

# Xpert<sup>®</sup> NPM1 Mutation

**REF** RNPM1-10

Instructions for Use  
For Use with GeneXpert Dx System  
Research Use Only (RUO)

**RUO**



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See Section 24, Revision History for a description of changes.

For Information Only - Not a Controlled Copy

# Xpert<sup>®</sup> NPM1 Mutation

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For Research Use Only. Not for use in diagnostic procedures.

## 1 Proprietary Name

Xpert<sup>®</sup> NPM1 Mutation

## 2 Common or Usual Name

Xpert NPM1 Mutation

## 3 Product Description

The Xpert NPM1 Mutation assay, performed on the Cepheid GeneXpert<sup>®</sup> Dx System, is a real-time RT-PCR (reverse transcription-polymerase chain reaction) for the quantification of mutant NPM1 mRNA transcripts (types A, B and D in exon 12) in EDTA peripheral blood specimens. The assay utilizes automated real-time RT-PCR and reports the percent ratio of mutant NPM1 to ABL1 endogenous control mRNA transcripts. The Xpert NPM1 Mutation assay is for Research Use Only. Not for use in diagnostic procedures.

## 4 Summary and Explanation

Acute myeloid leukemia (AML) is a cancer of the myeloid blood hematopoietic stem cells in the bone marrow<sup>1,2</sup> and is known to have various Nucleophosmin (NPM1) exon 12 mutations<sup>3</sup>. The insertion of nucleotides in exon 12 results in a frameshift mutation and creates a nuclear export signal (NES). The mutations in the NPM1 gene lead to aberrant cytoplasmic localization of NPM1 and NPM1-interacting proteins. NPM1 is one of the most commonly mutated genes in AML and the mutations occur in 28% to 35% of all AML cases. While several drugs targeting mutated NPM1 are currently under investigation, there are no FDA-approved targeted therapies presently available.<sup>4</sup>

The NPM1 gene encodes the nuclear shuttling protein that has a role in centrosome and ribosome biology, as well as regulation of other cellular systems, including tumor suppressor pathways. NPM1 is a nucleolar phosphoprotein that serves as a shuttle between the nucleus and the cytoplasm. It regulates the transport of ribosomal particles through the nuclear membrane. NPM1 mutations were first discovered in AML individuals following the observation of abnormal cytoplasmic location rather than the normal nuclear location. The genetic evaluation of leukemic blasts combined with the cytoplasmic NPM1 location has led to the knowledge of the known exon 12 frameshift mutations.<sup>3</sup> The most frequent NPM1 mutations are the type A (~75-80%), type B (~10%) and type D (~5%), all in exon 12, which result in a frameshift mutation from an insertion of four nucleotides. The mutation causes a loss of a nucleolar localization signal and an aberrant cytoplasmic localization of the protein in AML individuals.<sup>5</sup>

## 5 Principle of the Procedure

The Xpert NPM1 Mutation assay is an automated assay for quantifying the amount of NPM1 mutation transcripts as a ratio of NPM1 Mutation /ABL1. The assay is performed on Cepheid GeneXpert Dx System, which automates and integrates sample purification, nucleic acid amplification, and target sequence detection in simple or complex samples using real-time RT-PCR and nested PCR assays. The system consists of an instrument, computer, and pre-loaded software for running assays and viewing the results. The system requires the use of single-use, disposable GeneXpert cartridges that hold the RT-PCR and nested PCR reagents and host the RT-PCR and nested PCR processes. For a full description of the system, refer to the appropriate *GeneXpert Dx System Operator Manual*.

The Xpert NPM1 Mutation assay includes reagents to detect NPM1 mutation and the ABL1 transcript as an endogenous control in peripheral blood samples. The amount of NPM1 mutation transcript is quantified as the percent ratio of NPM1 Mutation/ABL1. There are two controls included in the Xpert NPM1 Mutation assay – the Endogenous Control (ABL1) and a Probe Check Control (PCC). The ABL1 endogenous control normalizes the NPM1 mutation target and ensures that sufficient sample is used in the assay. The PCC verifies reagent rehydration, PCR tube filling, and that all reaction components, including probes and dyes, are present and functional in the cartridge.

## 6 Reagents and Instruments

### 6.1 Materials Provided

The Xpert NPM1 Mutation kit (RNPM1-10) contains sufficient reagents to process 10 assay samples or quality control samples. The kit contains the following:

|  |                           |
|--|---------------------------|
| <b>Xpert NPM1 Mutation Reagents</b>  | <b>10 of each per kit</b> |
| <ul style="list-style-type: none"> <li>• Proteinase K (PK)</li> </ul>  | 10 x 130 µL per vial      |
| <ul style="list-style-type: none"> <li>• Lysis Reagent (LY) (Guanidinium Chloride)               <ul style="list-style-type: none"> <li>• Guanidinium Chloride</li> <li>• Urea</li> <li>• Sodium dodecyl sulphate</li> </ul> </li> </ul> | 10 x 5.3 mL per vial      |
| <ul style="list-style-type: none"> <li>• Wash Reagent               <ul style="list-style-type: none"> <li>• Ethanol</li> <li>• Guanidinium thiocyanate</li> </ul> </li> </ul>   | 10 x 2.9 mL per ampoule   |
| <b>Xpert NPM1 Mutation Cartridges with Integrated Reaction Tubes</b>   | <b>10 per kit</b>         |
| <ul style="list-style-type: none"> <li>• Bead 1, 2, 3 and 4 (freeze-dried)</li> </ul>  | 1 of each per cartridge   |
| <ul style="list-style-type: none"> <li>• Rinse Reagent</li> </ul>  | 2.0 mL per cartridge      |
| <ul style="list-style-type: none"> <li>• Elution Reagent</li> </ul>  | 2.5 mL per cartridge      |
| <b>CD</b>  | <b>1 per kit</b>          |
| <ul style="list-style-type: none"> <li>• Assay Definition File (ADF)</li> <li>• Instruction to import ADF into GeneXpert software</li> <li>• Instructions for Use (IFU)</li> </ul>   |                           |

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

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

## 6.2 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and operator manual.
- For GeneXpert Dx System: GeneXpert Dx software version 6.2 or higher.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Microcentrifuge (1000 x g minimum)
- Pipettes and aerosol filter pipette tips
- 50 mL conical tubes
- Reagent grade absolute ethanol
- 1X PBS, pH 7.4

## 6.3 Materials Available but Not Required

Xpert NPM1 Control Panel, catalog number C194 are quality controls from Maine Molecular Quality Controls, Inc.

## 7 Storage and Handling

- Store the Xpert NPM1 Mutation kit contents at 2 °C to 8 °C until the expiration date provided on the label.
- Do not open the cartridge lid until you are ready to perform the assay.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.
- The Wash Reagent is a clear, colorless liquid. Do not use the Wash Reagent if it has become cloudy or discolored.
- Twenty (20) minutes before starting the procedure, remove the blood sample, cartridge, and sample preparation reagents from storage to allow them to come to room temperature (20 °C to 30 °C).

## 8 Warnings and Precautions

### 8.1 General

- For Research Use Only. Not for use in diagnostic procedures.
- 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<sup>6</sup> and Clinical and Laboratory Standards Institute.<sup>7</sup>
- Follow safety procedures set by your institution for working with chemicals and handling biological samples.
- The assay function has been established with blood collected in EDTA tubes only. The assay function has not been evaluated with other sample types.
- 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 Instructions for Use and the *GeneXpert Dx System Operator Manual* are necessary to avoid erroneous results.
- Performing the Xpert NPM1 Mutation assay 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.<sup>8</sup>

## 8.2 Sample


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

## 8.3 Assay/Reagent

- Do not substitute Xpert NPM1 Mutation reagents with other reagents.
- Do not open the Xpert NPM1 Mutation cartridge lid except when adding sample and Wash Reagent.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- It is recommended that the Xpert NPM1 Mutation cartridges be at room temperature (20°C to 30°C) when used for testing.
- Each single-use Xpert NPM1 Mutation cartridge is used to process one assay. Do not reuse processed cartridges.
- Transfer the entire contents of one (1) Wash Reagent ampoule to the Wash Reagent Chamber. Missing adding Wash Reagent could cause a false **NOT DETECTED** result.
- Do not reuse pipette tips.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use the Xpert NPM1 Mutation cartridge if a reagent is added to the wrong opening.
- Do not open Xpert NPM1 Mutation cartridges after the assay is completed.
- Dedicate a set of pipettes and reagents exclusively to sample preparation.
- Wear clean lab coats and gloves. Change gloves between the handling of each sample.
- In the event of a spill of samples or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country. Allow a minimum of two minutes of contact time.
- Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Alternatively, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination.

## 9 Chemical Hazards

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

- CLP/GHS Hazard Pictogram: 
- Signal Word: DANGER
- UN GHS Hazard Statements
  - Highly flammable liquid and vapor H225.
  - Causes skin irritation H315.
  - Causes serious eye irritation H319.
  - May cause drowsiness or dizziness H336.
  - Suspected of causing genetic defects H341.
- UN GHS Precautionary Statements
  - **Prevention**
    - Refer to the Safety Data Sheet for special instructions before use.
    - Obtain special instructions before use.
    - Do not handle until all safety precautions have been read and understood.
    - Keep away from heat, sparks, open flames and/or hot surfaces. No smoking.

