Technical Training
Xpert® NPM1 Mutation

Catalog Number (GXNPM1-CE-10)
For CE-IVD Only

303- 0232 Rev. C February 2024

Training Objectives

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

- Properly store and handle the Xpert® NPM1 Mutation cartridge kit
- Follow proper laboratory safety precautions
- Collect and transport appropriate sample
- Prepare a cartridge and run the Xpert® NPM1 Mutation test
- Report the various software generated results
- Understand the Xpert® NPM1 Mutation control strategy

Training Agenda

1. Overview
2. Kit Handling
3. Sample Collection
4. Cartridge Preparation
5. Quality Controls
6. Results Interpretation
7. Troubleshooting

Overview

The Cepheid Solution

- Quantitative detection
- On-board internal controls for each sample
  - Probe Check Control (PCC)
  - ABL Endogenous Control
- Results in less than 3 hours
- Approximately 30 minutes sample preparation and less than 2.5 hours assay run time
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

Intended Use

- The Xpert® NPM1 Mutation test, performed on the Cepheid GeneXpert® Dx System is an *in vitro* diagnostic test for the quantification of mutant NPM1 mRNA transcripts (types A, B and D in exon 12) in peripheral blood specimens from patients with Acute Myeloid Leukemia (AML).
- The test utilizes automated real-time reverse transcription polymerase chain reaction (RT-PCR) and reports the percent ratio of mutant NPM1 to ABL1 endogenous control mRNA transcripts.
- The test is intended as an aid in monitoring patients with NPM1-mutated AML for the level of mutant NPM1 mRNA transcript. The test should be used in conjunction with other clinicopathological factors.
- The Xpert® NPM1 Mutation test does not differentiate between A, B or D type mutant NPM1 transcripts and does not detect or monitor other rare types of mutant NPM1.
- This test is not intended for the diagnosis of AML.

Intended User/Environment

- The Xpert® NPM1 Mutation test is intended for use by trained users in a laboratory setting.

Targets

- NPM1 mutations mRNA transcripts types A, B and D in exon 12
- ABL1 Endogenous Control

Xpert® NPM1 Mutation Requirements

GeneXpert® Systems

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

Test Kits

- Catalog Number (GXNPM1-CE-10)

Sample Collection

- Peripheral Blood collected in EDTA tubes

Other Materials

- Personal Protective Equipment (PPE)
- 1:10 Bleach / Sodium Hypochlorite (0.5% final concentration freshly prepared on daily basis)
- 70% ethanol or denatured ethanol
- Vortex mixer
- Microcentrifuge (1000 × g minimum)
- Pipettes and aerosol filter pipette tips
- 50 mL conical tubes
- Reagent grade absolute ethanol
- 1X PBS, pH 7.4

Other Materials

- Uninterruptible Power Supply /Surge Protector
- Printer If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

Good Laboratory Practice Review

Personal Protective Equipment (PPE)	• Wear clean lab coats, 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 Handling

Xpert® NPM1 Mutation Kit Contents

Catalog Number	GXNPM1-CE-10
Cartridges* Per Kit	10
Reagents vials (10 each)	Proteinase K (PK) Lysis Reagent (LY)(Guanidium Chloride) Wash Reagent
Kit CD	Xpert NPM1 Mutation Assay Definition File (ADF) Xpert NPM1 Mutation Import Instructions Instructions For Use (IFU)
Storage	2 - 8 °C

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

Kit Storage and Handling

- Store the Xpert® NPM1 Mutation Kit at 2–8˚C
- Do not open the cartridge lid until you are ready to perform the test.
- Do not use a cartridge that has leaked
- The Wash Reagent is a clear, colourless liquid. Do not use the Wash Reagent if it has become cloudy or discoloured.
- Twenty (20) minutes before starting the procedure, remove the blood sample, cartridge, and sample preparation reagents from storage to allow them to come to room temperature (20 ˚C to 30 ˚C).
- Do not use a cartridge past the expiration date.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Treat all biological samples, 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 samples should be treated with standard precautions.
- Guidelines for sample handling are available from U.S. Centers for Disease Control and Prevention[6] and Clinical and Laboratory Standards Institute.[7]
- Follow safety procedures set by your institution for working with chemicals and handling biological samples.
- Performance characteristics of this test have been established with blood collected in EDTA tubes only. The assay function has not been evaluated with other sample types.

6. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
7. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).

Warnings and Precautions continued

- Reliable results are dependent on adequate sample collection, transport, storage, and processing. Incorrect assay results may occur from improper sample collection, handling or storage, technical error, sample mix-up or because the target transcript in the sample is below the limit of detection of the assay. Careful compliance with this Instruction for Use and the *GeneXpert® Dx System Operator Manual* are necessary to avoid erroneous results.
- Performing the Xpert® NPM1 Mutation test outside the recommended kit or sample storage temperature ranges and time may produce erroneous or invalid results.
- Biological samples, 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 samples and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[8]

[8] Health-care Waste. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/health-care-waste.

Xpert® NPM1 Mutation Limitations

- The assay is not intended to be used with external calibrators.
- Modifications to these procedures may alter the function of the assay.
- This product was designed for use with blood collected in EDTA tubes only.
- Do not use heparin as the anticoagulant because it can inhibit the PCR reaction.
- Sodium citrate, buffy-coat and bone marrow sample types have not been validated.
- Erroneous assay results might occur from improper sample collection, handling or storage or sample mix-up. Careful compliance with the Instructions for Use is necessary to avoid erroneous results.
- 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.
- Excessively high white blood cell counts might cause pressure to build in the cartridge and lead to aborted runs or inaccurate results.
- Some samples 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 sample.

Specimen Collection, Storage and Transport

Sample Transport and Storage

- Peripheral blood samples should be collected in EDTA tubes following your institution's guidelines.
- Plasma should not be separated from cells

Specimen Type	Storage
Whole blood sample	2 - 8 ºC for up to 3 days

Cartridge Preparation

Proper Cartridge Handling Techniques

Correct

- Do not touch the reaction tube
- Keep the cartridge upright
- Do not tilt after sample is added

Incorrect

Before Starting the Procedure…

- Twenty (20) minutes before starting the procedure, remove the blood sample, sample 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.
- Start the assay within **1 hour** of adding the Sample Reagent-treated sample to the cartridge.
- Remove the cartridge from the cardboard packaging before preparing the sample.

Xpert® NPM1 Mutation Cartridge Preparation Sample with Unknown WBC count OR < than 30 million WBCs/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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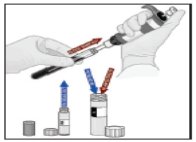
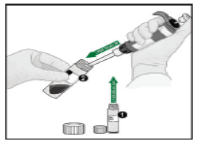
1 Remove EDTA whole blood and sample prep reagents from refrigerator. Place EDTA blood on rocker or invert 8 times prior to sampling.

