GeneXpert
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Xpert® NPM1 Mutation

REF **GXNPM1-CE-10**

Instructions for Use

IVD CE

Cepheid

In Vitro Diagnostic Medical Device

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Trademark, Patents and Copyright Statements

See Section 28, Revision History for a description of changes.

Xpert® NPM1 Mutation

For *In Vitro* Diagnostic Use.

1 Proprietary Name

Xpert® NPM1 Mutation

2 Common or Usual Name

Xpert NPM1 Mutation

3 Intended Purpose

3.1 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.

3.2 Intended User/Environment

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

4 Summary and Explanation

Acute myeloid leukemia (AML) is a cancer of the myeloid blood hematopoietic stem cells in bone marrow[1,2] and is known to have various Nucleophosmin (NPM1) exon 12 mutations[3]. 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 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.[4]

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.[3] The most frequent NPM1 mutations are the type A (~75-80%), type B (~10%) and type D (~5%), all in exon 12, which results 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 patients.[5]

5 Principle of the Procedure

The Xpert NPM1 Mutation test is an automated assay for quantifying the amount of NPM1 mutation transcripts as a ratio of NPM1 Mutation /ABL1. The test 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 test 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 test – 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 (GXNPM1-CE-10) contains sufficient reagents to process 10 assay samples or quality control samples. The kit contains the following:

Xpert NPM1 Mutation Reagents	**10 of each per kit**

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

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

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

Xpert NPM1 Mutation Cartridges with Integrated Reaction Tubes		10 per kit
Component	**Reagent Ingredient**	**Amount**
Bead 1 (freeze-dried)	Enzyme: Taq DNA polymerase < 50U/bead	1 per cartridge
	dNTPs < 0.05%	
Bead 2 (freeze-dried)	Primers and probes < 0.005%	1 per cartridge
Bead 3 (freeze-dried)	Primers and probes < 0.005%	1 per cartridge
Bead 4 (freeze-dried)	Enzyme: Taq DNA polymerase < 50U/bead	1 per cartridge
	dNTPs < 0.05%	
Rinse Reagent	Potassium chloride < 4%	2 mL per cartridge
	Sodium azide < 0.1%	
	Polyethylene glycol < 40%	
	Tween 20 < 0.2%	
Elution Reagent	Trizma base < 0.3%	2.5 mL per cartridge
	Trizma hydrochloride < 0.1%	
	Sodium azide < 0.05%	

CD **1 per kit**

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

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

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

7 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

8 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).

9 Warnings and Precautions

9.1 General

- 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.
- 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]

9.2 Specimen

- Maintain proper storage conditions to ensure the integrity of the sample (see Section 11, Specimen Collection and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Do not freeze EDTA peripheral blood specimen.
- Proper specimen collection, storage, and transport are essential for correct results.

9.3 Test/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. The 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.

10 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.

11 Specimen Collection and Storage

- Peripheral blood specimens should be collected in EDTA tubes following your institution's guidelines. Plasma should not be separated from cells.
- Specimens should be stored at 2 °C to 8 °C for no longer than 3 days (72 hours) prior to testing.
- Proper specimen collection and storage are critical to the assay function. Specimen stability under storage conditions other than those listed in Section 12, Procedure below have not been evaluated with the Xpert NPM1 Mutation test.

12 Procedure

12.1 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.

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

Important **Remove the cartridge from the cardboard packaging before preparing the sample. (Refer to Section 12.3, Preparing the Cartridge).**

12.2 Preparing the Sample

12.2.1 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 ETDA 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 ºC to 8 ºC for up to 48 hours or stored at -20 ºC 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.

12.2.2 Preparing the Sample with WBC Count 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.
2. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the ETDA 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 12.2.1 to make the final lysate.
7. Discard any remaining PK or LY reagents.

12.3 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.

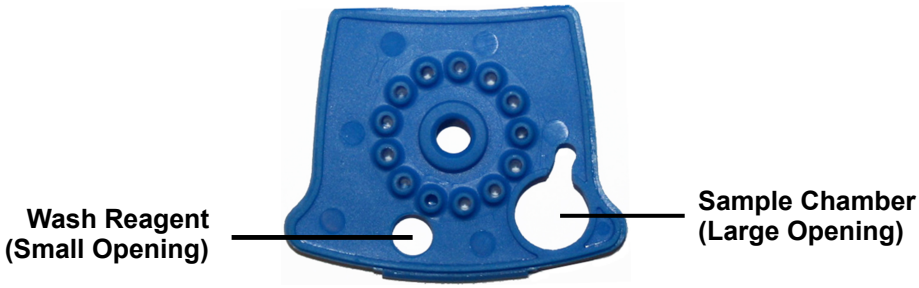

Figure 1. Xpert NPM1 Mutation Cartridge (Top View)

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

12.4 Starting the Assay

Important **Before you start the assay, ensure that the system is running GeneXpert Dx software version 6.2 or higher and that the correct 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 Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the **View Results** window and all the reports. The **Scan Sample ID Barcode** dialog box opens.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box opens.
6. 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. If you have scanned the cartridge barcode in the software and the Assay Definition File is not available, a screen will appear indicating the Assay Definition File is not loaded on the system. If this screen appears, contact Cepheid Technical Support.

7. Click **Start Test**. You may need to type your password in the dialog box that appears.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the assay is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door and removing the cartridge.
11. 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).

13 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.

14 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.

15 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 Test Results and Interpretation

Result	Interpretation

NPM1 Mutation DETECTED
See Figure 2, Figure 3, Figure 4
	NPM1 mutation transcript was detected.
	● 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.
	● Possible detected results:
	  ● NPM1 MUTATION DETECTED [#.##%]; Figure 2.
	  ● NPM1 MUTATION DETECTED [Above upper LoQ]; Figure 3.
	  ● NPM1 MUTATION DETECTED [Below LoD; <#.###%]; Figure 4.
	● ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and an endpoint above the threshold setting.
	● Probe Check PASS – all probe check results passed.

NPM1 Mutation NOT DETECTED See Figure 5	NPM1 mutation transcript was not detected. ● 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. ● ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and an endpoint above the threshold setting. ● Probe Check PASS – all probe check results passed.
INVALID 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 18, Troubleshooting Guide, for additional instructions for retesting the sample. ● NPM1 Mutation INVALID– NPM1 cycle threshold (Ct) was above zero and below the lower end of the valid range (Figure 8, Figure 9) ● 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 10) ● Probe Check – PASS; all probe check results passed.
ERROR See Figure 11	NPM1 Mutation transcript level cannot be determined. See Section 18, Troubleshooting Guide, for additional instructions for retesting the sample. ● NPM1 Mutation NO RESULT ● ABL NO RESULT ● Probe Check FAIL – All or one of the probe check results failed. ● Probe Check PASS or NA (not applicable) and Pressure Abort*. *If the probe check passed, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	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 18, Troubleshooting Guide, for additional instructions for retesting the samples. ● NPM1 Mutation NO RESULT ● ABL NO RESULT ● Probe Check NA (not applicable)

16 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 Test Results

Assay	NPM1 Mutant		ABL		Xpert NPM1 Mutation Test Results	Notes
	Ct	Result[a]	Ct	Result[a]		
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

[a] See the Analyte Results tab in the GeneXpert Dx System Software for details.

16.1 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 (ΔCt) 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; ΔCt = -2.6
$\% = 1.95^{(-2.6)} \times 100 \times 1.79 = 31.53\%$