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

## 10 Sample Collection, Transport and Storage

- Peripheral blood samples should be collected in EDTA tubes following your institution's guidelines. Plasma should not be separated from cells.
- Samples should be stored at 2°C to 8°C for no longer than 3 days (72 hours) prior to testing.
- Proper sample collection, storage, and transport are critical to the assay function. Sample stability under shipping and storage conditions other than those listed in Section 11, Procedure below have not been evaluated with the Xpert NPM1 Mutation assay.

## 11 Procedure

### 11.1 Before You Start

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.

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**Important** Start the assay within 1 hour of adding the Sample Reagent-treated sample to the cartridge.

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**Important** Remove the cartridge from the cardboard packaging before preparing the sample. (Refer to Section 11.3, Preparing the Cartridge).

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## 11.2 Preparing the Sample

### 11.2.1 Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL

1. To the bottom of a new, labelled 50 mL conical tube, add 100 µL of Proteinase K (PK).
2. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing PK, add 4 mL of blood sample.
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. To the same tube, add 2.5 mL of Lysis Reagent (LY).

**Note** Retain the remaining lysis reagent to use again in Step 13.

7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. Mix the sample by tapping the bottom of the tube 10 times.
12. Transfer 1 mL of the prepared lysate into a new, labelled 50 mL conical tube.

**Note** Remaining lysate can be stored at 2 °C to 8 °C for up to 48 hours or stored at -20 °C or lower for up to 1 month.

13. To the new conical tube containing lysate, add 1.5 mL of retained LY from Step 6.
14. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
15. Incubate at room temperature for 10 minutes.
16. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
17. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
18. Discard any remaining PK or LY reagents.

### 11.2.2 Preparing the Sample with WBC Count at Equal to or Greater than 30 Million/mL

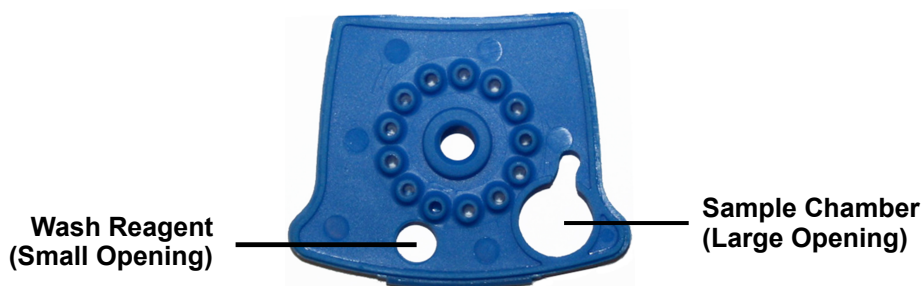
1. To the bottom of a new 50 mL conical tube, add 100 µL of PK.
2. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing PK, add 250 µL of blood sample and 3.75 mL of 1xPBS (pH7.4, provided by user).
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. Follow Steps 6-17 in Section 11.2.1 to make the final lysate.
7. Discard any remaining PK or LY reagents.

## 11.3 Preparing the Cartridge

To add the sample to the Xpert NPM1 Mutation cartridge:

1. Remove the cartridge from the cardboard packaging.
2. Inspect the cartridge for damage. If damaged, do not use it.
3. Open the cartridge by lifting the cartridge lid and transfer the entire contents of one (1) Wash Reagent ampoule to the Wash Reagent Chamber (with small opening). See Figure 1.
4. Pipette the entire contents of the prepared sample (4.5 mL) into the Sample Chamber (large opening). See Figure 1.





**Figure 1. Xpert NPM1 Mutation Cartridge (Top View)**

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

## 11.4 Starting the Assay

**Important** Start the assay within 1 hour of adding the sample to the cartridge.

**Important** Before you start the assay, ensure that the system is running GeneXpert Dx software version 6.2 or higher and that the Xpert NPM1 Mutation assay definition file is imported into the software. This section lists the default steps to operate the GeneXpert Dx System.

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

1. Turn on the GeneXpert system by first turning on the GeneXpert Dx instrument and then turning on the computer. The GeneXpert Dx software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log into the GeneXpert software using your username and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx). The Create Test window opens.
4. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the assay results.
5. Scan the barcode on the Xpert NPM1 Mutation cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

**Note** If the barcode on the Xpert NPM1 Mutation cartridge does not scan, then repeat the assay with a new cartridge.

6. Click **Start Test**. You may need to type your password in the dialog box that appears.
7. Open the instrument module door with the blinking green light and load the cartridge.
8. Close the door. The assay starts and the green light stops blinking. When the assay is finished, the light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.
10. Dispose of used cartridges in the appropriate sample waste container according to your institution's standard practices.

**Note** Time to result is less than 3 hours (approximately 30 minutes offboard sample preparation and less than 2.5 hours assay run time).

## 12 Viewing and Printing Results

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

- Click the **View Results** icon to view results.
- Upon completion of the assay, click the **Report** button of the **View Results** screen to view and/or generate a PDF report file.

## 13 Quality Control

Each cartridge includes an ABL1 Endogenous Control and Probe Check Control (PCC).

**ABL1 Endogenous Control** — The ABL1 Endogenous Control verifies that sufficient sample is used with the assay. Additionally, this control detects sample-associated inhibition of the real-time PCR assay. The ABL1 passes if it meets the assigned acceptance criteria.

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

## 14 Interpretation of Results

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

**Table 1. Xpert NPM1 Mutation Assay RUO Results and Interpretation**

| Result  | Interpretation  |
|---|---|
| <b>NPM1 Mutation DETECTED</b><br>See Figure 2, Figure 3, Figure 4       | NPM1 mutation transcript was detected. <ul style="list-style-type: none"> <li>NPM1 Mutation DETECTED – NPM1 mutation transcript was detected and has a cycle threshold (Ct) within the valid range and an endpoint above the threshold setting.</li> <li>Possible detected results:               <ul style="list-style-type: none"> <li>NPM1 MUTATION DETECTED [#.##%]; Figure 2.</li> <li>NPM1 MUTATION DETECTED [Above upper LoQ]; Figure 3.</li> <li>NPM1 MUTATION DETECTED [Below LoD; &lt;#.###%]; Figure 4.</li> </ul> </li> <li>ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.</li> <li>Probe Check PASS – all probe check results passed.</li> </ul> |
| <b>NPM1 Mutation NOT DETECTED</b><br>See Figure 5                       | NPM1 mutation transcript was not detected. <ul style="list-style-type: none"> <li>NPM1 Mutation NOT DETECTED [Sufficient ABL transcript] – NPM1 mutation transcript was not detected and has a cycle threshold (Ct) of zero or above the upper end of the valid range and/or an endpoint below the threshold setting.</li> <li>ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and an endpoint above the threshold setting.</li> <li>Probe Check PASS – all probe check results passed.</li> </ul>   |
| <b>INVALID</b><br>See Figure 6, Figure 7, Figure 8, Figure 9, Figure 10 | NPM1 Mutation transcript level cannot be determined due to sample containing excess NPM1 mutation transcript and/or excess or insufficient ABL transcript. See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. <ul style="list-style-type: none"> <li>NPM1 Mutation INVALID – NPM1 cycle threshold (Ct) was above zero and below the lower end of the valid range (Figure 8, Figure 9).</li> <li>ABL FAIL – ABL cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting (Figure 6, Figure 7, Figure 8, Figure 8).</li> <li>Probe Check – PASS; all probe check results passed.</li> </ul>  |
| <b>ERROR</b><br>See Figure 11   | NPM1 Mutation transcript level cannot be determined. See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. <ul style="list-style-type: none"> <li>NPM1 Mutation – NO RESULT</li> <li>ABL – NO RESULT</li> <li>Probe Check FAIL – All or one of the probe check results failed.</li> <li>Probe Check PASS or NA (not applicable) and Pressure Abort.*</li> </ul> <p>*If the probe check passed, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.</p>   |

| Result           | Interpretation  |
|------------------|---|
| <b>NO RESULT</b> | <p>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. See Section 17, Troubleshooting Guide, for additional instructions for retesting the samples.</p> <ul style="list-style-type: none"> <li>• NPM1 Mutation NO RESULT</li> <li>• ABL NO RESULT</li> <li>• Probe Check NA (not applicable)</li> </ul> |

## 15 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.

**Table 2. Examples of Xpert NPM1 Mutation Assay Results**

| Assay | NPM1 Mutation |                     | ABL  |                     | Xpert NPM1 Mutation Assay Results                    | Notes  |
|-------|---------------|---------------------|------|---------------------|--|--|
|       | Ct            | Result <sup>a</sup> | Ct   | Result <sup>a</sup> |  |  |
| 1     | 5.2           | INVALID             | 5.8  | FAIL                | INVALID [Too high NPM1 Mutation and ABL transcripts] | NA   |
| 2     | 9             | INVALID             | 5.5  | FAIL                | INVALID [Too high ABL transcripts]                   | NA   |
| 3     | 5.5           | INVALID             | 8.5  | PASS                | INVALID [Too high NPM1 Mutation transcripts]         | NA   |
| 4     | 25.0          | INVALID             | 21.8 | FAIL                | INVALID [Insufficient ABL transcript]                | NA   |
| 5     | 0             | INVALID             | 0    | FAIL                | INVALID [No ABL transcript]                          | NA   |
| 6     | 8.5           | POS                 | 13.6 | PASS                | NPM1 Mutation DETECTED [Above upper LoQ]             | NA   |
| 7     | 22.5          | POS                 | 14.8 | PASS                | NPM1 Mutation DETECTED [1.05%]                       | Reported value: 1.05%                            |
| 8     | 27.9          | POS                 | 14.0 | PASS                | NPM1 Mutation DETECTED [Below LoD; <0.030%]          | NA   |
| 9     | 0             | NEG                 | 14.6 | PASS                | NEGATIVE [Sufficient ABL transcript]                 | NA   |
| 10    | 0             | NO RESULT           | 0    | NO RESULT           | ERROR  | For example, Error 5017 [ABL] probe check failed |

<sup>a</sup> See the Analyte Results tab in the GeneXpert Dx System Software for details.

