Cepheid

Instructions for Use

Xpert® PML-RARA RUO

For use with GeneXpert® System with Touchscreen or GeneXpert® Dx System

Catalog Numbers

REF RPMLRARA-10

303-2876 | Rev. C | 2024-05

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

Trademark, Patents and Copyright Statements

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Product Information

Proprietary Name

Xpert® PML-RARA RUO

Common or Usual Name

Xpert PML-RARA RUO

Product Description, Summary, and Principle of Procedure

Product Description

The Cepheid Xpert® PML-RARA RUO assay, performed on the GeneXpert System with Touchscreen, and GeneXpert® Dx System, is a real-time RT-PCR (reverse transcriptase polymerase chain reaction) for the quantification of PML-RARA fusion transcript in EDTA peripheral blood samples as a normalized ratio to the ABL1 control gene transcript (%PML-RARA/ABL1). The product consists of the Xpert® PML-RARA RUO cartridge and the required off-board sample preparation reagents to run the assay.

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Summary and Explanation

PML-RARA is a fusion gene resulting from a translocation between chromosomes 15 and 17 [t(15;17)]. The PML-RARA acronym refers to Promyelocytes and the Retinoic Acid Receptor Alpha genes that fuse.[1] The Xpert®PML-RARA assay can detect bcr1, bcr2 and bcr3 isoforms.

Principle of the Procedure

Xpert® PML-RARA RUO is an automated assay for quantifying the amount of PML-RARA transcript as a ratio

of PML-RARA/ABL1. The assay is performed on Cepheid GeneXpert® Dx and Touchscreen Systems, 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 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 systems, refer to the appropriate *GeneXpert® Dx System Operator Manual* or *GeneXpert® System with Touchscreen Operator Manual*.

The Xpert® PML-RARA RUO assay includes reagents to detect PML-RARA fusion transcripts and the ABL1 transcript as an endogenous control in peripheral blood samples. The amount of PML-RARA transcript is quantified as the percent ratio of PML-RARA/ABL1. There are two controls included in the Xpert® PML-RARA RUO assay – the Endogenous Control (ABL1) and a Probe Check Control (PCC). The ABL1 endogenous control normalizes the PML-RARA 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 PML-RARA RUO kit (RPMLRARA-10) contains sufficient reagents to process 10 assay samples or quality control samples. The kit contains the following:

Xpert PML-RARA RUO Reagents	**10 of each per kit**
• Proteinase K (PK)	10 x 130 µL per vial
• Lysis Reagent (LY) (Guanidinium Chloride) ◦ Guanidium Chloride ◦ Urea ◦ Sodium Dodecyl Sulphate	10 x 5.3 mL per vial
• Wash Reagent (1) ◦ Ethanol ◦ Guanidinium thiocyanate	10 x 2.9 mL per ampoule
Xpert PML-RARA RUO 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 the /Cepheid OS software/GeneXpert software

- Instructions for Use

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) protein stabilizer 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.

Materials Required but Not Provided

- GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.1 or higher, and operator manual.
- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, GeneXpert Dx software version 6.4 or higher, and operator manual.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Microcentrifuge (1,000 x g minimum)
- Pipettes and aerosol filter pipette tips
- 50 mL conical tubes
- Reagent grade absolute ethanol

Materials Available but Not Required

Xpert® PML-RARA RUO External Controls, PML-RARA Control Panels catalog numbers (C215 and C221), are quality controls from Maine Molecular Quality Controls, Inc.

Warnings and Precautions

The assay is not designed to be used with other external controls.

General

- For Research Use Only. Not for use in diagnostic procedures.
- Treat all biological samples, including used cartridges and reagents, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological samples should be treated with standard precautions. Guidelines for sample handling are available from U.S. Centers for Disease Control and Prevention. [2]
- 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 the Instructions For Use and the *GeneXpert® Dx System Operator Manual or GeneXpert® System with Touchscreen Operator Manual* are necessary to avoid erroneous results.
- Performing the Xpert® PML-RARA RUO assay outside the recommended kit or sample storage temperature ranges and time may produce erroneous or invalid results.
- Do not allow Wash Reagent, which contains guanidinium chloride to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- 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.

