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| **F9C Factor IX Chromogenic Assay** |
| **Purpose** | This procedure provides instructions for Factor IX Chromogenic Assay in plasma.  |
| **Principle** | Factor IX in the sample is activated by human XIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Factor VIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined by the hydrolysis of a chromogenic FXa substrate. The FIX activity of the sample is assigned vs. a FIX plasma or FIX concentrate standard with FIX potency expressed in international units (IU). F.IX + F. XIa FIXa F.VIII + Thrombin F.VIIIa  F.X F.VIIIa F.Xa FIXa, PL, Ca²   F.Xa Z-D-Arg-Gly-Arg-pNA Z-D-Arg-Gly-Arg-OH+pNA (yellow) The Behring Coagulation System (BCS-XP) is a fully automated photometric instrument used to perform a wide range of coagulation assays rapidly and efficiently. It can be used to determine clotting, chromogenic, immunologic and agglutination-based assays. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, section supervisor, and pathologist.
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| **Materials** | **Equipment** | **Reagents** | **Supplies** |
|  | * **Behring Coagulation System (BCS-XP):** analyzer, personal computer, printer and associated non-disposable parts
* **Disposable 4 mL sample cups**, available from Allegiance OVIS31
* **Plastic transfer pipets**
* **BCS-XP disposable cuvettes**, available from Allegiance OVIP11
 | Factor IX Chromogenic Test Kit containing;**Reagent A** - lyophilized preparation containing human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor. Reconstitute with 1.4ml of water. Allow to stand 5 min.at room temperature with intermittent mixing for complete reconstitution.Stability is 72 hours at 2-8°C or 12 months at -70°C.**Reagent B** - lyophilized preparation containing human FXIa, human FII, calcium chloride and phospholipids.Reconstitute with 8.0ml of water. Allow to stand 5 min.at room temperature with intermittent mixing for complete reconstitution.Stability is 72 hours at 2-8°C or 12 months at -70°C.**FXa Substrate** -Liquid solution of chromogenic Xa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor. Ready for use.Opened vial is stable for 1 month at 2-8°C.**FIX Diluent Buffer, Stock Solution** – Liquid stock solution of diluents buffer, containing a heparin antagonist. For working solution prepare 1 part stock solution with 9 parts water.Open vial stability of stock solution is 1 month at 2-8°C.Working solution should be used the same day of preparation.**Standard Human Plasma (SHPL)** - lyophilized preparation of pooled human, normal citrated plasma and HEPES buffer solution (12 g/L)Reconstitute lyophilized SHPL with 1.0 ml distilled or deionized water.Mix carefully, let stand at 15-25°C for at least 15 minutes, mix again carefully before use.Stability 4 hours at 15 - 25°C.**Control Plasma N (BEN), Control Plasma P (BEP)** - lyophilized preparation of pooled normal plasma stabilized with HEPES buffer solution (12 g/L). Used for Quality Control (Normal and Pathological).Reconstitute lyophilized BEN and BEP with 1.0 ml distilled or deionized water.Mix carefully, let stand at 15-25°C for at least 15 minutes, mix again carefully before use.Stability 4 hours at 15 - 25°C.**● Low Control (AFAC)**  - Dilution of BEP (1:5) prepared by the analyzer. | * **Type I deionized water**
* **Washing Solution for Behring Coagulation Analyzers:** Siemens OWZC35. Contains hydrochloric acid and detergent
* **Barbicide**

**disinfectant solution**.King Research chc# 31111. Prepare working solution by diluting one 125ml bottle of concentrate to 2.0 L with deionized water.Working Barbicide solution is stable for 8 weeks.**Do not use this product for cleaning surfaces, lanes or racks on the analyzer.** |
| **Special Safety Precautions****Sample** | Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into a sink, flush with a large volume of water to prevent azide build up.[MSDS Search | MSDSonline](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True) Childrens Star Net1. Collect blood from a clean venipuncture; avoid foaming.
2. Mix nine parts of freshly collected blood with one part 3.2% (0.105 M) sodium citrate:
3. Add 1.8 mL whole blood to 0.2 mL 3.2% sodium citrate (blue-top Vacutainer tube)

- or -1. Add 2.7 mL whole blood to 0.3 mL 3.2% sodium citrate (blue-top Vacutainer tube)

- or -1. Special tubes must be prepared for patients whose hematocrit is > 55%. See procedure entitled *Citrate Concentration Adjustments.*
2. Invert to mix well; transport to lab at room temperature.
3. Check sample for clots with applicator sticks.
4. Centrifuge in Stat Spin for five minutes or 10 minutes at 3000 rpm at room temperature.