## 15.1 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 ( $\Delta Ct$ ) value is obtained from ABL Ct minus NPM1 Mutation Ct:

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

**Note** 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 (IVT-RNA) 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 example shown here.

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

**Result:** **NPM1 Mutation DETECTED [31.53%]**. See Figure 2.

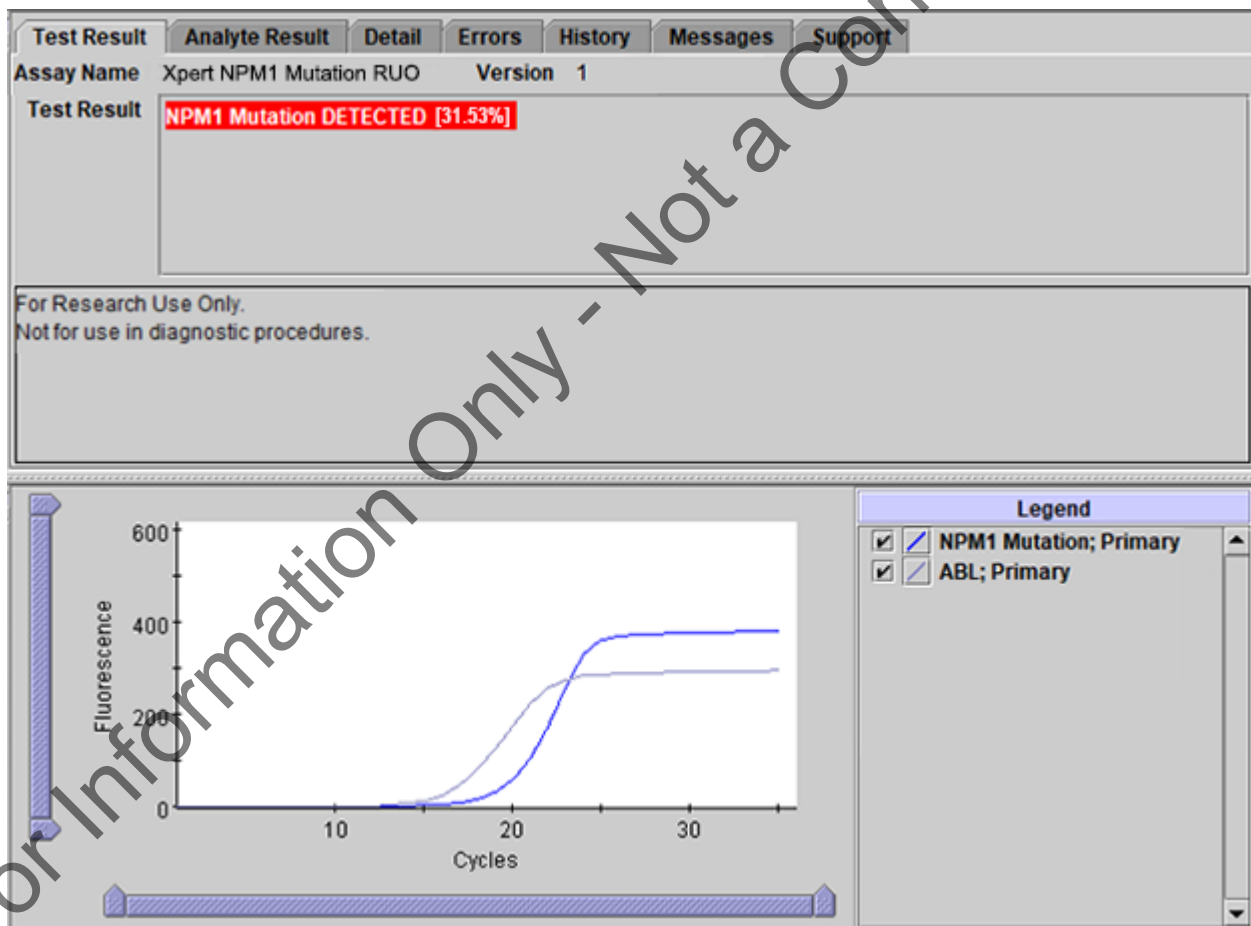


Figure 2. GeneXpert Dx View Results Window: NPM1 Mutation DETECTED [31.53%]

## 15.2 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 ( $\Delta Ct$ ) value is obtained from ABL Ct minus NPM1 Mutation Ct:

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

**Note** 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 (IVT-RNA) 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 example shown here.

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

**Result:** **NPM1 Mutation DETECTED [Above upper LoQ]**. See Figure 3.

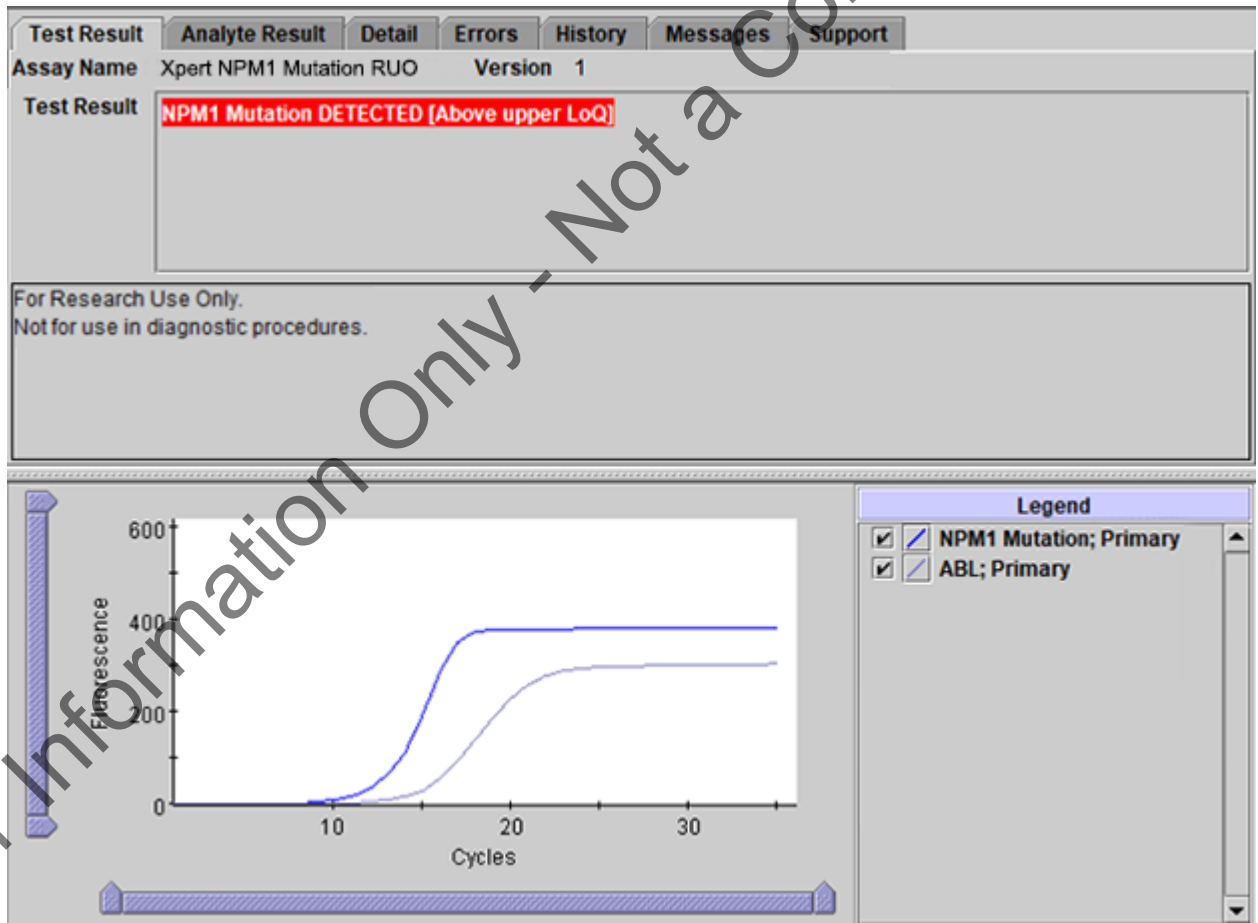


Figure 3. GeneXpert Dx View Results Window: NPM1 Mutation DETECTED [Above upper LoQ]

### 15.3 NPM1 Mutation DETECTED [Below LoD; <0.030%]

NPM1 mutation has been detected at a level < 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 ( $\Delta Ct$ ) value is obtained from ABL Ct minus NPM1 Mutation Ct:

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

**Note** 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 (IVT-RNA) 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 example shown here.

