Cepheid®

Instructions for Use

Xpert® NPM1 Mutation

For use with GeneXpert® System with Touchscreen

Catalog Numbers

REF RNPM1-10

303-0948 | Rev. A | 2023-07

RUO For Research Use Only. Not for use in Diagnostic procedures.

Trademark, Patents and Copyright Statements

See Revision History for a description of changes.

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Product Information

Proprietary Name

Xpert® NPM1 Mutation

Common or Usual Name

Xpert NPM1 Mutation

Product Description Summary and Principle of Procedure

Product Description

The Xpert NPM1 Mutation assay, performed on the Cepheid GeneXpert Instrument Systems, 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.

Summary and Explanation

Acute myeloid leukemia (AML) is a cancer of the myeloid blood hematopoietic stem cells in the 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 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.[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 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.[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 the GeneXpert system with touchscreen, 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 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.

Reagents, Instruments, and Materials

Reagents

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:

Xpert NPM1 Mutation Reagents	**10 of each per kit**
• Proteinase K (PK)	10 x 130 µL per vial

• Lysis Reagent (LY) (Guanidinium Chloride)
  ○ Guanidinium Chloride
  ○ Urea
  ○ Sodium dodecyl sulphate
	10 x 5.3 mL per vial

• Wash Reagent
  ○ Ethanol
  ○ Guanidinium thiocyanate
	10 x 2.9 mL per ampoule

Xpert NPM1 Mutation Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, 2, 3 and 4 (freeze-dried)	1 of each per cartridge
• Rinse Reagent	2.0 mL per cartridge
• Elution Reagent	2.5 mL per cartridge
CD	**1 per kit**
• Assay Definition File (ADF)	

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

Note Safety Data Sheets (SDS) are available at www.cepheid.com or 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.

Materials Required but Not Provided

- GeneXpert system with touchscreen running Cepheid OS software version 2.0 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

Materials Available but Not Required

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

Warnings and Precautions

General

- For Research Use Only. Not for use in diagnostic procedures. RUO
- 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.
- 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 operator manual are necessary to avoid erroneous results.

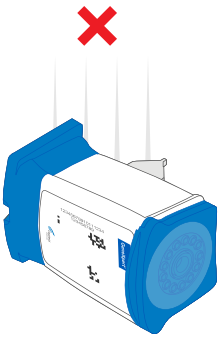
- Performing the Xpert NPM1 Mutation assay outside the recommended kit or sample storage temperature ranges and time may produce erroneous or invalid results.
- Biological samples, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard 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.[8]

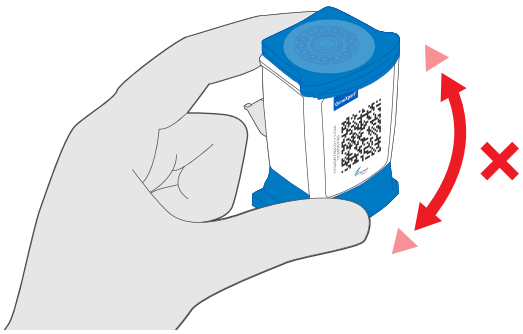
Sample

- Maintain proper storage conditions during sample transport to ensure the integrity of the sample (see Sample Collection, Transport and Storage, 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.

