**Cepheid GeneXpert *F2* & *F5* Assay Procedure**

1. **PRINCIPLE:**
   1. The Xpert Factor II & Factor V Assay is a qualitative in vitro diagnostic genotyping test for the detection of *F2* and *F5* alleles from EDTA anticoagulated whole blood. The test is performed on the Cepheid GeneXpert Dx System and is intended to provide results for *F2* (Prothrombin) and *F5* Leiden mutations as an aid in the diagnosis for individuals with suspected thrombophilia.
   2. *F2* (c.\*97G>A; alternative: 20210G>A) refers to the G to A transition at nucleotide 20210 in the 3’ untranslated region of the gene and is associated with increased plasma levels of prothrombin. This mutation is present in 2% of the general population.
   3. *F5* (c.1601G>A; alternative 1691G>A) is commonly referred to as Factor V Leiden. The mutation refers to the G to A transition at nucleotide position 1601 of the Factor V gene, resulting in the substitution of the amino acid arginine by glutamine in the Factor V protein, causing resistance to cleavage by Activated Protein C (APC). This mutation is present in 5% of the general population.
   4. The GeneXpert Dx system automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in whole blood using real-time Polymerase Chain Reaction (PCR) assays. The system consists of an instrument, personal computer, handheld barcode scanner, and preloaded software for running tests and viewing results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. The cartridges are self-contained, so cross-contamination between samples is eliminated.
   5. Each GeneXpert assay cartridge contains a Probe Check Control (PCC) that verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability. The primers and probes in the Xpert *F2*I & *F5* assay determine the genotype of the *F2* gene and *F5* gene.
2. **SAMPLE:**
   1. Whole blood in EDTA tube.
      1. Samples should be stored at 2-8˚C for no longer than 15 days.
      2. If necessary, blood samples can be stored at -80 ˚C for up to 3 months.
3. **REAGENTS AND STORAGE:**
   1. Xpert FII & FV Kit, Cat# GXFIIFV-10, store at 4°C.
4. **CONTROLS:**
   1. Internal Kit Controls:
      1. Probe Check Control (included in the assay cartridge).
         1. Measures the fluorescent signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability.
   2. External Controls:
      1. Xpert *Fll* & *FV* NOR/MUT Control, MMQCI Cat# G11601, store at 4°C.
         1. G116a01: FII Normal, FV Normal
         2. G116b01: FII Homozygous, FV Homozygous
      2. Xpert FII & FV HET, MMQCI Cat# G108-2H, store at 4°C.
         1. G10801: FII Heterozygous, FV Heterozygous
      3. Cepheid Environmental Control
         1. FII Normal, FV Normal
         2. Refer to the *Cepheid GeneXpert Instrument Procedure* for additional information.
5. **EQUIPMENT:**
   1. GeneXpert Dx System with GeneXpert instrument, computer, and barcode scanner
   2. GeneXpert Dx System software v6.4
   3. 100 uL Eppendorf Pipette
   4. 100 uL USA Scientific Pipette tips
6. **PROCEDURE:**
   1. **Create the worksheet: Factors – Test Worksheet Builder**
      1. Log into Soft Molecular.
      2. Open Factors – 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.
         1. **Note:** All Test Codes are attached to the F2F5CPHD worksheet.
      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.
         1. **Note:** If the tube has more than one test pending for the worksheet, all tests for the sample will be added to one well.
      7. Controls will be run with every new shipment, new lot of kits and every 30 days, whichever comes first. If necessary, add the negative, heterozygous, homozygous, and environmental controls to the worksheet.
         1. Select the correct control using the dropdown to the right of the Add Control button.
         2. Select **Add Control**.
         3. Repeat as needed for additional controls.
      8. Click the Plate View tab.
      9. If necessary, move the patient samples and control by clicking and dragging to the correct well.
      10. Switch to the Worksheet View tab.
      11. If necessary, verify the control lot numbers by clicking on the Sample ID field.
          1. If the lot needs to be changed, click on the dropdown arrow in the Sample ID field, and select the correct lot in the window that appears.
      12. Verify the reagent lot number by clicking on the vertical Settings tab on the 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.
      13. If necessary, verify and confirm the control test codes.
          1. Click the Test Code field and use the dropdown arrow to open the Test Code window.
          2. Click **Select All** in the test code window so F2BL2 and F5BL2 are selected for every control.
             1. Cepheid GeneXpert *Fll* & *FV* is a multiplex assay, so the Sample ID field must contain both targets for every control on the run.