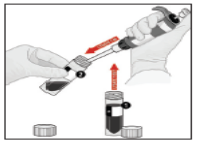
2 Briefly centrifuge PK reagent. To a 50mL conical tube, add 100uL of PK reagent. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.



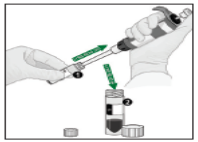
3 Add 2.5mL of lysis reagent (LY) to same tube, vortex 10 sec, and incubate 5 min at RT. Vortex again for 10 sec and incubate a 2nd time for 5 min. Mix by tapping tube 10x.



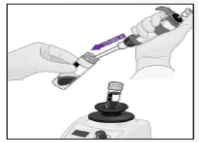
4 Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.



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.

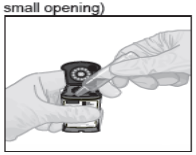


6 To the same conical tube, add 2mL of reagent grade absolute EtOH. Vortex for 10 sec and set aside. Discard remaining PK or LY reagents.

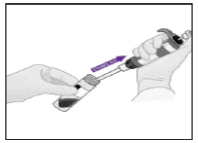


7 Open the Xpert test cartridge lid.

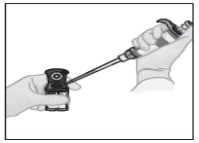
8 Transfer entire contents of Wash Reagent ampoule into Wash Reagent Chamber (with small opening)



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.

301-4954, Rev. E February 2023

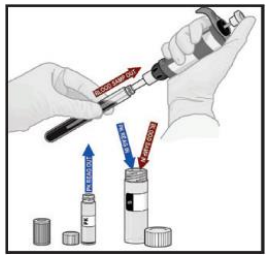
Xpert® NPM1 Mutation Cartridge Preparation- Sample with WBC at Equal to or Greater than 30 million WBCs/mL

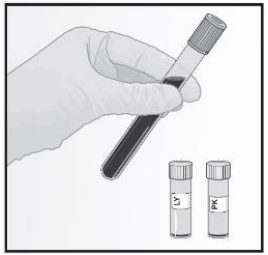
1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K). Ensure the blood specimen is well-mixed by inverting the EDTA blood collection tube 8 times immediately before pipetting

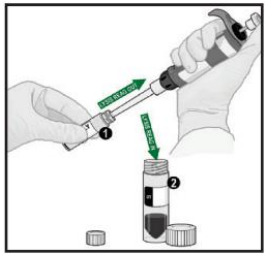
2. Add 250 µL of blood sample and 3.75 mL of 1xPBS (pH7.4, provided by user). Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min

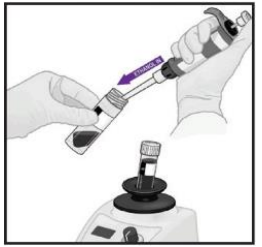
3. To the same tube, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Mix the sample by tapping the bottom of the tube 10 times. Transfer 1mL of prepared lysate into new 50 mL conical tube

4. To the same conical tube, add 1.5 mL of retained Lysis Reagent (LS). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 10 min



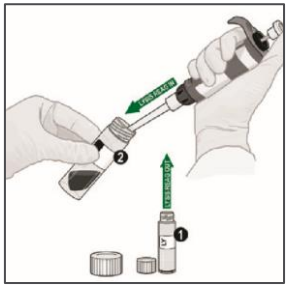






Xpert® NPM1 Mutation Cartridge Preparation- Sample with WBC at Equal to or Greater than 30 million WBCs/ mL continued

5. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user)



6.Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside at room temperature.

7. Remove the cartridge from the cardboard packaging

8. Inspect the cartridge for damage. If damaged, do not use it.

9. Open the cartridge Lid. Transfer the entire contents of Wash Reagent (1) ampoule into Wash Reagent Chamber (with small opening).

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

11. Close the Xpert® cartridge lid

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

Saving remaining lysate

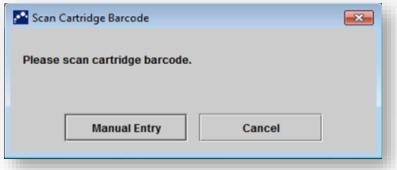
- Store remaining lysate at 2-8ºC for up to 48 hours OR store at -20ºC or lower for up 1 month

Run a Test on GeneXpert® Dx

1 Create a test.

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

2 Scan barcode for Patient and/or Sample ID.



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)

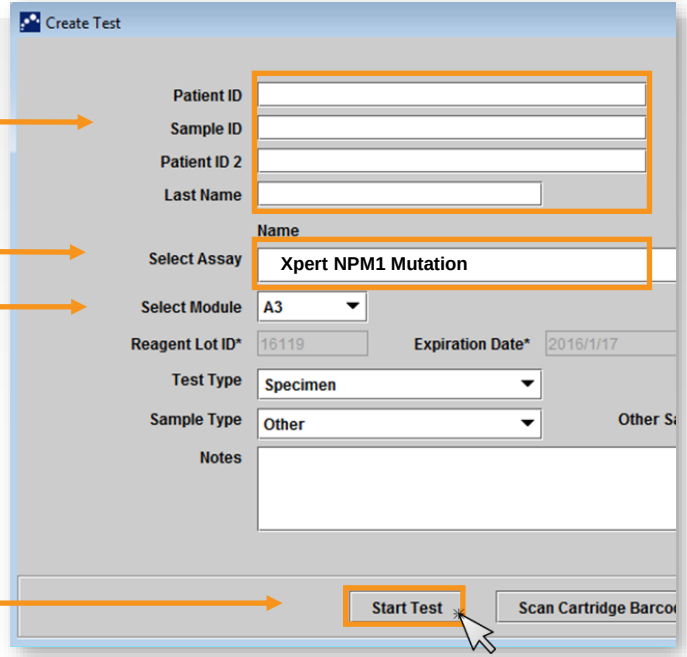
4 Complete the fields as required.

5 Xpert® NPM1 Mutation 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® NPM1 Mutation 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® NPM1 Mutation Control Strategy

CONTROL

- Xpert® NPM1 Mutation 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:
    - **Probe Check Controls (PCC)**
    - **ABL1 Endogenous Control**

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

Internal Quality Controls

- **ABL1 Endogenous Control**
  - Normalizes the NPM1 Mutation target
  - Ensures that sufficient sample is used in the assay
  - Detects sample-associated inhibition of the real-time PCR assay

- **Probe Check Controls (PCC)**
  - Before the PCR step, the fluorescence signal is measured from all probes and compared with default settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability
  - Checks if all reaction components are functional in the catridge
  - The PCC passes if it meets the assigned acceptable criteria

Commercially Available External Controls

CONTROL

- Contact Technical Support for inquiries about the External Controls on :

  Email: support@cepheideurope.com

- Contact information for all Cepheid Technical Support offices is available on our website:

http://www.cepheid.com/en/support/contact-us

Result Interpretation

Possible Results outputs

Result	Interpretation
NPM1 Mutation DETECTED	NPM1 mutation transcript was detected. ○ NPM1 MUTATION DETECTED [#.##%] ○ NPM1 MUTATION DETECTED [Above upper LoQ] ○ NPM1 MUTATION DETECTED [Below LoD; <#.###%]
NPM1 Mutation NOT DETECTED	NPM1 mutation transcript was not detected.
INVALID	NPM1 Mutation transcript level cannot be determined due to sample containing excess NPM1 mutation transcript and/or excess or insufficient ABL transcript
ERROR	NPM1 Mutation transcript level cannot be determined.
NO RESULT	NPM1 Mutation transcript level cannot be determined. Insufficient data were collected to produce an assay result.

