**TITLE: HIPA Screen (Heparin Induced Platelet Antibody---HIT)**

**PRINCIPLE:**

The risk of heparin-induced thrombocytopenia is greatly increased in patients with recent exposure to heparin. Immune-mediated heparin-induced thrombocytopenia (HIT) is often caused by platelet-activating antibodies that recognize complexes of Heparin/PF4.

**PERSONNEL:**

Medical Technologist

# CLINICAL SIGNIFICANCE:

# HIT is caused by heparin-dependent antibodies (HIT antibodies) formed to the heparin/PF4 complex. HIT antibodies are most frequently induced by Unfractionated Heparin (UFH) use following Cardiopulmonary Bypass Surgery (50%) and major orthopedic Surgery (15%); ultimately HIT is observed in 1-to5% of patients treated with UFH. HIT antibodies are initially formed when a patient has been on heparin therapy for five or more days. An immune response to a heparin dose may be observed sooner (1-to-2 days) if the patient has had previous exposure to heparin. The hallmark symptoms of HIT are a drastic fall in platelet count and thrombosis. Other symptoms may include cutaneous reactions, from a simple allergic reaction to lesions or necrosis.

## REAGENTS AND EQUIPMENT:

 One (1) PLUSS PF4 Assay; packaged in individual sealed pouches.

 Each sealed pouch contains the following components:

 One (1) PIFA PLUSS™ PF4 MiniReactor Devise

 One (1) sera STAT™ Blood Cell Separator

Tube Rocker

**Materials Required But Not Provided**

Timing Device

Pipettor capable of delivering 150µl and disposable tips

Positive and Negative Controls; stored in Microbiology freezer (see Quality Control)

**SAMPLE:**

**Collection**

Only fresh, anticoagulated blood samples collected in Sodium Citrate (blue top tube) can be used. No other collection tubes are recommended for use.

Do not use frozen and/or thawed specimens.

**Preparation**

The seraSTAT blood cell separator must be used to extract the appropriate volume of patient sample for introduction into the Mini-Reactor.

Patient samples that remain at room temperature after collection must be introduced to the sera blood separator within a maximum of TWO (2) hours from the draw.

**Storage**

Properly collected anticoagulated whole blood specimens that cannot be tested immediately, should be stored refrigerated (2-8C) for no longer than 24 hours.

**CALIBRATION:**

None Indicated

**QUALITY CONTROL:**

PF4 is a single-use/unit use test system. An external positive and negative control will be run with each new shipment. If values are not within range, repeat controls. Contact a senior tech if Quality Control is still out of control after repeat testing.

The PIFA PLUSS™ PF4 contains an internal device control with each test run. The appearance of RED in the CONTROL Window indicated that the device has functioned as designed. If RED does not develop in the CONTROL Window within a maximum of 10 minutes after performing the test procedure, the test result is considered invalid.

The appearance of YELLOW or blue-GREEN in the TEST Result window indicates that a sufficient sample has been introduced into the reaction mixture.

Controls should be assayed using the same test procedure used for patient specimens. Use only confirmed Heparin/PF4 antibody positive and negative specimens. SEE CONTROL PACKAGE INSERT FOR STORAGE, HANDLING AND PREPARATION OF CONTROL MATERIAL (Insert can be found in the box with control and a copy follows this procedure).

**STEPWISE PROCEDURE:**

PRE-TEST PREPARATION

**Complete in the following order:**

1. Remove the PIFA PLUSS™ PF4 sealed pouch from refrigeration.
2. Visually inspect the pouch to confirm date of use is prior to expiration date.
3. The sealed pouch must remain at an ambient temperature (18 to 27ºC; 64 to 81ºF) for a minimum of thirty (30) minutes.
4. Keep the 2 blue top tubes on the tube rocker until ready for testing.
5. Open the pouch. Remove the PIFA PLUSS™ PF4 MiniReactor and place on a flat surface. Remove the seraSTAT™ Blood Cell Separator packer; open packet and place seraSTAT™ on a flat surface. Ensure that both components are NOT COOL to the touch. If they are COOL, allow to warm to an ambient temperature.
6. Label the PIFA PLUSS™ PF4 MiniReactor with the patient’s identification. Ensure that the PIFA PLUSS™ PF4 logo on the device label is facing you.
7. Set timing device to 65 seconds; DO NOT initiate timing.

### TEST PROCEDURE

**NOTE:** The seraSTAT™ blood cell separator MUST be used to both extract a measured volume of the liquid fraction from the patient’s FRESH, Whole Blood specimen and dispense it into the PIFA PLUSS™ PF4 MiniReactor.

**STEP 1:**

Ensure that the Whole Blood specimen is thoroughly mixed.

Using a calibrated pipettor, dispense 150 µL of the specimen

 onto the seraSTAT™ sample well.

Keep seraSTAT™ stationary until YELLOW reaches the LOWER EDGE of the membrane. Most samples will separate within five (5) minutes; occasionally separation may take up to eight (8) minutes.

**STEP 2:**

Keep seraSTAT™ flat and gently slide clear, plastic sleeve

 over the sample well until the top edge of the sleeve is flush

with the top edge of the seraSTAT™.