      14. Mark the Completed checkbox and **Save**.
          1. **NOTE:** Q numbers will generate for the control upon saving.
      15. Click the **Print Worksheet** button to open the worksheet preview window.
      16. Select the **printer** icon; verify the correct printer is selected, and **Print**.
      17. Close the Print Preview window.
      18. Select **Back** in the Factors – Test Worksheet Builder window.
      19. Exit Soft Molecular application.
   2. **Preparing the Cartridge:**
      1. If any samples are frozen, allow to thaw completely at room temperature prior to assay setup.
      2. **NOTE:** EDTA whole blood should not be freeze/thawed more than one time. Remove the cartridge from the kit being careful not to touch or agitate the PCR tube.
      3. Check that the correct assay cartridge was selected, then inspect the cartridge for any damage and verify the kit has not expired.
         1. **NOTE:** The GeneXpert Dx System will not allow expired cartridges to be run on the instrument.
      4. Mix the sample and/or controls by inverting the tube(s) 5 times, until homogenous.
      5. Open the cartridge lid.
         1. **NOTE:** Do not open the cartridge lid until the cartridge can be loaded onto the GeneXpert. The test must be started within 15 minutes of loading the sample into the Sample Chamber.
      6. Using a 100uL pipette, transfer 50 uL of EDTA anticoagulated whole blood or control sample to the bottom wall of the sample opening of the Xpert Factor II & Factor V Assay cartridge.
      7. Close the cartridge lid.
   3. **Starting the Test:**
      1. If necessary, turn on the GeneXpert Dx instrument using the toggle switch located in the back of the instrument, then turn on the computer. The GeneXpert software will launch automatically.
      2. Log on to the GeneXpert Dx System 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. Place the cursor in the Patient ID field and in the window that appears, click **Manual Entry**. Enter the patient Soft Molecular Order Number.
         2. Place the cursor in the Sample ID field, and in the window that appears, select **Manual Entry**, then type the patient’s full name.
         3. Refer to the F2 F5 Cepheid worksheet to determine which tests were ordered for each patient.
         4. In the Select Assay dropdown, choose the applicable assay for each patient.
            1. If the incorrect test is run, the sample will need to be repeated.
      5. For controls:
         1. Place the cursor in the Patient ID field, and in the window that appears, select **Manual Entry**, then manually type the control name.
            1. Example: YYYYMMDD\_Heterozygous Control
         2. When the Sample ID window appears, click **Cancel**.
         3. When loading controls onto the instrument, remember to leave the assay set as Xpert FII & FV Combo.
      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.
   4. **Process the worksheet: Load Cepheid**
      1. Log into Soft Molecular.
      2. Open Factors – Test Worksheet Processing by using the tile on the dashboard.
      3. Scan the barcode of the F2 F5 Cepheid worksheet into the Worksheet# field and select **Find**.
      4. Mark the Completed checkbox for the Load Cepheid action and select **Save**.
      5. Select **Back** in the Factors – Test Worksheet Processing window.
      6. Open Factors – Tasklist by using the icon on the dashboard.
      7. Change the date range to one month.
      8. Scan the barcode of the F2F5CPHD worksheet into the Worksheet# field and select **Find**.
      9. Click **Select All**.
      10. Click **OK**, followed by **Save**.
      11. Select **Back** in the Factors – Tasklist window.
      12. Exit Soft Molecular.
   5. **Transferring Results:**
      1. Once the test is finished, the green light turns off and the system releases the door lock.
      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.
         3. Select **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.
            1. Example: MOL-22-1234\_LastName
         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 a folder labeled with the F2 F5 Cepheid Worksheet name.
         1. Example: 04.06.22-F2F5CPHD-1
   6. **Tasklist Processing: Upload Tasklist Documents**
      1. Log into Soft Molecular.
      2. Open Factors – Tasklist by using the icon on the dashboard.
      3. Select the Built Tasklist Search tab.
      4. Scan the barcode on the F2F5CPHD worksheet in the Worksheet# field.
      5. Click **Find**. The built tasklist will open automatically.
      6. If necessary, open the QC Data tab.
         1. Click 2 times on the first control.
         2. Navigate to the Documents tab.
         3. Select the Add File tab, then click the add file (folder) icon.
         4. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
         5. Select the Instrument Documents Template TQC in the Template dropdown.
         6. Select the green check icon to add the file(s).
         7. Click **Save** in the TQC window.
         8. Click **OK** in the QC Components window that appears.
         9. Repeat as necessary for all controls.
      7. Click on the Assigned Tests tab.
      8. Highlight the first order for the first patient sample (parent level).
      9. Verify the Test Results window populates with the correct patient information.
      10. Open the Analysis Images tab.
      11. Select the Add File tab, then select the add file (folder) icon.
      12. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
      13. Choose Instrument Documents from the Template dropdown.
      14. Select the green check icon to add file(s).
      15. 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 each patient sample. Rerun documents should be labeled with the suffix \_RERUN#.