Quantitative Results

- Xpert® NPM1 Mutation quantitative outputs are provided as a percent ratio of NPM1 Mutation/ABL1. Kits are assigned lot-specific Efficiency ($E_{\Delta Ct}$) and Scaling Factor (SF) values that tie the quantitation of NPM1 Mutation (A,B and D) and ABL1 transcripts to copy numbers of synthetic NPM1 Mutation and ABL1 in vitro transcribed RNA (IVT-RNA) primary standards.

NPM1 Mutation DETECTED [#.##]%

NPM1 mutation has been detected at a level of #.##%.

- For a "**NPM1 Mutation DETECTED [#.##%]"** result, NPM1 mutation is detectable with NPM1 Mutation Ct greater than or equal to "6" and less than or equal to "32" and ABL Ct greater than or equal to "6" and less than or equal to "20".
- The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus NPM1 Mutation Ct:

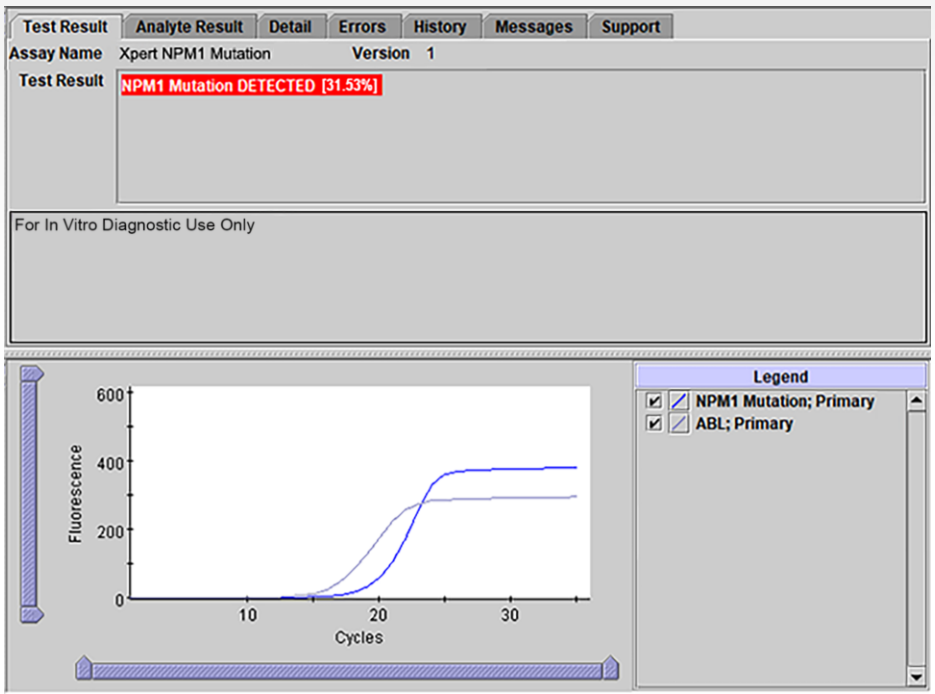
$$\% = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times \text{Scaling Factor}$$

The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using primary standards calibrated to the copy numbers of synthetic NPM1 mutation and ABL1 *in vitro* transcribed RNA (IVTRNA) calibrators for quantitation of NPM1 mutation transcript. The $E_{\Delta Ct}$ is set for 1.95 and *SF* value is set for 1.79 in the use in the example below:

Example: Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79
Assay's ABL Ct = 14.5; NPM1 Mutation Ct = 17.1; ΔCt = -2.6
$\% = 1.95^{(-2.6)} \times 100 \times 1.79 = 31.53\%$

Result: NPM1 Mutation DETECTED [31.53%].

NPM1 Mutation DETECTED [#.##]% continued



- NPM1 Mutation – Detected [#.##]%
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 32, and Endpoint above threshold (Example: NPM1 Mutation Ct = 17.1)
- ABL – PASS
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 20, and Endpoint above threshold (Example: ABL Ct = 14.5)
- Probe check control – PASS
  - All probe check results passed

NPM1 Mutation DETECTED [Above upper LoQ]

NPM1 mutation has been detected at a level > 500%.

- For a "**NPM1 Mutation DETECTED [Above upper LoQ]**" result, NPM1 mutation is detectable with NPM1 Mutation Ct greater than or equal to "6" and less than or equal to "32" and ABL Ct greater than or equal to "6" and less than or equal to "20".
- The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus NPM1 Mutation Ct:

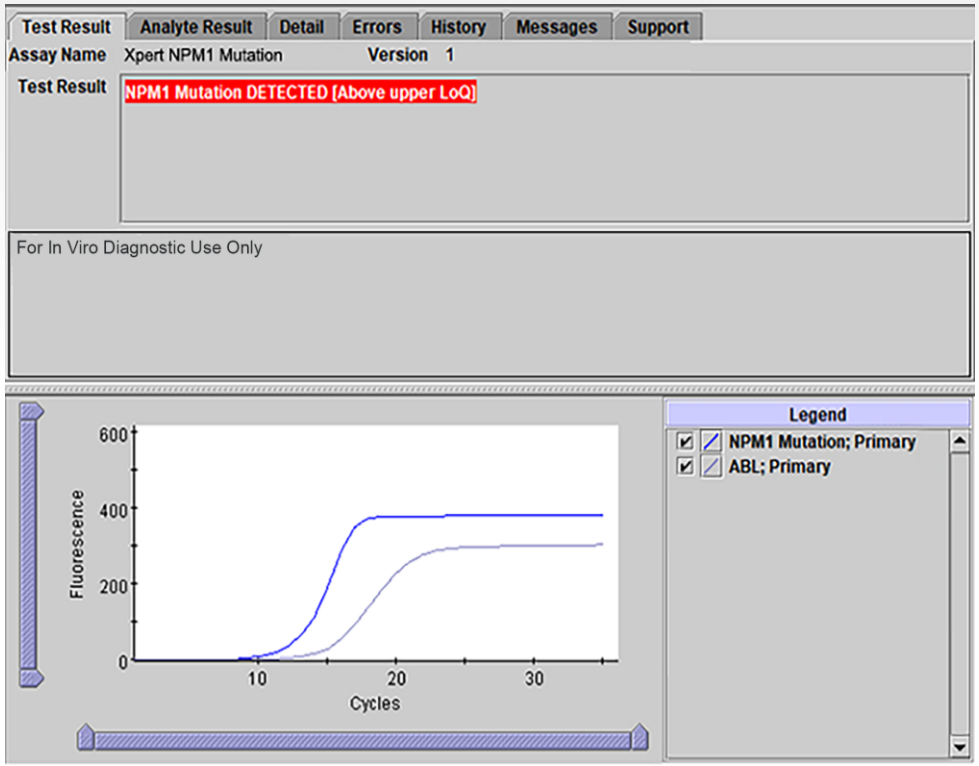
$$\% = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times \text{Scaling Factor (SF)}$$