NOTE: YELLOW color may not be uniformly distributed throughout the membrane and may appear mottled

**STEP 3:**

Pick up the seraSTAT™ at the clear plastic sleeve.

 Rotate the seraSTAT™ so the YELLOW membrane is hanging

 in a downward position and the SAMPLE WELL is facing

AWAY from you. Locate the seraSTAT™ above the TOWER,

 making sure that the YELLOW membrane can be inserted

through the middle of the SLOT. Continue inserting into the SLOT

 until the seraSTAT™ reaches a seated position.

**STEP 4:**

With your flat palm(s), apply pressure to the seraSTAT™

 and push DOWN until the seraSTAT™ and tower REACH

A full seated POSITION. You will hear a “CRACK” as the

 reagents contained with the ampoule are released into the

 Reaction Chamber.

NOTE: The small but visible gap present when the Tower/seraSTAT™ has reached a FULL SEATED position.

**STEP 5:**

Initiate 65-second timer. Vigorously, slide the device from

 side-to-side on the flat surface for 5 seconds (approximately 12 to 15 times). PIFA PLUSS must be slid in a very vigorous manner in a horizontal fashion.  It is better to error on the side of excessive sliding speed than it would be to error on the mild side.

 Stop motion when timer reaches “60”.

**STEP 6:**

1. Keep device stationary for the remaining 60 seconds.

 When timer sounds, IMMEDIATELY Pull the

 TOWER UP to the STOP position.

1. Tilt the MiniReactor 45º so the TOWER portion is elevated.

 Tap the Results Flange with finger until a BLUE/GREEN color

 appears to fill approximately 50% of the Reagent Window.

**STEP 7:**

Return device to a flat surface. When a RED color appears in the

 CONTROL Window; interpret the result in the TEST Window in well-lit conditions.

**REPORTING AND INTERPRETING RESULTS:**

**All** HIPA Screens are called to the unit and record who you called the results to in the Call List.

**NEGATIVE/Non-Reactive:**

TEST Window = BLUE-GREEN

NOTE: May contain various hues of

 BLUE-GREEN as the color is sample dependent.

**POSITIVE/ Reactive:**

TEST Window = YELLOW

1. When a positive HIT is found in the screening procedure, a Heparin Induced Platelet Aggregation test is done.

The LIS will automatically order the platelet aggregation reflex on the same order as HIPA Screen.

1. To prepare specimen to be sent out:
2. Print label.
3. Take the processing label to the send out bench with the sample.
4. They will handle the sending of this specimen to Coagulation Consultants laboratory.
5. When the result returns, LCC will bring it to Serology for resulting.
6. Call PLAGG results and make a note of who was called on result form from Coag Consultants. Scan PLAGG report into Soft Media using “BW Ref. Lab” printer/ Test PLAGG. Result “See Report” in Soft.
7. File Coagulation Consultants Report in the HIPA book in the ledge above the

Serology work bench.

 **If result is Inconclusive**:

 Report as Inconclusive with comment sent to Coagulation Consultants for confirmation.

 Send to Coagulation Consultants for Heparin Induced Platelet Aggregation test

ALL HIPA AND PLATELET AGGREGATION MUST BE CALLED

**NOTE:**

* Flow rate is sample dependent. The time interval for RED to develop in the CONTROL Window varies and ranges from 1-to-10 minutes.
* If RED fails to appear in the CONTRAOL Window, beyond the 10-minute mark, the TEST result is considered INVALID.
* Test Result is stable for thirty (30) minutes.

**PROCEDURAL NOTES:**

**Warnings and Precautions**

* For *in vitro* Diagnostic Use.
* Do not expose the PIFA PLUSS™ PF4 MiniReactors to temperatures greater than 40ºC (104ºF) or below 0ºC (32ºF).
* Allow each PIFA® MiniReactor to warm to an ambient temperature (18 to 27ºC; 64 to 81ºF), in the individual sealed pouch, for a minimum of 30 minutes prior to performing the test.
* If possible, perform test on a white background to enhance readability.
* Do not initiate testing with the PIFA PLUSS PF4 assay if the membrane extending from the accompanying seraSTAT™ is torn or creased.

**Limitations**

* The PIFA PLUSS™ PF4 should be used for the qualitative detection of any antibody directed against the PF4 complex and should be used as a screening test. There may be some antibodies reactive to the Heparin/PF4 complexes that are not reactive with this test. Test result should not be relied upon solely to identify an antibody to the PF4 complex.
* A positive test result may be indicative of a Heparin/PF4-related antibody in the test sample. However, the presence of these antibodies does not confirm the diagnosis of HIT or HITT. Therefore, results obtained from the PIFA PLUSS™ PF4 should be interpreted along with clinical findings and/or other serological tests.

**Performance Characteristics**

Akers Biosciences, Inc. has conducted a series of evaluation to determine the performance of the PIFA® Heparin/PF4 Rapid Assay for the detection of antibodies for the Heparin/PF4 complex.

Studies were performed by outside laboratories to determine the performance of the PIFA® Heparin/PF4 Rapid Assay compared to standard laboratory methods using samples originating from field sources. The standard laboratory method was a commercially available ELISA technique.

**REFERENCE:**

Akers Biosciences P1FA Plus SS PF4 Current Package Insert

Current CAP checklist