

PIFA® Heparin / Platelet Factor 4 Rapid Assay

Intended Use

The PIFA® Heparin/Platelet Factor 4 Rapid Assay is a qualitative *in vitro* diagnostic device designed for the detection of antibodies to Platelet Factor 4 (PF4) sensitized microspheres. These antibodies are found in some patients undergoing heparin therapy.

Summary and Explanation

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The risk of heparin induced thrombocytopenia (HIT) is greatly increased in patients with recent exposure to heparin. HIT is often caused by platelet-activating antibodies that recognize complexes of Heparin/PF4. As a result, antibody detection is rapidly becoming a standard of care in hematology and cardiology. Currently available laboratory tests for HIT are classified by CLIA as high complexity, take many hours to perform, and often provide confirmation of HIT or HIT and thrombosis (HITT) after the symptoms are seen in a patient. As a result, there is a need for an easily performed, rapid test that helps clinicians identify and treat patients at risk for HIT or thrombosis.

Explanation

HIT is caused by heparin-dependent antibodies formed to the heparin/platelet factor 4 complex, and 1-5% of adults exposed to heparin develop these antibodies⁽¹⁾. These 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 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 to necrosis.

Studies have determined that the antibodies associated with Type II HIT recognize sites on a platelet protein designated “platelet factor 4” (PF4) that are created when PF4 is complexed with heparin or another linear polyanionic compound^(2,3).

Currently, there are three laboratory testing methods that are most commonly used to identify HIT antibodies: C-14 Serotonin Release Assay, Platelet Aggregation Studies, and Enzyme-Linked Immunoassay. However, these tests are used primarily as a confirmation of HIT after the symptoms are seen in a patient and take many hours to perform.

The PIFA® Heparin/PF4 Rapid Assay is a manual assay that can easily be performed in minutes and is CLIA-classified as moderate complexity.

Principle of Test

The PIFA® Heparin/PF4 Rapid Assay is based upon principles of the Particle ImmunoFiltration Assay (PIFA®). Dyed microparticles coated with purified Platelet Factor-4 (PF4) protein derived from platelet-rich plasma provide the visual signal for the results of the assay. The ability of matrixed or non-matrixed particles to move through a filter medium is the measure of the reactivity/non-reactivity of the test sample.

The PIFA® Heparin/PF4 Rapid Assay consists of a MiniReactor device that contains a membrane filtration system, a TEST Result window, CONTROL window, and a push button reagent dispensing system, referred to as the Tower, that contains microparticle-based reaction reagents.

The MiniReactor contains a reaction chamber that allows the reagents to react with the sample. The reagent contained in the reagent dispenser is added to the reaction chamber followed by the sample. The reagents contain microparticles coated with purified PF4 protein as well as additional enhancing agents designed to promote rapid aggregation or matrix formation of the particles in the presence of specific antibodies in the test sample.

Once the reagents have reacted with the sample in the reaction chamber, the reaction mixture automatically collects over the membrane filtration system. This system acts to filter matrixed particles, while allowing non-matrixed particles to pass through. Thus, a matrixed, reactive sample will be trapped within the membrane. Since the dyed particles are trapped on this filter, no color and hence no particles are able to migrate into the TEST Result window. Conversely, a non-matrixed, non-reactive sample will pass through the membrane filter and into the wicking layers, and color will migrate into the TEST Results window.

Materials Provided

(1) Kit containing:

- 6 MiniReactor Devices
- 1 PIFA® Heparin/PF4 Package Insert
- 1 Pictorial / Procedure Guide

Materials Required But Not Provided

Disposable Lab Gloves
Timing Device
Positive and Negative Controls

Storage Conditions

The tests must be stored refrigerated at 2-8°C (36-46°F).

Please note:

- Do not freeze tests; if the test is frozen, results will be invalid.
- Do not use any tests beyond their expiration date.

Warnings and Precautions

- All specimens should be handled in accordance with good laboratory practices, including Universal Precautions for the handling and proper disposal of potentially bio-hazardous materials.
- Do not expose the tests to temperatures greater than 40°C (104°F) or below 0°C (32°F).
- Allow test materials to warm to ambient temperature for a minimum of 30 minutes prior to performing the test.
- Anticoagulated samples are not suitable for test with this assay and **must not be used**.
- Caution must be exercised so that the appropriate sample of fresh serum is used in the assay. Frozen/thawed, hemolyzed, icteric, or bacterial-contaminated specimens should not be used and can produce erroneous results.
- Inadequate incubation time, incomplete mixing, or improperly performed test procedures can result in erroneous results.
- See also “Limitations of the Procedure”, below.