Sample

- Maintain proper storage conditions during sample transport to ensure the integrity of the sample (see 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® PML-RARA RUO reagents with other reagents.
- Do not open the Xpert® PML-RARA RUO 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® PML-RARA RUO cartridges be at room temperature (20 °C – 30 °C) when used in the assay.
- Each single-use Xpert® PML-RARA RUO 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 the cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use the Xpert® PML-RARA RUO cartridge if a reagent is added to the wrong opening.

- Do not open the Xpert® PML-RARA RUO cartridge 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 sample or control spill, wear gloves and absorb the spill with paper towels. Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Final active chlorine concentration should be 0.5%. After the work area is dry, follow by wiping the surface with 70% ethanol. For equipment, follow the manufacturer's recommendations for decontamination of equipment. Alternately, follow your institution's standard procedures for a contamination or spill event.

Chemical Hazards, Storage and Handling

Chemical Hazards[3]

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

- GHS US Hazard Pictogram: 
- Signal Word: DANGER
- **GHS US Hazard Statements**
  - Harmful if swallowed H302
  - Highly flammable liquid and vapor H226
  - Causes skin irritation H315
  - Causes serious eye irritation H318
  - May cause drowsiness or dizziness H336
  - Suspected of causing genetic defects H341
- **GHS US Precautionary Statements**
  - **Prevention**
    - Refer to 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, or 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 SPILLED: Immediately remove contaminated clothing. If on skin or hair, rinse with water/shower.
- 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.
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

Storage and Handling

- Store the Xpert® PML-RARA RUO 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).

Sample Collection, Testing, and Results

Sample 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 using.
- Proper sample collection, storage, and transport are critical to the assay function. Sample stability under shipping and storage conditions other than those listed below have not been evaluated with the Xpert PML-RARA RUO 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 **Remove the cartridge from the cardboard packaging before preparing the sample. (See Preparing the Cartridge).**

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

Preparing the Sample

Preparing a Sample with Unknown White Blood Cell (WBC) Count or with No Greater than 30 Million WBC/mL

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

3. To the tube already containing Proteinase K, 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 50 mL conical tube.
    Note Remaining lysate can be stored at 2–8 °C for up to 72 hours or stored at -20 °C or lower for up to 2 months.
13. To the new conical tube containing lysate, add 1.5 mL of retained Lysis Reagent (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 a Sample with WBC Count Greater than 30 Million cells/mL

1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K).
2. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing Proteinase K, add 50 µL 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).
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. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
12. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
13. Discard any remaining PK or LY reagents.

Preparing the Cartridge

To add the sample to the Xpert PML-RARA RUO cartridge:

1. Remove the cartridge from the cardboard packaging.
2. Inspect the cartridge for damage. Do not use if damaged.
3. Lift the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent Chamber (small opening). See Figure 1.
4. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.

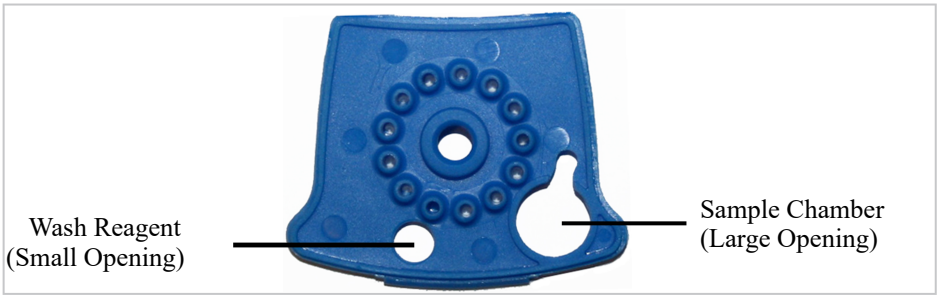


Figure 1 Xpert PML-RARA RUO Cartridge (Top View)

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

Starting the Assay

- For GeneXpert System with Touchscreen, see Starting the Assay: GeneXpert System with Touchscreen.
- For GeneXpert Dx System, see Starting the Assay: GeneXpert Dx System.