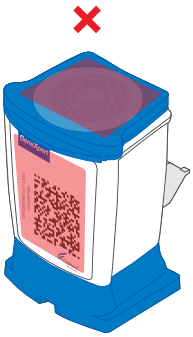
Assay/Reagent

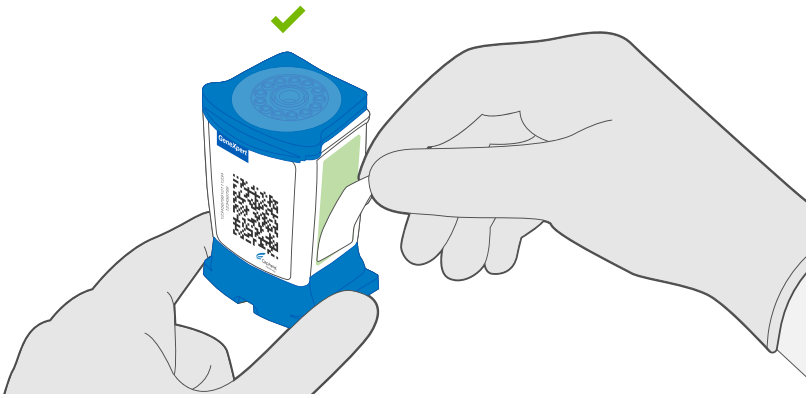
- Do not substitute Xpert NPM1 Mutation reagents with other reagents.
- Do not open the Xpert NPM1 Mutation cartridge lid except when adding sample and Wash Reagent.
- Do not use a cartridge that has been dropped after removing it from the packaging.





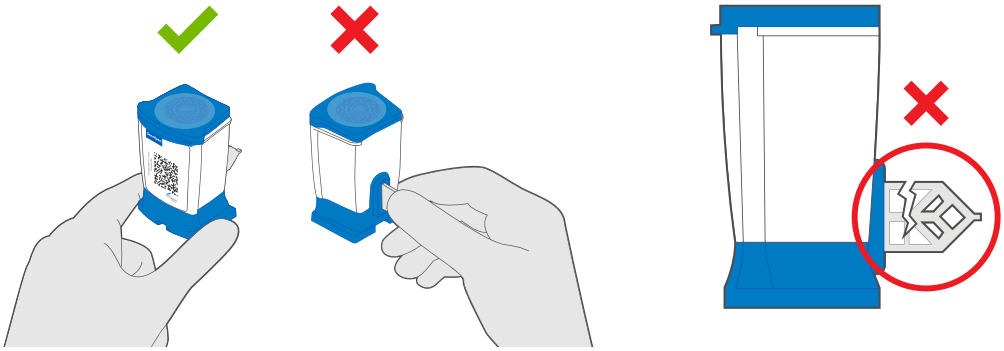
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge.





- 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.

Chemical Hazards, Storage and Handling

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.

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).

Specimen Collection, Testing, and Results

Specimen Collection

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 Procedure, Procedure below have not been evaluated with the Xpert NPM1 Mutation assay.

Procedure

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 Preparing the Cartridge, Preparing the Cartridge).**

Preparing the Sample

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 °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.

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 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 Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL to make the final lysate.
7. Discard any remaining PK or LY reagents.

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.

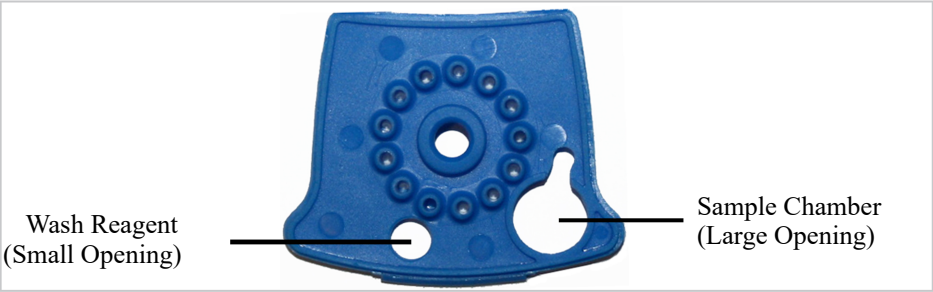


Figure 1 Xpert NPM1 Mutation Cartridge (Top View)