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

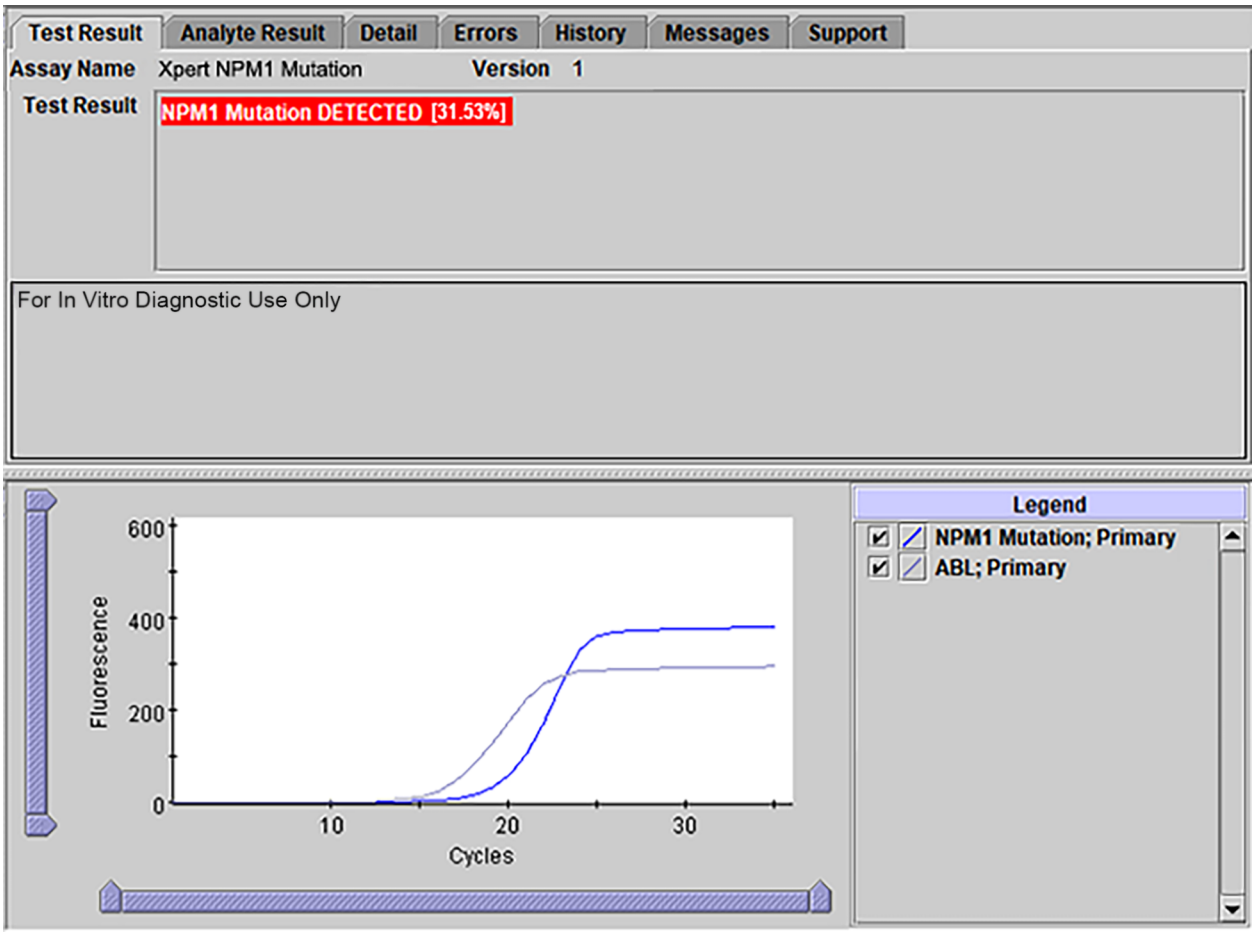

Figure 2. GeneXpert Dx View Results Window: NPM1 Mutation DETECTED [31.53%]

16.2 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 (ΔCt) value is obtained from ABL Ct minus NPM1 Mutation Ct:

$\% = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times$ 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; 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]**. See Figure 3.

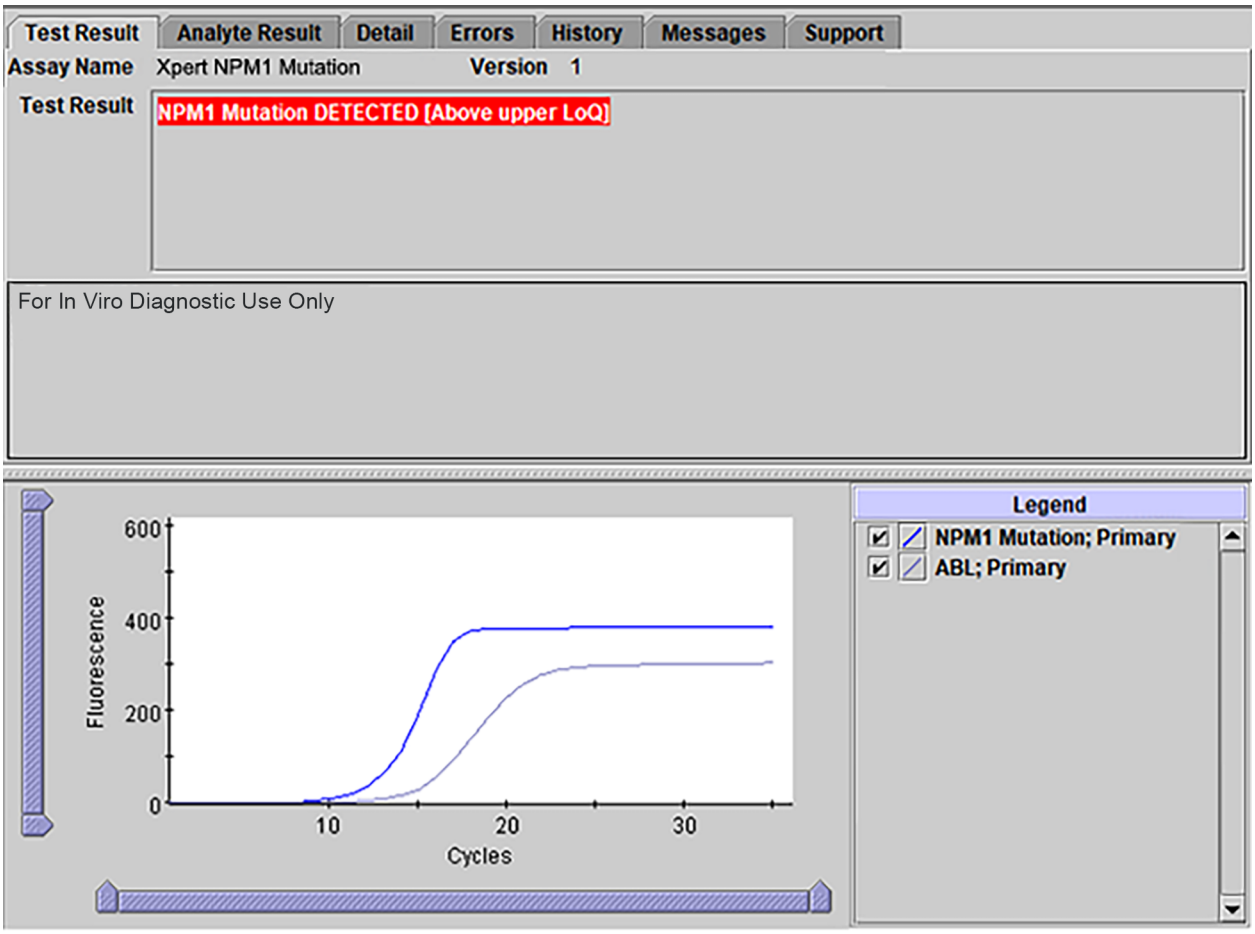

Figure 3. GeneXpert Dx View Results Window: NPM1 Mutation DETECTED [Above upper LoQ]

16.3 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 (ΔCt) 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; 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%]**. See Figure 4.

