PIFA® Heparin/PF4 Rapid Assay

Akers Biosciences, Inc.

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

Summary

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. The majority of 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 (1-2 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 to necrosis.

There are two categories of laboratory methods used to identify HIT antibodies: Functional/Activation Assays and Antigen Assays/Immunoassays. Functional Assays include the C-14 Serotonin Release Assay (SRA) and the Platelet Aggregation Test (PAT). The Antigen Assay class consists of the Enzyme-Linked Immunoassay (ELISA) and the Particle ImmunoFiltration Assay (PIFA®). SRAs, PATs and ELISAs are CLIA-classfied high complexity, take many hours to perform, and are used primarily as a confirmation of HIT after the symptoms are seen in a patient[2].

The PIFA® Heparin/PF4 Rapid Assay is the only 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 REAGENT Window, 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 patient's fresh serum sample. The reagent contained in the Tower is added to the reaction chamber followed by the sample. The reagent contains 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.

IVD

Once the reagent has 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 reactive sample will form a matrix of particles that will be trapped within the membrane. Since the dyed particles are trapped within this membrane filter, no particles and hence, no BLUE color, are able to migrate into the TEST Result Window. Conversely, a non-reactive sample will not form a matrix of particles. These non-matrixed particles will pass through the membrane filter into the wicking layers, and BLUE color will migrate into the TEST Result Window.

Materials Provided:

(1) Kit containing:
- 6 MiniReactor Devices
- 1 PIFA® Heparin/PF4 Package Insert

Materials Required But Not Provided

- Pipettor capable of delivering 30µl and disposable tips
- Timing Device
- Positive and Negative Controls (see Quality Control)

Storage Conditions

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

Devices that have been removed from refrigeration and stored at an ambient temperature for a maximum of 8 hours may be returned to refrigeration for future use.

Please note:
- Do not freeze tests.
- If the test is frozen upon receipt, results will be invalid.
- Do not use any tests beyond their expiration date.

Warnings and Precautions

- Do not expose the PIFA® MiniReactors to temperatures greater than 40°C (104°F) or below 0°C (32°F).
- Allow each PIFA® MiniReactor to warm to an ambient temperature (18-27° C; 64-81°F), in the individual foil sealed pouch, for a minimum of 30 minutes prior to performing the test.
- The PIFA® Heparin/PF4 Rapid Assay MUST be performed using FRESH Patient Serum ONLY. Anticoagulated samples are not suitable for test with this assay and must not be used.
- All Fresh Serum specimens should be collected using aseptic technique and should be handled in accordance with good laboratory practices.
- Frozen, thawed, hemolyzed, icteric, lipemic (of an excessive nature), bacterial contaminated specimens, or controls from other test kits should not be used, and can produce erroneous results.
- Inadequate incubation time, incomplete mixing, or improperly performed test procedures can produce erroneous results.
- See also "Limitations."

Quality Control

The PIFA® Heparin/PF4 Rapid Assay is a single-use/unit use test system. Akers Biosciences, Inc. recommends that the laboratory analyzes a sample representative of the lot for Quality Control. It is also recommended that a positive and negative control be utilized in the following circumstances:

1) during the initial use of a new lot of PIFA® Heparin/PF4 Rapid Assay devices,
   and
2) after running 100 tests of the same lot of devices.

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 has functioned as designed. If RED does not develop in the CONTROL Window within a maximum of 50 minutes after performing the test procedure, the test result is considered invalid.

Controls should be assayed as required by your laboratory's standard quality control procedures using the same procedure as the specimens. Use only confirmed Heparin/PF4 antibody positive and negative samples as controls. Akers Biosciences, Inc. supplies FDA-approved Heparin/PF4 Antibody Serum Panels for use with the PIFA® Heparin/PF4 Rapid Assay. For information on product availability contact 1-800-451-TEST.

Limitations

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 Heparin/PF4 complex that are non-reactive with this test. Test results 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® Heparin/PF4 Rapid Assay should be interpreted along with other serological tests and clinical findings.

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

		ELISA	
		Positive	**Negative**
PIFA®	**Positive**	**21**	**3**
	Negative	**2**	**153**

• Specificity: 98.1% • Sensitivity: 91.3% • Overall Agreement: 97.2%

Reproducibility

The reproducibility of the PIFA® Heparin/PF4 Rapid Assay in detecting Heparin/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 Daneschvar, H.Leon, MD, Daw, Hamed, MD: How to Interpret and Pursue an Abnormal Complete Blood Cell Count in Adults. Mayo Clinic Proc. 2005;80:1389-1394 2005 Mayo Foundation for Medical Education and Research.

