HEALTH-CHEM DIAGNOSTICS LLC

3341 SW 15th St, Pompano Beach, FL 33069 – (954) 979-3845 – Fax: (954) 979-7997 – www.healthchemdiagnostics.com

HealthTest™ PIFA Heparin / PF4 Rapid Assay

Clinical Laboratory Background

Heparin-Induced Thrombocytopenia (HIT) Type II

Heparin is one of the most widely prescribed medications in the United States, with an estimated 12 million patients receiving the drug annually.1 Although the most common adverse event associated with heparin is bleeding, a significant number of patients develop a prothrombotic state known as Heparin-Induced Thrombocytopenia, the clinical course of which can be as mild as an asymptomatic decrease in platelet count (Type I) or as severe and potentially devastating as a thromboembolic complication (Type II).

Type I or Heparin-Associated Thrombocytopenia (HAT), is mild and transient, and is usually not associated with thrombosis. Normally, there is no need to stop heparin therapy, since platelet counts will usually return to normal even if therapy is continued[2]. Type II, commonly referred to as HIT, is an immune-mediated condition that occurs in approximately 1% to 5% of patients receiving heparin therapy. Typically, HIT occurs between days 5 and 10 after initiation of Heparin therapy, but patients may present with clinical symptoms within minutes or hours of a Heparin re-challenge if the prior exposure was within the previous 100 days[3]. There have also been reports of delayed-onset HIT that begin several days to weeks after heparin has been discontinued[4,5].

The major determinant in the pathogenesis of HIT appears to be antibodies to the heparin/platelet factor-4 complex [6,7,8]. These antibodies are most frequently induced by Unfractionated Heparin (UFH) use following Cardiopulmonary Bypass Surgery (50%) and major Orthopedic Surgery (15%)[9]. Although HIT is more common in patients receiving large doses of heparin therapy intravenously, HIT can occur following incidental exposure to heparin through Heparin flushes, subcutaneous administration, or Heparin-coated catheters and prostheses, such as those used in chronic dialysis patients[10].

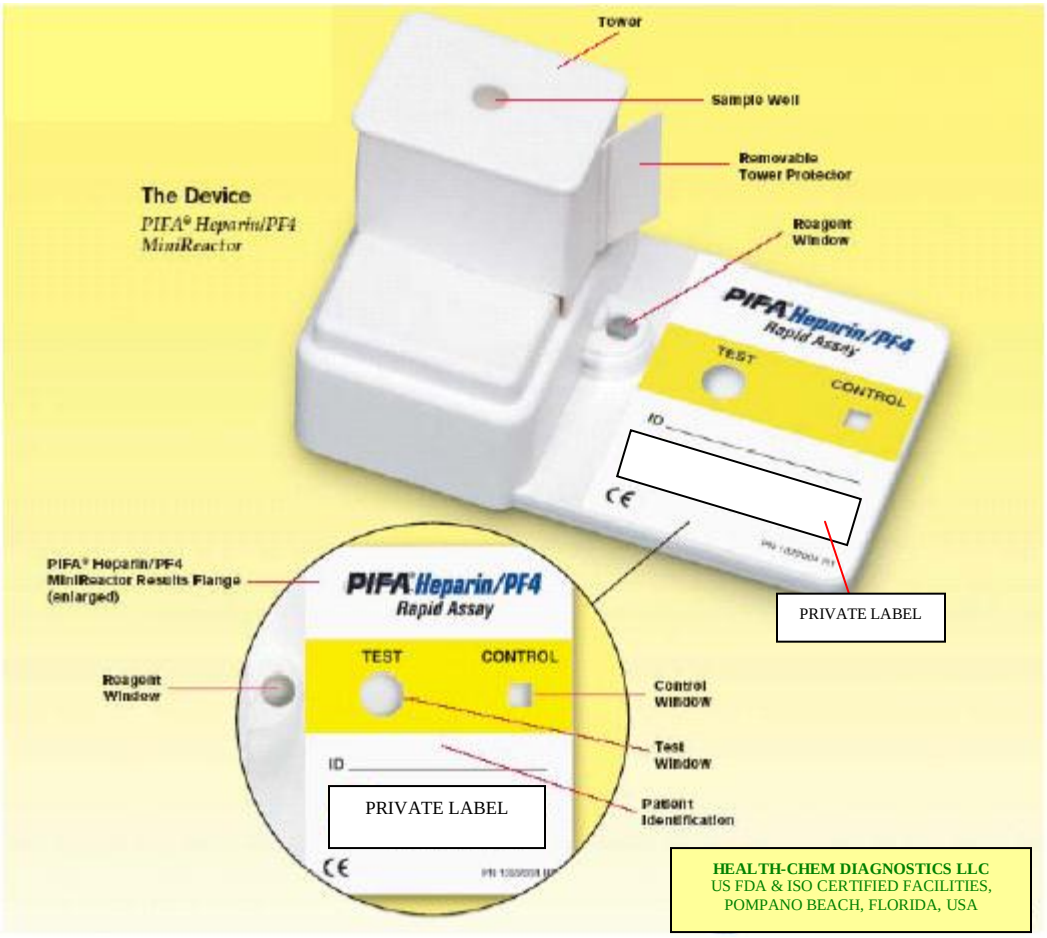
Currently, there are three routine testing methods for identifying patients with HIT antibodies: C-14 Serotonin Release Assay, Platelet Aggregation Studies, and Enzyme-Linked Immunoassay. These tests, however, take many hours to perform and are used primarily to help confirm a diagnosis of HIT after a patient presents with symptoms. Additionally, these methods are CLIA classified as high complexity, require special instrumentation, and are not conducive to cost effectively or efficiently processing single patient samples. As a result, there is a need for an easily performed, rapid test to provide clinicians with HIT antibody determinations that can be integrated into time-sensitive, therapeutic decisions.