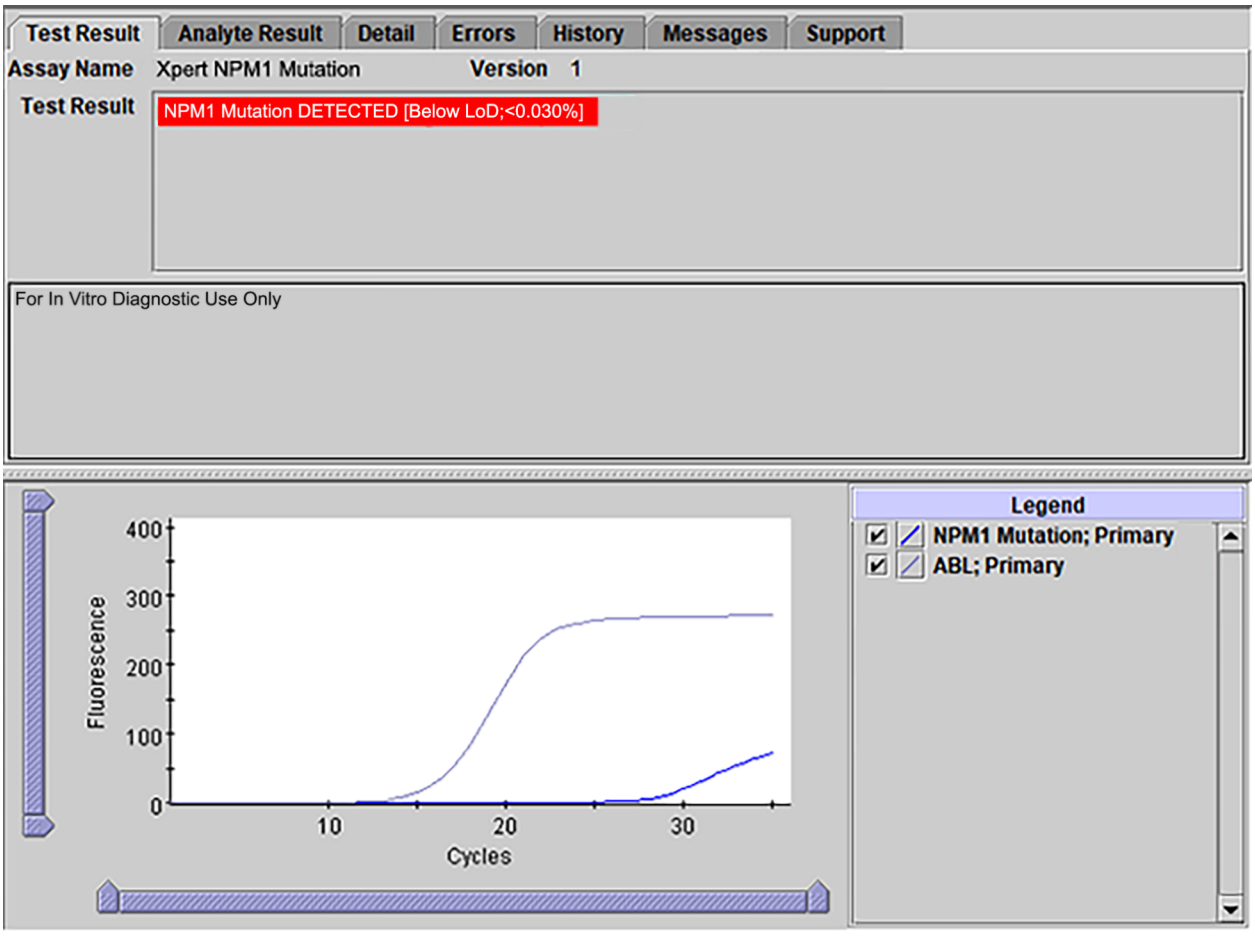

Figure 4. GeneXpert View Results Window: NPM1 Mutation DETECTED [Below LoD; <0.030%]

16.4 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 test to ensure having “Sufficient ABL transcript”. See Section 15, 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.

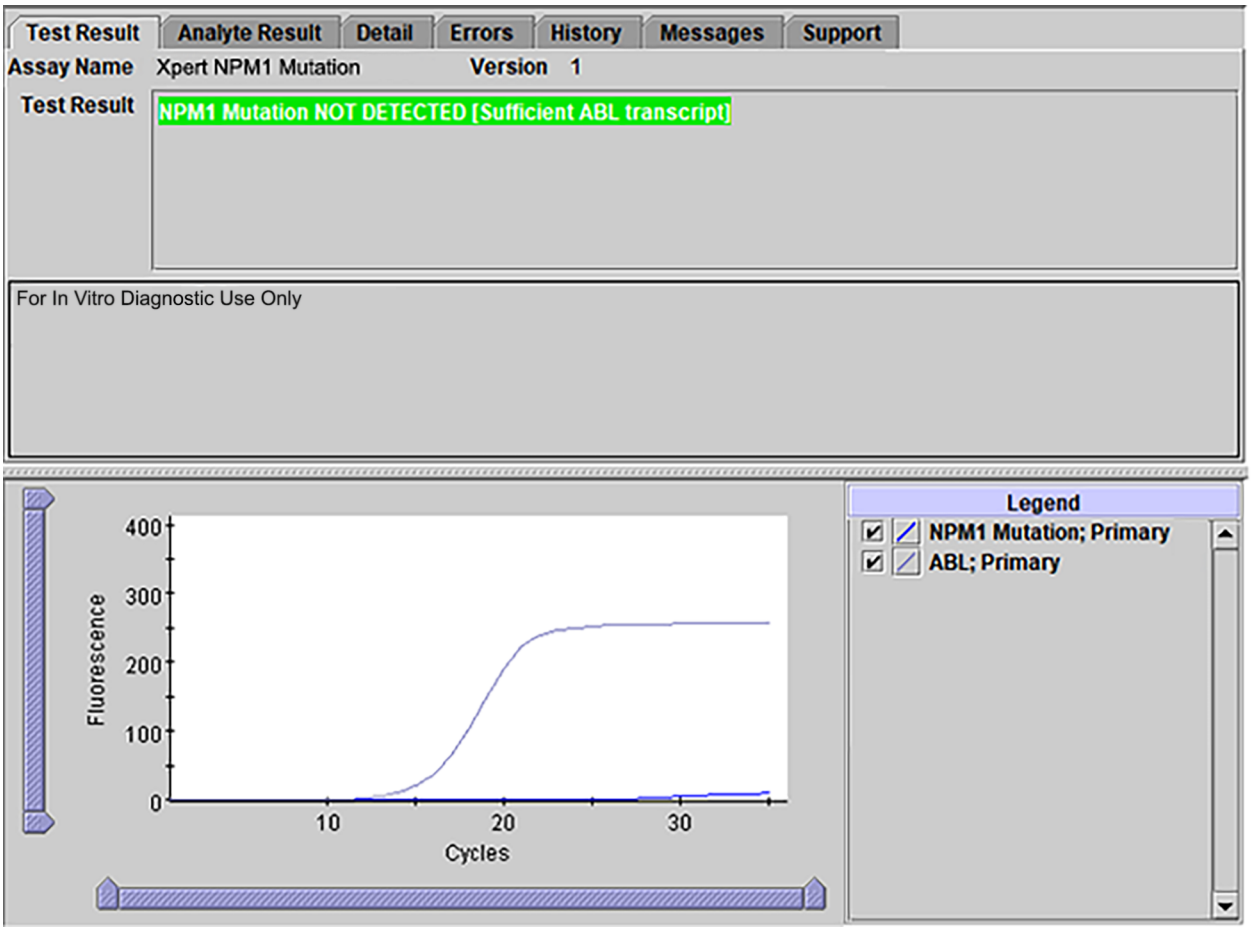

Figure 5. GeneXpert View Results Window: NPM1 Mutation NOT DETECTED [Sufficient ABL transcript]

16.5 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 test to ensure having “Sufficient ABL transcript”. Refer to Section 18, Troubleshooting Guide.