Specimen Collection and Preparation

The PIFA® Heparin/PF4 Rapid Assay must be performed using FRESH patient specimens of SERUM within 72 hours after draw only.

Serum that cannot be tested immediately should be stored at 2-8°C for no longer than 72 hours.

Serum should be separated from clot when stored

Reminder: Do not use frozen and thawed specimens.

Important Pre-Test Preparation:

Before beginning the test procedures:

1. Remove the PIFA® Heparin/PF4 MiniReactor from refrigeration and allow to sit at an ambient temperature (18-27°C; 64-81°F) for a minimum of 30 minutes.
2. Visually inspect device to confirm date of use is prior to expiration date.
3. Label the device with the patient’s identification number and place on a level surface.
4. Remove the Tower Protector.

Test Procedure:

1. Push the Tower down completely to release the reagents into the Reaction Chamber.
2. Insert the pipettor with the 20µL specimen of the patient’s FRESH Serum into the Sample Well of the Tower. When the pipettor tip makes contact with the bottom of the Well, pull up slightly and dispense the sample.
3. Slide the device from side-to-side for 5 seconds, then keep it stationary for 1 minute.

- 4a. Pull the Tower up to the stop position.
- 4b. Tilt the Mini-Reactor 45° so that the Tower portion is elevated. Tap the Results Flange with finger until a blue color appears in the Reagent Window.
5. Lay unit on the table and wait approximately 10 minutes or until a RED color appears in the CONTROL window. Read and record result in the TEST window. The test result will be stable for one (1) hour.

NOTE: If RED fails to appear in the CONTROL window, the TEST result is invalid.

Interpretation of Results:

The following PIFA® Interpretation Guide is provided to assist in the determination of the results.

| TEST Window | CONTROL Window | RESULT |
|-------------|----------------|-----------------------|
| NO Blue | ANY Red* | Reactive/Positive |
| ANY Blue* | ANY Red* | Non-reactive/Negative |
| NO Blue | NO Red | Invalid |
| ANY Blue* | NO Red | Invalid |

* Intensity of color in TEST Window may vary

Test Disposal:

Dispose of the test in accordance with applicable, standard laboratory biohazard procedures.

Quality Control

The PIFA®Heparin/PF4 Rapid Assay provides an internal device control with each test run. The appearance of RED in the CONTROL Window indicates that the device functioned as designed. If RED does not develop in the CONTROL Window, the test result is considered invalid

Controls should be assayed routinely or as otherwise required by your laboratory's standard quality control procedures using the same procedures as the specimens. Use only confirmed Heparin PF4 antibody positive and negative samples as controls. For guidance on obtaining controls, contact Akers Technical Assistance.

Limitations of the Procedure

The PIFA®Heparin/PF4 Rapid Assay 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 PF4: heparin complex that are non-reactive with this test.

Test results should, therefore, not be relied upon solely to identify an antibody to the PF4 complex. Positive or negative test results obtained from the PIFA®Heparin/PF4 Rapid Assay should be interpreted along with clinical findings or serological tests.

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.

Performance Characteristics

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

Specificity and Sensitivity

| AKERS | ELISA | |
|----------|----------|----------|
| | Positive | Negative |
| Positive | 21 | 3 |
| Negative | 2 | 153 |

Specificity = 98.1%
Sensitivity = 91.3%
Overall Agreement = 97.2%

Reproducibility

The reproductibility of the PIFA® Heparin/PF4 Rapid Assay in detecting H/PF4 antibodies was demonstrated by testing 10 aliquots of 5 specimens for inter-day evaluation and 10 aliquots for intra-day evaluation. Reproducibility of the PIFA® Heparin/PF4 Rapid Assay was determined to be 100% in both studies.

References

- ¹ Warkentin, Theodore; "Heparin Induced Thrombocytopenia: A Ten Year Retrospective" Annual Review in Medicine. 1999: Vol. 50 pp 129-47.
- ² Visentin GP, Moghaddam M, Collins JL, McFarland JG, Aster RH: Antibodies Associated with Heparin-Induced Thrombocytopenia (HIT) Report Conformational Changes in Platelet Factor 4 (PF4) Induced by Linear, Polyanionic Compounds. Blood (Supplement) 90:460a, 1997.
- ³ Visentin GP, et al. Heparin is not Required for the Detection of Antibodies Associated with Heparin-Induced Thrombocytopenia/Thrombosis. J. Lab. Clin. Med. 138:22-31, July 2001.



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