**Title: HIT-Ab(PF4-H) – ACL TOP® Family**

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# REVISION HISTORY

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**Title: HIT-Ab(PF4-H) – ACL TOP® Family**

1. Purpose

This procedure provides instructions for the analysis of Heparin associated antibodies, using HemosIL HIT-Ab(PF4-H) on the ACL TOP® Family.1,2,3

Heparin-induced thrombocytopenia (HIT) is an immune complex mediated disorder that can cause morbidity and mortality in patients receiving heparin therapy. Anticoagulant is administered to prevent thrombosis; however the major clinical event in HIT is an increased risk for venous and/or arterial thrombosis. HIT is suspected when patients treated with unfractionated heparin (UFH) or low molecular weight heparin (LMWH) show a decrease of platelet counts greater than 50% from baseline, typically between days 5 and 10 from the initiation of the anticoagulant treatment. Cases of early-onset HIT and delayed-onset HIT have also been reported. Prompt diagnosis is very important because heparin treatment must be suspended and alternative anticoagulants used in case of confirmed HIT.

Type II HIT, the immunoallergic type, is caused by the development of platelet-activating antibodies, mostly directed against Platelet Factor 4 when complexed with Heparin (PF4/H). These antibodies are the primary cause for inducing thrombosis both venous and arterial. Development of pathology is mainly associated with heparin-dependent antibodies of the IgG isotype. However, the presence of only IgM and/or IgA was observed in some patients, including cases showing a thrombotic complication.

PF4/H antibody testing combined with an appropriate clinical assessment has been proven to be very useful as an aid in the management of HIT suspected patients. Particularly, a negative result for a PF4/H antibody test can support the clinical decision to exclude the presence of HIT, and therefore continue heparin treatment. A weak positivity for PF4 antibodies may indicate that the antibodies are non-platelet activating, while a strong positivity may indicate a higher risk for HIT. In both cases, confirmation with a functional test is recommended. A clinical reassessment supported by laboratory data should be performed before confirmation or exclusion of the diagnosis.

A monoclonal antibody, that mimics human HIT (Heparin Induced Thrombocytopenia) antibodies, is coated onto latex particles. In the presence of PF4 from human platelets, complexed to polyvinyl sulfonate (PVS), and the patient sample, a competitive agglutination reaction occurs. The degree of agglutination is inversely proportional to the concentration of antibodies in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.1,2,3

1. Scope

This procedure will be used by URMC Hematology and Chemistry Lab staff for the qualitative presence of HIT antibodies.

1. Responsibilities

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| --- | --- |
| Roles | Responsibilities |
| Quality | Ensure that procedure is followed when performing the HIT-Ab(PF4-H) test |
| Medical Director | Approval of the HIT-Ab(PF4-H) test |
| Management | Ensure that procedure is followed when performing the HIT-Ab(PF4-H) test |
| Technologists | Follow procedure |

1. Acronyms/Definitions

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| --- | --- |
| URMC | University of Rochester Medical Center |
| HIT | Heparin Induced Thrombocytopenia |
|  |  |

1. Specimens
   1. Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to the most recent Clinical and Laboratory Standards Institute (CLSI) Document H21-A5 for further instructions on specimen collection, handling and storage. No other anticoagulant is acceptable.
   2. Centrifugation: Centrifuge specimens for 12 minutes at 4000 rpm (RCF = 3756g).
   3. Plasma storage: 4 hours at 20ºC ± 5ºC

2 weeks at -20ºC or below

2 freeze/thaw cycles at -70ºC

* 1. Thaw frozen specimens rapidly at 37ºC and centrifuge plasma before testing. After thawing the assay must be performed within 2 hours.
  2. Sample volume is CRITICAL to obtain accurate coagulation results. A 90% draw is the minimum volume acceptable for accurate testing. Do not run or report these samples. Place “.ND” in the result field with the canned text comment, “Sample inadequate for testing. Minimum volume not received.”
  3. Each specimen is checked visually for the presence of clots prior to analysis.  If a clot is suspected, the tube is uncapped, and checked with a pair of applicator sticks.  *ANY* clot present in the specimen makes it inadequate for ALL coag testing. Clotted specimens are rejected and a new specimen should be requested.