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

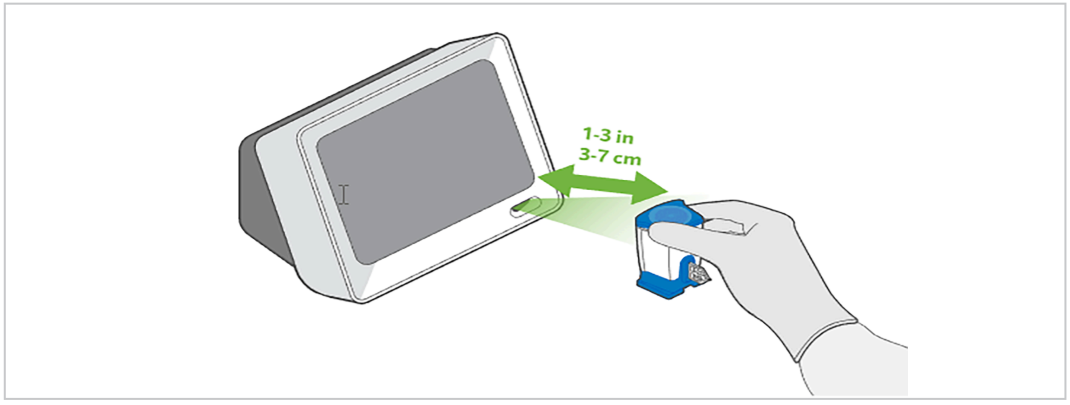
Starting the Test: GeneXpert System with Touchscreen

Important **Before you start the test, make sure that:**

- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

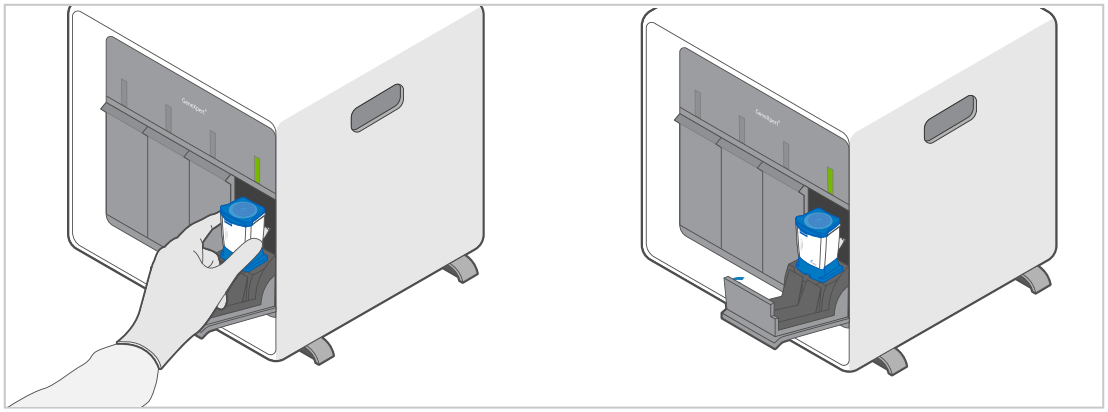
Note The default workflow is shown. Your system administrator may alter the workflow.

1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue**.

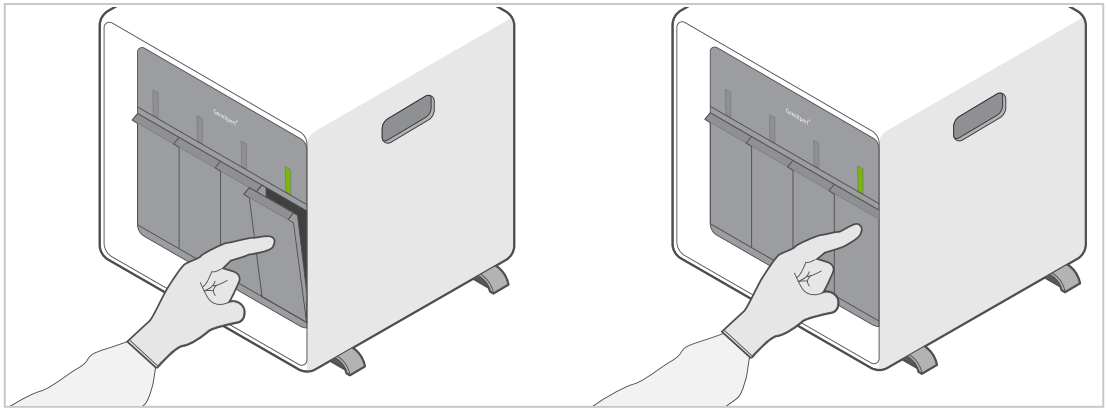


6. Select the desired test and touch **Continue**.

7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm**.
9. Open the module door under flashing green light and insert the cartridge.



