**Xpert BCR-ABL1 Major (p210) Quantitative Assay Procedure**

1. **PRINCIPLE:**
   1. Chronic myelogenous leukemia (CML) is one of the most common hematologic malignancies and accounts for 15-20% of all cases of leukemia. More than 95% of patients with CML have the distinctive Philadelphia chromosome (Ph1) that results from a reciprocal translocation between the long arms of chromosome 9 and 22. The translocation involves the transfer of the Abelson or *ABL1* gene on chromosome 9 to the breakpoint cluster region (BCR) of chromosome 22, resulting in a fused *BCR::ABL1* gene. The fusion produces BCR::ABL1, a tyrosine kinase with deregulated activity that plays a key role in the development of CML. The Xpert BCR-ABL Ultra assay detects the chromosomal translocation mRNA transcripts for the p210 form resulting from two major breakpoints, translocations e13a2/b2a2 and e14a2/b3a2.

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* 1. The clinical utility of monitoring BCR::ABL1 mRNA levels by real time polymerase chain reaction (RT-PCR) was established in the International Randomized Study of Interferon and STI571 (IRIS), in which patients received interferon and/or tyrosine kinase inhibitor (TKI) treatment.
  2. The Xpert BCR-ABL Ultra assay results are expressed in international scale (*IS*), and Major Molecular Response (MMR) which is defined as a 3-log reduction from the standardized baseline which represents 0.10% (*IS*)/MR3. A 3-log reduction is associated with favorable survival outcome. In this fashion, IS-standardized molecular testing provides an essential aid for clinicians to manage their CML patients’ disease. The assay quantifies BCR::ABL1 mRNA level as % (IS) via calibration of the assay to the first World Health Organization (WHO) international genetic reference panel for quantification of BCR::ABL1 mRNA.
  3. The GeneXpert system automates and integrates sample purification, nucleic acid amplification, and target sequence detection in simple or complex samples using RT-PCR and PCR assays. The system consists of an instrument, computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use, disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the reactions.