The Scaling Factor (SF) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using primary standards calibrated to the copy numbers of synthetic NPM1 mutation and ABL1 in vitro transcribed RNA (IVTRNA) calibrators for quantitation of NPM1 mutation transcript. The $E_{\Delta Ct}$ is set for 1.95 and SF value is set for 1.79 for use in the next example shown:

Example: Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79
Assay's ABL Ct = 13.4; NPM1 Mutation Ct = 10.2; ΔCt = 3.2
$\% = 1.95^{(3.2)} \times 100 \times 1.79 = 1516.92\%$ is greater than the defined assay upper LoQ at 500%

Result: NPM1 Mutation DETECTED [Above upper LoQ].

NPM1 Mutation DETECTED [Above upper LoQ] continued



NPM1 Mutation has been detected at level > 500%

- NPM1 Mutation – Detected [Above upper LoQ]
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 32, and Endpoint above threshold (Example: NPM1 Mutation Ct = 10.2)
- ABL – PASS
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 20, and Endpoint above threshold (Example: ABL Ct = 13.4)
- Probe check control – PASS
  - All probe check results passed

NPM1 Mutation DETECTED [Below LoD, < 0.030%]

NPM1 mutation has been detected at a level of < 0.030%.

- For a "**NPM1 Mutation DETECTED [Below LoD, < 0.030%]"** result, NPM1 mutation is detectable with NPM1 Mutation Ct greater than or equal to "6" and less than or equal to "32" and ABL Ct greater than or equal to "6" and less than or equal to "20".
- The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus NPM1 Mutation Ct:

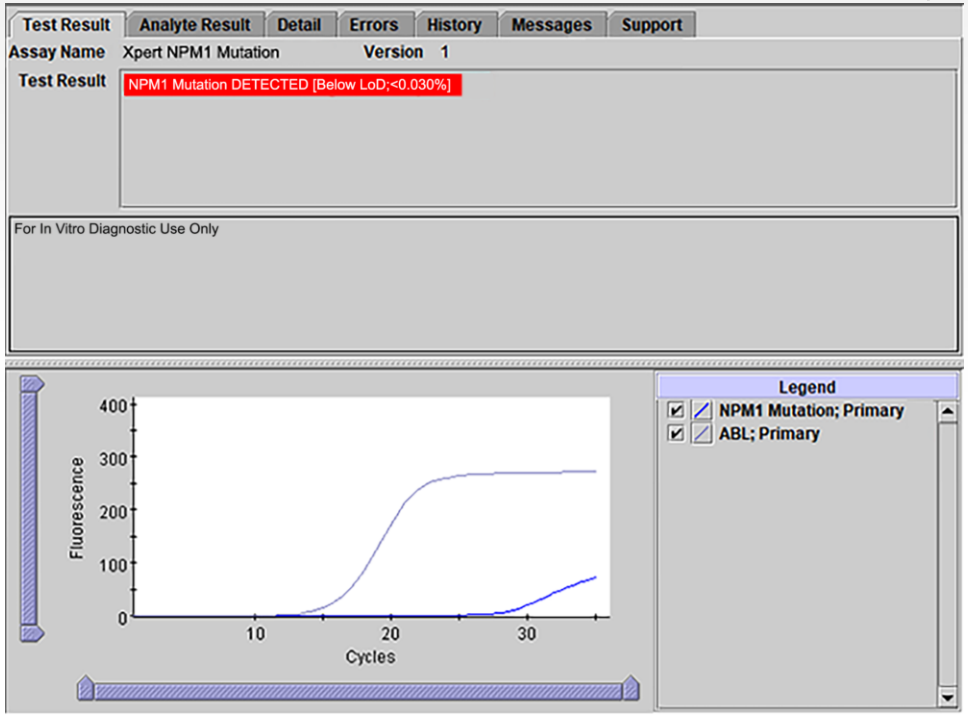
$$\% = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times \text{Scaling Factor}$$

The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using primary standards calibrated to the copy numbers of synthetic NPM1 mutation and ABL1 *in vitro* transcribed RNA (IVTRNA) calibrators for quantitation of NPM1 mutation transcript. The $E_{\Delta Ct}$ is set for 1.95 and *SF* value is set for 1.79 in the use in the example below:

Example: Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79
Assay's ABL Ct = 14.3; NPM1 Mutation Ct = 28.8; ΔCt = -14.5
% = $1.95^{(-14.5)} \times 100 \times 1.79$ = 0.011% is less than the defined assay LoD at 0.030%

Result: NPM1 Mutation DETECTED [Below LoD; <0.030%]

NPM1 Mutation DETECTED [Below LoD, < 0.030%] continued



NPM1 Mutation has been detected at level < 0.030%

- NPM1 Mutation – Detected [Above upper LoQ]
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 32, and Endpoint above threshold (Example: NPM1 Mutation Ct = 28.8)
- ABL – PASS
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 20, and Endpoint above threshold (Example: ABL Ct = 14.3)
- Probe check control – PASS
  - All probe check results passed

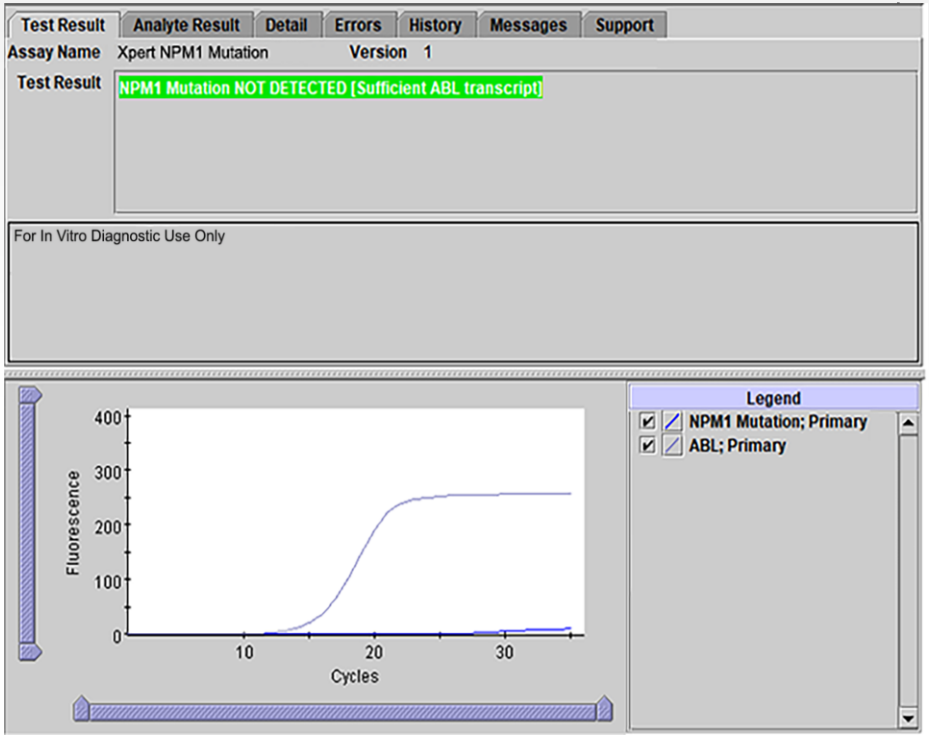
NPM1 Mutation NOT DETECTED [Sufficient ABL Transcript]