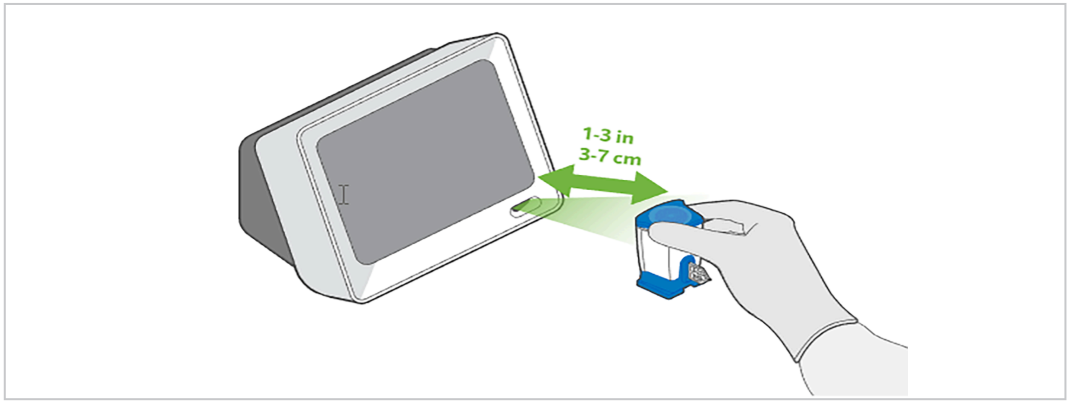
Starting the Assay: GeneXpert System with Touchscreen

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

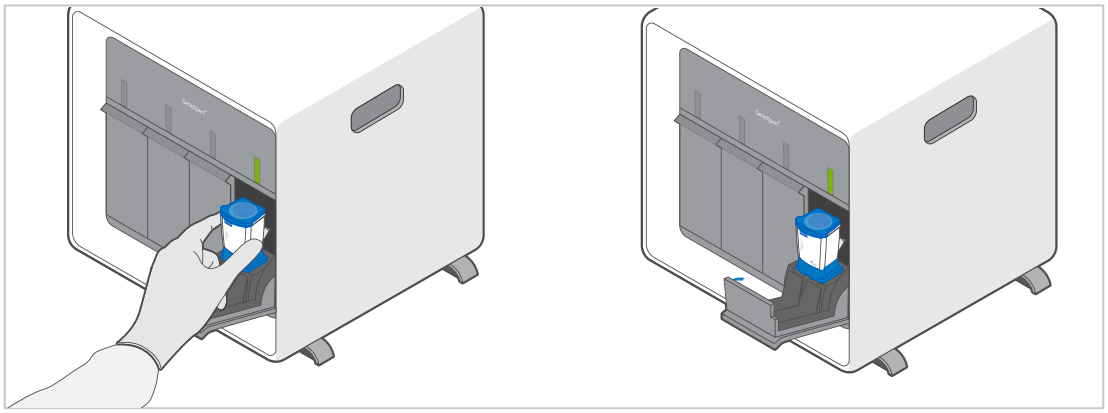
- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

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

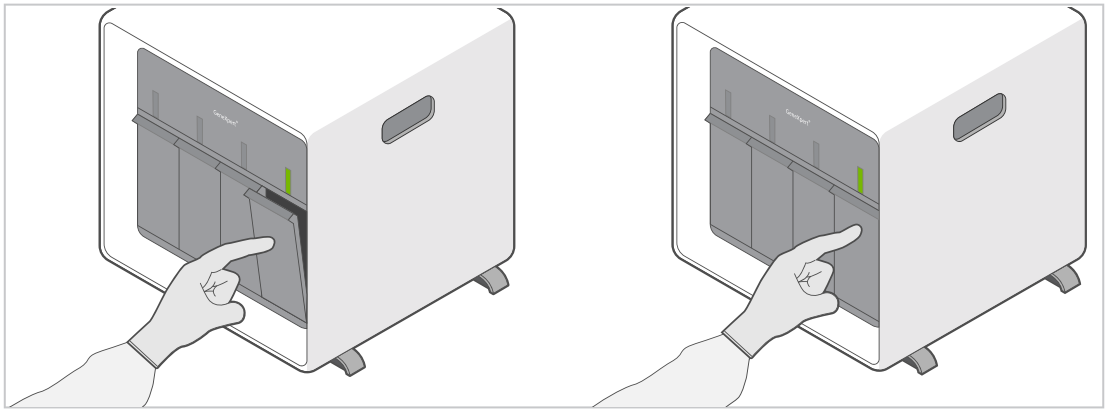
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new assay and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If 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 assay 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 assay starts.