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

**Result:** **NPM1 Mutation DETECTED [Below LoD; <0.030%]**. See Figure 4.

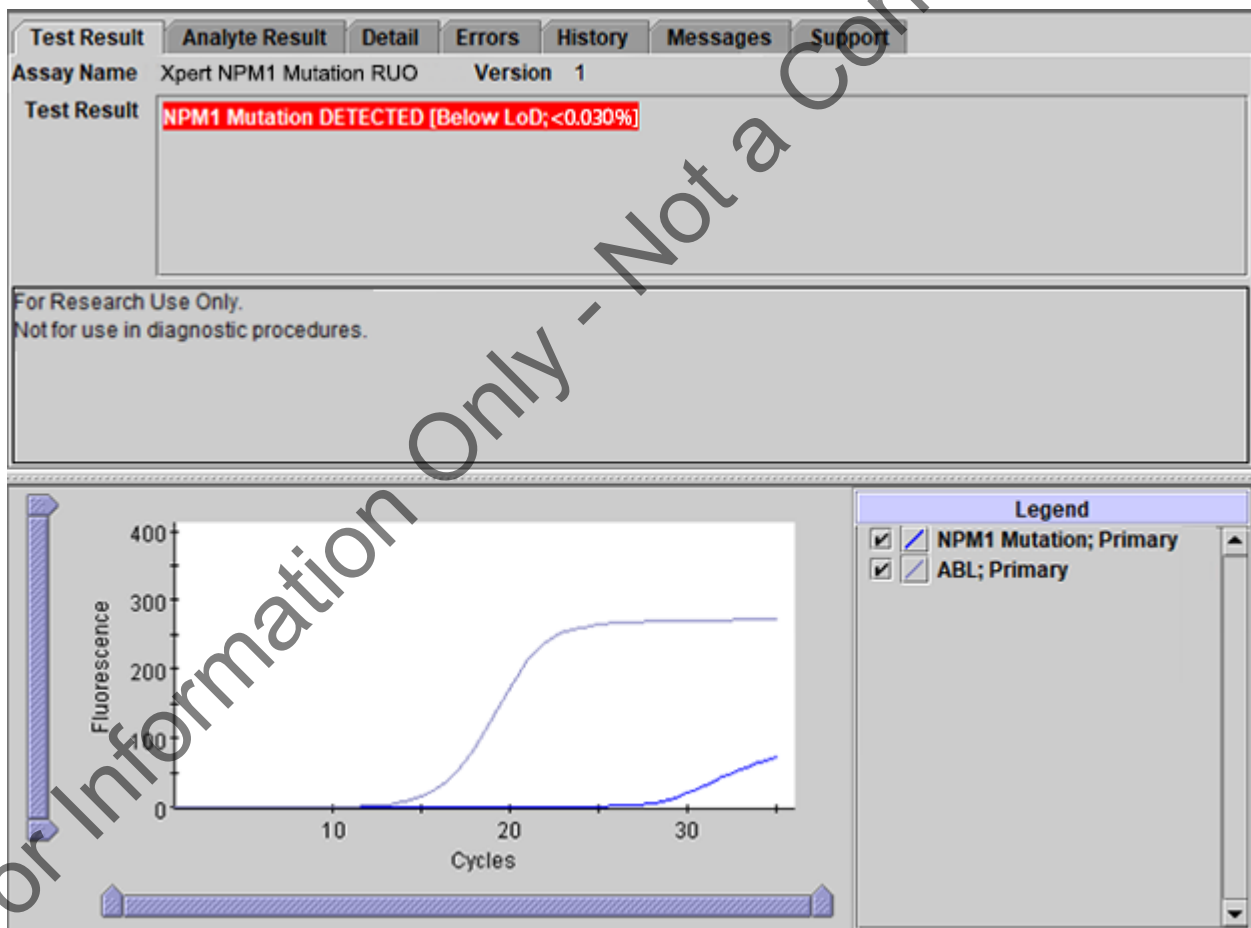


Figure 4. GeneXpert View Results Window: NPM1 Mutation DETECTED [Below LoD; <0.030%]

## 15.4 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 assay to ensure having “Sufficient ABL transcript”. See Section 14, Interpretation of Results, Table 1.

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

**Result:** **NPM1 Mutation NOT DETECTED [Sufficient ABL transcript]**. See Figure 5.

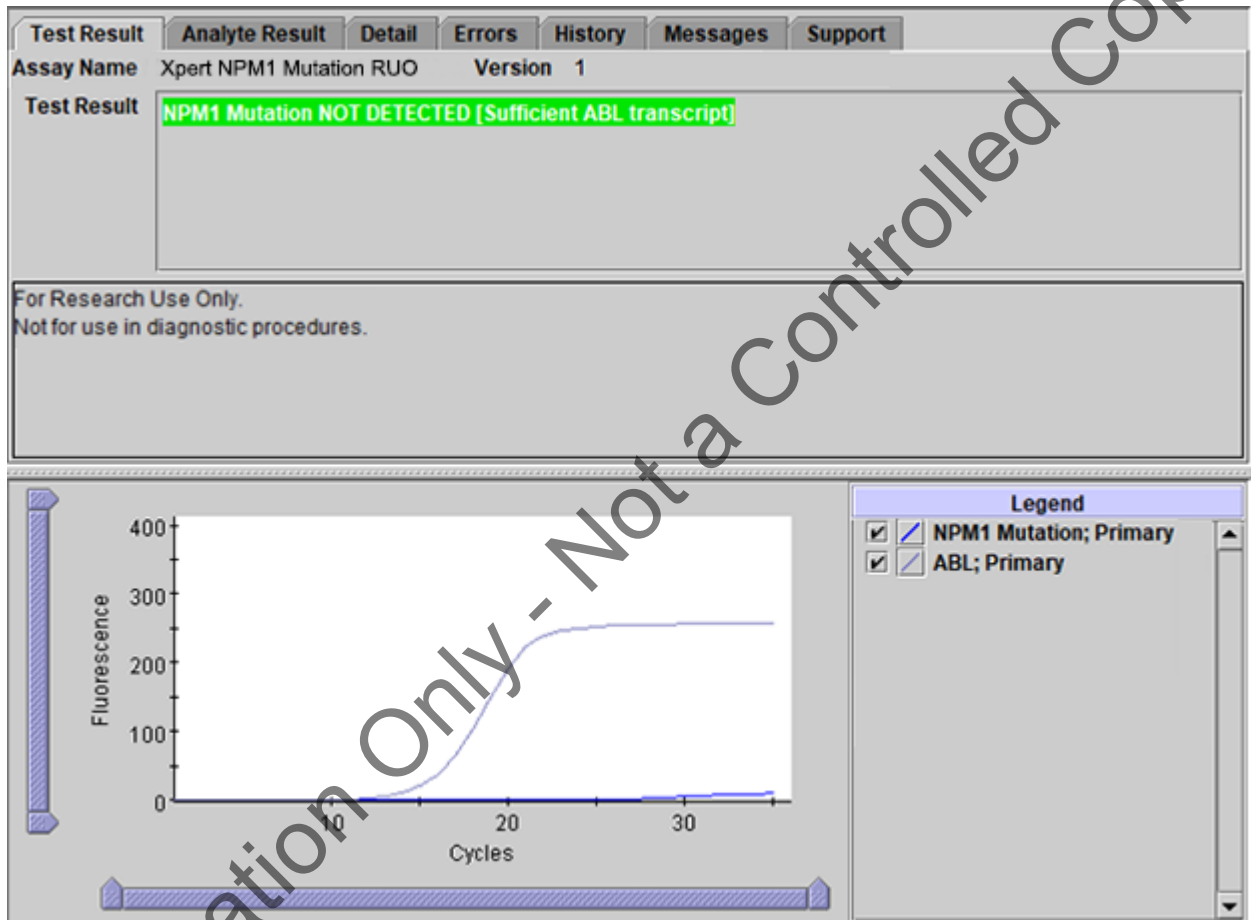


Figure 5. GeneXpert View Results Window: NPM1 Mutation NOT DETECTED [Sufficient ABL transcript]

## 15.5 INVALID [No ABL transcript]

NPM1 mutation was detected or not detected with ABL Ct equal to “0”.

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 assay to ensure having “Sufficient ABL transcript”. Refer to Section 17, Troubleshooting Guide.