- NPM1 mutation was not detected with NPM1 Ct equal to “0” or greater than “32” and ABL Ct greater than “6” and less than or equal to “20”.
- The GeneXpert software requires the ABL Ct to be greater than or equal to “6” and less than or equal to “20” for the Xpert NPM1 Mutation test to ensure having “Sufficient ABL transcript”.

Example: Assay’s NPM1 Mutation Ct = 0; ABL Ct = 14.0 is between "6" and "20".

Result: NPM1 Mutation NOT DETECTED [Sufficient ABL transcript].

NPM1 Mutation NOT DETECTED [Sufficient ABL Transcript] continued



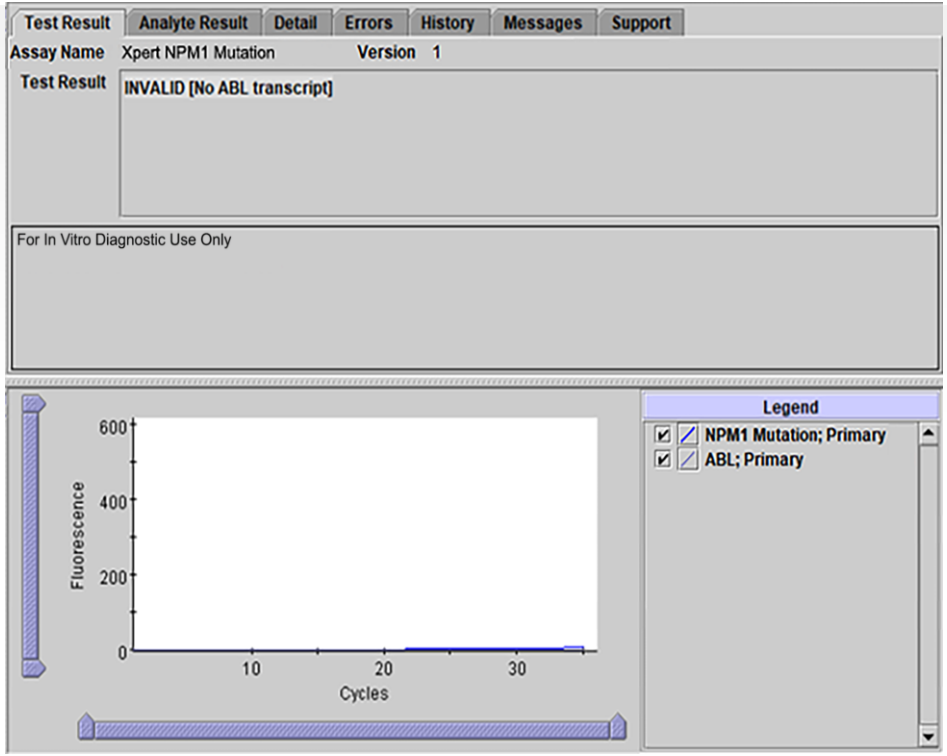
- NPM1 Mutation – Not Detected
  - No cycle threshold (Ct) or Ct = 0, or Endpoint lower than threshold setting (Example: NPM1 Mutation Ct = 0)
- ABL – PASS
  - Cycle threshold (Ct) within valid range: 6 ≤ Ct ≤ 20, and Endpoint above threshold (Example ABL Ct = 14.0)
- Probe check control – PASS
  - All probe check results passed

Troubleshooting

Factors That Negatively Affect Results

- Improper sample collection.
  - The performance of this assay with other specimen types or samples has not been evaluated.
- 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.

INVALID [No ABL transcript]



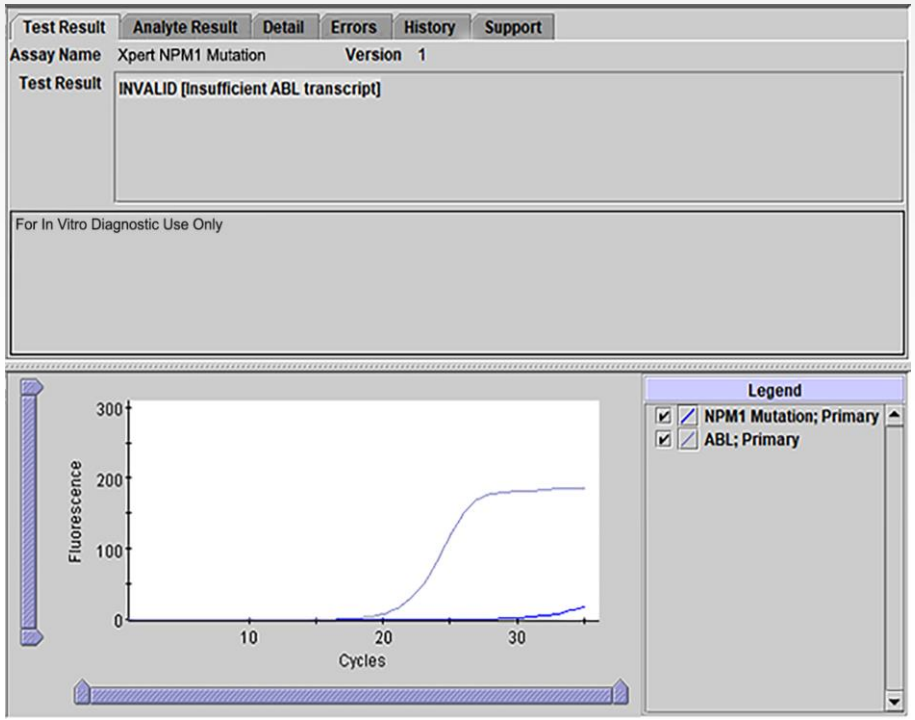
- NPM1 mutation was detected or not detected with ABL Ct equal to 0
- GeneXpert software requires ABL Ct to be greater than OR equal to “6” AND less than or equal to “20”.