References

1. Mahaffey KW. Anticoagulation for acute coronary syndromes and percutaneous coronary intervention
in patients with heparin-induced thrombocytopenia. Curr Cardiol Rep. 2001;3:362-370.
2. Fahey VA. Heparin-induced thrombocytopenia. J Vasc Nurs. 1995;13:112-116.
3. Lubenow N, Kempf R, Eichner A, Eichler P, Carllson LE, Greinacher A. Heparin-induced thrombocytopenia: temporal pattern of Thrombocytopenia in relation to initial use and reexposure to heparin. Chest 2002; 122:37-42.
4. Warkentin TE, Kelton JG. Delayed-onset heparin-induced thrombocytopenia and thrombosis. *Ann Intern Med*. 2001;135:502-50
5. Rice L, Attisha WK, Drexler A, Francis JL. Delayed-onset heparin-induced thrombocytopenia. *Ann Intern Med*. 2002;136:210-215.
6. Amiral, J., F. Bridey, M. Dreyfus, A. M. Vissoc, E. Fressinaud, M. Wolf, and D. Meyer. 1992. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. Thromb. Haemost. 68:95-96.
7. Amiral, J., F. Bridey, M. Wolf, C. Boyer-Neumann, E. Fressinaud, A. M. Vissac, E. Peynaud-Debayle, M. Dreyfus, and D. Meyer. 1995. Antibodies to macromolecular platelet factor 4-heparin complexes in heparin-induced thrombocytopenia: a study of 44 cases. Thromb. Haemost. 73:21-28.
8. Greinacher, A., and T. E. Warkentin. 2001. Treatment of heparin-induced thrombocytopenia: an overview, p. 291-322. *In* T. E. Warkentin and A. Greinacher (ed.), Heparin-induced thrombocytopenia., 2nd ed. Marcel Dekker, New York, N.Y.
9. Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia, 3rd ed. New York: Marcel Dekker, Inc. 2004, 53, 205, 271, 295.
10. Nand S, Wong W, Yuen B et al. Heparin-induced thrombocytopenia with thrombosis: incidence, analysis of risk factors, and clinical outcomes in 108 consecutive patients treated at a single institution. *Am J Hematol*. 1997; 56:12-6.

Test Overview

The HealthTest™ PIFA Heparin-PF4 Rapid Assay is a MiniReactor device that consists of a push button reagent dispensing system, referred to as the Tower, and a Results Flange. The tower houses the sample well, micro particle-based reagents, and a reaction chamber, and the Results Flange contains the PIFA membrane filtration system and TEST, CONTROL, and reagent windows.



The HealthTest™ PIFA Heparin/PF4 Rapid Assay is a rapid manual assay that is CLIA-classified as Moderate Complexity and can easily be performed in minutes. The test is based on principles of the **P**article **I**mmuno**F**iltration **A**ssay or **PIFA** Technology.

Fresh Serum is introduced into the Sample Well and reacts with the ***PIFA Reagent*** which consists of:

- ✓ Dyed micro particles coated with purified PF4 protein
- ✓ Enhancing agents promote the rapid matrix formation of micro particles in the presence of H/PF4 antibodies

The Blue reaction mixture automatically collects over the membrane filtration system to provide the visual TEST Result.

Positive/Reactive sample:

- ✓ Matrixed micro particles are trapped within the membrane filter, and **NO BLUE** color migrates into the TEST Result window

Negative/Non-reactive sample:

- ✓ Non-matrixed micro particles pass through the membrane filter, and **BLUE** color migrates into the TEST Result window

Performance Characteristics

Health-Chem Diagnostics LLC has conducted a series of evaluations to determine the performance of the HealthTest™ PIFA Heparin/PF4 Rapid Assay for the detection of antibodies to the Heparin/PF-4 complex. Studies were performed by outside laboratories to determine the performance of the HealthTest™ 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.

PIFA® vs. GTI ELISA[12]

Study - Serum

		GTI ELISA	
		POSITIVE	NEGATIVE
PIFA®	POSITIVE	21	3
	NEGATIVE	2	153

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

Time and Effort Comparison

	Time	Procedure
PIFA®	Approximately 10 minutes	5 Steps
GTI ELISA	At least 3 hours	26 Steps

Reproducibility
The reproducibility 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.

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 attached.