**Example:** Assay’s NPM1 Mutation Ct = 0; ABL Ct = 0.

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

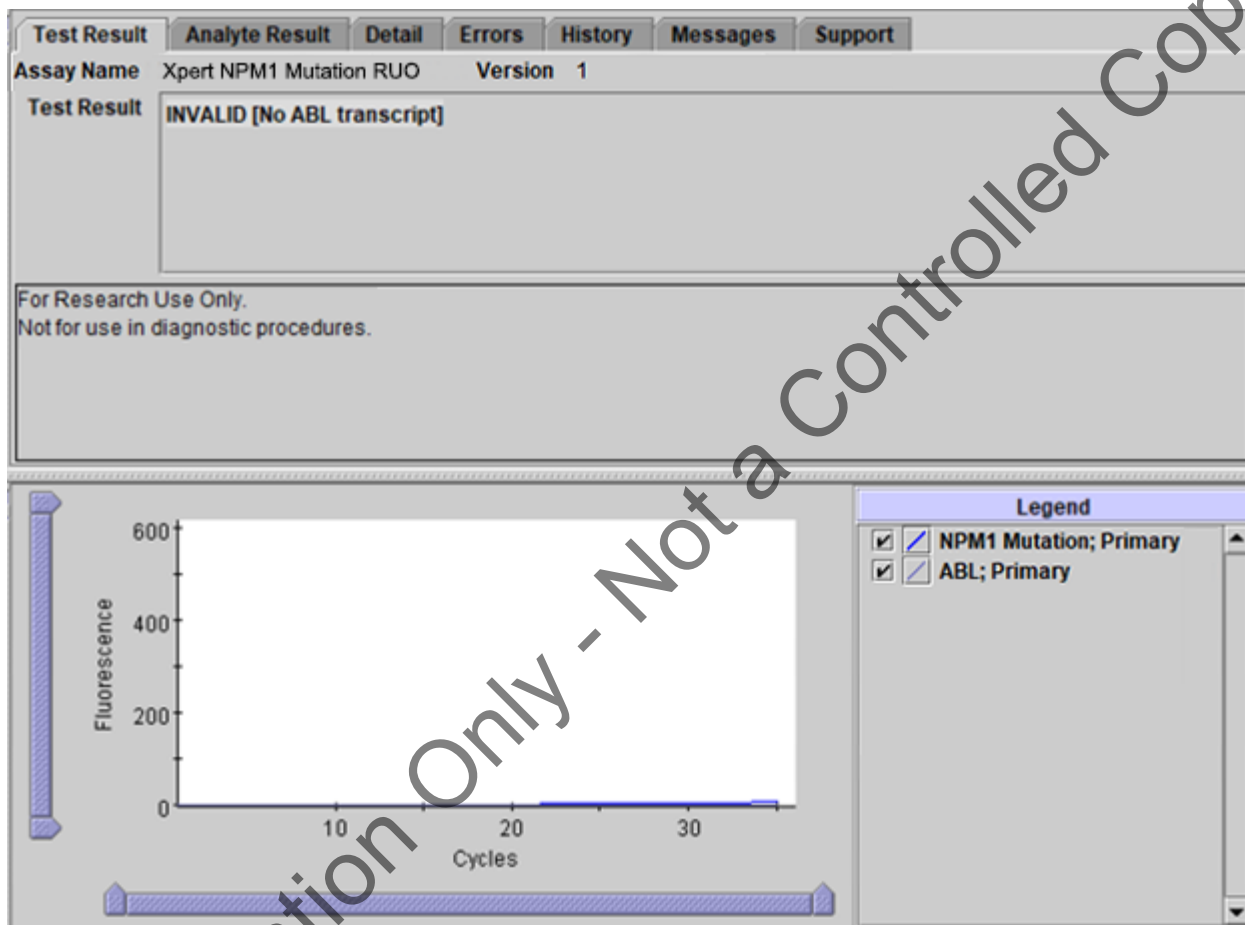


Figure 6. GeneXpert View Results Window: INVALID [No ABL transcript]



## 15.6 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 assay to ensure having "Sufficient ABL transcript". Refer to Section 17, Troubleshooting Guide.

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

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

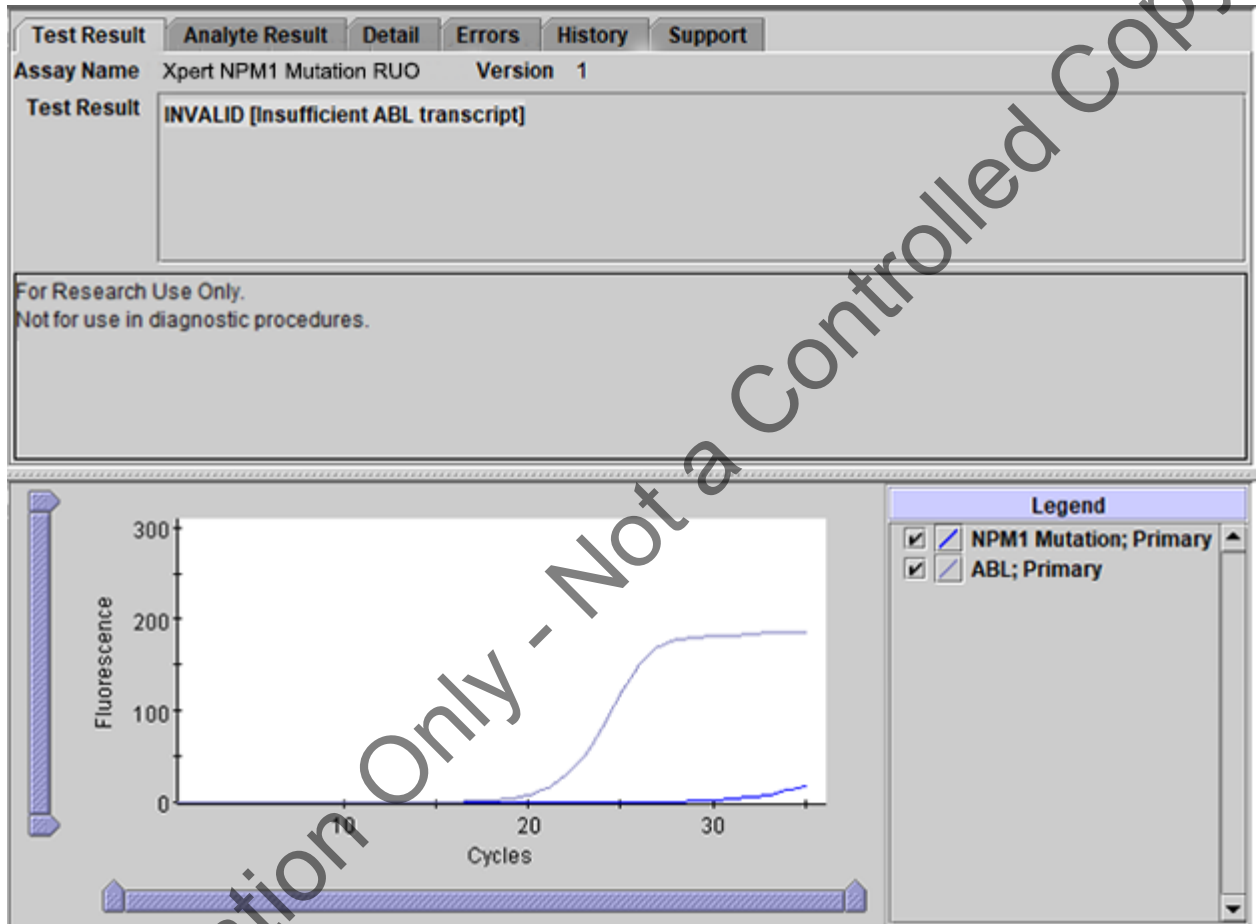


Figure 7. GeneXpert View Results Window: INVALID [Insufficient ABL transcript]

## 15.7 INVALID [Too high NPM1 Mutation and ABL transcript]

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 assay to ensure having "Sufficient ABL transcript". Refer to Section 17, Troubleshooting Guide.