1. **SAMPLE:**
   1. 4 mL whole blood in EDTA tube.
      1. 2mL whole blood in EDTA tube can be used if necessary.
         1. **NOTE:** Any sample with volume less than 2mL is considered insufficient.
      2. Samples should be stored at 2-8˚C for no longer than 3 days (72 hours) from draw date.
2. **REAGENTS:**
   1. Xpert BCR-ABL Ultra Kit, Cat# GXBCRABL-US-10, store at 4 ˚C.
      1. Proteinase K
      2. Lysis Reagent
      3. Wash Reagent
      4. Xpert BCR-ABL Ultra Cartridges with Integrated Reaction Tubes
         1. Bead 1, 2, 3, and 4 (freeze dried)
         2. Rinse Reagent
         3. Elution Reagent
   2. 100% Ethanol – Reagent Grade, store at room temperature.
3. **CONTROLS:**
   1. Internal Controls:
      1. ABL Endogenous Control
      2. Probe Check Control
   2. External Controls:
      1. MMQCI Xpert BCR-ABL IS Panel, Cat# C130, store at -20˚C.
         1. BCR-ABL 0% IS
         2. BCR-ABL 0.0032% IS
         3. BCR-ABL 0.01% IS
         4. BCR-ABL 0.1% IS
         5. BCR-ABL 1% IS
         6. BCR-ABL 10% IS
      2. MMQCI Xpert BCR-ABL IS p210 Linearity Panel, Cat# C207, store at -20°C.
         1. Xpert BCR-ABL 0.01% IS
         2. Xpert BCR-ABL 0.1% IS
         3. Xpert BCR-ABL 1% IS
         4. Xpert BCR-ABL 10% IS
         5. Xpert BCR-ABL 20% IS
         6. Xpert BCR-ABL 50% IS
4. **MAJOR EQUIPMENT:**
   1. GeneXpert Dx System with GeneXpert instrument, computer, and barcode scanner
   2. GeneXpert Dx System software v6.5
   3. Vortex Mixer
   4. Microcentrifuge
   5. Pipettes and aerosol filter pipette tips
   6. 50 mL conical tubes
5. **QUALITY CONTROL:**
   1. Three weekly controls will be run every Wednesday with a test patient, alternating group one, and group two each week.
      1. Group one: 0.000%BCR\_CONTROL, 0.01%BCR\_CONTROL, 1%BCR\_CONTROL
      2. Group two: 0.0032%BCR\_CONTROL, 0.1%BCR\_CONTROL, 10%BCR\_CONTROL
   2. Six linearity controls will be run with each new lot number of Cepheid cartridges or every 6 months, and after any major maintenance to the Cepheid instrument including software updates.
      1. BCRMQ\_0.01%IS
      2. BCRMQ\_0.1%IS
      3. BCRMQ\_1%IS
      4. BCRMQ\_10%IS
      5. BCRMQ\_20%IS
      6. BCRMQ\_50%
6. **QUALITY CONTROL PROCEDURE**:
   1. Order a Test patient in Soft Lab:
      1. Log in to Soft Lab LIVE7
      2. Open **Order Entry** tile.
      3. Input TEST in the Last Name field, and BCRABL in the First Name field.
      4. Select the **Next** button in the Search window.
      5. Select the **Finish** button in the Search window.
      6. Input a collection time in the required field.
      7. Place the cursor in the ID field. In the Keypad window, click the 9 Molec tab.
      8. Click the **Blood** folder and select the BCR-ABL1 Major (p210) Quant test.
      9. Click the Specimen tab on the right side of the screen.
      10. Click on the order, then **Coll/Rec** and OK the verify popup.
      11. Click the save icon in the menu bar at the top of the screen.
      12. Verify the correct label printer is selected and click **Print** in the Print Label popup.
   2. Receive the Test patient in Soft Molecular:
      1. Log into Soft Molecular LIVE7.
      2. Open the **Specimen Receiving Worklist** by selecting the tile on the dashboard.
      3. Place the cursor in the Barcode# field. Scan the Soft Lab specimen label.
      4. Mark the Received checkbox and click **Save**.
      5. Open **Order Entry** using the tile on the dashboard.
      6. Highlight the Barcode# field. Scan the Soft Lab specimen label.
      7. Open the child level using the + sign in the Code field.
      8. Make sure the **Protocol** field is set to **ATST** and **Att Tests** is set to **BCRMQ**.
      9. Select the Internal Notes tab.
      10. Click the Add button.
      11. Input ‘No patients pending for BCRMQ, test patient ordered for control run only.’
      12. In the dropdown menu in the Type column, mark Select All and uncheck Employee Specific.
      13. Mark the Request checkbox.
      14. Select BCRMQ in the Test dropdown menu, then click the Save button in the Order Entry Home menu.
   3. Worksheet Builder:
      1. Log into Soft Molecular.
      2. Open BCR-ABL Quant – Test Worksheet Builder using the tile on the dashboard.
      3. Click the Find button.
      4. Highlight the Test patient and click the **Add** button**.**
         1. For weekly QC, select controls from group one or group two as described in the Quality Control section above. Click the **Add Control** button.
         2. For linearity testing, select the six controls described in the Quality Control section above. Click the **Add Control** button.
      5. Mark the Completed checkbox and save.
      6. Click the **Print Worksheet** button to open the worksheet preview window.
      7. Select the **printer** icon; verify the correct printer is selected and click **Print**.
      8. Close the Print Preview window.
      9. Select **Back** in the BCR-ABL Quant – Test Worksheet Builder window.
      10. Proceed to section **Preparing the sample(s)** below**.**
7. **PROCEDURE:**
   1. **Create the worksheet: BCR-ABL1 Major Quant Test Worksheet Builder**
      1. Log into Soft Molecular.
      2. Open BCR-ABL1 Major Quant – Test Worksheet Builder using the tile on the dashboard.
      3. Select **Find**.
      4. In the Found Activities tab, click **OK** or double click any row.
      5. If applicable, double click **New** on the Pending Worksheets tab.
      6. Highlight the Barcode# field. Scan the Soft Lab label of the sample to be added to the worksheet. Select Enter on the keyboard.
      7. Mark the Completed checkbox and **Save**.