 1. Sample for testing: Remove plasma from RBCs and place in a 4 mL plastic

Cup, spin again and remove plasma leaving a small amount in the bottom of the first cup. Allow for 100 l of dead space in the sample cup for testing.1. Specimen Stability:
2. Plasma must be frozen if testing cannot be completed within four (4) hours.
3. Plasma two (2) weeks when stored -20°C.
4. Plasma six (6) months when stored -70°C (rapidly frozen).
5. Thaw frozen plasmas at 37°C for three (3) minutes, test immediately.
6. If there is a delay in sample transport:
7. Notify supervisor or pathologist.
8. If approval is given to run test, append one of the following to the result:
* “-DELA” (transport delayed)
1. Reject specimen if:
2. Clotted
3. Tubes insufficiently filled (tubes may vary by no more than +/-10%, see comparison tubes by centrifuge).
4. Incorrect ratio of anticoagulant to blood.
5. Grossly hemolyzed specimens should be rejected unless a new specimen cannot be drawn without causing the patient trauma or a non-hemolyzed sample is unobtainable (post-op heart, ECMO, etc.).

**If a hemolyzed sample is tested, add one of the following comments to the result depending on the amount of hemolysis:*** + “-HP” (hemolysis present may affect results)

 or – * + “-GRH” (gross hemolysis may interfere with testing)
1. Notify unit or physician of unacceptable specimens; enter appropriate comment in the computer.
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| **Calibration****Quality Control** | 1. Open Calibration dialog by clicking on it on the main screen. 2. Select “New” to request a new reference curve.3. Place Standard Human Plasma (SHPL) in a 5 mL rack, any lane 5 through 14, with bar code facing left.4. Click on the Calibration button.5. Click on New.6. Click the FIX.CH.SHPL or FIX.CH.Low. SHPL assay from the selection box on the left side of the screen.7. Select the correct lot number for all of the reagents.Click on the inverted triangle of the lot number selection box (right side of screen).Highlight the correct lot number from the pop-up menu.8. Click on Measure Curve.9. Click on Close.10. View the curve when completed:Click on the Calibration button.Highlight the curve in the Curve Overview box.Click on Show Curve.Print the curve.11. View individual points on the curve:Highlight the curve in the Curve Overview box.Click on the Info buttonHighlight the point in the Individual Results box.Each measurement can be viewed in the Individual Measurement box.12. If any point is flagged, the curve will be labeled invalid and the point must be rerun.Close the Info box being viewed.Click on Show CurvePoint and click on the invalid pointClick on the Repeat buttonNOTE: The request to repeat a point must be made within 30 minutes of obtaining the initial curve. After the point has been repeated, the curve will be updated.13. To activate a specific curve when several curves of the same assay are present Click on the specific calibration curve Click on the Reactivate button on the bottom left side of the screen.**Auto Calibration**1. Load the new/old reagents into the appropriate racks (cooler and 15 mL racks); place on the BCSXP.2. Load appropriate calibrator ( SHPL as defined above) into a 5.0 mL rack; place on the BCS-XP.3. Request control or patient samples tests first.4. Once processing is complete, the BCS-XP will perform an AutoCalibration for a Factor IX chromogenic assay.5. When the calibration is complete, the patient and control results will be displayed.6. Check curve and repeat appropriate points as discussed above (Manual Calibration).1. Assayed Control Plasmas (Control Plasma N, Control Plasma P) should have their ranges verified when there is a change in lot number of reagent or control material.2. Assayed Control Plasmas (Control Plasma N, Control Plasma P, AFAC) are run:At the beginning of each shift or once every eight (8) hours (AFAC if low curve was needed).Each time a reagent is changedCodes for controls (BEN, BEP, AFAC) are listed on the appropriate worklistPlace controls on the BCS in their original vial using a 5.0 mL (small) bottle rackOrder controls by:* Click CONTROL JOURNAL button
* Highlight FIX.CH or FIX.CH.Low on both controls
* Click New
* Analysis will begin

3. Patient results cannot be reported unless control values are within expected tolerance limits.1. If values do not fall within the expected range, test new controls then new reagents.
2. If QC is still out of range, notify the supervisor.

4. Control values are recorded daily.5. All control values must be entered into Sunquest whether in or out of control range. Out of control  values must have an appropriate modifier appended.6. When QC data is entered, it is reviewed using Westgard rules.* If a Westgard rule fails in Sunquest, the computer displays the result’s standard deviation from the mean.

7. To enter corrective action in Sunquest; after the standard deviation is displayed, the prompt ENTER QC MODIFIER is displayed, use the QC modifier that best describes the action taken from [Table A - Exclusion Comment Codes](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/200705.pdf) |
| **Procedure** | Follow the activities in the table below for FIX.CH or FIX.CH.Low (Factor IX, CHROMOGENIC) IN PLASMA. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | Load Reagent A, Reagent B, Factor Xa Substrate, Factor IX Diluent Buffer (working solution) in either cooler rack (lane 1 - 4) with bar codes facing left. |  |
|  | 2 | Load Saline onto BCS-XP in any available lane (3 through 14). |  |
|  | 3 | Place controls in a 5.0mL rack, load onto BCS-XP in any available lane (5-14). |  |
|  | 4 | To load patients:a. Insert rack loaded with barcoded samples in any available lane (6 through 14).b. The barcodes are read and the sample numbers are entered on the Job List.c. Click on the Job List button; all patient sample numbers will appear on the job list with an analyzer symbol preceding the sample number and a red X in the appropriate test cell.d. The run will begin. |  |
|  | 5 | Results appear on the job list when completed. Copy the results on the FAC worklist.1. *If the instrument is online*, the results are transmitted to Sunquest and appear dark green on the Joblist.
2. *If the instrument is offline*, enter result in computer following

