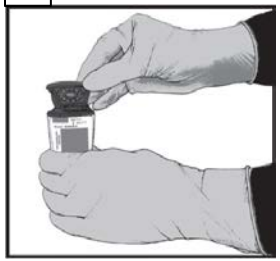
6



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.

GeneXpert BCR-ABL V2 Cartridge Preparation

7

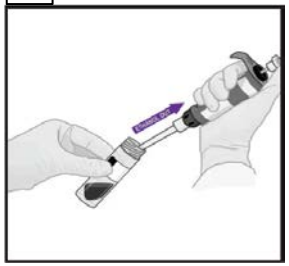


Open the Xpert cartridge lid.

8

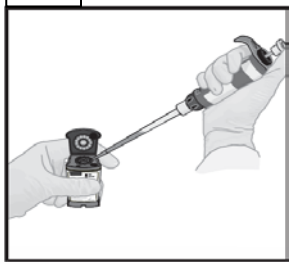
Transfer entire contents of Wash Reagent ampoule into Chamber 1.

9



Pipette entire contents of final prepared lysate from the 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.

Automated GeneXpert BCR-ABL V2 Test Steps

1. Add the sample to the cartridge
2. Place the cartridge into the instrument.
3. Nucleic acids are purified.
4. Purified nucleic acids mix with PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.

Quality Control

Refer to the Package Insert for complete details

Instrument System Control – Check Status

- System control checks the optics, temperature of the module, and mechanical integrity of each cartridge.
  - If the system controls fail, an ERROR test result will be reported.

Cepheid Assay Control Strategy

- Each GeneXpert cartridge is a self-contained test device.
  - Cepheid designed specific molecular methods including internal controls, that enable the system to detect specific failure modes within each cartridge.
    - Instrument system control: Check status
    - Reagent control: Probe Check
    - Endogenous control: ABL
    - Amplification control: ABL

Probe Check Control - PCC

- After sample preparation, bead reconstitution, and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures.
- The readings are compared to default settings established by Cepheid.
- The Probe Check feature controls for:
  - Missing Target Specific Reagent (TSR) beads, which contain all primers, probes, and internal control template
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Taqman probe degradation
- If the Probe Check fails, an ERROR test result will be reported.

Endogenous Control - ABL

- The Endogenous Control (ABL) normalizes the BCR-ABL target and ensures that sufficient sample is used in the assay.
- Serves as normalization control
- ABL control checks for adequate processing and quality of the sample
  - Missing primer/probe or enzyme beads
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Enzyme degradation
  - Inhibition of the RT-PCR or nested PCR reactions
- If the ABL fails in a sample, an INVALID test result will be reported.

Results Analysis

Refer to the Package Insert for complete details

Definitions

- 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 $E_{\Delta Ct}$ are determined in quality control testing of each assay lot using secondary standards derived from the World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript.
- Together, the secondary standards and the lot-specific $E_{\Delta Ct}$ and *SF* values calibrate the quantitative output of the assay to the *IS* (International Scale).

Quantitative Results

- A data sheet is supplied with each GeneXpert BCR-ABL V2 Assay kit that contains a lot-specific standard curve for the GeneXpert BCR-ABL V2 kit and an Efficiency Value ($E_{\Delta Ct}$).
- The Efficiency Value is embedded in the barcode of the GeneXpert BCR-ABL V2 cartridge. See the data sheet 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*).

Data Analysis and Results Reporting

BCR-ABL Detected

- For a “BCR-ABL has been detected at a level of...” result, the GeneXpert software calculates the % BCR-ABL/ABL (*IS*) using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL Ct:

  $$\%\ \text{BCR-ABL/ABL } IS = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times \text{Scaling Factor (SF)}^*$$

- Example:

  Lot-specific $E_{\Delta Ct} = 1.96$

  *Lot-specific Scaling Factor = 1.22

  Assay’s ABL Ct = 11.3; BCR-ABL Ct = 27.4 ; ΔCt = -16.1

  % BCR-ABL/ABL *IS* = 1.96 (-16.1) x 100 x 1.22 = 0.0024% (*IS*)

  Result: BCR-ABL has been detected at a level of 0.0024% (*IS*).