**NOTE:** Q numbers will generate upon saving.

* + 1. Click the **Print Worksheet** button to open the worksheet preview window.
    2. Select the **printer** icon; verify the correct printer is selected and click **Print**.
    3. Close the Print Preview window.
    4. Select **Back** in the BCR-ABL1 Major Quant – Test Worksheet Builder window.
    5. Open **Order Entry** from the dashboard tile.
    6. Scan the SoftLab label on the specimen tube or enter the MOL# of the patient. Press **Find** to open the patient record.
    7. Open the print tab and click **Print Specimen Label.** Print two labels. Repeat for each patient being tested.
    8. Exit Soft Molecular application.
  1. **Preparing the Sample(s):**
     1. 20 minutes before starting the procedure, remove the blood specimen(s) or controls, cartridge, and sample preparation reagents from storage to allow them to come to room temperature.
     2. Verify the Wash Reagent is a clear, colorless liquid. Do not use the Wash Reagent if it has become cloudy or discolored.
     3. Verify the cartridges are not expired.
        1. **NOTE:** Do not open the lid until you are ready to perform the assay.
     4. In a microcentrifuge, briefly spin down the appropriate number of Proteinase K tubes to collect contents.
     5. Label the appropriate number of 50mL conical tubes with the Soft Molecular specimen label. Each specimen will have two 50ml tubes. On the cap, write the first three letters of the patient’s last name and the number ‘1’. On the second tube, label the cap with the same three letters and the number ‘2.’
     6. Prepare the sample according to the following ratio included in **Table 1**:

**Table 1:** Reagent Volumes based on Starting Sample Volume

|  |  |  |
| --- | --- | --- |
| **Sample Volume** | **Proteinase K** | **Lysis Reagent** |
| 4 mL | 100 uL | 2.5 mL |
| 2 mL | 50 uL | 1.25 mL |
| **Control Volume** |  |  |
| 4 mL | 100 uL | 2.5 mL |

* + 1. Once the reagents are at room temperature, add the appropriate volume of Proteinase K to the bottom of each 50mL conical tube labelled “1”.
    2. Invert each whole blood specimen 8 times immediately before pipetting to ensure the specimen is well-mixed.
       1. Controls: Vortex controls on max setting for 10 seconds immediately before pipetting to ensure it is well mixed.
    3. Add 2mL or 4mL of whole blood to the tubes containing Proteinase K
       1. Controls: always add 4 mL to the tubes containing Proteinase K
    4. Vortex the sample at the maximum setting for 10 seconds.
    5. Incubate at room temperature for 1 minute.
    6. To the same tube, add the appropriate volume of Lysis Reagent.
    7. Vortex the sample at the maximum setting for 30 seconds.
    8. Incubate at room temperature for 5 minutes.
    9. After the first 5-minute incubation, vortex the sample at the maximum setting for 30 seconds.
    10. Incubate at room temperature for 5 minutes a second time.
    11. Mix the sample by tapping the bottom of the tube 10 times.
    12. Transfer 1 mL (500uL for a 2mL specimen) of the prepared lysate into the second 50mL conical tube labelled ‘2’.