      16. Repeat as necessary for all patient samples on the run.
          1. **NOTE:** The PDF report only needs to be added once per patient sample.
      17. Press the **Select All** button.
      18. Complete the Upload Tasklist Documents action by marking the Completed checkbox, found on the parent row for each patient sample, and select **Save**.
7. **INTERPRETATION:**
   1. The results are interpreted by the GeneXpert Dx System from measured fluorescent signals and embedded algorithms to identify genotypes. A report is generated including the Final Result, which is uploaded into Soft Molecular for review.
   2. The results include:
      1. Normal – Wildtype (no mutation detected)
      2. Homozygous – Homozygous mutant (mutation detected in both alleles)
      3. Heterozygous – Heterozygous mutant (mutant detected in one allele)
      4. Invalid – Sample was not properly processed, or PCR was inhibited.
         1. Probe Check: PASS; all probe check results pass
            1. Repeat assay according to Molecular Genomic Pathology Laboratory Cepheid GeneXpert F2 & F5 Assay procedure.
      5. Error – The Probe Check control failed, and the assay was aborted possibly due to an improperly filled reaction tube, maximum pressure limits were exceeded, or a reagent probe integrity problem was detected.
         1. Probe Check: FAIL; one or more of the probe check results failed.
            1. Repeat assay according to Molecular Genomic Pathology Laboratory Cepheid GeneXpert F2 & F5 Assay procedure.
         2. Probe Check: PASS; Error is caused by system component failure
            1. Repeat assay according to Molecular Genomic Pathology Laboratory Cepheid GeneXpert F2 & F5 Assay procedure.
      6. No Result - Insufficient data was collected to produce an assay result. Most likely due to the operator stopping a test that was in progress.
         1. Probe Check: N/A
8. **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 tasklist number.
   4. Click **No** in the window that appears.
   5. Open the QC Data tab on the left side of the screen.
      1. Click 2 times on a control.
      2. Navigate to the Document tabs.
      3. Click the Dual View icon, so the uploaded PDF report is available when entering results.
      4. Select the Results tab.
      5. Enter results in the result field, then click **Verify All**.
      6. Click **Save**.
      7. Click **OK** to close the QC Components window.
      8. Click **Yes** when asked to save changes.
      9. Repeat as necessary for additional controls.
   6. Open the Assigned Tests tab.
      1. For each patient sample:
         1. Click the Dual View icon, so the uploaded PDF report is available when entering results.
         2. Enter result under Result column for each test code.
         3. Complete the Result Review action by marking the Completed checkbox.
         4. Click **Save**.

Close the Tasklist Entry 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.
   8. Verify the information on the report is accurate or edit, as needed.
   9. Click **Complete Sign Out**.
   10. Select **Edit** in the Sign Out Entry window and repeat steps as necessary for all tests ordered on each specimen.
   11. Close Sign Out Entry.
2. **REPEAT TESTING:**
   1. During the testing process, testing for 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.
3. **ASSAY LIMITATIONS:**
   1. Rare Factor V mutations (A1696G, G1689A, and A1692C) and any additional SNPs in the probe binding region may interfere with the target detection and yield an INVALID result.
   2. Other rare Factor II mutations in the probe binding region may interfere with the target detection and could yield an INVALID result, or a false HOMOZYGOUS mutant result when occurring concordantly with the Factor II c.\*97G>A (G20210A) mutation.
   3. The performance of the Xpert Factor II & Factor V Assay has not been evaluated with samples from pediatric patients.
   4. Patients on heparin therapy and blood transfusion patients may have blood specimens that potentially interfere with the PCR results and lead to invalid or erroneous results.
4. **REFERENCES:**
   1. Xpert Factor II & Factor V Product Insert, #301-0590 Rev. C, August 2020
   2. MMQCI Xpert FII & FV Genotype Panel Product Insert, #G109 Rev. 1, August 2016
   3. MMQCI Xpert FII & FV HET Product Insert, #G108-2H Rev. 2, September 2016
   4. MMQCI Xpert FII & FV NOR/MUT Control Product Insert, #G11601 Rev. 2, March 2019