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|  | **WORK INSTRUCTION** |
| **J-W-CG-2140-00** |
| ACL TOP - Fibrinogen - Q.F.A. (FIB) | |
| **St. Joseph Medical Center Tacoma, WA**  **St. Clare Hospital Lakewood, WA**  **St. Elizabeth Hospital Enumclaw, WA**  **St. Francis Hospital Federal Way, WA**  **St. Anthony Hospital Gig Harbor, WA**  **PSC** | |

**PURPOSE**

This procedure provides instructions for the quantitative determination of fibrinogen, based on the Clauss method, in human citrated plasma on the ACL TOP Family analyzers.

**PRINCIPLES**

In 1957 Clauss developed a quantitative assay using thrombin to measure fibrinogen in plasma. In this procedure an excess of thrombin is added to diluted plasma and the resulting clotting time value is measured. The log of the clotting time value is inversely proportional to the log of the fibrinogen concentration.

**BACKGROUND**

**Clinical Significance**

When unexplained bleeding or abnormal clotting occurs it may be of clinical importance to quantitate fibrinogen. Fibrinogen is also a useful marker in the evaluation of several disease states including Disseminated Intravascular Coagulation, liver disease and inflammatory diseases.

**Methodology**

The fibrinogen in the diluted test sample is converted to fibrin by the addition of an excess of thrombin and the resulting clotting time value is measured. The log of the clotting time value is inversely proportional to the log of the fibrinogen concentration. A fibrinogen reference curve is plotted from the clotting time results of the known referenced plasma dilutions having different fibrinogen values. The concentration of fibrinogen in patient plasma samples is determined by comparing clotting time values to the reference curve.

**RELATED DOCUMENTS**

R-PO-CH-0810 Quality Control Program General Laboratory

R-PO-CH-0809 Quality Control Westgard Rules Statistics

R-W-CG-2300 Coagulation-Specimen Integrity

R-PR-AD-0540 Specimen Rejection/Cancellation Protocol

R-F-CG-1130 ACL TOP - Reagent Stability and Reconstitution

R-W-CG-2090 ACL TOP - Sample Analysis-Barcoded Specimens

R-W-CG-2091 ACL TOP - Sample Analysis-Non-Barcoded Specimens

R-W-CG-1131 ACL TOP - Reagent Management-Assay Reagents

R-W-CG-1132 ACL TOP - Reagent Management-Bulk Reagents

R-W-CG-2200 ACL TOP - QC Analysis & Review

**SPECIMEN**

**Specimen Requirement**

Citrated blood (9:1 blood to anticoagulant) 3.2% sodium citrate. Follow CLSI NCCLS guidelines H3-A5 and H21-A5. No other anticoagulant is acceptable.

Specimen Storage and Stability

Specimen is stable for 2 hours at room temperature (20 +/- 5º C). If unable to complete testing in 2 hours, freeze at -20º C. Frozen plasma should be thawed only once at 37º C for 5 minutes.

**Specimen Handling**

Samples that are short-filled (refer to B-D Vacutainer fill chart), over-filled or clotted should be rejected.

Centrifugation of sample to achieve platelet poor plasma (<10,000 platelet count) is required.

**REAGENTS**

**Contents of Kit**

The Q.F.A. Thrombin (Bovine) kit consists of:

* **Q.F.A. Thrombin (Bovine)**: 10 x 2 mL vials of lyophilized bovine thrombin (approx 100 UNIH/mL) containing buffer, an antiheparin agent and a preservative.
* Package Insert

**Reagent Preparation**

Q.F.A. Thrombin (Bovine)

1. Allow the vial of reagent to equilibrate at 15-25º C for at least 15 minutes before reconstitution.
2. Dissolve the contents of each vial with 2 mL of NERL reagent grade water or equivalent..
3. Replace the stopper and swirl gently.
4. Let sit for 30 minutes at 15-25º C and invert to mix before use.

**Reagent Storage and Stability**

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8ºC.

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| **Stability after Reconstitution** | |
| Q.F.A. Thrombin | 7 days at 2-8°C, |
| 7 days at 15°C on the ACL TOP |

**Additional Materials Needed**

HemosIL Normal Control

HemosIL Calibration Plasma

HemosIL Low Fibrinogen Control

NERL water

Factor Diluent

HemosIL Cleaning Agent (Clean B diluted): Dilute Cleaning Agent 1:8 with NERL Reagent Grade water (or equivalent) (2 mL Cleaning agent +14 mL of NERL water)

**CALIBRATION**

Calibration and storage of a valid Fibrinogen QFA calibration curve are required to obtain Fibrinogen results. Calibration is performed:

* Per manufacturer recommendation
* Every 3-6 months
* With a change of reagent lot numbers
* After major parts replacement
* To satisfy local regulatory requirements
* At laboratory discretion
* When indicated by Quality Control data
* To validate the analytical measurement range

**Calibrator Preparation**

1. Reconstitute one vial of Calibration Plasma with 1.0 mL of NERL reagent grade water or equivalent.
2. Replace the stopper and swirl gently.
3. Keep Calibration Plasma at 15-25ºC for 30 minutes. Ensure the complete reconstitution of the calibrator.
4. Gently swirl and invert to mix before use. Do not shake. Avoid foam formation.

**Note:** After reconstitution the Calibration plasma should be handled in the same manner as fresh citrated plasma.

**Calibrator Storage and Stability**

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8ºC

Reconstituted calibrator is stable for 24 hours at 2-8ºC in the original vial.