**NOTE:** Store the remaining lysate at -20˚C or lower for up to 24 hours.

* + 1. To the conical tube labelled ‘2’, add 1.5mL (750uL for 2mL specimen) of Lysis Reagent.
    2. Vortex the sample at the maximum setting for 30 seconds.
    3. Incubate at room temperature for 10 minutes.
    4. After the 10-minute incubation, add 2mL of 100% Ethanol.
    5. Vortex the sample at maximum setting for 30 seconds, then set aside.
  1. **Process the worksheet: Sample Prep and Lysis**
     1. Log into Soft Molecular.
     2. Open BCR-ABL1 Major Quant – Test Worksheet Processing by using the tile on the dashboard.
     3. Scan the barcode of the BCR-ABL1 Major Quant worksheet in the Worksheet# field and select **Find**.
     4. Verify the reagent lot number by clicking on the General Settings tab on the lower left side of the screen.
        1. If the reagent lot needs to be changed, click on the dropdown arrow in the Stock# column and select the correct lot in the window that appears.
     5. Mark the Completed checkbox for the Sample Prep and Lysis action, then click **Save**.
     6. Select **Back** in the BCR-ABL1 Major Quant – Test Worksheet Processing window.
  2. **Preparing the Cartridge:**
     1. Remove the cartridge from the packaging.
     2. Inspect the cartridge for damage. If damaged, do **NOT** use.
     3. Label the side of the cartridge with the Soft Molecular order number, or appropriate control name.
     4. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent ampoule to the Wash Reagent chamber. Be careful not to transfer any bubbles that may be present. See **Figure 1**.
     5. Pipette the entire contents of the prepared sample into the Sample Chamber.

Be careful not to transfer any bubbles that may be present**.** See **Figure 1**.

* + - 1. Close the cartridge lid, ensuring that the lid snaps firmly shut.

**NOTE:** The test must be started within 1 hour of adding the reagent-treated sample to the cartridge.

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**Figure 1**: Xpert BCR-ABL Ultra Cartridge (Top View)