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

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

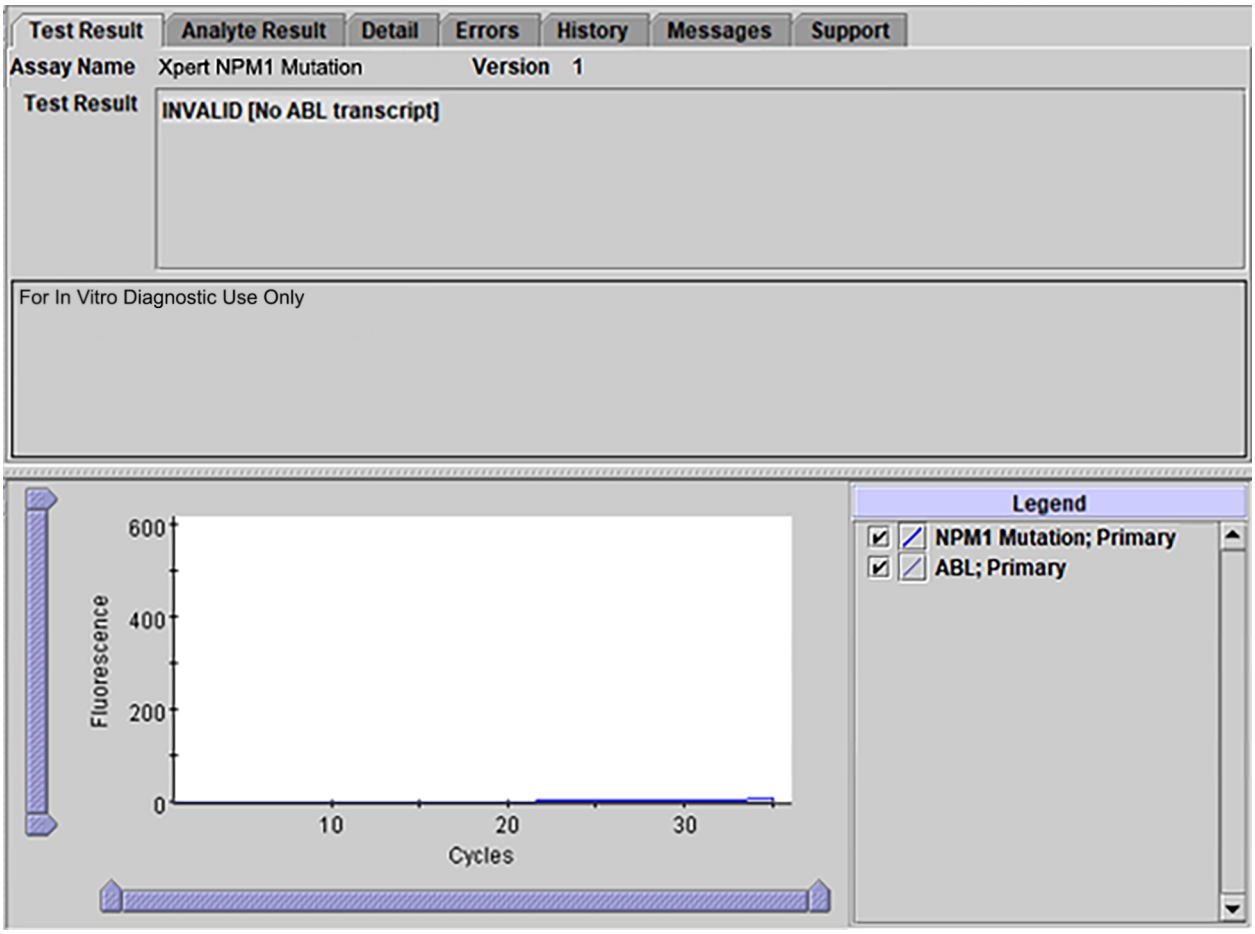

Figure 6. GeneXpert View Results Window: INVALID [No ABL transcript]

16.6 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 test to ensure having “Sufficient ABL transcript”. Refer to Section 18, 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.

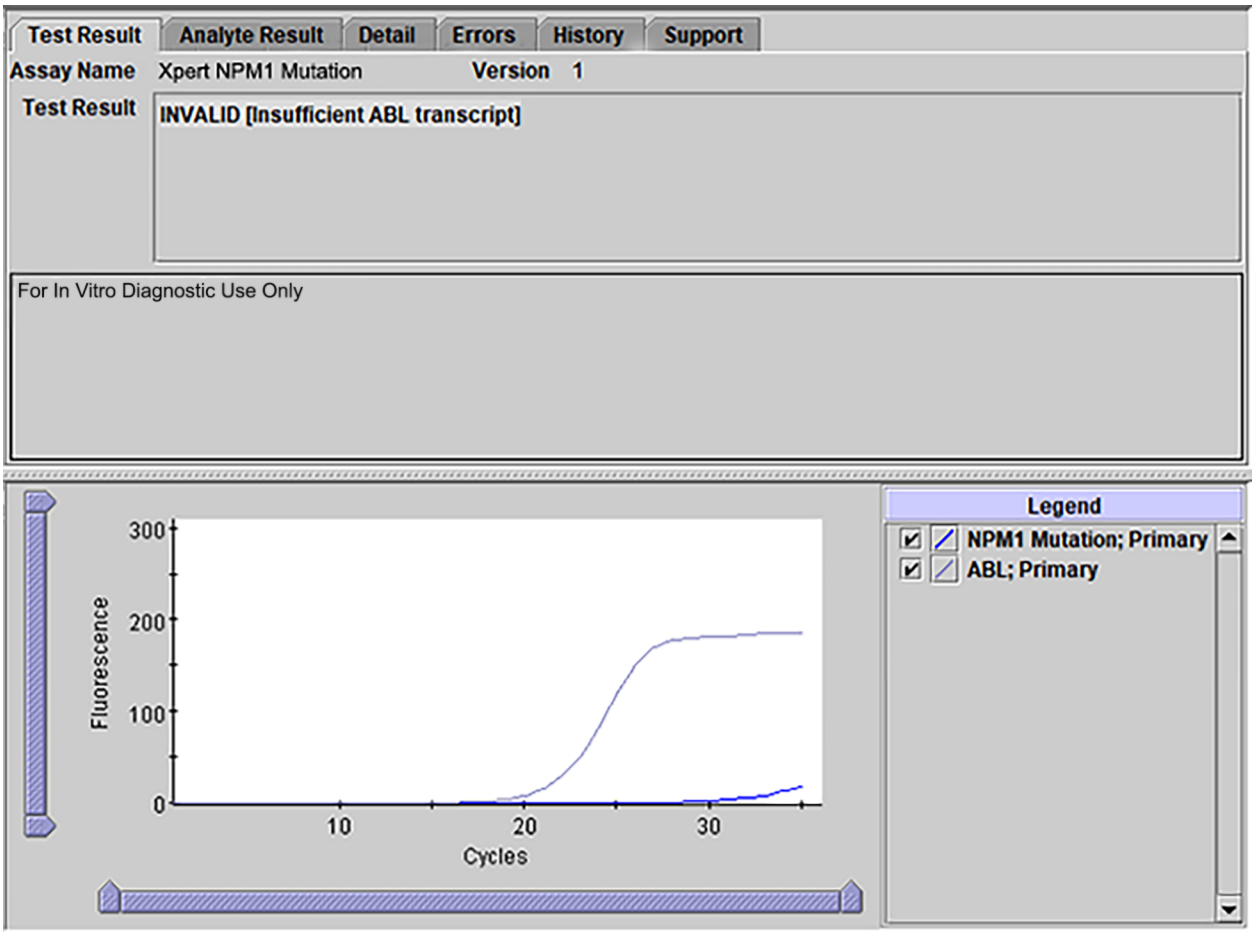

Figure 7. GeneXpert View Results Window: INVALID [Insufficient ABL transcript]

16.7 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 test to ensure having “Sufficient ABL transcript”. Refer to Section 18, Troubleshooting Guide.

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 and ABL transcript]**. See Figure 8.

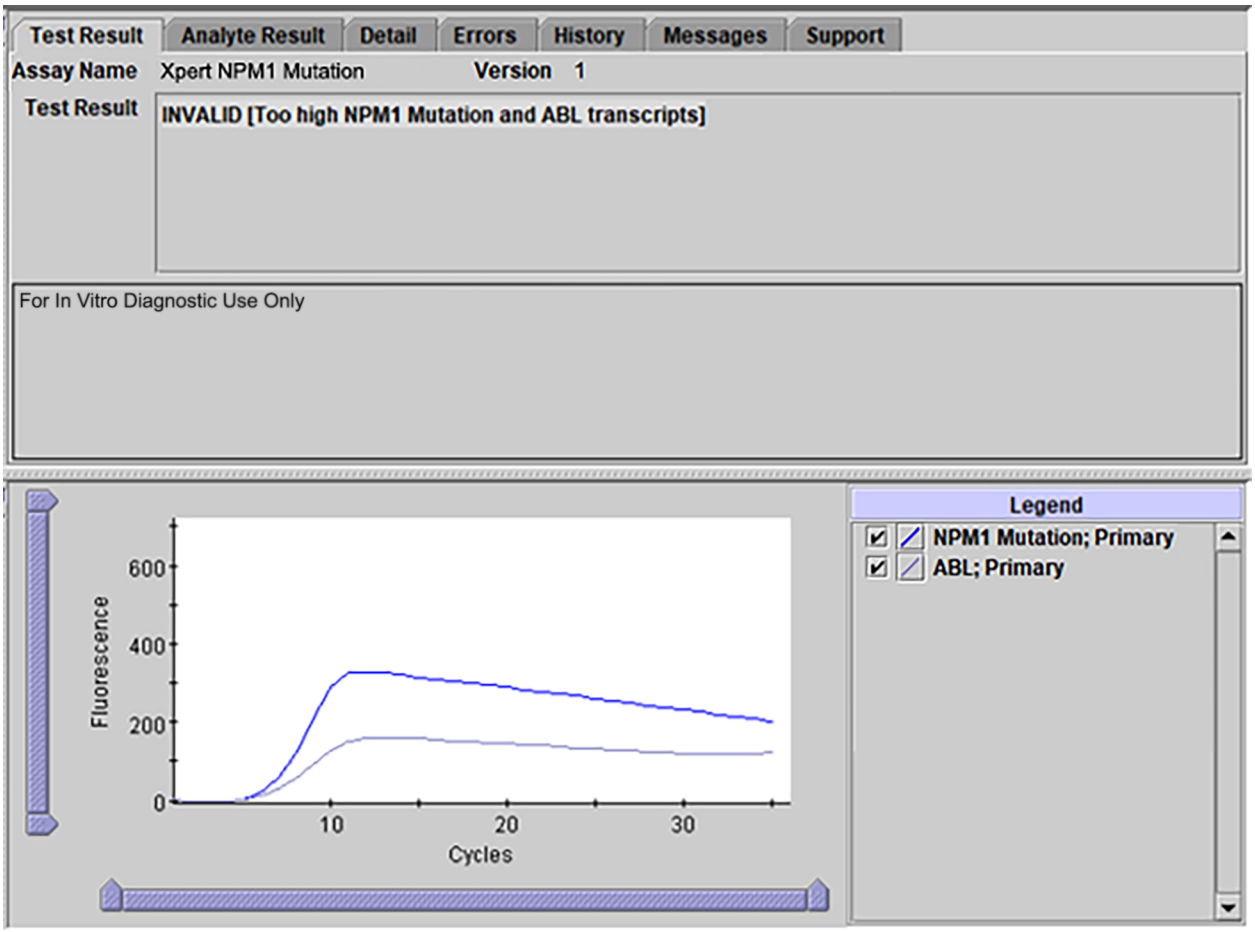

Figure 8. GeneXpert Dx View Results Window: INVALID [Too high NPM1 Mutation and ABL transcript]