**Method for Calibration (if necessary):**

1. Define Results Units and Rerun Rules in the Q.F.A. Thrombin Test Definition if necessary (Setup, Test List, Test Code, Result Units and Rerun Rules).
2. Choose Setup, Materials List.
3. Double-click on the appropriate calibration plasma to open the Materials Definition screen.
4. Choose the Lot Specific Information tab and enter the Calibration Plasma lot number and Expiration Date.
5. Enable Lot Management from the Lot Specific Information tab.
6. Select the Save icon to store the lot number. Once the lot number is saved, the Assign Values icon becomes available.
7. Select the Assign Values icon.
8. Enter the calibration value from the Calibration Plasma package insert. Press OK.
9. Choose the Previous Screen icon to exit.
10. Load the Q.F.A. Thrombin (Bovine) reagent, Calibration Plasma, Diluted Clean B and Factor Diluent on to the ACL TOP.
11. Select Calibration, Status List.
12. Double-click on Q.F.A. Thrombin Test Code to open the Calibration Details screen.
13. Choose the Run icon.
14. Select OK at the “Do you confirm the operation?” prompt.
15. Choose the Previous Screen icon to exit.
16. Verify the Job Status for the Q.F.A. Test says Active.

Once the calibration is complete, review the calibration results. An acceptable r2 - value is >0.975. If there are no errors/failures and the calibration is acceptable, choose the Validate icon to validate the calibration curve.3

**Note:** The QFA Low calibration curve must also be validated.

**QUALITY CONTROL**

Normal and abnormal controls are recommended for a complete quality control program. HemosIL controls are designed for this program and must be performed every 8 hours. Controls should also be performed following loading of new reagents and after calibration.

The HemosIL controls are:

* HemosIL Normal Control
* HemosIL Low Fibrinogen Control

**Quality Control Preparation**

1. Dissolve the contents of each vial with 1 mL of NERL reagent grade water or equivalent.
2. Replace the stopper and swirl gently.
3. Keep the control at 15-25°C for 30 minutes and invert to mix before use. Do not shake. Avoid foam formation.

**Quality Control Storage and Stability**

Unopened control is stable until the expiration date shown on the vial when stored at 2-8°C.

Stability after reconstitution at 2-8°C in the original vial is 24 hours.

Stability at 15-25°C in the original vial on-board the ACL TOP is 24 hours.

**PROCEDURE STEPS**

**Running QC and Patient Specimens**

1. Load reagent and Clean B Diluted onto the ACL TOP.

Reagent Mapping

In order for accurate test result, a reagent map has been established for specific reagents. The following reagents **must** be in specific positions:

* PT Recombiplastin
* Q.F.A Thrombin (Fibrinogen)
* Thrombin time
* Heparin Anti-Xa
* Clean B Diluted

PT Recombiplastin, Q.F.A Thrombin (Fibrinogen), Thrombin time, Heparin Anti-Xa reagent and Clean B diluted **must** be in the same rack.

Clean B diluted must be in position 6 of that rack.

1. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the ACL TOP in a Diluent track.
2. Choose QC from the Main Menu and select Test Status List.
3. Double-click on a Test Code to reveal the Test Materials Definition tree.
4. Select any Q.F.A. Thrombin QC Control and choose the Program QC icon. This will run all QC levels for that test.
5. Place sample tubes in a sample rack with barcodes facing outwards.
6. Select an available sample track and load the sample rack when the barcode reader is in position.
7. Verify the samples have been identified and have a test ordered. If not, program the sample ID manually and/or order the test manually from the test and programming window.

**Note:** Q.F.A. Low test should only be used for samples that are below the linear range for the standard tests.

1. Choose the Run icon if the ACL TOP is not currently running.

**CALCULATIONS**

The ACL TOPautomatically converts the results in seconds from a standard curve to mg/dL.

# PERFORMANCE CHARACTERISTICS

# Reference Range

180 – 402 mg/dL

**Critical Value**

< 50 mg/dL (Refer to FHS Critical Value Reporting Protocol)

Analytic Measurement Range (AMR)

Reportable Range: 35-1000 mg/dL

For low or high fibrinogen results, report with Alpha responses of <35 mg/dL or >1000 mg/dL

**LIMITATIONS**

**Interference Substances**

Fibrinogen assay results may be affected by degradation (fibrin or fibrinogen) in the plasma assayed.

No interference on the ACL TOP Family by heparin up to 2 U/mL, hemoglobin up to 375 mg/dL, triglycerides up to 880 mg/dL, and bilirubin up to 23 mg/dL.

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| **Condition** | **Effect** | **Action/Additional Info** |
| Lipemia | May affect | If lipemic may have to airfuge. See Coagulation-Specimen Integrity. |
| Hct ≥55% | False Prolongation | See Anticoagulant Adjustment for High Hematocrits. |
| Hemolysis | May affect | See Coagulation-Specimen Integrity. |

**REFERENCES**

ACL TOP® Family On-Line Help Manual Rev 2.0, Instrumentation Laboratory.

Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition, CLSI Document H21-A5; Vol. 28 No. 5

HemosIL Q.F.A. Thrombin (Bovine) (PN 0020301700) package insert issued 05/2012 Instrument Laboratory.

HemosIL Q.F.A. Thrombin (Bovine) (PN 0020301800) package insert issued 05/2012 Instrument Laboratory

Reference Clinical and Laboratory Standards Institute.Preparation and Testing of Reagent Water in the Clinical Laboratory;Approved Guideline. Fourth Edition, CLSI Document C3-A4;Vol.26 No.22

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| **DOCUMENT APPROVAL Purpose of Document / Reason for Change:** | | | |
| New Document for new test method/analyzer | | | |
| *No significant change to process in above revision.  Per CAP, this revision does not require further Medical Director approval.* | | | |
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