* 1. **Loading the Cartridge onto the GeneXpert:**
     1. If necessary, turn on the GeneXpert Dx instrument using the toggle switch located at the back of the instrument, then turn on the computer. The GeneXpert software will launch automatically.
     2. Login to the GeneXpert software using the appropriate username and password.
     3. In upper left corner of the GeneXpert Dx System window, click **Create Test**.
     4. For patient samples:
        1. In the Patient ID popup, select **Manual Entry**. Enter the patient’s Soft Molecular Order Number.
        2. In the Sample ID popup, select **Manual Entry**. Enter the patient’s full name. (Last name, First name)
     5. For controls:
        1. In the Patient ID popup, select **Manual Entry**. Enter the name of the control.
           1. Example: BCRMQ\_0.0032%IS
        2. In the Sample ID popup, select **Manual Entry**. Enter the date using the format YYYYMMDD.
     6. Scan the barcode on the cartridge when prompted by the software.
     7. Click **Start Test**.
        1. **NOTE:** A dialog box may appear after selecting Start Test. If so, enter your password, click **OK** and proceed.
     8. Open the instrument module door with the blinking green light and load the cartridge so that the QR code on the face of the cartridge is facing outwards.
     9. Push the module door closed until it locks. If the door is closed correctly, the blinking green light will change to a continuous green light.
     10. Verify that a run time populates for the correct module in the GeneXpert software.
  2. **Process the worksheet: Load Cepheid** 
     1. Log into Soft Molecular.
     2. Open BCR-ABL1 Major Quant – Test Worksheet Processing by using the tile on the dashboard.
     3. Scan the barcode of the BCR-ABL1 Major Quant worksheet into the Worksheet# field and select **Find**.
     4. Mark the Completed checkbox for the Load Cepheid action, then select **Save**.
     5. Select **Back** in the BCR-ABL1 Major Quant – Test Worksheet Processing window.
     6. Open BCR-ABL1 Major Quant – Tasklist by using the icon on the dashboard.
     7. Change the date range to one month.
     8. Scan the barcode of the BCR-ABL1 Major Quant worksheet into the Worksheet# field and select **Find**.
     9. Click **Select All**.
     10. Click **OK**, followed by **Save**.
     11. Select **Back** in the BCR-ABL Quant – Tasklist window.
     12. Exit Soft Molecular.
  3. **Transferring Results:**
     1. Once the test is finished, the green light turns off and the system releases the door lock.
        1. If an error occurs during processing, please refer to **Repeat Testing** section below, for the appropriate retest procedure.
     2. Verify the run completed without any errors.
     3. Open the module door, remove the cartridge, and dispose in the nearest sharps container.
     4. Plug the Secure Key flash drive into the Cepheid computer.
     5. In upper middle of the GeneXpert Dx System window, click **View Results** icon.
        1. At the bottom of the View Results window, click **Report**.
        2. Mark the checkbox for the first sample to be transferred onto the flash drive.
           1. **NOTE:** Verify no other samples or standards/controls are marked before clicking Generate Report File.
        3. Click **Generate Report File**.
        4. In Window Explorer, navigate to the Secure Key flash drive.
        5. In the file name field, enter the Soft Molecular Order number and the patient’s last name. For controls, enter the %IS followed by “Control”, or "Linearity” as appropriate.
           1. Example: MOL-22-1234\_LastName
           2. 0.1%IS\_Control
           3. 0.1%IS\_Linearity
        6. Click **Save**.
        7. Uncheck the checkbox for the sample transferred onto the flash drive.
        8. Repeat this process for all applicable samples and controls.
     6. Remove the Secure Key flash drive from the Cepheid computer.
     7. Transfer the data to the CMB\_Tests folder on the RICMBLAB$ shared drive in the appropriate BCR-ABL Quant run folder.
     8. Create your run folder on the G: drive by scanning the barcode on the worksheet.
        1. Example: 06.09.23-BCRMJCPHD-1
  4. **Tasklist Processing: Upload Tasklist Documents**
     1. Log into Soft Molecular.
     2. Open BCR-ABL1 Major Quant – Tasklist by using the icon on the dashboard.
     3. Select the Built Tasklist Search tab.
     4. Scan the barcode on the BCR-ABL1 Major Quant worksheet in the Worksheet# field.
     5. Click **Find**. The built tasklist will open automatically.
     6. Highlight the order for the patient sample (parent level).
        1. Verify the Test Results window populates with the correct patient information.
        2. Open the Analysis Images tab.
        3. Select the Add File tab, then select the add file (folder) icon.
        4. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
        5. Choose Instrument Documents from the template dropdown.
        6. Select the green check icon to add file(s).
        7. If a patient sample required rerun on the Cepheid instrument and multiple PDF documents were generated:
           1. Attach all PDFs to the first order for the patient sample. Rerun documents should all be labeled with the suffix RERUN.
        8. Repeat for all patient samples on the tasklist.
     7. Click the **Select All** button, followed by **Collapse/Expand**.
     8. If this is a control run:
        1. Open the QC data tab.
        2. Click two times on the first control.
        3. Open the Documents Tab
        4. Select the Add File tab, then click the add file (folder) icon.
        5. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
        6. Select Instrument Documents TQC in the Template dropdown.
        7. Click the green check icon to add the file(s).
        8. Click **Save** in the TQC window.
        9. Click OK in the QC Components window that appears.
        10. Repeat as necessary for all controls.
     9. Return to the Assigned Tests Tab
     10. Complete the Upload Tasklist Documents action by marking the Completed checkbox, found on the parent row for the Test Patient sample, and click **Save**.
     11. Exit SoftMol and inform the Director that control results are ready for review.
     12. For Linearity Panel Testing:
         1. Open the *Xpert BCR-ABL IS p210 Linearity Resulting* *Template* in G:\QC\IQCP's\Xpert BCR-ABL p210 Linearity.
         2. Enter the appropriate data regarding lot #s, date, and observed IS% results.
         3. The linearity graph will populate and display the R correlation value. If this value is within tolerance, the cell will turn green indicating a successful QC. If this value is out of tolerance, the cell will turn red indicating a failed QC.
         4. Save the linearity results by clicking file>save as in the menu bar. Save the worksheet with the date and title. Example “20240311\_BCR-ABL\_Linearity”
         5. Print the linearity resulting sheet and give to the Senior Tech for review.
         6. Inform the director that linearity testing results are ready for review. In the event of a failed QC, the director will request a repeat of the entire linearity panel.