1. Quality Control
   1. Two levels of controls are recommended for a complete quality control program. HemosIL HIT-Ab(PF4-H) Controls Low and High are designed for this program. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice before testing patient samples.
   2. QC data is transmitted to the LIS and manually entered into Unity QC. Remedial action for out-of-range values is documented in the LIS and on the Coagulation QC Log. When a value is out of range, the operator should:
      1. Repeat the control using the same control material – if acceptable document in LIS
      2. If still not acceptable, reconstitute a new control and rerun – if acceptable document in LIS
      3. If still not acceptable reconstitute a new reagent for the out-of-range test and rerun – if acceptable document in LIS.
      4. If still not acceptable notify the supervisor or assistant supervisor
   3. CAP proficiency testing will be rotated between appropriate instruments to monitor performance against peer laboratories.
2. Special Safety Precautions
   1. All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC (Center for Disease Control) recommendations and in compliance with the Federal OSHA (Occupational Safety and Health Administration) Blood-borne Pathogen Standard, 29 CFR (Code of Federal Regulations) part 1910.1030. All animal products should be treated as potentially infectious. Avoid contact with skin and eyes. Do not empty into drains. Wear suitable protective clothing. Follow specimen handling as outlined by Laboratory Safety Policy, (SH.CP.AU.gen.0005.0002).
   2. The human material used in this product was tested by FDA approved test methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), Anti-HCV and HIV ½ antibodies. Handle as if potentially infectious.
   3. These reagents except the Complex contain sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures. Please refer to the Material Safety Data sheet for this product for more detailed safety information.
3. Materials
   1. Equipment
      1. ACL TOP® Family Coagulation Analyzer
      2. Centrifuge
   2. Supplies
      1. Pipettes and pipette tips
      2. CLSI CLRW Type (or equivalent) water
   3. Reagents found in HIT-Ab(PF4-H) kit1,2
      1. Latex Reagent: 2 vials x 1.8 mL of a suspension of polystyrene latex particles coated with purified mouse monoclonal anti-PF4/Heparin in Tris buffer, containing bovine serum albumin, stabilizers, and preservative
      2. Stabilizer: 2 vials x 3.2 mL of PBS buffer containing bovine serum albumin, stabilizers and preservatives
      3. Complex: 2 vials x 0.8 mL of a solution of PF4-PVS complex (PF4 from human platelets complexed to PVS), in PBS buffer containing bovine serum albumin, stabilizers and preservative. Contains 0.02% BronidoxTM as a preservative.
      4. Calibrator: 2 vials x 1mL of a lyophilized solution of a monoclonal anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers and preservative1.
   4. The following are not supplied with the HIT-Ab(PF4-H) kit and may be purchased separately:
      1. HemosIL Cleaning Agent PN 9823700
      2. HemosIL Cleaning Solution PN 9831704
      3. HemosIL ACL TOP Rinse Solution PN 20302400
      4. HemosIL Factor Diluent PN 9757600
   5. Controls
      1. HemosIL HIT-Ab(PF4-H) Controls (PN 20014700) consists of8:
         1. Low HIT-Ab(PF4-H) Control (PN 20014710): 3 vials x 1mL of a solution of humanized monoclonal anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers and preservative
         2. High HIT-Ab(PF4-H) Control (PN 20014720): 3 vials x 1mL of a solution of humanized monoclonal anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers and preservative
4. Procedure

**NOTE:** Please refer to the ACL TOP® Family onboard help manual for additional information on the procedures below.

* 1. Reagent/Control Preparation and Stability

Unopened reagents, controls and calibrator are stable until the expiration date shown on the vial when stored at 2-8ºC. For optimal stability, remove reagents from the system and store them at 2-8ºC in the original vial.

**NOTE:** Avoid foam formation when homogenizing reconstituted calibrators or controls. Bubbles on top of the liquids may interfere with the instrument liquid sensors1,2.