Example : Assay’s NPM1 Mutation Ct =0

ABL Ct = 0

Result : INVALID [No ABL transcript]

INVALID [Insufficient ABL transcript]



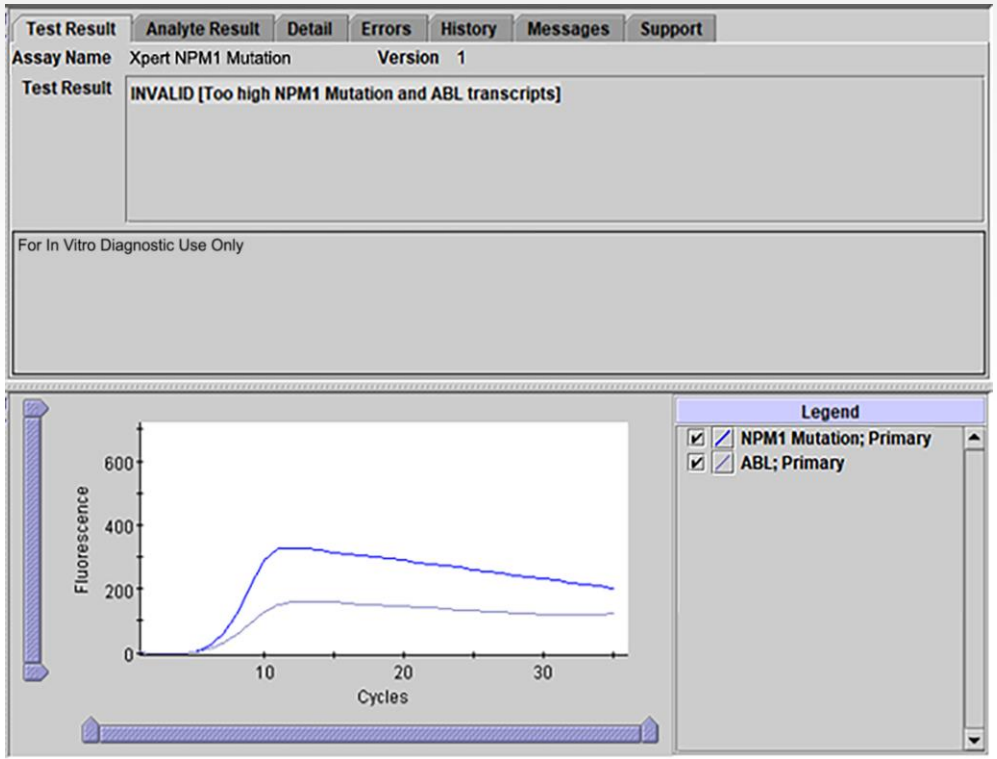
NPM1 mutation was detected or not detected with ABL Ct greater than "20".

- The GeneXpert software requires the ABL Ct to be greater than or equal to “6” and less than or equal to “20” for the Xpert NPM1 Mutation test to ensure having “Sufficient ABL transcript”.

Example: Assay’s NPM1 Mutation Ct = 33.3; ABL Ct = 20.2 is greater than "20".

Result: INVALID [Insufficient ABL transcript].

INVALID [Too high NPM1 Mutation and ABL transcripts]



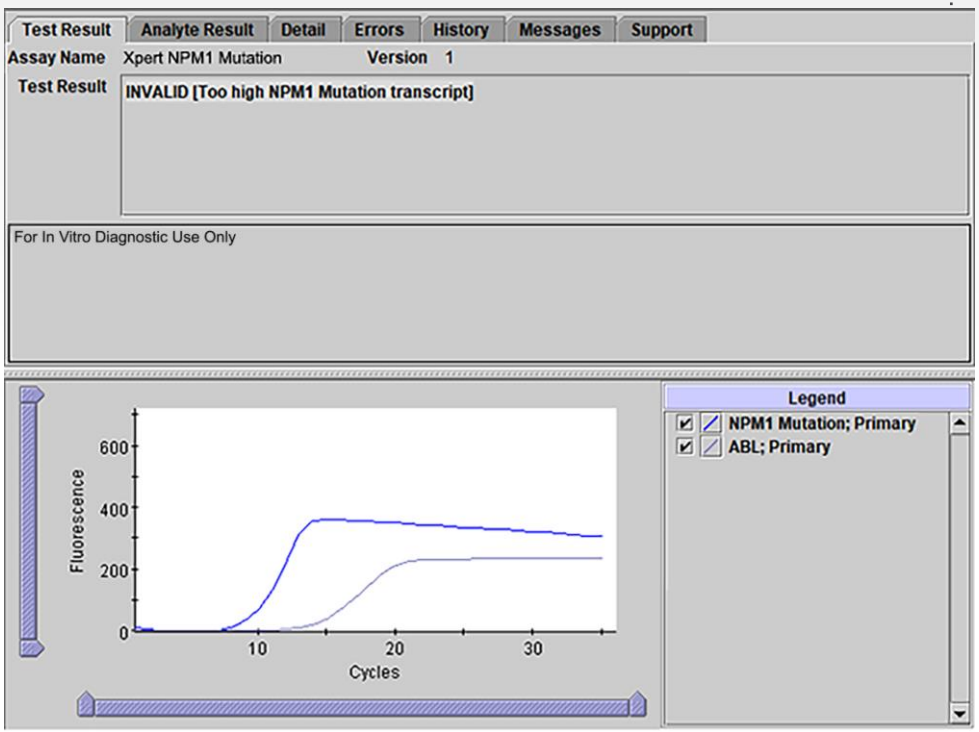
NPM1 mutation was detected with both NPM1 Mutation and ABL Cts greater than "0" and less than "6" .

The GeneXpert software requires the ABL Ct to be greater than or equal to “6” and less than or equal to “20” for the Xpert NPM1 Mutation test to ensure having “Sufficient ABL transcript”.

Example: Assay’s NPM1 Mutation Ct = 5.4 is greater than "0" and less than "6";
ABL Ct = 5.9 is less than "6".

Result: INVALID [Too high NPM1 Mutation transcript].

INVALID [Too high NPM1 Mutation transcripts]