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

**Result:** **INVALID [Too high NPM1 Mutation and ABL transcript]**. See Figure 8.

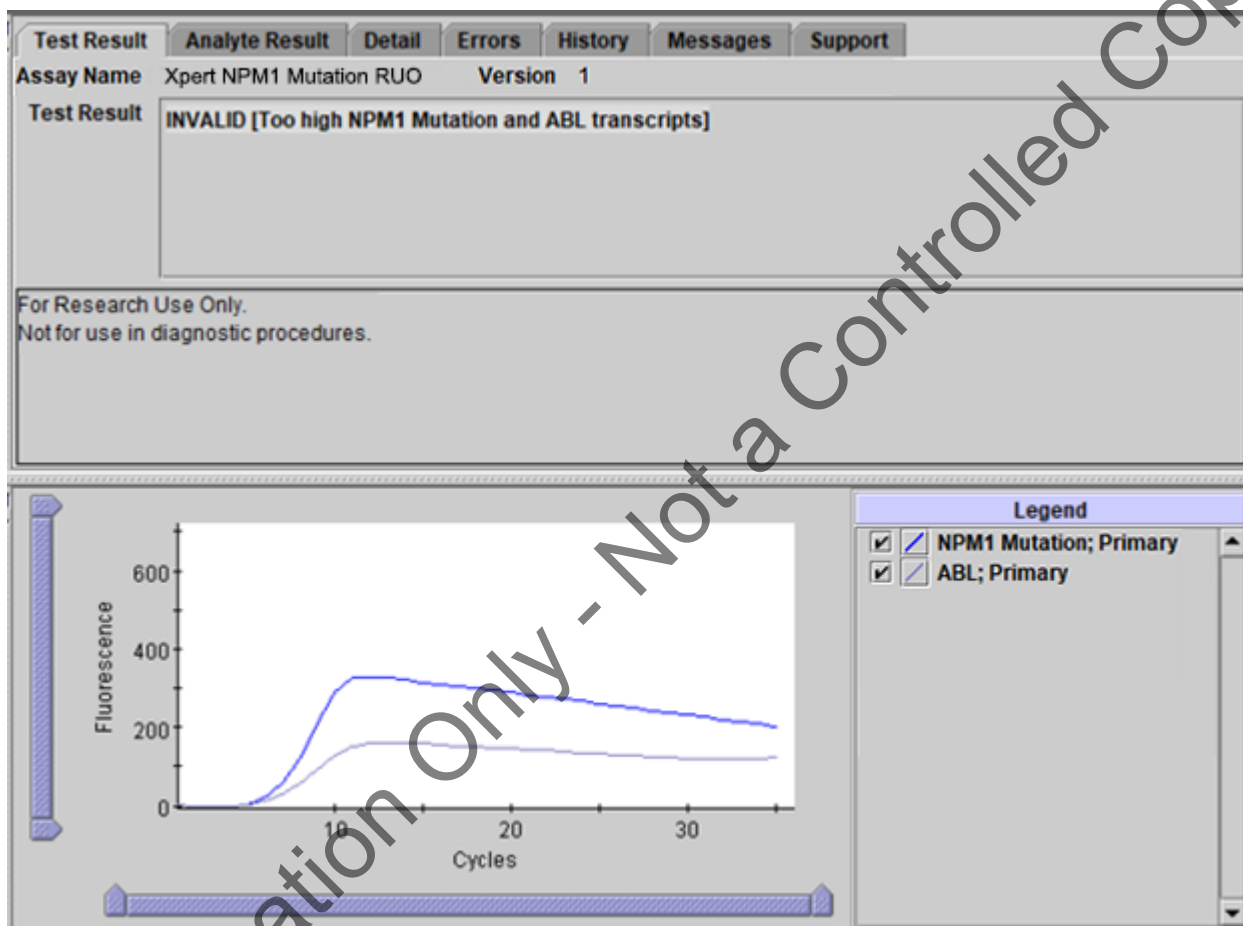


Figure 8. GeneXpert Dx View Results Window: INVALID [Too high NPM1 Mutation and ABL transcript]

## 15.8 INVALID [Too high NPM1 Mutation transcript]

NPM1 mutation was detected with NPM1 Mutation Ct greater than "0" and less than "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 assay to ensure having "Sufficient ABL transcript". Refer to Section 17, Troubleshooting Guide.

**Example:** Assay's NPM1 Mutation Ct = 5.8 is between "0" and less than "6"; ABL Ct = 13 is between "6" and "20".

**Result:** **INVALID [Too high NPM1 Mutation transcript]**. See Figure 9.

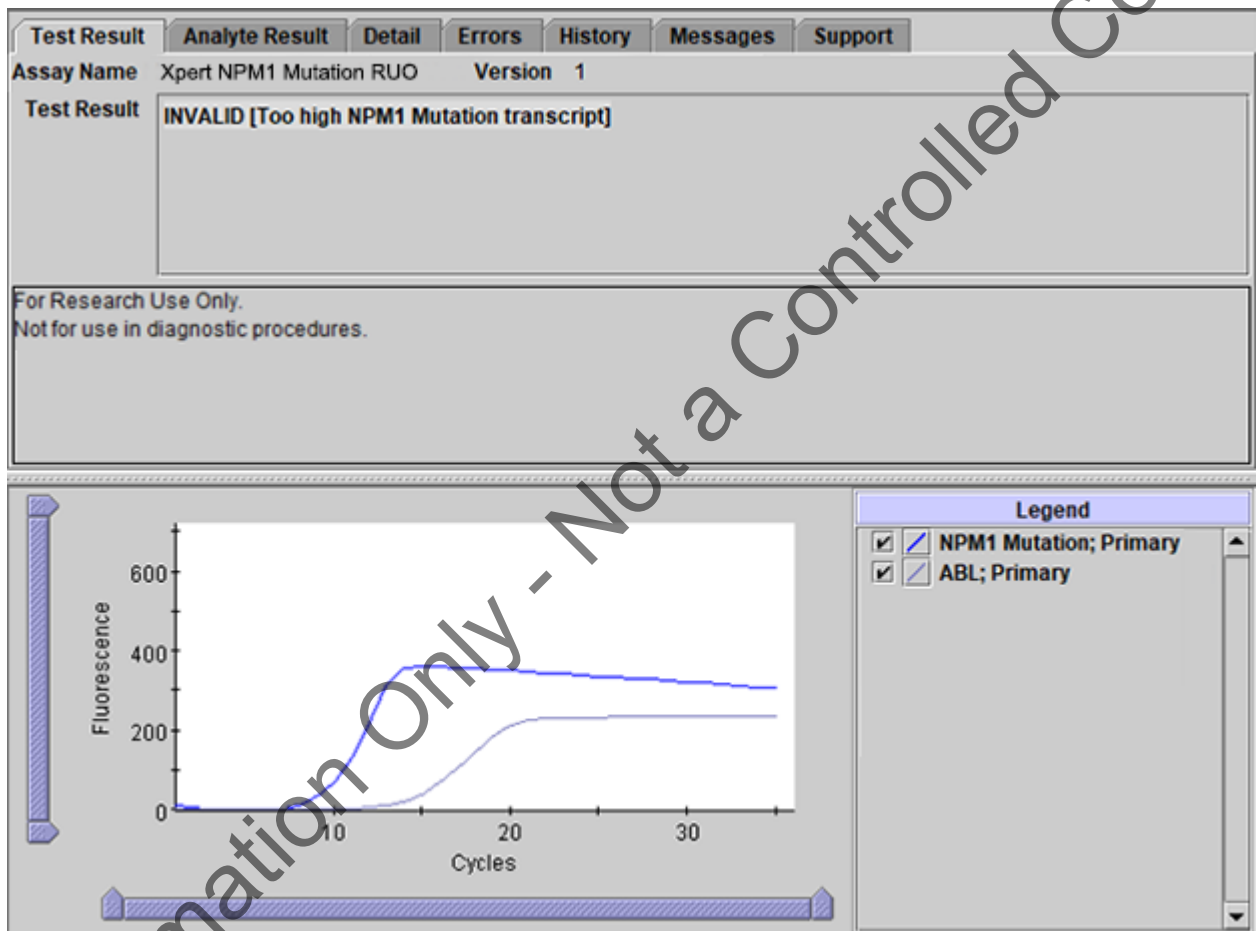


Figure 9. GeneXpert View Results Window: INVALID [Too high NPM1 Mutation transcript]

## 15.9 INVALID [Too high ABL transcript]

NPM1 mutation was detected with NPM1 Mutation Ct greater than “6” and less than or equal to “32” and ABL Ct 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 assay to ensure having “Sufficient ABL transcript”. Refer to Section 17, Troubleshooting Guide.

**Example:** Assay’s NPM1 Mutation Ct = 13.2; ABL Ct = 5.8 is between “0” and less than “6”.

**Result:** **INVALID [Too high ABL transcript]**. See Figure 10.

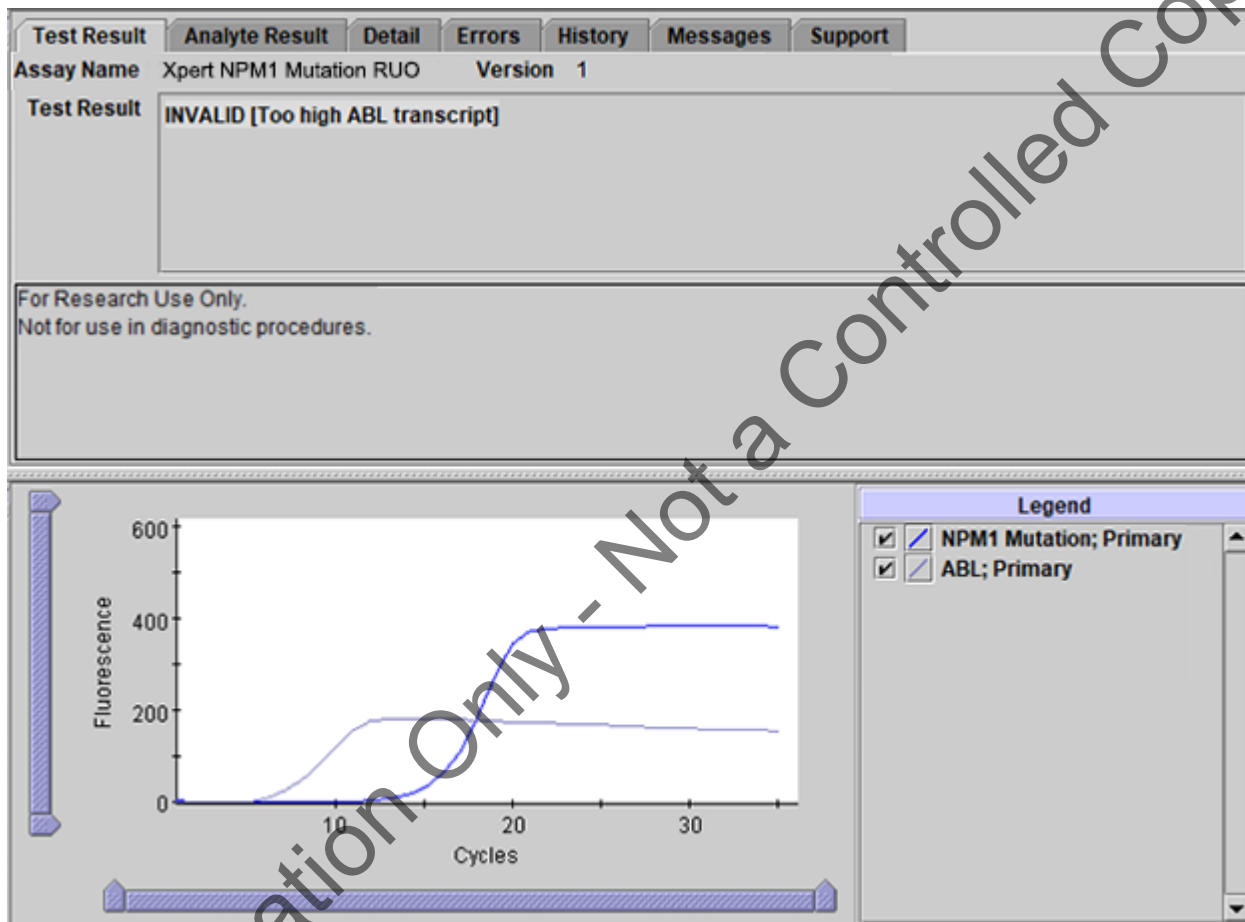


Figure 10. GeneXpert View Results Window: INVALID [Too high ABL transcript]