11. When the assay 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.

Starting the Assay: GeneXpert Dx System

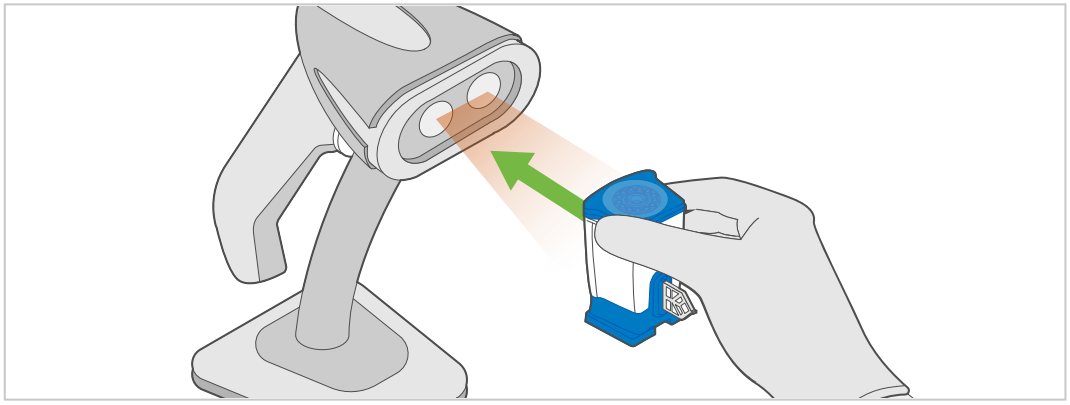
Important **Before you start the assay, make sure that:**

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

This section lists the basic steps for running the assay. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

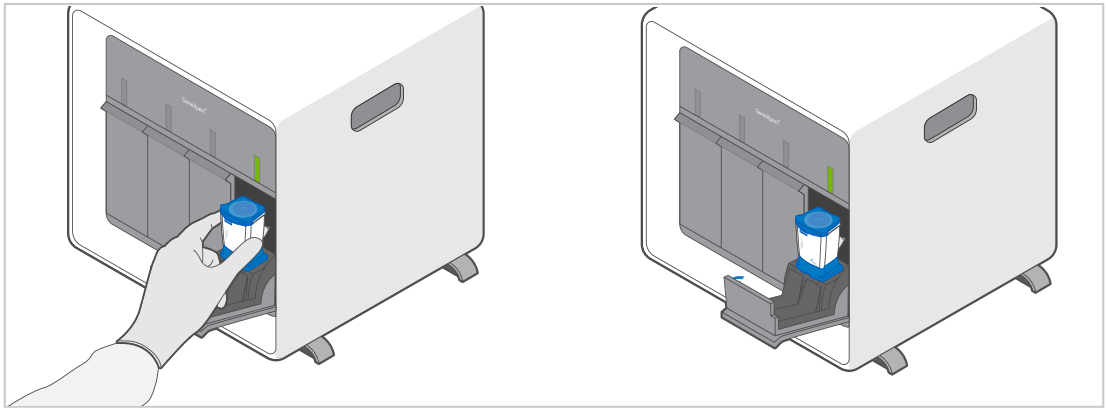
Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**. The **Create Test** window displays.
4. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the assay results and displays in the **View Results** window. The **Scan Cartridge Barcode** dialog box displays.
5. Scan the barcode on the cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