10. Close cartridge module door completely by pressing until it latches. The test starts.



11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution's standard practices.

Viewing Results: GeneXpert System with Touchscreen

The GeneXpert system with touchscreen results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**. More detailed instructions for viewing and uploading results are available in your system operator manual.

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.

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

NPM1 Mutation DETECTED	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 [#.##%]
	  ○ NPM1 MUTATION DETECTED [Above upper LoQ]
	  ○ NPM1 MUTATION DETECTED [Below LoD; <#.###%]
	• ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
	• Probe Check PASS – all probe check results passed.

NPM1 Mutation NOT DETECTED	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	NPM1 Mutation transcript level cannot be determined due to sample containing excess NPM1 mutation transcript and/or excess or insufficient ABL transcript. See Troubleshooting Guide, 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 . • ABL FAIL – ABL cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting • Probe Check – PASS; all probe check results passed.

Result	Interpretation
ERROR	NPM1 Mutation transcript level cannot be determined. See Troubleshooting Guide, 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 Troubleshooting Guide, Troubleshooting Guide, for additional instructions for retesting the samples. • NPM1 Mutation NO RESULT • ABL NO RESULT • Probe Check NA (not applicable)

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[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.

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:

% = $E_{\Delta Ct}^{(\Delta Ct)}$ x 100 x 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.

Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79

Example: Assay’s ABL Ct = 14.5; NPM1 Mutation Ct = 17.1; ΔCt = -2.6

% = $1.95^{(-2.6)}$ x 100 x 1.79 = 31.53%

Result: `NPM1 Mutation DETECTED [31.53%]`.

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:

% = $E_{\Delta Ct}^{(\Delta Ct)}$ x 100 x 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.

Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79

Example: Assay’s ABL Ct = 13.4; NPM1 Mutation Ct = 10.2; ΔCt = 3.2

% = $1.95^{(3.2)}$ x 100 x 1.79 = 1516.92% is greater than the defined assay upper LoQ at 500%

Result: `NPM1 Mutation DETECTED [Above upper LoQ]`.

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:

% = $E_{\Delta Ct}^{(\Delta Ct)}$ x 100 x 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.

Lot-specific $E_{\Delta Ct}$ = 1.95; *SF* = 1.79

Example: Assay's ABL Ct = 14.3; NPM1 Mutation Ct = 28.8; ΔCt = -14.5

% = $1.95^{(-14.5)}$ x 100 x 1.79 = 0.011% is less than the defined assay LoD at 0.030%

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

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 Results, 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]`**.

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 Troubleshooting Guide, Troubleshooting Guide.

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

Result: **`INVALID [No ABL transcript]`**.

INVALID [Insufficient ABL transcript]

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

The GeneXpert software requires the ABL Ct to be greater than or equal to "6" and less than or equal to "20" for the Xpert NPM1 Mutation assay to ensure having "Sufficient ABL transcript". Refer to Troubleshooting Guide, Troubleshooting Guide.

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

Result: **`INVALID [Insufficient ABL transcript]`**.

INVALID [Too high NPM1 Mutation and ABL 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 Troubleshooting Guide, 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]`**.

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 Troubleshooting Guide, 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* .

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 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 Troubleshooting Guide, 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]`**.

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

Assay Result	Possible Causes	Suggestions
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 Retest Procedure for ERROR (Code 2008) or INVALID (Type 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 Retest Procedure for ERROR or INVALID (Type 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 Retest Procedure for ERROR or INVALID (Type 1), Retest Procedure for ERROR or INVALID (Type 1).

Retests

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 Troubleshooting Guide, Troubleshooting Guide.

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

   -OR-

   If blood sample volume is insufficient, re-test can be performed with the retained lysate from Preparing the Sample with Un

   a. If retained lysate from Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL, 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 Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL 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 , Starting the Assay).