## 15.10 ERROR

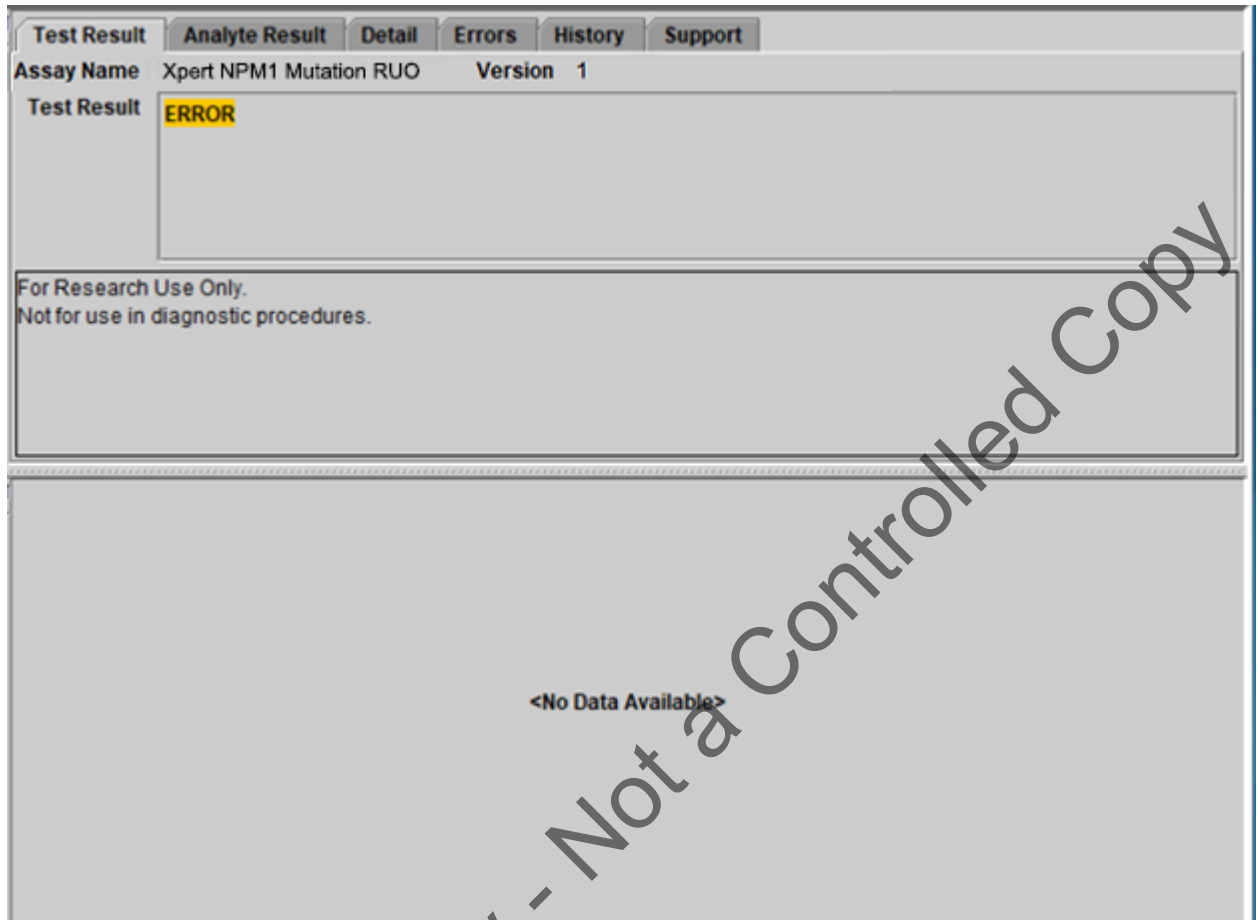


Figure 11. GeneXpert View Results Window: ERROR

## 16 Limitations of the Assay

- For Research Use Only. Not for Use in Diagnostic Procedures.
- 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.

## 17 Troubleshooting Guide

Table 3. Troubleshooting Guide

| Assay Result   | Possible Causes  | Suggestions  |
|--|--|--|
| <b>INVALID</b>   | Type 1: Endogenous control ABL failure: <ul style="list-style-type: none"> <li>Poor sample quality</li> <li>RT-PCR inhibition</li> <li>ABL Ct &gt; 20, and/or endpoint &lt; 100</li> </ul> | <ul style="list-style-type: none"> <li>Check the sample quality (e.g., exceeded sample storage requirement including time and temperature).</li> <li>Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).</li> </ul> |
|  | Type 2: NPM1 Mutation transcript level cannot be determined due to sample containing excess NPM1 Mutation and/or ABL transcripts (Ct < 6)  | Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).   |
| <b>ERROR</b> (Code 2008)   | Pressure exceeding limit (error message 2008)  | <ul style="list-style-type: none"> <li>Check the sample quality</li> <li>Check for grossly elevated WBC count</li> <li>Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).</li> </ul>                   |
| <b>ERROR</b> (Code 5006, 5007, 5008, and 5009*)<br>*This is not an exhaustive list of ERROR codes. | Probe check failure  | Repeat the assay with original sample (if available) or from retained lysate and with a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).  |
| <b>NO RESULT</b>   | Data collection failure. For example, the operator stopped an assay that was in progress or a power failure occurred.  | Repeat the assay with original sample (if available) or from retained lysate and with a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).  |

## 18 Retests

### 18.1 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). Also refer to Section 17, Troubleshooting Guide.

1. If sufficient blood sample volume is available, re-test from original blood sample collection tube following the procedure in Section 11.2.

-OR-

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

- a. If retained lysate from Section 11.2.1, Step 12 is stored frozen, thaw to room temperature before use.
  - b. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle.
2. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.
  3. Follow Steps 13-17 in Section 11.2.1 to make the final lysate.
  4. Open the cartridge by lifting the cartridge lid and transfer the entire contents of one (1) Wash Reagent ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
  5. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
  6. Close cartridge lid. Initiate assay (see Section 11.4, Starting the Assay).

### 18.2 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 (Ct > 0 and Ct < 6) and/or when pressure limit is exceeded. Also refer to Section 17, Troubleshooting Guide.

1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K).
2. Ensure blood sample or left-over lysate from Section 11.2, Step 12 is well-mixed by inverting the tube 8 times immediately before pipetting.
3. To the tube already containing Proteinase K, add 250 µL of blood sample and 3.75 mL of PBS (pH 7.4, provided by user), if available, or 60 µL of retained lysate from Section 11.2.1, Step 12.
  - a. If retained lysate from Section 11.2.1, Step 12 is stored frozen, thaw to room temperature before use.
  - b. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle.
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. For the retest sample of blood with PBS, follow Steps 6-17 in Section 11.2.1, to make the final lysate. For the retest sample of retained lysate, follow Steps a-g below to make the final lysate.
  - a. To the tube with retest sample of retained lysate, add 2.5 mL of LY.
  - b. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
  - c. Incubate at room temperature for 5 minutes.
  - d. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
  - e. Incubate at room temperature for 5 minutes.
  - f. To the same tube, add 2 mL of reagent grade absolute ethanol (provided by user)
  - g. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
7. Open the cartridge by lifting the cartridge lid and transfer the entire contents of one (1) Wash Reagent ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
8. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
9. Close cartridge lid. Initiate assay (see Section 11.4, Starting the Assay).

## 19 Analytical Data

The data was collected from internal studies only.

### 19.1 Linearity/Dynamic Range

Linearity was determined for each of the three NPM1 mutant subtypes, mutA, mutB and mutD, using cell lysates that contain high levels of each subtype transcript. Such lysates were diluted in a background lysate prepared from presumably NPM1 mutation-negative donors to targeted ranges of ~0.01–2500% NPM1 Mutation/ABL. All levels were tested on one reagent lot in quadruplicate. Testing and statistical analyses were conducted in accordance with CLSI EP06-A<sup>9</sup> 6. Regression curves for each subtype are shown in Figure 12, Figure 13, and Figure 14. Linear range of each subtype and their linear model coefficients are summarized in Table 4.