 directions listed for manual entry mode under Result Reporting  section of this procedure. |  |
| **Procedure Notes** |  | Additional Notes:1. Linearity: Approximately 10.0 - 150.0 (lowest and highest points on the calibration curve). Factor IX values below 10.0 should be measured on the low curve which measures between 1.0 – 25.0. 2. Heparin concentrations of up to 2 IU/ml do not interfere with the Factor IX Chromogenic assay.3. Elevated Factor IX values should be diluted with saline, tested and the result multiplied by the appropriate dilution factor. 4. Lupus anticoagulants (LA) do not interfere with the chromogenic assay. In the presence of a LA, markedly falsely decreased FIX activity may be evident with a one stage clotting assay, but the FIX activity appears normal with the chromogenic assay. |  |
| **Interpretation/****Results/Alert Values** | 1. Certain modified recombinant FIX replacement products demonstrate variable and clinically significant differences in post infusion recovery (that is, the amount of factor measured vs. the actual concentration present), based on the activated partial thromboplastin time (APTT) reagent used in the one stage clotting assay. Overestimation of post infusion plasma factor activity can lead to underdosing of the replacement factor and an increased risk of bleeding. Conversely, underestimation of factor activity in a post infusion sample may lead to overdosing of the replacement factor, which not only has cost implications but may also place the patient at risk for thrombosis. Most recombinant FIX products may be accurately measured using a chromogenic assay, even when this is performed with a plasma calibrator rather than a product specific calibrator.2. All results will be appended with the coded comment “ASR” in Sunquest translated as the following:"This test was developed and its performance characteristics determined by Children's Hospitals and Clinics. It has not been cleared or approved by the U.S. Food and Drug Administration. Analyte Specific Reagents(ASR's) are used in many laboratory tests necessary for standard medical care andgenerally do not require FDA approval." |
| **Reference Intervals****Result Reporting** | 1. [Table - QQ Factor Assays Reference Intervals](https://starnet.childrenshc.org/References/labsop/coag/res/table-qq-factor-assays-reference-intervals.pdf)Sunquest:1. On-line mode (OEM): MPLS- See procedure “Autoverification of Coagulation”

Function: OEM <CR>Device: XP1 or XP3<CR>Workload data for - <CR>Last Cup Received = xxxx Last Cup Processed = xxxxxStart at Cup Enter cup # if appropriate (same as sequence #)WAITING (ENTER \* TO EXIT ‘OE’)Accession numbers appear as results are transmitted. Check flagged results on the BCS-XP, if all results are acceptable:Accept (A), Modify (M), or Reject (R): A <CR>If results are unacceptable:Accept (A), Modify (M), or Reject (R): R <CR>1. Manual entry mode (MEM):

Function: MEM <CR>Worksheet: FAC <CR>Test-1: <CR>Test-2: <CR>CAP Method: M <CR>Lots of tests appear one at a time Enter XP1 or XP3 (A)ccept, (M)odify or (R)eject: A <CR>Workload data for - <CR>Acc. No.: Enter ##### <CR>F9C Enter results (xxx.x) <CR>Accept (A), Modify (M), or Reject (R): A <CR> |
| **Maintenance** | 1. Night Shift performs daily maintenance:
2. See procedure on the back side of the BCS-XP Maintenance form
3. Document on the BCS-XP Maintenance form
4. Day Shift performs weekly, monthly, and “as needed” maintenance:
5. See procedures in the front of the BCS-XP Logbook
6. Document on the BCS-XP Maintenance form
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| **Troubleshooting** | 1. Reoccurring problems are documented in the BCS-XP Action Log.
2. Call Siemens Technical Services (TAC) 1-877-457-4BCS, be prepared to give the following:
* Serial number
* What was going on at time of instrument malfunction
 |
| **References** | 1. BCS®XP System Instruction Manual 1 000 767.0506 Manual Version 1.0, Siemens Diagnostics Inc., Marburg Germany, Copyright 2006.
2. Rossix Chromogenic Factor IX product insert, ROX FACTOR IX – 90 00 20,

Rossix AB SE-431 53 Molndal, Sweden Revision 04/2014.1. Control Plasma N package inserts, Siemens Healthcare Diagnostics, Newark, DE, December 2007.
2. Control Plasma P package inserts, Siemens Healthcare Diagnostics, Newark, DE, November

2007.1. Standard Human Plasma package insert, August 2008.
2. ROX FIX Test Definition, BCS-XP, ML-00-00172 Rev01.

7. The value of the chromogenic activity assay in diagnosis and therapeutic monitoring of hemophilia. By Dorothy Adcock, Stefan Tiefenbacher, Rajiv Pruthi 01/23/2017.   |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 08/01/19 | Initial Version |