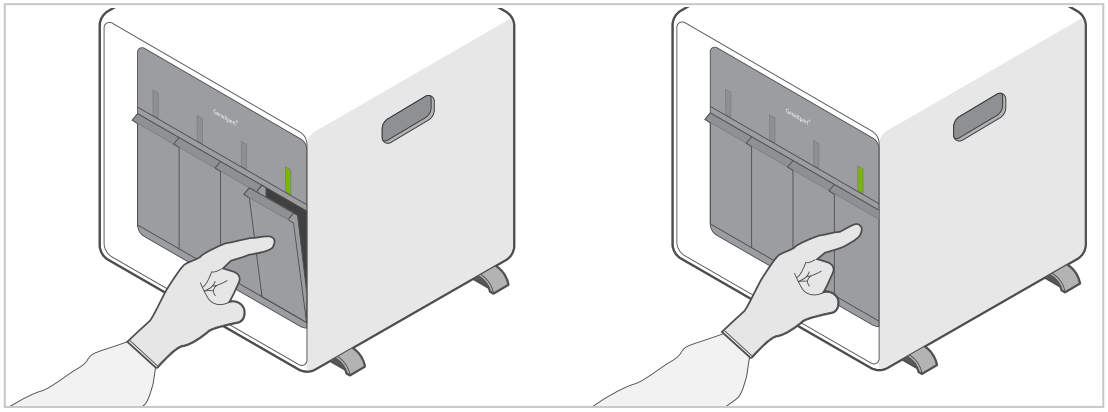


Note If the barcode on the cartridge does not scan, then repeat the assay with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

6. Click **Start Test**. In the dialog box that displays, type your password, if required.
7. Open the instrument module door with the blinking green light and load the cartridge.



8. Close the door. The assay starts and the green light stops blinking.



When the assay is finished, the light turns off.

9. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
10. Dispose of the used cartridges in the appropriate sample waste containers according to your institution's standard practices.

Viewing and Printing Results

- For the GeneXpert System with Touchscreen, see Viewing and Printing Results on GeneXpert System with Touchscreen.
- For the GeneXpert Dx System, see Viewing and Printing Results on GeneXpert Dx System.

Viewing and Printing Results on GeneXpert System with Touchscreen

The Cepheid OS 2.1 results screen will automatically interpret assay results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the assay to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**.

5. To print a report, touch the  (Print) icon. More detailed instructions for viewing and uploading results are available in your system operator manual.

Viewing and Printing Results on GeneXpert Dx System

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*.

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

Quality Control

Each cartridge includes an ABL 1 Endogenous Control and a Probe Check Control (PCC).

ABL 1 Endogenous Control — The ABL 1 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 ABL 1 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.

Retests

Retest Procedure for ERROR and INVALID (Type 1)

Retest samples with **ERROR** or **INVALID** results due to the ABL cycle threshold (Ct) exceeding the maximum valid Ct cut-off (Ct >21) or the endpoint is below the threshold setting (< 50). Also refer to Table 3 of the Troubleshooting Guide.

1. Measure blood sample volume:
   - If *sufficient* blood sample volume is available, retest from original blood sample collection tube following the procedure in **Preparing a Sample with Unknown White Blood Cell (WBC) Count with No Greater than 30 Million WBC/mL**.

   -OR-

   - If blood sample volume is insufficient, re-test can be performed with the retained lysate from Step 12 of **Preparing a Sample with Unknown White Blood Cell (WBC) Count or with No Greater than 30 Million WBC/mL**.

     a. If retained lysate from , 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. Go to Step 2.
2. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.
3. To the new conical tube containing lysate, add 1.5 mL of Lysis Reagent (LY).
4. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.

5. Incubate at room temperature for 10 minutes.
6. To the same conical tube, add 2 mL of reagent grade absolute ethanol (not provided).
7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
9. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
10. Close cartridge lid. Initiate assay (see Starting the Assay).

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

Retest samples with PML-RARA RUO and/or ABL transcript levels below the valid minimum Ct cut-off (Ct < 8) and/or when pressure limit is exceeded. Also refer to Table 3.

1. To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).
2. Measure blood sample volume:
   - If *sufficient* blood sample volume is available, re-test from original blood sample collection tube. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. Go to Step 3 and add the 50 µL of blood sample.