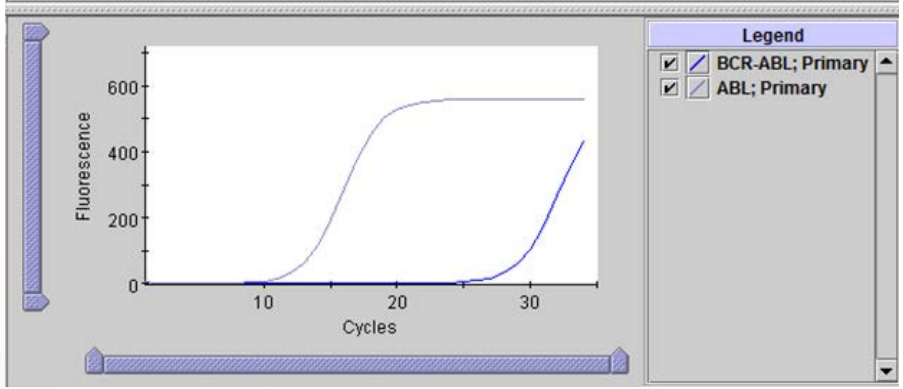
BCR-ABL Detected

Test Result	BCR-ABL has been detected at a level of 0.0024% (IS)

- BCR-ABL has been detected at a level of 0.0024% (IS)

Test Result | Analyte Result | Detail | Errors | History | Messages | Support

Analyte Name	Ct	EndPt	Interpretation Result	Reason	Analyte Result	Probe Check Result	Target Delta Ct
BCR-ABL	27.4	429	POS		POS	PASS	-16.1
ABL	11.3	562	PASS		PASS	PASS	



BCR-ABL transcript not detected at a detection limit of….

- BCR-ABL was not detected – BCR-ABL1 transcript was not detected within the valid Ct range or above the endpoint (EndPt) threshold setting.
- ABL – PASS; ABL1 transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting
- Probe Check – PASS; all probe check results passed.

Data Analysis and Results Reporting

BCR-ABL Not Detected

- When BCR-ABL is not detected, the GX software calculates the ΔCt by subtracting 32 from the ABL Ct (ABL Ct - 32)
- Example:

  Lot-specific $E_{\Delta Ct}$ = 1.96

  Assay's ABL Ct = 12.2

  Lot-specific Scaling Factor = 1.22

  Theoretical ΔCt = (12.2-32) = -19.8

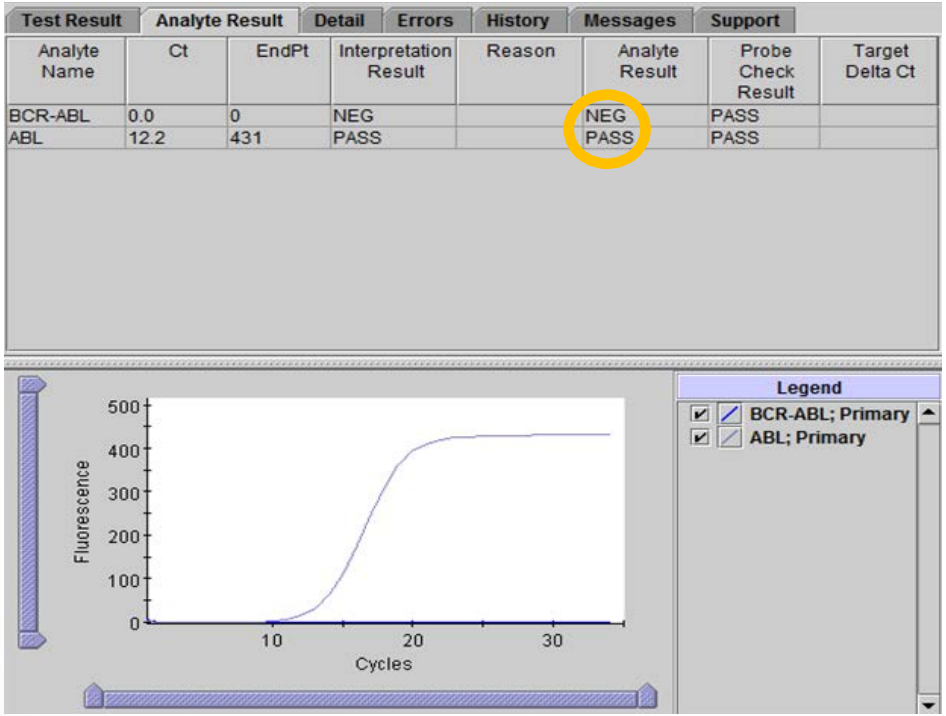
Theoretical % BCR-ABL/ABL (*IS*) = 1.96 (-19.8) x 100 x 1.22 = 0.0002% (*IS*)