Figure 12. Regression Curves for mutA

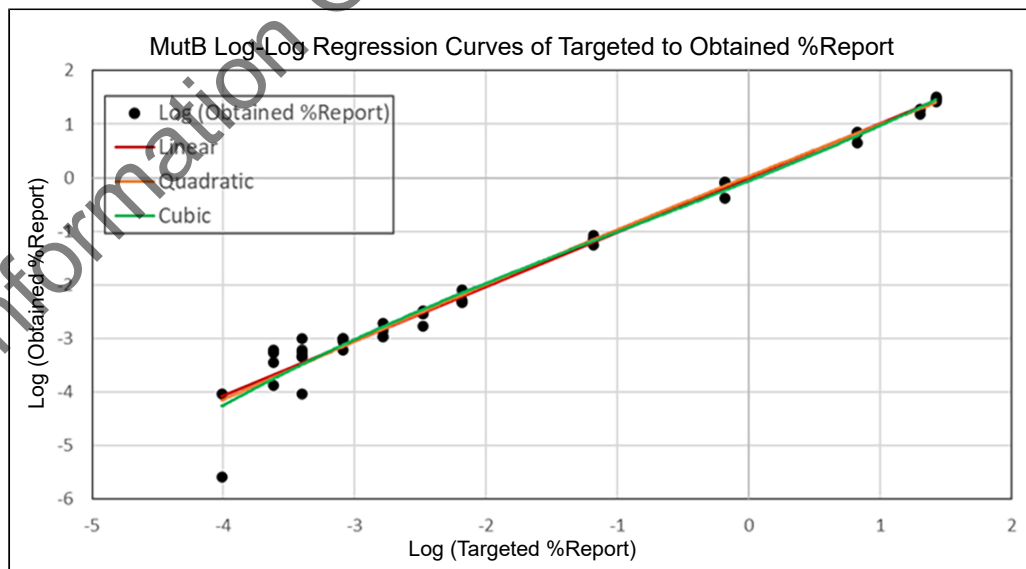


Figure 13. Regression Curves for mutB



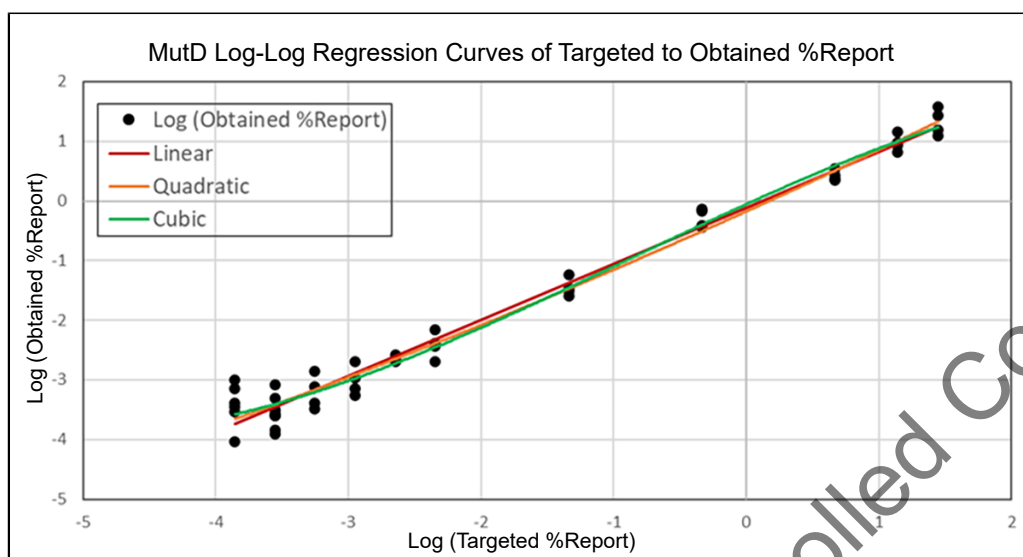


Figure 14. Regression Curves for mutD

Table 4. Summary of Linear Ranges and Linear Model Coefficients

| Subtype | Linear Range | Intercept | Slope  | R <sup>2</sup> |
|---------|--------------|-----------|--------|----------------|
| mutA    | 0.010–2020%  | -0.0223   | 1.0134 | 0.989          |
| mutB    | 0.010–2673%  | -0.0061   | 1.0174 | 0.978          |
| mutD    | 0.014–2783%  | -0.1163   | 0.9389 | 0.981          |

Collectively, the Xpert NPM1 Mutation assay demonstrated linearity within 0.014–2020% NPM1 Mutation/ABL. Bounded by the LoQ and the software upper limit, the reportable dynamic range is 0.030–500%.

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

The limit of detection (LoD) is the lowest NPM1 Mutation/ABL level at which 95% of samples are consistently reported as “NPM1 Mutation DETECTED [##.##%]”. LoD was determined for mutA, mutB, and mutD subtypes individually by testing serial dilutions of NPM1-mutation-positive cell lysates and clinical lysates harboring each mutation subtype. The corresponding LoDs were estimated and verified in accordance with CLSI EP17-A2<sup>7</sup>. The resulting analyses yielded an LoD of 0.025% for mutA, 0.023% for mutB, and 0.030% for mutD (Table 5). The highest LoD among the three subtypes at 0.030% is taken as the overall LoD of the Xpert NPM1 Mutation assay.

The limit of quantitation (LoQ) is the lowest NPM1 Mutation/ABL level above which samples can be quantified with a standard deviation  $\leq 0.36$  log reduction (LR) for mean LRs above 3.5. In accordance with CLSI EP17-A2<sup>7</sup>, the LoQs were estimated and verified at 0.025% for the mutA subtype, 0.023% for the mutB subtype, and 0.030% for the mutD subtype (Table 5). The highest LoQ among the three subtypes at 0.030% is taken as the overall LoQ of the Xpert NPM1 Mutation assay.

The limit of blank (LoB) is the highest NPM1 Mutation/ABL result expected among 95% of blank samples from presumably NPM1-mutation-negative donors. In accordance with CLSI EP17-A2<sup>7</sup>, the LoB of the Xpert NPM1 Mutation assay was estimated and verified at 0.0085% (Table 5).

**Table 5. Limit of Detection, Limit of Quantitation and Limit of Blank of the Xpert NPM1 Mutation assay [% NPM1 Mutation/ABL]**

| Subtype | LoD<br>[%NPM1 Mutation/ABL] | LoQ<br>[%NPM1 Mutation/ABL] | LoB<br>[%NPM1 Mutation/ABL] |
|---------|-----------------------------|-----------------------------|-----------------------------|
| mutA    | 0.025%                      | 0.025%                      | 0.0085%                     |
| mutB    | 0.023%                      | 0.023%                      |                             |
| mutD    | 0.030%                      | 0.030%                      |                             |

## 20 References

1. Saultz JN, Garzon R. Acute myeloid leukemia: A concise review. *J Clin Med.* 2016; 5(3). doi:10.3390/jcm5030033
2. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med.* 2015; 373(12): 1136-1152. doi:10.1056/NEJMra1406184
3. Diagnostic Molecular Pathology. A Guide to Applied Molecular Testing. <https://www.medic4arab.com/2017/01/diagnostic-molecular-pathology-guide-to.html>. Accessed September 16, 2020.
4. Kunchala P, Kuravi S, Jensen R, McGuirk J, Balusu R. When the good go bad: Mutant NPM1 in acute myeloid leukemia. *Blood Rev.* 2018; 32(3): 167-183. doi:10.1016/j.blre.2017.11.001
5. Heath EM, Chan SM, Minden MD, Murphy T, Shlush LI, Schimmer AD. Biological and clinical consequences of NPM1 mutations in AML. *Leukemia.* 2017; 31(4): 798-807. doi:10.1038/leu.2017.30
6. CLSI EP06-A:2003 Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, 1st Edition
7. CLSI EP17-A2:2012 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition

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## 21 Cepheid Headquarters Locations

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## 22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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















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## 23 Table of Symbols

| Symbol  | Meaning                           |
|---|-----------------------------------|
|    | Catalog number                    |
|    | Research Use Only                 |
|    | Batch code                        |
|    | Do not reuse                      |
|    | Consult instructions for use      |
|    | Manufacturer                      |
|    | Country of manufacture            |
|    | Contains sufficient for $n$ tests |
|    | Control                           |
|    | Expiration date                   |
|    | Temperature limitation            |
|   | Biological risks                  |
|  | Caution                           |
|  | Warning                           |



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## 24 Revision History

**Description of Changes:** 302-5822, Rev. A to Rev. B

**Purpose:** Updates to the Research Use Only (RUO) Instructions for Use

| Section   | Description of Change   |
|-----------|---|
| 3         | Updated product description.  |
| 6.1       | Added additional reagent details.   |
| 6.2       | Added "1X PBS, pH 7.4".   |
| 8.3       | Updated Assay/Reagent warnings to include "Transfer the entire contents of one (1) Wash Reagent ampoule to the Wash Reagent Chamber. Missing adding Wash Reagent could cause a false <b>NOT DETECTED</b> result." |
| 9         | Added references to Safety Data Sheet.  |
| 11.2.2    | Updated the procedure to prepare the sample.  |
| 11.3      | Specified the volume of the prepared sample.  |
| 15        | Updated Table 2, Examples of Xpert NPM1 Mutation Assay Results.   |
| 15.3      | Updated "[Below LoD;<0.033%]" to "Below LoD;<0.080%" throughout.  |
| 15.4-15.9 | Updated content for clarity.  |
| 18.1      | Updated threshold setting from "<200" to ">100" and replaced call-outs to specific sections with hyperlinks.  |
| 18.2      | Updated the retest procedure for Error (Code 2008) or Invalid (Type 2).   |
| 19.1      | Replaced section with new content and new figures.  |
| 19.2      | Replaced section with new content and updated Table- 5.   |
| 20        | Updated list of references to add references 6 and 7.   |
| 24        | Updated Revision History table.   |