   -OR-

   - If blood sample volume is *insufficient*, re-test can be performed from the retained lysate from Step 12 of Preparing a Sample with Unknown White Blood Cell (WBC) Count or with No Greater than 30 Million WBC/mL.

     a. If retained lysate from Step 12 is stored frozen, thaw to room temperature before use. If refrigerated lysate is used, allow to come to equilibrate 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. Go to Step 3 and add 80 µL of left-over lysate from Step 12 of **Preparing a Sample with Unknown White Blood Cell (WBC) Count with No Greater than 30 Million WBC/mL**.
3. To the tube already containing Proteinase K, add 50 µL of blood sample, if available, or 80 µL of left-over lysate.
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 the Steps 6-13 of **Preparing a Sample with Unknown White Blood Cell (WBC) Count with Greater than 30 Million WBC/mL** to make the final lysate.
7. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) 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**).

Interpretation of Results

Xpert PML-RARA RUO quantitative outputs are provided as a percent ratio of PML-RARA/ABL1. Examples of

possible results and interpretations are presented in Table 1.

Table 1. Xpert PML-RARA RUO Possible Results and Interpretation

Result	Interpretation

PML-RARA DETECTED	PML-RARA transcript was detected.
	• PML-RARA DETECTED – PML-RARA transcript was detected and has a cycle threshold (Ct) within the valid range and an endpoint above the threshold setting.
	• Possible detected results:
	  ○ PML-RARA DETECTED [#.##%]; .
	  ○ PML-RARA DETECTED [Above upper LoD];.
	  ○ PML-RARA 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.

PML-RARA NOT DETECTED	PML-RARA transcript was not detected. • PML-RARA NOT DETECTED [Sufficient ABL transcript] – PML-RARA transcript was not detected and has a cycle threshold (Ct) of zero or the Ct is within the valid range but the endpoint is 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	PML-RARA transcript level cannot be determined due to sample containing excess PML-RARA transcript and/or excess or insufficient ABL transcript. See for additional instructions for retesting the sample. • PML-RARA INVALID – PML-RARA 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 endpointwas below the threshold setting. • ProbeCheck – PASS; all probe check results passed.
ERROR	PML-RARA transcript level cannot be determined. See for additional instructions for retesting the sample. • PML-RARA– 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	PML-RARA 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 for additional instructions for retesting the samples. • PML-RARA NO RESULT • ABL NO RESULT Probe Check NA (not applicable)

Quantitative Results

Xpert PML-RARA RUO quantitative outputs are provided as a percent ratio of PML-RARA/ABL1. Kits are assigned lot-specific Efficiency (EΔCt) and Scaling Factor (SF) values that tie the quantitation of PML-RARA (bcr1, bcr2, and bcr3) and ABL1 transcripts to copy numbers of synthetic PML-RARA and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) primary standards.

Table 2. Examples of Xpert PML-RARA RUO Assay Results

Assay	PML-RARA		ABL		Xpert PML-RARA RUO Assay Results	Notes
	Ct	Result[a]	Ct	Result[a]		
1	21.0	POS	12.1	PASS	PML-RARA DETECTED (1.66%)	Reported value: 1.66%
2	12.5	POS	12.3	PASS	PML-RARA DETECTED (Above upper LoQ)	NA
3	28.8	POS	12.1	PASS	PML-RARA DETECTED (Below LoD; <0.012%)	NA
4	0	NEG	12.6	PASS	PML-RARA NOT DETECTED (Sufficient ABL transcript)	NA
5	0	INVALID	0	FAIL	INVALID [No ABL transcript]	NA
6	0	INVALID	7.6	FAIL	INVALID [Too high ABL transcript]	NA
7	7.9	INVALID	7.4	FAIL	INVALID [Too high PML-RARA and ABL transcripts]	NA
8	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.