Result: BCR-ABL was not detected at a detection limit of 0.0002% (*IS*).

BCR-ABL Not Detected

Test Result	BCR-ABL was not detected at a detection limit of 0.0002% (IS)

- BCR-ABL was not detected at a detection limit of 0.0002% (IS).



Reasons to Repeat the Assay

- An INVALID result indicates that the endogenous ABL control failed.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted due to an improperly filled reaction tube, a reagent probe integrity problem, or because the maximum pressure limits were exceeded. Other mechanical errors can also cause this result.
- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, a load error occurred, or the software was closed prior to completing the test.

INVALID

BCR-ABL1 transcript level cannot be determined because the sample contains excess BCR-ABL1 and/or ABL1 transcripts.

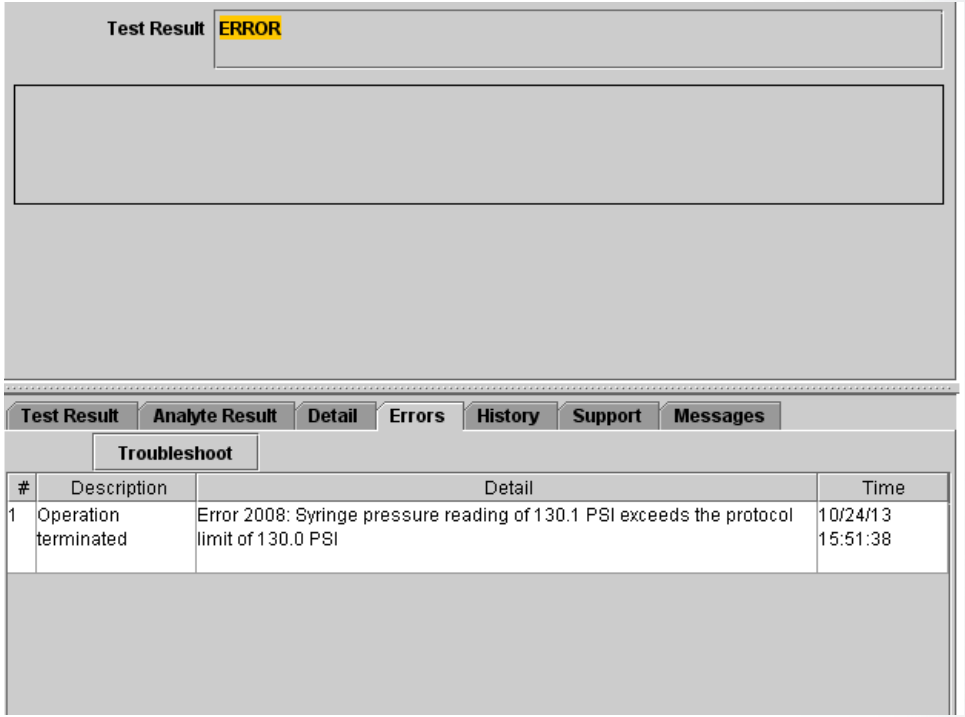
- Sample contains excess BCR-ABL1 and/or ABL1 transcript.
- Endogenous control ABL failure:
  - ABL – FAIL – ABL1 cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting.
  - Probe Check – PASS, all probe check results passed.
- Poor sample quality
- RT-PCR inhibition
- If ABL Ct > 18, and/or endpoint <200

ERROR

BCR-ABL1 transcript level cannot be determined.

- BCR-ABL – NO RESULT
- ABL – NO RESULT
- Probe Check – PASS*/FAIL; all or one of the probe check results failed.

* If the probe check passed, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.