16.8 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 test to ensure having “Sufficient ABL transcript”. Refer to Section 18, Troubleshooting Guide.

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

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

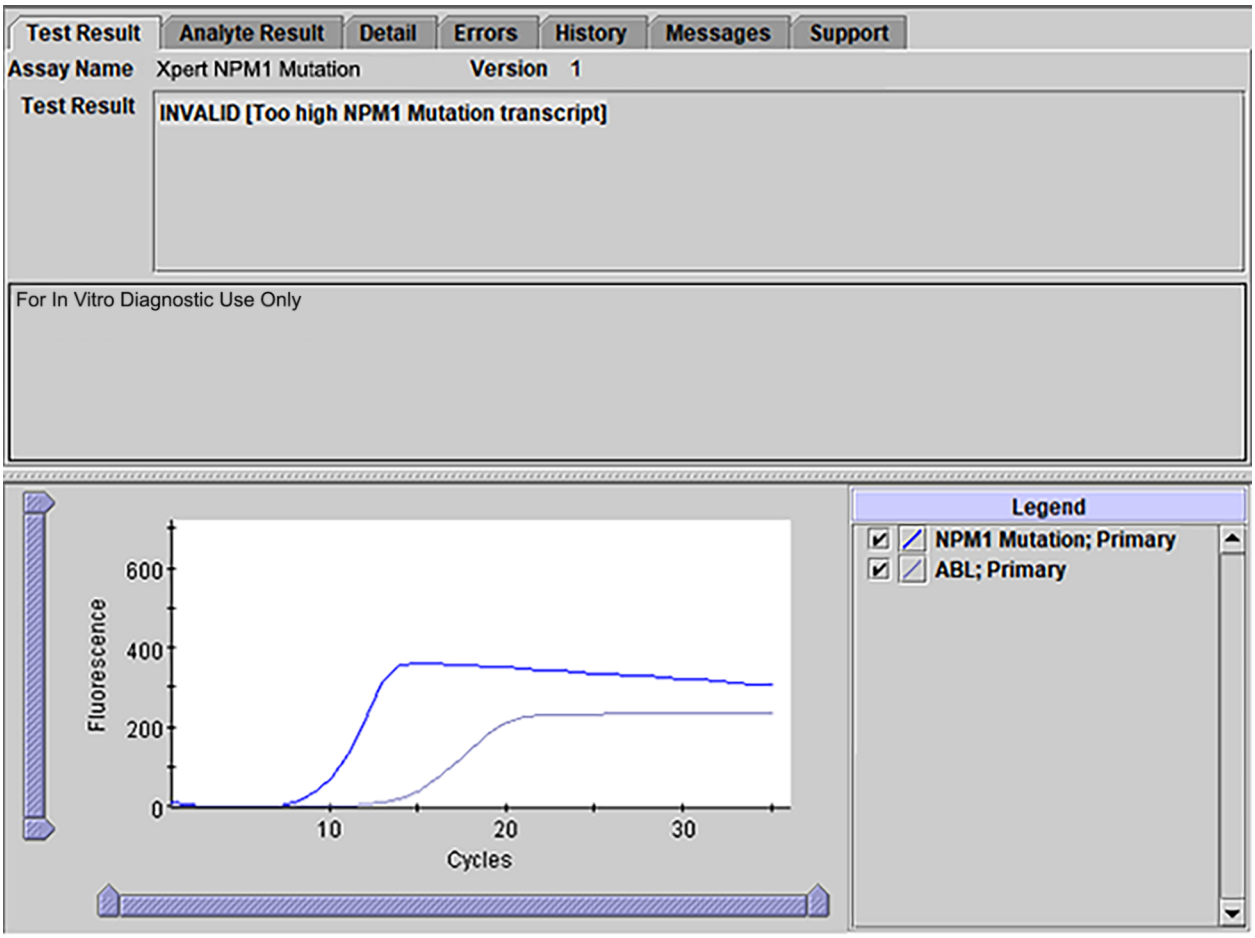

Figure 9. GeneXpert View Results Window: INVALID [Too high NPM1 Mutation transcript]

16.9 INVALID [Too high ABL Mutation transcript]

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”. Refer to Section 18, Troubleshooting Guide.

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

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

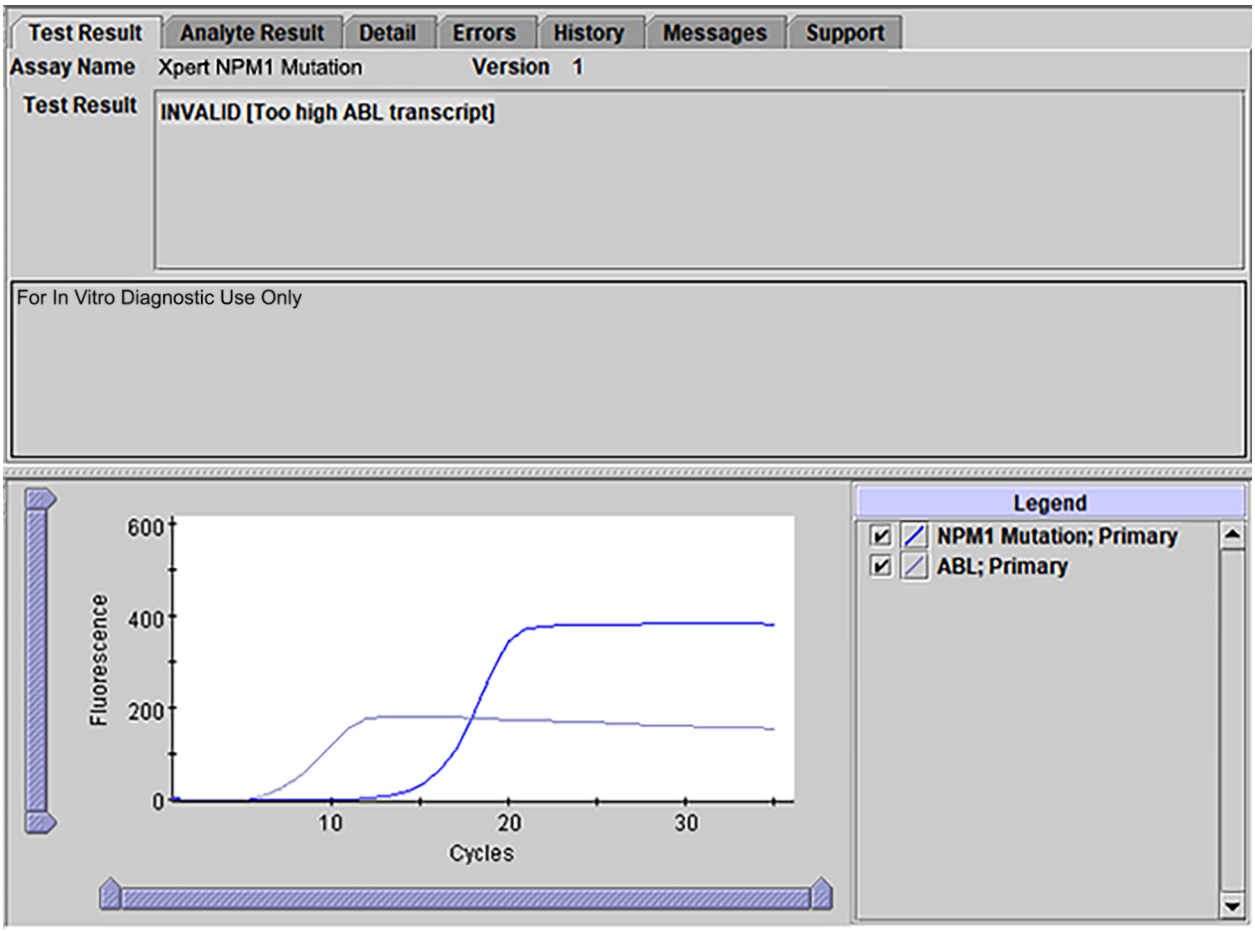

Figure 10. GeneXpert View Results Window: INVALID [Too high ABL transcript]