* + 1. Latex Reagent: Gently invert several times to mix before use. Do not shake. Avoid foam formation. Opened reagents are stable for 2 months at 2-8ºC in the original vial. Stable continuous on-board for 24 hours.
    2. Stabilizer: Gently mix several times before use. Do not shake. Avoid foam formation. Opened reagents are stable for 2 months at 2-8ºC in the original vial. Stable continuous on-board for 24 hours.
    3. Complex: Gently mix several times before use. Do not shake. Avoid foam formation. Opened reagents are stable for 2 months at 2-8ºC in the original vial. Stable continuous on-board for 24 hours.

**NOTE:** The HemosIL HIT-Ab(PF4-H) Latex Reagent, Stabilizer and Complex have the same stability claims and should be used as pairs on the IL Coagulation System. If one vial is replaced the other components should also be replaced at the same time regardless of the residual content in the vial1.

* + 1. Calibrator: Dissolve the contents of each vial with 1 mL of CLSI Type CLRW water or equivalent. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25ºC for 30 minutes and gently mix several times before use. Do not shake. Avoid foam formation. Use immediately after reconstitution. Opened calibrator is stable for 3 hours on-board and ACL TOP® Family.
    2. Controls: The HIT-Ab(PF4-H) Controls should be mixed several times before use. Do not shake. Avoid foam formation. Opened controls are stable for 2 months at 2-8ºC. Stable continuous on-board for 24 hours. When controls are used for 1 hour on-board per test session and the vials are returned well capped to 2-8ºC between sessions, the controls are stable for an on-board cumulative time of up to 8 hours.
  1. Calibration

Calibration and storage of valid HIT-Ab(PF4-H) calibration curve are required to obtain results. Calibration is performed:

* With a change of reagent lot numbers
* After major parts replacement
* To satisfy local regulatory requirements
* At laboratory discretion

Method for Calibration (if necessary):

* + - 1. Define Materials if necessary (**Setup, Material List)** Select the appropriate materials from the Material List.
      2. Add HIT-Ab test code to the Test/Profiles Programming Window, if necessary (**Setup, Display, and Test Programming Window**).
      3. Load HIT-Ab reagents (HIT Latex Reagent, HIT Stabilizer, HIT Complex, and HIT Calibrator) and Factor Diluent onto ACL TOP® Family instrument.
      4. Choose **Setup, Materials List.**
      5. Double-click on the appropriate calibrator (HIT Cal) to open the **Materials Definition** screen.
      6. Enable **Lot Management** from the **Lot Specific Information tab**.
      7. Enter the Calibrator lot number and Expiration Date.
      8. Select the **Save** icon to store the lot number. Once the lot number is saved, the **Assign Values** icon becomes available.
      9. Select the **Assign Values** icon.
      10. Enter the calibrator value from the package insert. Press **OK**.
      11. Choose the **Previous Screen** icon to exit.
      12. Load HIT Latex Reagent, HIT Stabilizer, HIT Complex, HIT Calibrator and Factor Diluent onto the ACL TOP® Family instrument.
      13. Select **Calibration, Status List**.
      14. Double-click on the HIT-Ab test code to open the **Calibration Details** screen.
      15. Choose the **Run** icon.
      16. Select **OK** at the “Do you confirm the operation?” prompt.
      17. Choose the **Previous Screen** icon to exit.
      18. Once the calibration is complete, review calibration results. This calibration uses a spline curve fit, therefore there are no slope, intercept or r2 values displayed. If there are no errors/failures and the calibration is acceptable, choose the **Validate** icon to validate the calibration curve.
  1. Quality Control

Controls should be analyzed at least once every 8 hour shift, in accordance with good laboratory practice1.