Note

GeneXpert systems calculate results automatically based upon *cycle threshold* (Ct) values generated by the assay, and lot-specific parameters assigned during manufacturing. The software applies the following algorithm, wherein the ΔCt (Delta Ct) value is obtained from ABL Ct minus PML-RARA Ct, and Efficiency (*E*) and Scaling Factor (*SF*) are lot specific values:

$$\text{Percent ratio} = \text{Efficiency}^{(\Delta Ct)} \times \text{Scaling Factor} \times 100$$

Note Efficiency and Scaling Factor values calibrate the quantitation of PML-RARA and ABL1 transcripts to copy numbers of synthetic PML-RARA and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) primary standards. Efficiency and Scaling Factor values are embedded within each cartridge barcode. Lot Specifications Data Sheets are available through Cepheid Technical Support.

Limitations

Limitations of the Assay

- For Research Use Only. Not for use in diagnostic procedures.
- This product is designed for use with blood collected in EDTA tubes only.
- Heparin as the anticoagulant can inhibit the PCR reaction.
- Xpert PML-RARA RUO assay is designed to detect the PML-RARA transcripts: bcr1, bcr2, and bcr3. The assay does not detect rare mutations.
- Samples with high white blood cell counts (greater than 30 million cells/mL) tested may yield inaccurate results, **INVALID** results, or aborted runs due to pressure build-up within the cartridge. See Troubleshooting Guide for additional information.
- 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 white blood cells in the sample.

Troubleshooting Guide

Table 3. Troubleshooting Guide

Assay Result	Possible Causes	Suggestions
INVALID	Type 1: Endogenous control ABL failure: • Poor sample quality • RT-PCR inhibition • If ABL Ct > 21, and/or endpoint <50	• 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).
	Type 2: PML-RARA transcript level cannot be determined due to sample containing excess PML-RARA and/or ABL transcripts (Ct < 8)	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).
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).
ERROR (Code 5006, 5007, 5008, and 5009[a])	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).
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).

a. This is not an exhaustive list of ERROR codes.

Specific Performance Characteristics

Analytical Data

The data was collected from internal studies only.

Assay Linearity/Dynamic Range

Linearity was determined for each of the three PML-RARA subtypes, bcr1, bcr2 and bcr3.

Table 4. Summary of Linear Ranges and Linear Model Coefficients

Subtype	Linear Range	Intercept	Slope	R^2
bcr1	0.0064–862%	0.1189	0.9994	0.9964
bcr2	0.0099–835%	0.0529	0.9603	0.9984
bcr3	0.0124–1750%	0.0177	0.9694	0.9990

Collectively, the Xpert® PML-RARA RUO assay demonstrated linearity within 0.0124–835% PML-RARA/ABL. Bounded by the LoQ and the software upper limit, the reportable dynamic range is 0.012–500%.

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

The limit of detection (LoD) was estimated for each of the PML-RARA subtypes bcr1, bcr2 and bcr3 by testing serial dilutions of PML-RARA positive samples [>1% PML-RARA/ABL]. The highest LoD among the three subtypes is 0.011%. However, the lower LoQ is 0.012% and the LoD is restrained by the LoQ. Therefore, 0.012% taken as the overall LoD of the Xpert PML-RARA assay.

The limit of quantitation (LoQ) was estimated with the data obtained from the LoD studies. The mean and standard deviation for the % PML-RARA/ABL values were calculated for replicates at levels equal to the LoD 0.011% PML-RARA/ABL. The LoQ of the assay was determined to be 0.012% PML-RARA/ABL.

The limit of blank (LoB) was determined with PML-RARA negative blood samples. No measurable PML-RARA values were observed for any of the assays. Thus the overall LoB was determined to be 0.00% PML-RARA/ABL. (Table 5).

Table 5. Limit of Detection, Limit of Quantitation, and Limit of Blank of the Xpert PML-RARA RUO Assay [%PML-RARA-ABL]

Subtype	LoD [%PML-RARA/ABL]	LoQ [%PML-RARA/ABL]	LoB [%PML-RARA/ABL]
bcr1	0.0052%	0.012%	0.00%
bcr2	0.011%		
bcr3	0.0082%		

Appendix

Bibliography

1. *FS26 Acute Promyelocytic Leukemia Facts* September 2015 (refer to latest edition).
2. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition).
3. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

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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

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Email: techsupport@cepheid.com

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
	Caution
	Warning

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