16.10 ERROR

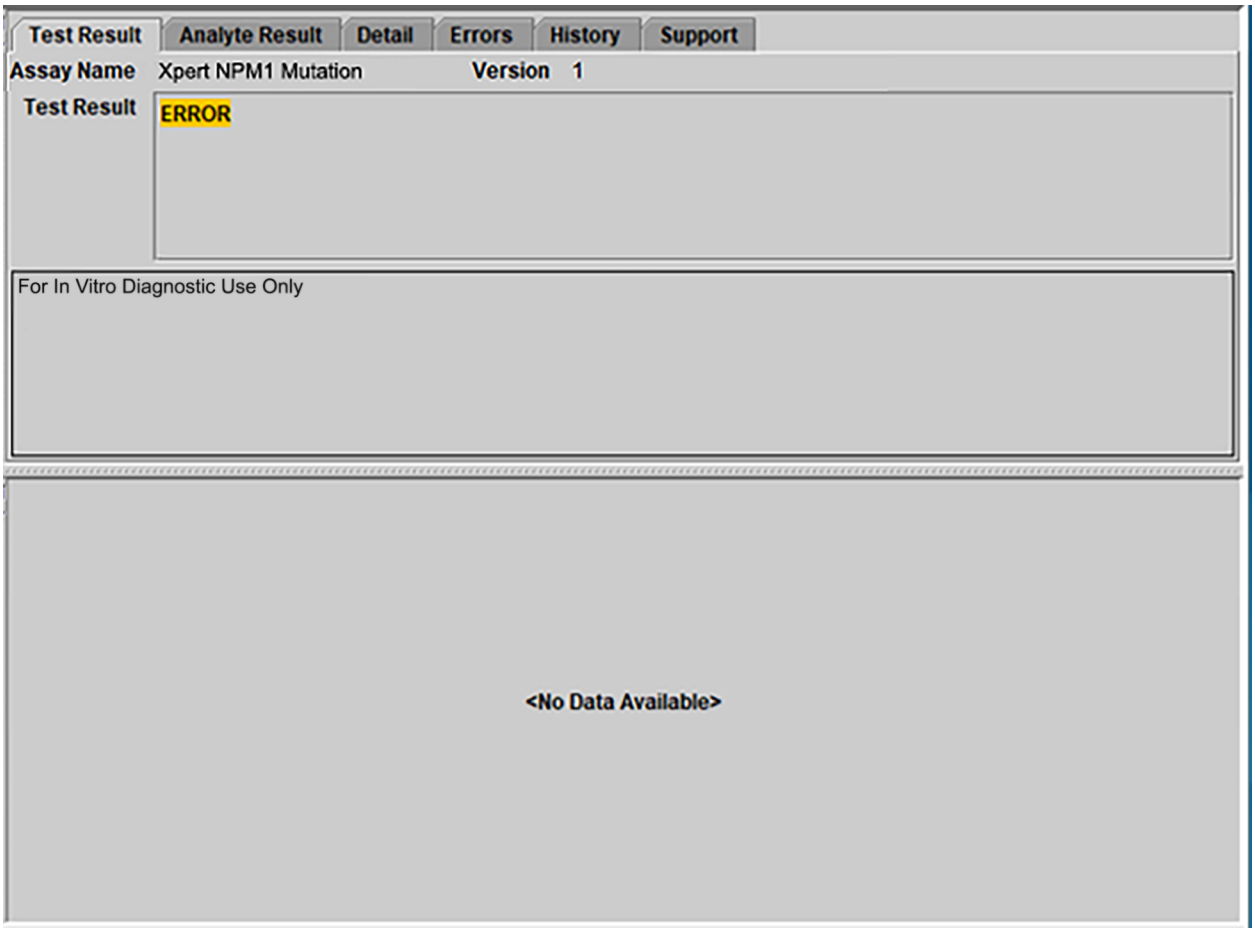

Figure 11. GeneXpert View Results Window: ERROR

17 Limitations of the Assay

- 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.

18 Troubleshooting Guide

Table 3. Troubleshooting Guide

Assay Result	Possible Causes	Suggestions
INVALID	Type 1: Endogenous control ABL failure: ● Poor sample quality ● RT-PCR inhibition ● ABL Ct > 20, and/or endpoint < 100	● Check the sample quality (e.g., exceeded sample storage requirement including time and temperature). ● Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 19.1, Retest Procedure for ERROR or INVALID (Type 1).
	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 19.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 2008)	Pressure exceeding limit (error message 2008)	● Check the sample quality ● Check for grossly elevated WBC count ● Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 19.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 5006, 5007, 5008, and 5009*) *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 19.1, Retest Procedure for ERROR or INVALID (Type 1).
NO RESULT	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 19.1, Retest Procedure for ERROR or INVALID (Type 1).

19 Retests

19.1 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 18, Troubleshooting Guide.

1. If sufficient blood sample volume is available, re-test from original blood sample collection tube following the procedure in Section 12.2.

   -OR-

   If blood sample volume is insufficient, re-test can be performed with the retained lysate from Section 12.2.1, Step 12.

   a. If retained lysate from Section 12.2.1, 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 12.2.1 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 12.4, Starting the Assay).

19.2 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 18, 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 12.2, 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 12.2.1, Step 12.

   a. If retained lysate from Section 12.2.1, 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 12.2.1, 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 12.4, Starting the Assay).

20 Expected Values

The Xpert NPM1 Mutation range covers key clinical decision points for monitoring of AML. Expected values are expressed as percent ratio of NPM1 Mutation mRNA to the ABL mRNA and range between 0.030% and 500%. Measurements below this range are reported as undetected or below the limit of detection (LoD). Measurements above this range are reported as above the limit of quantitation (LoQ). Refer to Section 15 for details.

21 Clinical Performance

A multi-site, observational method comparison study was conducted at three sites in the United States and one site outside of the United States. Specimens from 40 discrete AML patients with NPM1 mutation from one timepoint and across the dynamic range of the Xpert NPM1 Mutation test were enrolled into the study. Age and gender were collected for the patients from which the samples were obtained. The gender distribution was 11 males (27.5%) and 29 females (72.5%). All samples were from patients between 16 and 81 years of age with mean age of 59.7 years.

All 40 samples yielded valid tests results. Thirty-six of the 40 samples yielded results within the quantitative ranges of both tests. Four samples were excluded from the Deming regression since the samples were negative on the Xpert NPM1 Mutation and/or the comparator test. An additional sample was excluded because it was an outlier. In total 35 samples were included in the Deming regression analysis.

The performance of the Xpert NPM1 Mutation test versus the comparator assay was evaluated using a Deming Regression to determine the slope and intercept. Figure 12 presents the results of the Deming Regression analysis including the slope, intercept, and line of identity on the 35 samples. The 95% confidence bounds were calculated using the jackknife method and the Pearson's correlation coefficient is displayed.