3 CLSI (Clinical and Laboratory Standards Institute) H18-A3: Procedures for the Handling and Processing of Blood Specimens; Approved Guideline -- 3rd ed. (2004) (ISBN 1-56238-555-0).

Akers Biosciences, Inc.
201 Grove Road • Thorofare, NJ 08086 USA

Tel: +1-856-848-2116
Fax: +1-856-848-0269
E-mail: info@akersbiosciences.com

Visit us on the web at www.akersbiosciences.com

Index of Symbols

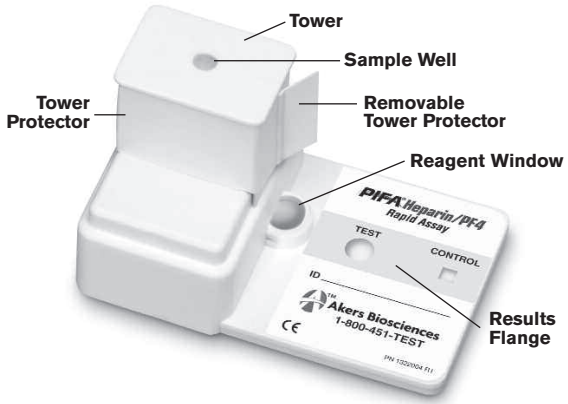
	Attention, see instructions for use		Tests per kit		Manufacturer
IVD	For in vitro diagnostic use		Use by		Do not reuse
	Store between 5-30° C	LOT	Lot Number	REF	Catalog #

EC	REP

MDSS
Schiffgraben 41
D-30175 Hannover
Germany

PN 1322003 R5 01/11

PIFA® Heparin/PF4 MiniReactor



Specimen Collection, Preparation, and Storage

Specimen Collection

FRESH Serum must be used with the PIFA® Heparin/PF4 Rapid Assay. Do not use frozen and/or thawed specimens.

In accordance with CLSI Guideline H18-A3, "Procedures for the Handling and Processing of Blood Specimens", serum should be separated from contact with cells within a maximum of 2 hours of the draw[3].

Whole Blood specimens must be collected in Red Top Tubes ONLY. Plain, Glass Red Top tubes OR Plastic, Red Top Tubes, with Clot Activator must be used to obtain patient specimens; no other collection tubes are recommended for use, including gel serum separation tubes (SST).

Specimen Preparation

1. **Clot Activation:** Patient specimens must be allowed to clot before centrifugation.
   - **Plastic, Red Top Tubes with Clot Activator:** Consult Collection Tube Manufacturer's instructions for mixing protocols and recommended clotting times (clotting times range from thirty (30) to sixty (60) minutes).
   - **Plain, Glass Red Top Tubes:** Allow specimen to clot for approximately 30 minutes.
2. **Centrifugation:** The specimen should be centrifuged at the end of the clotting period in strict accordance with the Collection Tube Manufacturer's instructions for speed and duration of centrifugation.

   IMPORTANT: Patient specimen should not stand on the clot for longer than 60 minutes after centrifugation or the PIFA® test result may be invalid.

Specimen Storage

Properly prepared serum that has been removed from the clot, but cannot be tested immediately, should be stored refrigerated (2-8°C; 36-46°F) for no longer than 72 hours.

Note: Any refrigerated serum should reach an ambient temperature prior to performing the test.

Pre-Test Preparation:

Before beginning the test procedure:

1. Remove the PIFA® Heparin/PF4 MiniReactor from refrigeration and allow to sit in the foil sealed pouch at an ambient temperature (18-27° C; 64-81° F) for a minimum of 30 minutes.

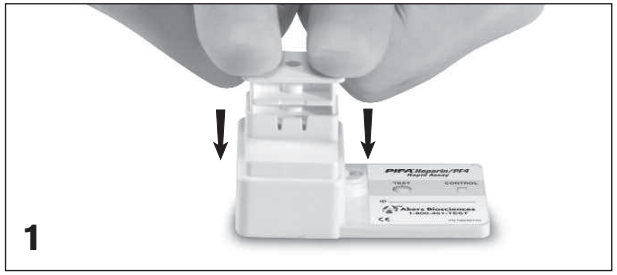
   Make sure the device is not cold to the touch.