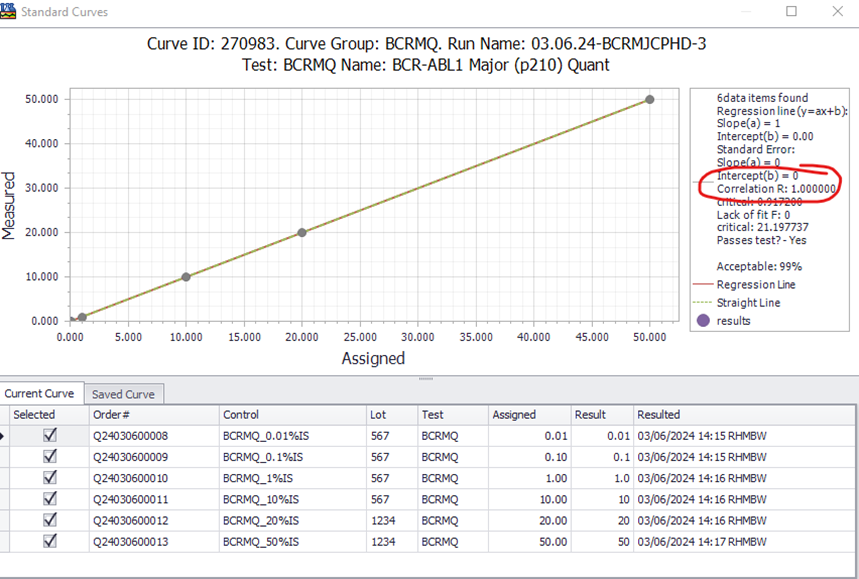
1. **INTERPRETATION:**
   1. The results are interpreted automatically by the GeneXpert Dx system from measured fluorescent signals and embedded calculation algorithms that are shown in the View Results window.
   2. The results include:
      1. Positive: BCR::ABL1 transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
         1. Possible positive results:
            1. POSITIVE [#.##% (*IS*) and MR#.##]
            2. POSITIVE [Above upper LoQ]
            3. POSITIVE [Below LoD; >MR4.52/<0.0030% (*IS*)]
         2. ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
            1. When ABL Ct value is below 18, a minimum of 32,000 ABL copy number was present in the reaction.
         3. Probe Check PASS – All probe check results passed.
      2. Negative: BCR::ABL1 transcript was not detected and has a cycle threshold (Ct) above the valid cycle.
         1. ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
            1. When ABL Ct is below 18, a minimum of 32,000 ABL copy number was present in the reaction.
         2. Probe Check PASS – All probe check results passed.
      3. Invalid: BCR::ABL1 transcript level cannot be determined.
         1. INVALID – BCR::ABL1 transcript level cannot be determined due to sample containing excess BCR::ABL1 and/or ABL transcripts.
         2. ABL FAIL – ABL cycle threshold (Ct) was not within the valid range, or the endpoint was below the threshold setting.
         3. Probe Check – PASS; all probe check results passed.
      4. Error: BCR::ABL1 transcript cannot be determined.
         1. BCR::ABL1 – NO RESULT
         2. ABL – NO RESULT
         3. Probe Check FAIL – All or one of the probe check results failed.
         4. Probe Check PASS or NA (not applicable) and Pressure Abort.
            1. If the probe check passed or shows IS, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
      5. No Result: BCR::ABL1 transcript level cannot be determined. Insufficient data was collected to produce a test result. This can happen if the operator stops a test that is in progress.
         1. BCR::ABL1 NO RESULT
         2. ABL NO RESULT
         3. Probe Check NA (not applicable)
   3. Quantitative Results:
      1. A certificate of analysis is supplied with each Xpert BCR-ABL Ultra test kit and contains a lot-specific standard curve for the Xpert BCR-ABL Ultra kit and an Efficiency value (*E*ΔCt). The Efficiency Value is embedded in the barcode of the Xpert BCR-ABL Ultra cartridge.
      2. Each kit contains a lot-specific scaling factor (*SF*) embedded in the barcode that ties the quantitative test output to the International Scale (*IS*). Test results are provided with quantitative test output in both % (*IS*) and molecular response (MR) scales. See **Table 2** for Log Reduction, International Scale (*IS*), and Molecular Response (MR) correlation.