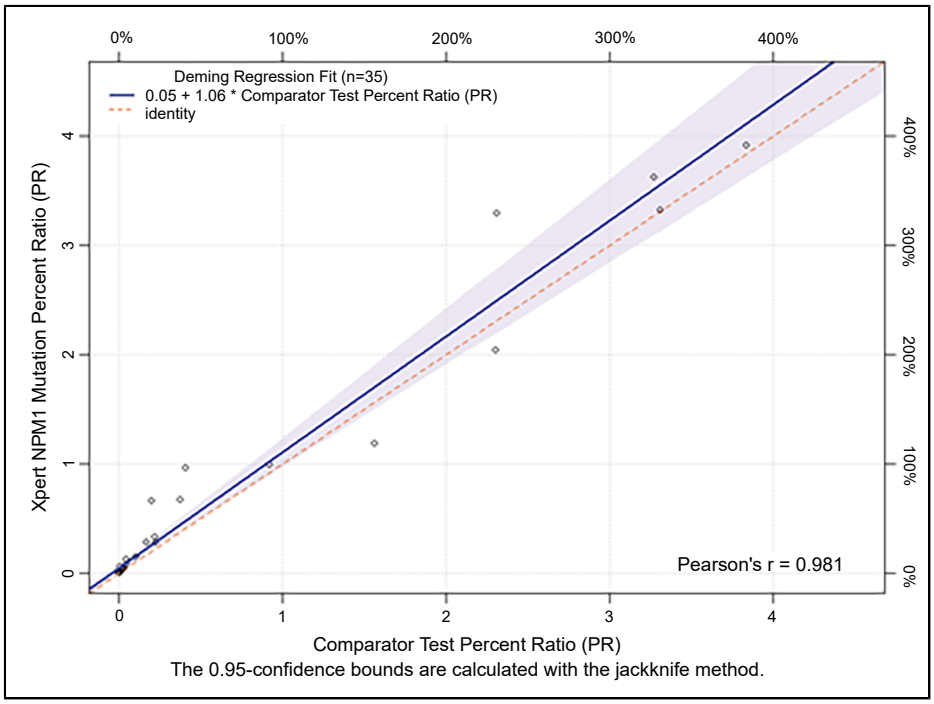

Figure 12. Deming Regression for Percent Ratio

The slope and intercept for the percent ratio from the Deming Regression analysis were 1.06 and 0.05, respectively, and the Pearson's correlation was 0.981 between the Xpert NPM1 Mutation test and comparator test measurements.

A Bland-Altman analysis for difference in percent ratio was evaluated for the 35 samples with quantitative results that were within the linear range of the Xpert NPM1 Mutation and the comparator test. Figure 13 shows the Bland-Altman plot with the difference in percent ratio between the two tests versus the average percent ratio results for each sample. The plot also shows the upper and lower two standard deviation (2SD) of the mean difference that was observed in the study.

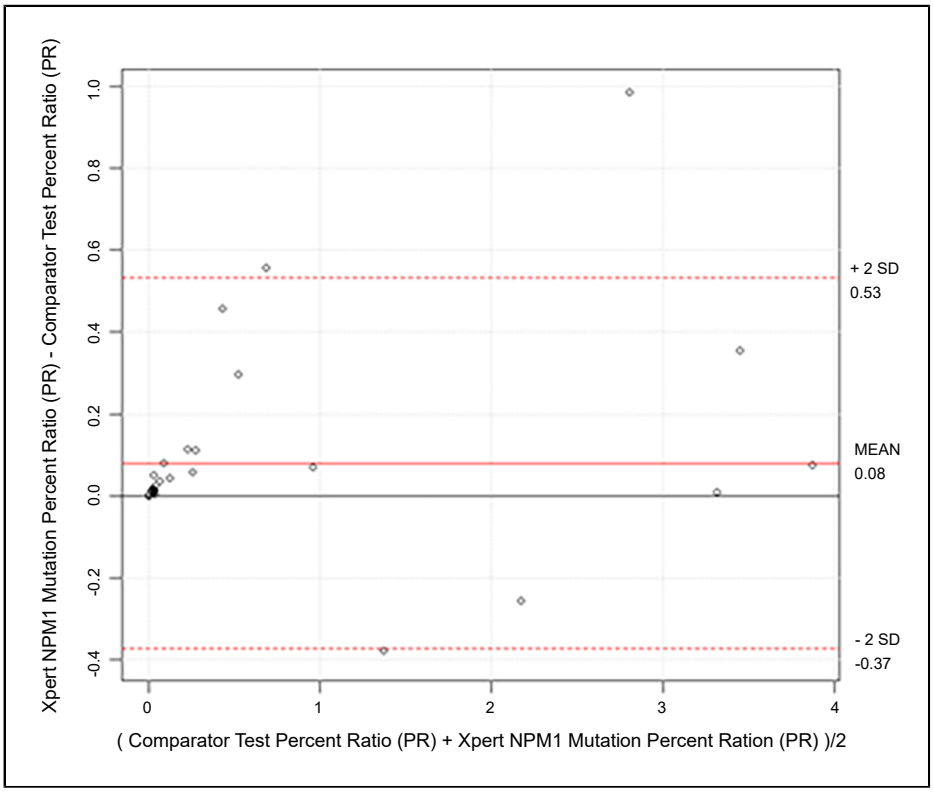

Figure 13. Bland-Altman Plot for Xpert NPM1 Mutation & Comparator Test Percent Ratio

The mean difference was 0.08 in percent ratio between the Xpert NPM1 Mutation and the comparator test result. The majority (91.4%, 32/35) of the results were within the 2SD of the mean difference.

22 Analytical Data

22.1 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[9] [6]. Regression curves for each subtype are shown in Figure 14, Figure 15, and Figure 16. Linear range of each subtype and their linear model coefficients are summarized in Table 4.

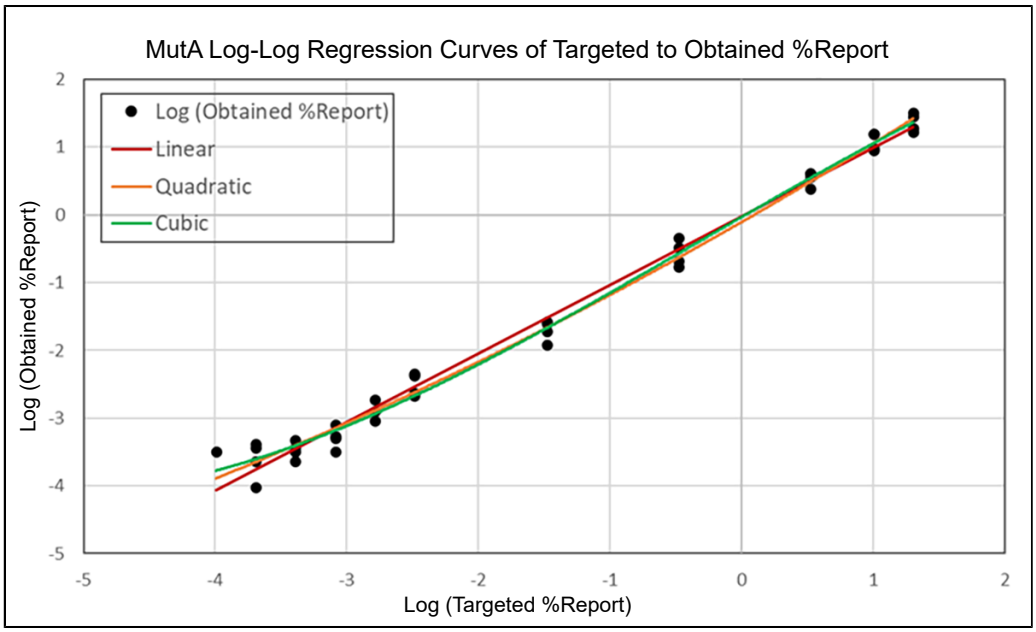

Figure 14. Regression Curves for mutA

Figure 15. Regression Curves for mutB

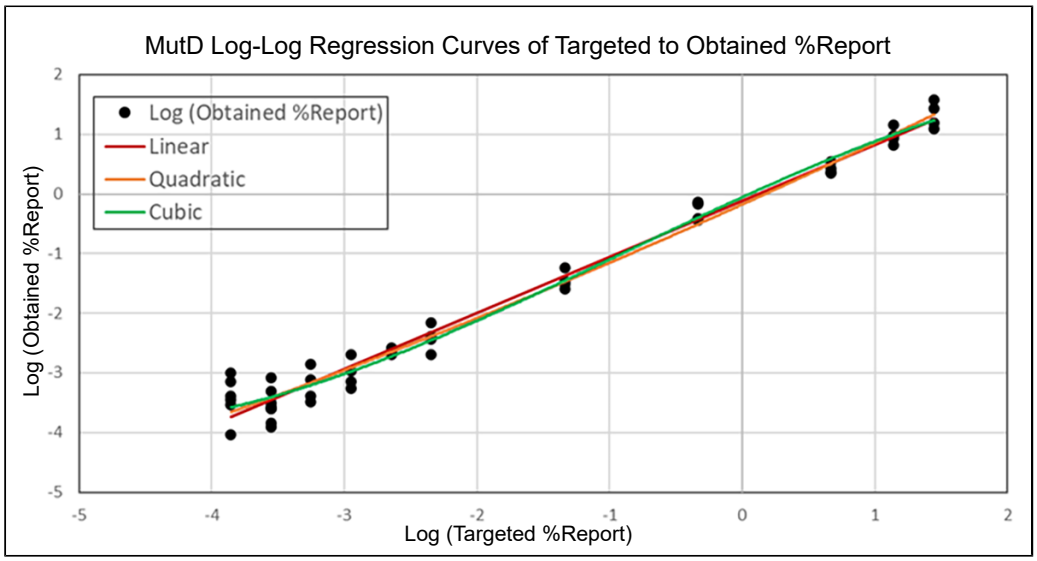

Figure 16. Regression Curves for mutD

Table 4. Summary of Linear Ranges and Linear Model Coefficients

Subtype	Linear Range	Intercept	Slope	R^2
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%.