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 Troubleshooting Guide, 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 Preparing the Sample, 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 Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL, Step 12.
   a. If retained lysate from Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL, 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 Preparing the Sample with Unknown White Blood Cell (WBC) Count or Samples with Less than 30 million/mL, 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 , Starting the Assay).

Limitations

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.

Specific Performance Characteristics

Analytical Data

The data was collected from internal studies only.

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 [6]. Regression curves for each subtype are shown in Figure 2, Figure 3, and Figure 4. Linear range of each subtype and their linear model coefficients are summarized in Table 4.

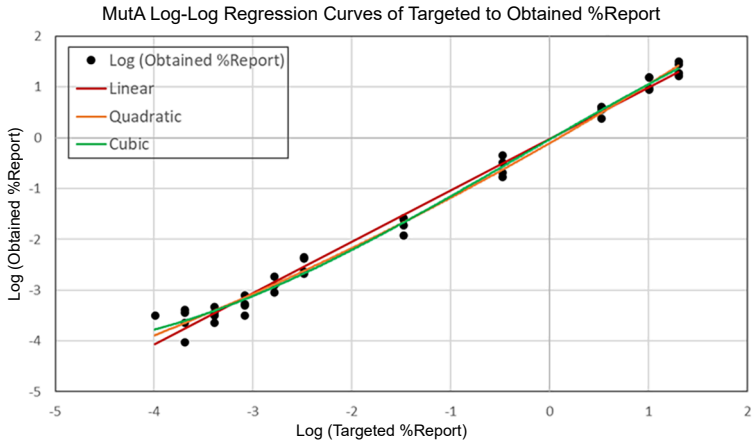


Figure 2 Regression Curves for mutA

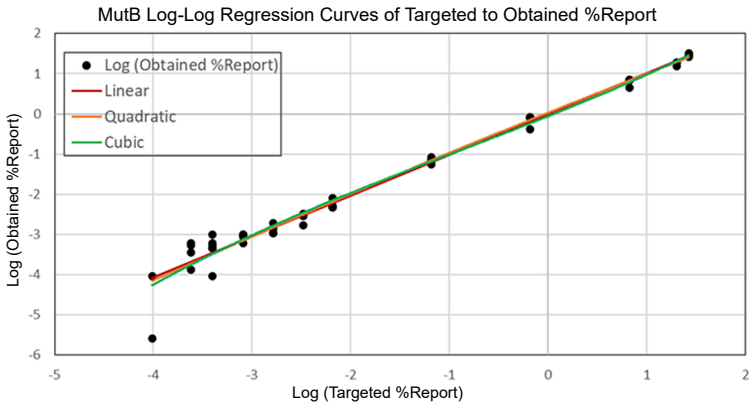


Figure 3 Regression Curves for mutB

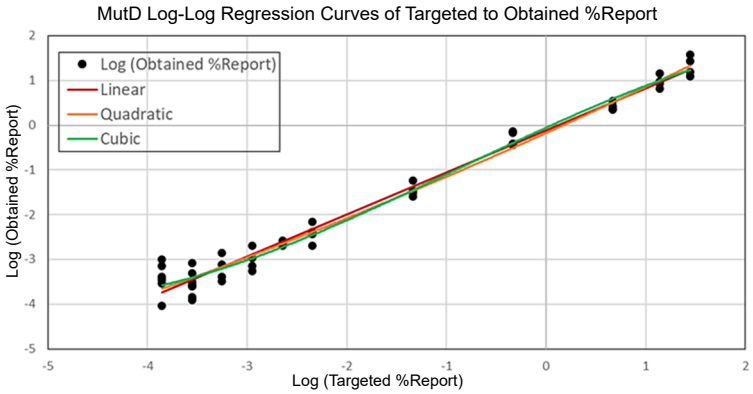


Figure 4 Regression Curves for mutD

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%.

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[7]. 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 ≤ 0.36 log reduction (LR) for mean LRs above 3.5. In accordance with CLSI EP17-A2[7], 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[7], 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 [%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%	

Appendix

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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 Technical Support

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

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

Symbol	Meaning
	Caution
	Warning

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Revision History

Description of Changes: 303-0948 Rev. A

Purpose: Initial release.