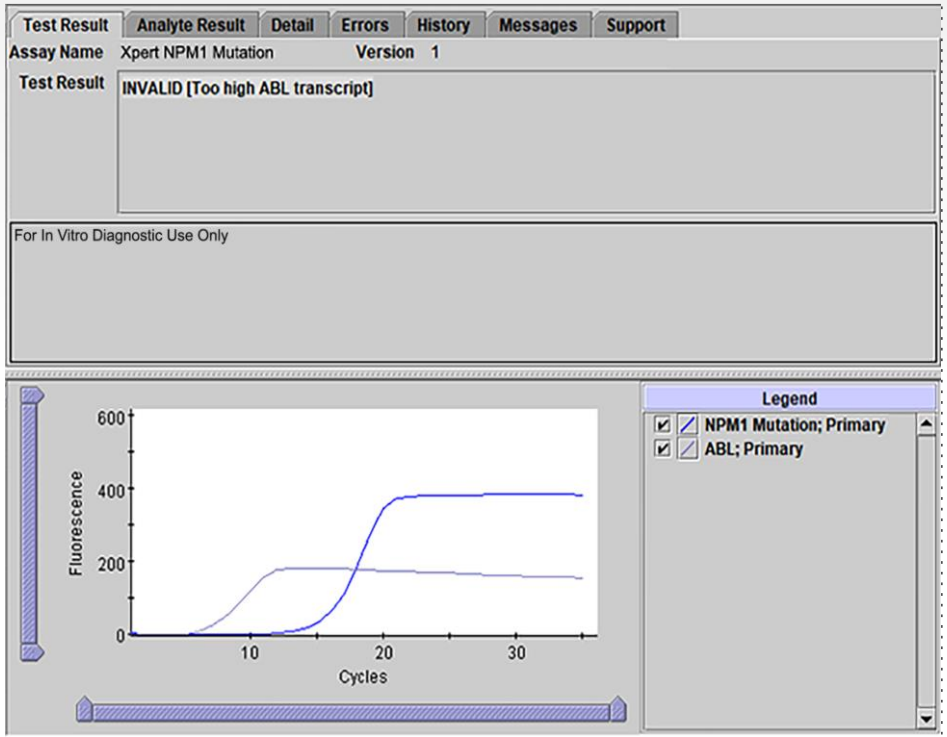
NPM1 mutation was detected with NPM1 Mutation Ct greater than “0” and less than or equal to “6” and ABL Ct greater than “6” and less than or equal to “20”

- The GeneXpert software requires the ABL Ct to be greater than or equal to “6” and less than or equal to “20” for the Xpert NPM1 Mutation test to ensure having “Sufficient ABL transcript”.

Example: Assay’s NPM1 Mutation Ct = 5.8; is greater than "0" and less than "6"; ABL Ct = 13 between “6” and “20”.

Result: INVALID [Too high NPM1 transcript].

INVALID [Too high ABL mutation transcripts]



NPM1 mutation was detected with NPM1 Mutation Ct greater than “6” and less than or equal to “32” and ABL Ct not equal to "0" and less than “6”.

- The GeneXpert software requires the ABL Ct to be greater than or equal to “6” and less than or equal to “20” for the Xpert NPM1 Mutation test to ensure having “Sufficient ABL transcript”.

Example: Assay’s NPM1 Mutation Ct = 13.2;

ABL Ct = 5.8 is less than “6”.

Result: INVALID [Too high ABL transcript].

ERROR – code 2008, 5006,5007,5008,5009 etc

BCR-ABL transcript level cannot be determined

Possible Causes

- Probe check failure
- Pressure exceeding limit (error message 2008)

Solution

- Check sample quality
- Check for grossly elevated WBC count
- Repeat the assay with original sample (if available) or from retained lysate and a new cartridge.
- Retest following procedure for
  Error 2008/Invalid ➔ **Type 2** OR
  Error 5006,5007,5008,5009,/Invalid ➔ **Type 1**

NO RESULT

NPM1 Mutation transcript level cannot be determined. Insufficient data were collected to produce an assay result. For example, this could occur if the operator stopped an assay that was in progress.

- NPM1 Mutation NO RESULT
- ABL NO RESULT
- Probe Check NA (not applicable)

Solution

- Repeat the assay with original sample (if available) or from retained lysate and a new cartridge.
- Follow Retest Procedure for Error OR Invalid **(Type 1)**

Retest Procedure for ERROR or INVALID (Type 1)

- Retest samples with ERROR or INVALID results due to the ABL cycle threshold (Ct) exceeding the maximum valid Ct (Ct >20) or the endpoint is below the threshold setting (<100).

Retest Procedure for ERROR or INVALID (Type 1) Sufficient Sample

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

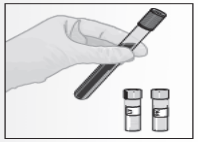
Cepheid Technical Support
US office (888) 838-3222, Option 2
techsupport@cepheid.com
European office +33 563 82 53 19

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

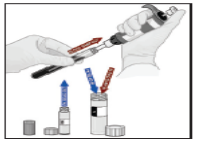
Cepheid. A better way.

Start here

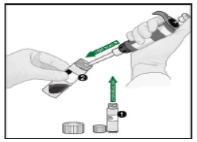
1 Remove EDTA whole blood and sample prep reagents from refrigerator. Place EDTA blood on rocker or invert 8 times prior to sampling.



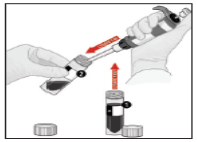
2 Briefly centrifuge PK reagent. To a 50mL conical tube, add 100uL of PK reagent. Then add 4mL of well-mixed EDTA whole blood to the same 50mL conical tube. Vortex for 3 sec and incubate for 1 min at RT.



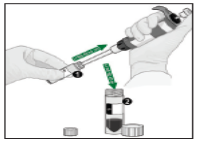
3 Add 2.5mL of lysis reagent (LY) to same tube, vortex 10 sec, and incubate 5 min at RT. Vortex again for 10 sec and incubate a 2nd time for 5 min. Mix by tapping tube 10x.



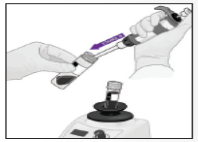
4 Transfer 1mL prepared lysate to new 50mL conical tube. Save remaining lysate for possible retest.



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.

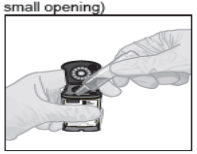


6 To the same conical tube, add 2mL of reagent grade absolute EtOH. Vortex for 10 sec and set aside. Discard remaining PK or LY reagents.

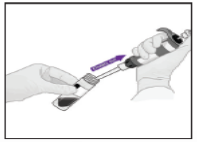


7 Open the Xpert test cartridge lid.

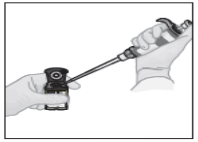
8 Transfer entire contents of Wash Reagent ampoule into Wash Reagent Chamber (with small opening)



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.