NOTE: *A PIFA® MiniReactor that is removed from the foil sealed pouch should be used within 60 minutes.*

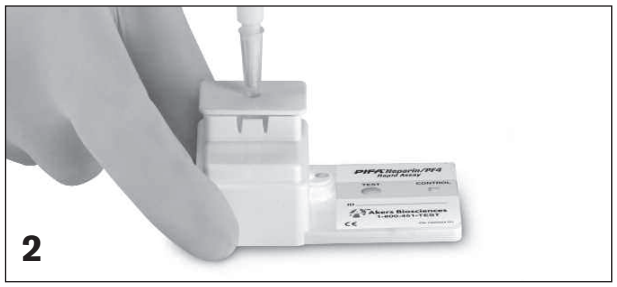
2. Visually inspect device to confirm date of use is prior to expiration date.
3. Label the device with the patient's identification number and place on a level surface.
4. Remove the Tower Protector.

Test Procedure:

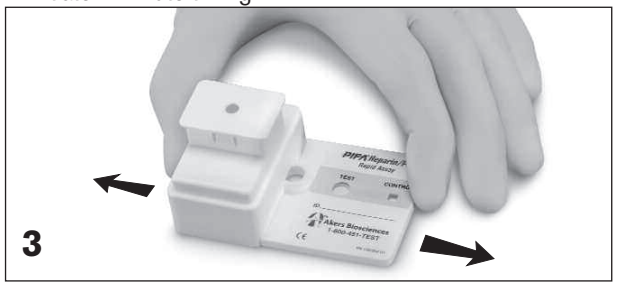
1. Push the Tower down **completely** to release the reagents into the Reaction Chamber.



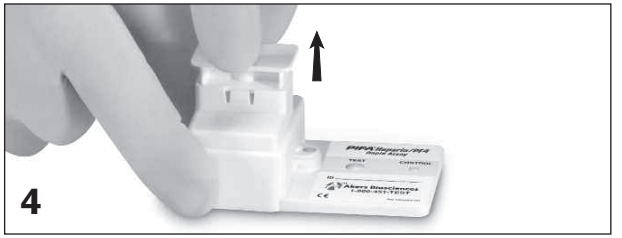
2. Using a pipettor, obtain a 30µl sample of the patient's FRESH Serum. Insert the pipettor tip into the Sample Well in the Tower. When the pipettor tip makes contact with the bottom of the Well, pull up slightly and dispense the sample.



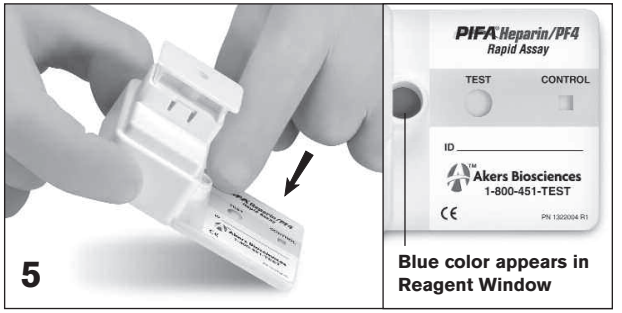
3. Slide the device vigorously from side-to-side for 5 seconds (approximately 12 to 15 times), then keep it stationary and initiate 1 minute timing.



4. At the end of the 1 minute timing, pull tower up to the stop position.



5. Tilt the MiniReactor 45° so that the Tower portion is elevated. Tap the Results Flange with finger until a BLUE color appears in the Reagent Window.



6. Lay unit on a level surface. When a RED color appears in the CONTROL Window, interpret the result in the Test Window in well-lit conditions. The test result is stable for one (1) hour.

NOTE: *Since flow rate is sample dependent, the time interval for a RED color to develop in the CONTROL Window varies, ranging from 1 minute to 10 minutes.*

If RED fails to appear in the CONTROL Window, beyond the 10 minute mark, the TEST result is considered 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	Positive/Reactive
ANY Blue**	ANY Red	Negative/Non-reactive
NO Blue*	NO Red	Invalid
ANY Blue**	NO Red	Invalid

NOTE:

***Reactive/Positive Result**: The TEST Window may appear to be a PALE GREY or "WET WHITE" color as the wicking material will have been moistened by the Reagent/Serum mixture.

****Non-Reactive/Negative Result**: The intensity of the BLUE color in the TEST Window may range from a PALE BLUE to a VIBRANT BLUE as the color is sample dependent.

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