22.2 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[10]. 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 test.

The limit of quantitation (LoQ) is the lowest NPM1 Mutation/ABL level above which samples can be quantified with a standard deviation ≤ 0.36 log reduction (LR) for mean LRs above 3.5. In accordance with CLSI EP17-A2[10], 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 test.

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[10], the LoB of the Xpert NPM1 Mutation test was estimated and verified at 0.0085% (Table 5).

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

Subtype	LoD [%NPM1 Mutation/ABL]	LoQ [%NPM1 Mutation/ABL]	LoB [%NPM1 Mutation/ABL]
mutA	0.025%	0.025%	0.0085%
mutB	0.023%	0.023%	
mutD	0.030%	0.030%	

22.3 Analytical Specificity

The analytical specificity of the Xpert NPM1 Mutation test was determined by testing EDTA-treated peripheral blood specimens drawn from twenty-five healthy donors.

No NPM1 Mutation **DETECTED** result was obtained from any of the presumably NPM1-mutation-negative specimens evaluated in this study. Thus, the Xpert NPM1 Mutation test is specific to the mutant NPM1 mRNA transcripts (types A, B and D in exon 12) associated with AML and has an analytical specificity of 100% for EDTA peripheral blood specimens.

22.4 Evaluation of Carry-over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination from cartridges run sequentially in the same instrument module. A presumably NPM1-mutation-negative sample was tested following a high NPM1-mutation-positive sample in the same GeneXpert module. The testing scheme was repeated 10 times on two GeneXpert modules (22 negatives and 20 positives in total). All runs of the positive sample returned the expected result of "**NPM1 Mutation DETECTED [#.##%]**", and all runs of the negative samples returned the expected result of "**NPM1 Mutation NOT DETECTED [Sufficient ABL transcript]**".

22.5 Potentially Interfering Substances

This study evaluated five substances that may be present in EDTA peripheral blood specimens with the potential to interfere with test performance. The compounds and levels tested (see Table 6) were based on guidance from CLSI EP07-ED3[11]. Interferents were tested in EDTA peripheral blood specimens contrived with lysates of cultured NPM1-mutation-positive cells, representing three levels: > 1%, 0.1–0.5%, and negative. Test controls consisted of the same samples without the potentially interfering substances. Each level was tested in the absence and presence of the five individual interferents at 4 replicates per condition. A substance was considered non-interfering if in its presence, the mean percent ratio observed was within 3-fold difference when compared to the control.

No clinically significant inhibitory effects on the Xpert NPM1 Mutation test were observed with any of the interfering substances evaluated in this study. No statistically significant differences (p-value < 0.05) in any test conditions were observed and the reported percent ratios between test and control conditions were within the acceptable 3-fold range.

Table 6. Potentially Interfering Substances Tested Using Xpert NPM1 Mutation

Interfering Substances	Concentration Tested
Unconjugated Bilirubin	20 mg/dL
Cholesterol, Total	500 mg/dL
Triglycerides, Total (Lipids)	3000 mg/dL
Heparin	3500 U/L
EDTA (short draw)	930 mg/dL

23 Reproducibility and Precision

The study was designed in accordance with general principles espoused in CLSI EP05-A3 standard for Multifactor Studies. It was conducted at three sites. The study design incorporated sample panel members that included mutations A, B, and D at two concentrations. Seven panel members were tested in duplicate, two runs per day, for a total of 6 days by each of the two operators at three different sites (3 Sites × 2 Operators × 3 Lots × 2 days × 2 Run × 2 Replicates = 144 test results/panel member). The reproducibility and precision panels were prepared by Cepheid and consist of seven panel members as shown in Table 7. The panels were contrived in a simulated EDTA peripheral blood (PB) matrix.

Table 7. Reproducibility and Precision Panels

Panel Member	Target	Level Percent Ratio (PR)
1	Negative	NA
2	NPM1 Mutation A	Moderate Positive (~5%)
3	NPM1 Mutation A	Low Positive (~0.2%)
4	NPM1 Mutation B	Moderate Positive (~5%)
5	NPM1 Mutation B	Low Positive (~0.2%)
6	NPM1 Mutation D	Moderate Positive (~5%)
7	NPM1 Mutation D	Low Positive (~0.2%)

The number of samples with valid results for each panel member analyzed by each of the two operators across three sites is shown in Table 8.

Table 8. Reproducibility and Precision: Number of Samples with Valid Results

Panel Member		Site 1			Site 2			Site 3			Total Samples
		Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
1	Negative	24/24[a]	(24/24)	(48/48)[a]	(24/24)[b]	(24/24)	(48/48)[b]	(24/24)	(24/24)	(48/48)	(144/144)
2	LR1.3: mut A (~5% ratio)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
3	LR2.7: mut A (~0.2% ratio)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
4	LR1.3: mut B (~5% ratio)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
5	LR2.7: mut B (~0.2% ratio)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
6	LR1.3: mut D (~5% ratio)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(24/24)	(24/24)	(48/48)	(144/144)
7	LR2.7: mut D (~0.2% ratio)	(24/24)	(24/24)	(48/48)	(24/24)[c]	(24/24)	(48/48)[c]	(24/24)	(24/24)	(48/48)	(144/144)

[a] Two negative specimens had valid but detected results (FP)

[b] One negative specimen had a valid but detected result (FP)

[c] One LR 2.7: mut D (~0.2% ratio) specimen had a valid but not detected result (FN)

The quantitative results were analyzed by nested analysis of variance (ANOVA) with random effects and the coefficient of variation (CV). The results from the ANOVA calculations for standard deviation and variance for each positive sample are provided in Table 9. The variance and percent of the total variance contributed by each component (Site/Instrument, Operator, Lot, Day, Run) is indicated as SD and percent contribution of each component.

Table 9. Results from Coefficient of Variation (CV): Percent Ratio (PR)

Panel Member	N	Mean	Site		Op		Lot		Day		Run		Within Assay		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LR1.3: mut A (~5% ratio)	144	4.3%	0.00	6.14	0.00	0.00	0.00	4.29	0.00	8.91	0.00	4.36	0.01	17.83	0.01	21.74
LR2.7: mut A (~0.2% ratio)	144	0.2%	0.00	0.00	0.00	12.43	0.00	0.00	0.00	23.71	0.00	0.00	0.00	74.56	0.00	79.22
LR1.3: mut B (~5% ratio)	144	5%	0.00	8.24	0.00	0.00	0.01	11.50	0.00	7.19	0.00	0.00	0.01	20.88	0.01	26.23
LR2.7: mut B (~0.2% ratio)	144	0.2%	0.00	0.00	0.00	0.00	0.00	7.48	0.00	0.00	0.00	0.00	0.00	19.28	0.00	20.68
LR1.3: mut D (~5% ratio)	144	4.2%	0.00	5.15	0.00	0.00	0.01	12.91	0.00	8.78	0.00	0.00	0.01	18.30	0.01	24.60
LR2.7: mut D (~0.2% ratio)	143[a]	0.2%	0.00	10.86	0.00	0.00	0.00	12.91	0.00	6.77	0.00	0.00	0.00	22.83	0.00	29.18

[a] One sample was not detected by Xpert NPM1 and was excluded from the analysis because there was no quantitative measurement.

The total coefficient of variation (CV) percent of the percent ratio reporting quantitative values for the moderate positive samples LR1.3: mut A, mut B and mut D (~5% ratio) ranged from 21.74 to 26.23 and for the low positive samples LR2.7: mut A, mut B and mut D (~0.2% ratio) ranged from 20.68 to 79.22.

24 References

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25 Cepheid Headquarters Locations

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26 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

United States

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

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

27 Table of Symbols

Symbol	Meaning
	Catalog number
	CE marking – European Conformity
	*In vitro* diagnostic medical device
	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
	Flammable liquids
	Reproductive and organ toxicity
	Warning
	Authorized Representative in the European Community
	Authorized Representative in Switzerland
	Importer

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28 Revision History

Section	Description of Change
23	Corrected error in "Reproducibility and Precision" section.