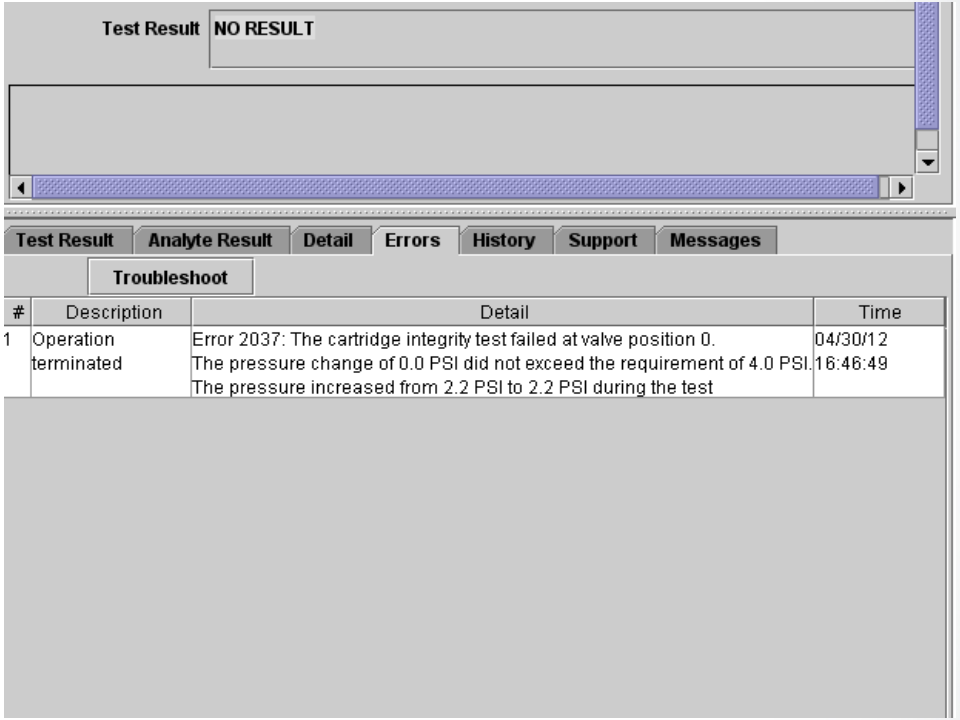
No Result

- Presence or absence of BCR-ABL or ABL target RNAs cannot be determined.
- Repeat the test according to the instructions in the Retest Procedure section in the package insert.
- BCR-ABL: NO RESULT
- ABL: NO RESULT

- Probe Check: NA (not applicable)

* If the probe check passed, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.

Example:



GeneXpert BCR-ABL V2 Retest Procedure

1

Discard used cartridge.

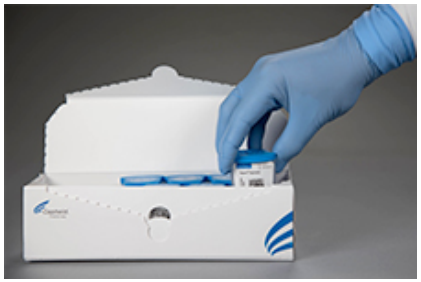
Follow your institution's safety guidelines for disposal of cartridges.

2

Obtain the original blood sample collection tube or the retained lysate.

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample.

3



Obtain a new cartridge.

Process the sample per the package insert.

4

Close the cartridge lid.

Run the test on the system.

Factors That Negatively Affect Results

- Improper specimen collection
  - Performance with other collection devices and specimen types has not been assessed.
- Improper transport or storage of collected specimen
  - Storage and transport conditions are specimen specific.
  - Refer to the package insert for the appropriate handling instructions.
- Improper testing procedure
  - Modification to the testing procedures may alter the performance of the test.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the package insert is necessary to avoid erroneous results.
- Interfering substance
  - False negative test results or invalid results may be observed in the presence of an interfering substance.
- Excessively high white blood cell counts might cause pressure to build in the cartridge and lead to aborted runs

Limitations

- Refer to the Package Insert for a complete list of limitations.

Technical Support

Cepheid provides technical support in the field, on the phone, by fax, and by email.

- **Contact information for Cepheid offices is available on our website at http://www.cepheid.com/support**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