**Table 2**: Log Reduction, International Scale (*IS*), and Molecular Response (MR) Correlation

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1. **RESULT REVIEW:**
   1. Open My Orders by using the icon on the dashboard.
   2. Click on the Director Review tab.
   3. Click two times on the appropriate tasklist number.
   4. Click **No** in the window that appears.
   5. Highlight the order for the first patient sample (parent level).
      1. Verify the Test Results window populates with the correct patient information in the Analysis Images tab, Click the Dual View icon, so the uploaded PDF report is available when entering results.
      2. Click the Test Results Tab. Choose the correct BCR::ABL1 Major result from the dropdown menu under **Result** column.
      3. Enter numerical results for BCR::ABL1 IS% and BCR::ABL1 Major MR number.
   6. Complete the Result Review action by marking the Completed checkbox.
   7. Click **Save**.
   8. Close the Tasklist Entry window.
   9. If this is a weekly control run:
      1. Open the QC Data tab on the left side of the screen.
      2. Click two times on the first control.
      3. Navigate to the Documents tab.
      4. Click the Dual View icon, so the uploaded PDF report is available when entering results.
      5. Select the Results tab on the left side of the screen.
      6. Enter numerical results for BCR::ABL1 %IS in the result fields, press enter, then click Verify All.
      7. Click Save.
      8. Click OK to close the QC Components window that appears.
      9. Click Yes when asked to save changes.
      10. Repeat as necessary for additional controls.
      11. Click the Assigned Tests tab.
      12. Complete the Result Review action by marking the Completed checkbox.
      13. Click Save, then close the Results Review window.
   10. If this is a linearity control run:
       1. Open the QC Data tab on the left side of the screen.
       2. Click two times on the first control.
       3. Navigate to the Documents tab.
       4. Click the Dual View icon, so the uploaded PDF report is available when entering results.
       5. Select the Results tab on the left side of the screen.
       6. Enter numerical results for BCR::ABL1 %IS in the result fields, press enter, then click **Verify One**.
       7. Select **No** when asked to proceed with the standard curve graph.
       8. Click **Save** in the QC order window.
       9. Click **OK** in the QC popup window that appears.
       10. Click **Yes** in the save changes popup that appears.
       11. Repeat for the remaining 5 linearity controls.
       12. When the last control is saved, open any control, and click **Std Curves.**
       13. Click yes when asked to proceed with standard curve graph.
       14. Evaluate the value for Correlation R. If this value is within the lab designated tolerance, linearity has passed. If this value is out of tolerance, the director will reject the results and request a repeat of the linearity panel.



* + 1. Click the Assigned Tests tab.
    2. Complete the Result Review action by marking the Completed checkbox.
    3. Click Save, then close the Results Review window.