* + - 1. Create/Edit QC files, if necessary (**Setup, QC List**, **Test Code**, to allow access to the **QC Definition Screen.**
      2. Add HIT-Ab test code to the Test/Profiles Programming Window, if necessary (**Setup, Display, Test Programming Window**.
      3. Load HIT-Ab reagents (HIT Latex Reagent, HIT Stabilizer and HIT Complex) onto the ACL TOP® Family instrument.
      4. Calibrate, if necessary (see calibration section of this procedure).
      5. Place QC materials with the barcodes facing out in a Diluent Rack and load onto the ACL TOP® Family instrument in a Diluent track. (If running the QC from the sample rack refer to **Quality Control, Performing a QC Test** in the ACL TOP® Family On-Line Help Manual)..
      6. Choose **QC** from the Main Menu and select **Test Status List**.
      7. Double-click on the HIT-Abtest code to reveal the Test Materials Definition tree.
      8. Select any HIT-Ab QC Control and choose the **Program QC** icon. This will run all QC levels for that test.
      9. Verify that both controls pass before running patient samples by entering QC results into Unity QC.
  1. Patients

For instructions on loading samples without barcodes or LIS, please refer to **Samples Analysis, Managing Patient Samples, Programming** **Bar Coded Samples** and **Programming Non-Bar Coded Samples** in the ACL TOP® Family On-Line Help Manual.

* + - 1. Place sample tubes in a sample rack with barcodes facing outwards.
      2. Select an available sample track and load the sample rack when the barcode reader is in position.
      3. 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.
      4. Choose the **Run** icon if the ACL TOP® Family instrument is not currently running.
      5. If the sample is above linearity and requires an on-board sample dilution, Factor Diluent must be loaded.

1. Limitations

HIT-Ab(PF4-H) results on the ACL TOP® Family are not affected by:

* 1. Hemoglobin up to 500 mg/dL
  2. Bilirubin up to 19 mg/dL
  3. Triglyceride up to 375 mg/dL
  4. Rheumatoid Factor up to 1000 IU/mL
  5. Human anti-mouse antibodies (HAMA) up to 1 µg/mL1

1. Calculations

NA

1. Interpretation
   1. The presence of PF4/H antibodies in a normal population is not expected. Based on the studies outlined on the package insert, it has been determined that on heparin treated patient samples, **HemosIL HIT-Ab(PF4-H) results equal to or higher than 1.0 U/mL may indicate the presence of HIT antibodies.** The assay results should be used with other information, including the clinical context, in forming a diagnosis1,2,3.
2. Result Reporting
   1. Lower linearity: Results may be flagged as below linearity (<0.6 U/mL)1. These results should be reported <0.6 U/mL.
   2. Upper linearity: Results may be flagged as above linearity (>5.7 U/mL). The instrument will perform an on-board sample dilution and correct the final result for the dilution factor thereby expanding the measuring range to 16.0 U/mL1. If the result still exceeds the expanded range, the result should be reported >16.0 U/mL.
   3. A result that is ≥1.0 U/mL will reflex a POS result in the HITAR field. Results less than 1.0 U/mL will reflex a NEG result in the HITAR field.
   4. All patient results will be emailed to the coagulation lab director, the coagulation resident, and any applicable laboratory techs as designated by the coagulation lab director.
   5. All results ≥1.0 U/mL that reflex to POS should be treated as a critical value. See UR.CP.GL.Gen.0004.0001 for general guidelines on reporting critical values.
3. Training

Staff are trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

1. References
   * + 1. HemosIL HIT-Ab(PF4-H) (PN 0020014600) package insert
       2. HemosIL HIT-Ab(PF4-H) (PN 0020302700) package insert
       3. HemosIL HIT-Ab(PF4-H) (PN 0020301200) package insert
       4. ACL TOP® Family On-Line Help Manual
       5. Westgard JO and Barry PL. Cost-Effective Quality Control: Managing the Quality and Productivity of Analytical Process, AACC Press 1986
       6. HemosIL HIT-Ab(PF4-H) Controls (PN 0020013200) package insert
       7. HemosIL HIT-Ab(PF4-H) Controls (PN 0020013300) package insert
       8. HemosIL HIT-Ab(PF4-H) Controls (PN 0020014700) package insert