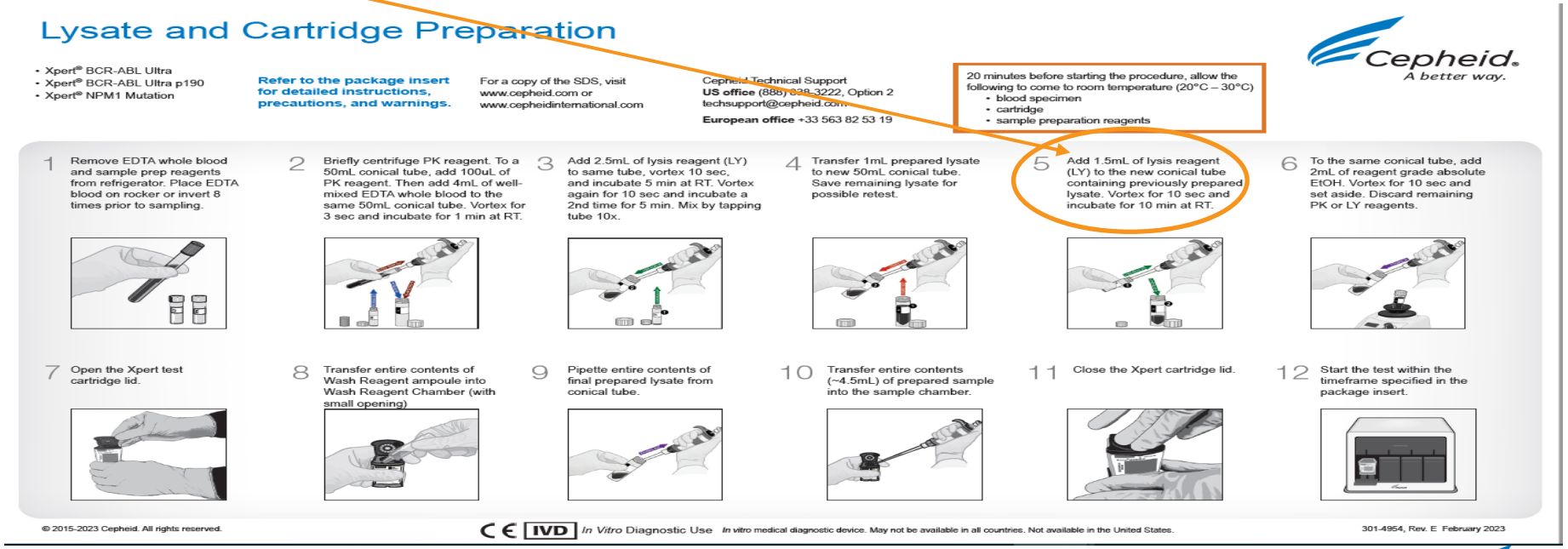
12 Start the test within the timeframe specified in the package insert.

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

301-4954, Rev. E February 2023

Retest Procedure for ERROR or INVALID (Type 1) Insufficient Sample

- If retained lysate is stored frozen, 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. Transfer 1 mL of the “remaining lysate into a new 50mL conical tube. Then start here



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.

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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

Cepheid. 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. 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.

301-4954, Rev. E February 2023

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

- Retest samples with NPM1 mutation and/or ABL transcript levels below the valid minimum (Ct > 0 and Ct < 6) or when the pressure limit is exceeded.

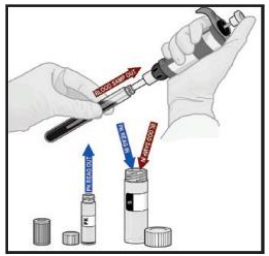
Retest Procedure for ERROR (code 2008) or INVALID(Type 2)- Sufficient Blood available

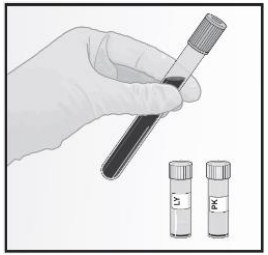
1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K). Ensure the blood specimen is well-mixed by inverting the EDTA blood collection tube 8 times immediately before pipetting

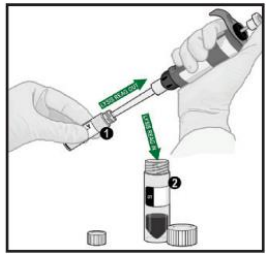
2. Add 250uL of blood specimen and 3.75mL.of PBS (Ph 7.4 provided by user). Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min

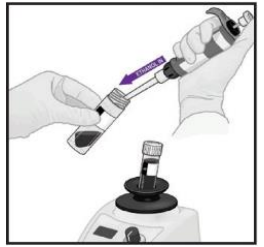
3. To the same tube, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Mix the sample by tapping the bottom of the tube 10 times. Transfer 1mL of prepared lysate into new 50 mL conical tube.

4. To the same conical tube, add 1.5 mL of retained Lysis Reagent (LS). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 10 min.



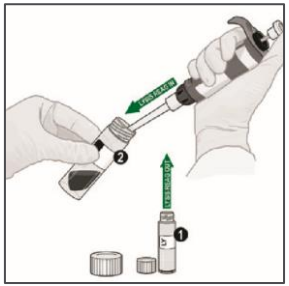






Retest Procedure for ERROR (code 2008) or INVALID(Type 2) Sufficient Blood available continued

5. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user)



6.Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.

7. Remove the cartridge from the cardboard packaging

8. Inspect the cartridge for damage. If damaged, do not use it.

9. Open the cartridge Lid. Transfer the entire contents of Wash Reagent (1) ampoule into Wash Reagent Chamber (with small opening).

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

11. Close the Xpert® cartridge lid

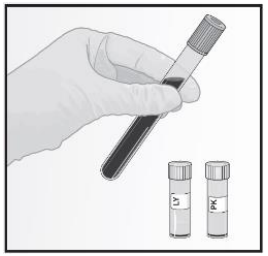
12. Start the assay in the timeframe specified in the Package Insert

Retest Procedure for ERROR (code 2008) or INVALID(Type 2)- Lysate

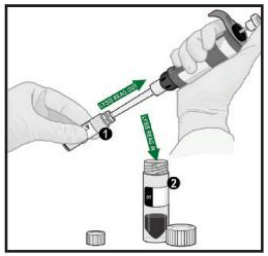
- If retained lysate is stored frozen, thaw lysate to room temperature before use.
- If retained lysate is refrigerated, allow to come to equilibrate to room temperature before use.

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.

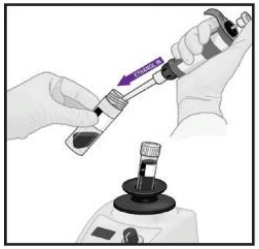
1.To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).



2. To the tube already containing Proteinase K, add 60 µL of left-over lysate. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds. Incubate at room temperature for 1 min

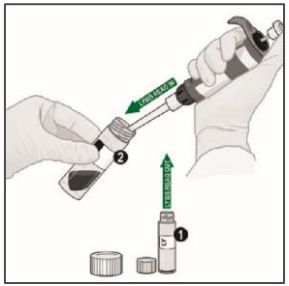


3. To the new conical tube containing lysate, add 2.5 mL of Lysis Reagent (LY). Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Incubate at room temperature for 5 min.



Retest Procedure for ERROR (code 2008) or INVALID (Type 2)- Lysate continued

4. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user)



5.Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside

6. Open the cartridge Lid. Transfer the entire contents of Wash Reagent (1) ampoule into Wash Reagent Chamber (with small opening).

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

8. Close the Xpert® cartridge lid

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

Retest Procedures

1

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

2

Refer to Instructions For Use for directions on retest procedure Type 1 and Type 2.

Retest can be done on left over blood sample or retained lysate.

3

Obtain a new cartridge.

Process the sample per the Instructions For Use.

4

Run the test on the system.

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*

Cepheid
A better way.

Thank You

www.Cepheid.com