1. **SIGN OUT ENTRY:**
   1. Open My Orders by using the icon on the dashboard.
   2. Verify the Molecular Pathologist tab is displayed.
   3. Click two times on the appropriate order.
   4. Click **No** in the window that appears.
   5. Verify RBS rules triggered correctly for the Result, Interpretation, Methodology and Disclaimer sections.
   6. Mark the Completed checkbox.
   7. Click the **Sign Out** button at the top of the screen, then again in the pop-up that appears.
   8. Verify the information on the report is accurate or edit, as needed.
   9. Click **Complete Sign Out**.
   10. Close Sign Out Entry.
2. **REPEAT TESTING:** 
   1. During the testing process, some samples must be repeated for a variety of technical or analytical reasons.
   2. Samples will be rerun if failures occur, and both PDF reports will be uploaded into Soft Molecular for documentation.
   3. If the sample results with ERROR or INVALID due to the ABL cycle threshold exceeding the maximum valid Ct cutoff (Ct >18) or the endpoint is below the threshold setting (<200):
      1. If sufficient blood specimen volume is available, re-test from original blood specimen collection tube following the standard procedure.
      2. If the blood specimen volume is insufficient, re-test can be performed with the retained lysate.
         1. If lysate has been frozen, thaw to room temperature before use.
      3. Ensure lysate is well-mixed by vortexing the sample at maximum setting for 10 seconds, then set aside for 3 minutes to allow bubbles to settle.
      4. Transfer 1 mL of the prepared lysate into the new 50 mL conical tube.
         1. Ensure the tube is labeled with the MOL# and patient last name.
      5. To the 50 mL conical tube, add 1.5 mL of Lysis Reagent.
      6. Vortex the sample at the maximum setting for 30 seconds.
      7. Incubate at room temperature for 10 minutes.
      8. To the same conical tube, add 2 mL of 100% ethanol.
      9. Vortex the sample at the maximum setting for 30 seconds.
      10. Prepare and load the cartridge following standard procedure.
   4. If the sample results with ERROR (Code 2008) or INVALID due to excess BCR-ABL and/or ABL transcripts (Ct <8):
      1. If sufficient blood specimen volume is available, re-test from original blood specimen collection tube.
      2. If blood specimen volume is insufficient, re-test can be performed using the retained lysate.
      3. Ensure blood specimen or lysate are thawed to room temperature and adequately mixed prior to setup.
      4. To the bottom of a new 50mL conical tube, add 100uL of Proteinase K.
      5. To the tube containing Proteinase K, add 50uL of blood specimen OR 80 uL of retained lysate.
      6. Vortex the sample at the maximum setting continuously for 10 seconds.
      7. Incubate at room temperature for 1 minute.
      8. To a new conical tube, add 2.5mL of Lysis Reagent.
      9. Vortex the sample at the maximum setting continuously for 30 seconds.
      10. Incubate at room temperature for 10 minutes.
      11. To the same conical tube, add 2mL of 100% ethanol.
      12. Vortex the sample at the maximum setting continuously for 30 seconds.
      13. Prepare and load the cartridge following standard procedure.
3. **ASSAY LIMITATIONS:**
   1. The precision of this assay is not demonstrated or assured below MR4.5.
   2. This assay is not indicated for determining discontinuation from TKI treatment nor for monitoring after discontinuation.
   3. This assay has been validated for whole blood collected in EDTA tubes only.
      1. Heparin cannot be used as an anticoagulant because it can inhibit the PCR reaction.
      2. Sodium citrate, buffy-coat and bone marrow sample types have not been validated.
   4. Erroneous test results might occur from improper specimen collection, handling or storage or sample mix-up. Careful compliance with the assay procedure is necessary to avoid erroneous results.
   5. The Xpert BCR-ABL Ultra test is designed to detect, but not distinguish between the p210 BCR-ABL fusion transcripts e13a2/b2a2 and e14a2/b3a2. The ability to detect other fusion transcripts has not been evaluated beyond those described in these instructions for use. The test does not detect minor or micro breakpoints, microdeletions, or mutations.
   6. The Xpert BCR-ABL Ultra is not intended to detect the e1a2 (p190), e19a2 (p230) or other minor translocations that may be present in a peripheral blood sample from a patient with leukemia.
   7. The Xpert BCR-ABL Ultra will not detect aberrant e13a2/b2a2 fusion transcripts in which parts of the sequence adjacent to the breakpoint are deleted.
   8. For some specimens with extremely high white blood cell counts (higher than 30 million cells/mL), Xpert BCR-ABL Ultra may report INVALID results due to excess BCR-ABL or ABL levels in the sample.
   9. Some specimens with extremely low levels of ABL transcript or with white blood cells lower than 150,000 cells/mL may be reported as INVALID. A non-determinate result does not preclude the presence of extremely low levels of leukemic cells in the patient.
   10. CML p230 transcript with e19a2 micro breakpoint may report a BCR-ABL positive result below the assay LoD (0.0030% (IS)/MR4.52) when tested at high target levels.
   11. 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.
   12. Some patients with extremely low levels of BCR::ABL1 transcript (i.e., below LoD 0.0030% IS or higher than MR4.52) may be reported as NEGATIVE (Sufficient ABL transcript). Hence, an undetected result does not preclude the presence of very low levels of leukemic cells in the patient.
4. **REFERENCES:**
   1. Xpert BCR-ABL Ultra SOP Template, #GXBCRABL-US-10, extracted from package insert 302-0738, Rev A.