Specimen Collection and Preparation

- ✓ The HealthTest™ 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.

Assay Procedure

(Please see Pictorial Guide attached)

Test Disposal

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

Limitations of the Procedure

The HealthTest™ 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.

HealthTest™ PIFA Heparin / PF4 Rapid Assay

Frequently Asked Questions

Sample Collection, Preparation & Storage

Q. What type of tube should I collect the blood sample in?

A. The HealthTest™ PIFA Heparin/PF4 Rapid Assay is designed for use with **Serum only**. As a result**, only Red Top serum collection tubes** should be used to obtain patient specimens. Plain, glass Red Top tubes OR plastic, Red Top Tubes, with or without Clot Activator, may be utilized with the HealthTest™ PIFA test; SST Tubes are not recommended.

Caution must be exercised so that the appropriate 20µL sample volume of fresh serum is used in the assay.

NOTE: Plasma should not be used with the HealthTest™ PIFA assay. Anticoagulated samples are not suitable for testing with this assay and must not be used. Hemolyzed, icteric, lipemic (of an excessive nature), bacterial contaminated specimens, controls from other test kits, or samples from patients with multiple myeloma, should not be used, and can produce erroneous results. It is not recommended that frozen patient samples be used with the test; Extreme care must be taken when preparing, freezing and thawing a serum specimen in order to mitigate the formation of particulates and prevent protein break down and other factors that will influence the validity of the test result.

Q. Should a freshly drawn blood sample, collected in a Red Top serum collection tube, sit before spinning down?

A. Yes. When using plastic, Red Top tubes with clot activator, allow the sample to sit approximately 15 minutes to facilitate clotting. If plain, Red Top glass tubes are used, the sample should sit for approximately 30 minutes. After the specimens have clotted, spin down for 10 minutes at 1500 g.

Q. How should I store patient serum that cannot be tested immediately?

A. Serum that cannot be tested immediately should be stored at 28°C (36-46°F) for no longer than 72 hours, and must not be stored on the clot. Serum stored at 28 C (36-46°F) for less than 72 hours should always be checked visually for bacterial growth and/or a cloudy appearance. Samples with bacterial growth will produce erroneous results as they may clog the pores in the membrane filter system in the test device, and could cause a negative sample to produce a positive test result.

Do not freeze serum samples; Do not use serum that has been frozen. Freezing samples may cause at least two problems.

1. *If not done meticulously, freezing and thawing will decrease antibody activity, and could cause a positive sample to produce a negative test result.*

2. Freezing/thawing may also cause certain proteins to precipitate out of solution, and cause other micro particulates or debris to form. These particulates can clog the pores in the membrane filter system in the test device, and could cause a negative sample to produce a positive test result.

Q. If too much or not enough specimen sample was added to the device, is the result still reliable?

A. No, an incorrect amount of specimen sample can affect the test result. Caution must be exercised so that the appropriate 20µL sample volume of fresh serum is used in the assay.

Q. Can plasma be used with the HealthTest™ PIFA test?

A. No, the HealthTest PIFA Heparin/PF4 Rapid Assay is designed for use with Serum only. Plasma is obtained when blood is collected in a tube containing various anticoagulants. The chemical makeup of anticoagulants will interfere with the reaction between the reagent and the sample. Additionally, plasma contains fibrinogen which may initiate micro particle aggregation nonspecifically.

Quality Control

Q. How do Health-Chem Diagnostics recommend our laboratory initially validate the test?

A. It is the responsibility of the Laboratory to define internal validation protocols, as applicable, in accordance with good clinical and scientific laboratory practice. In lieu of established lab procedure, Akers would recommend that 2030 test evaluations be performed using well characterized, fresh serum samples.

Well characterized frozen samples, for the purposes of validation ONLY, can be used with the test as long as the sample preparation and freezing processes mitigate the formation of particulates and protein break down. Samples should be flash frozen and stored at –70°C (94 °F), and should only be subjected to one freeze thaw cycle. A quick thaw procedure should be used (thaw in a water bath with temperatures between 30-37°C (86-98°F)).

Health-Chem offers a serum panel, designed for Research Use Only, to help facilitate the validation process.

Q. How often do I have to run a confirmatory test since the HealthTest™ PIFA Heparin /PF4 Rapid Assay is a screening test?

A. It is the responsibility of the Laboratory to define protocols, as applicable, in accordance with good clinical and scientific laboratory practice. The laboratory may choose to run confirmatory tests

when a positive PIFA test result is obtained, or when a PIFA test result is inconsistent with other clinical findings.

The HealthTest™ PIFA Heparin/PF4 Rapid Assay should be used for the qualitative detection of antibodies directed against the Heparin/PF4 complex, and should be used as a screening test. Test results should, therefore, not be relied upon solely to identify antibodies to the Heparin/PF4 complex. Positive or negative test results obtained from the HealthTest™ PIFA Heparin/PF4 Rapid Assay should be interpreted along with clinical findings or other serological tests.

Q. How does one know that the test has been performed properly?

A. The Laboratory Technician must follow all 5 Steps in the Test Procedure noted within the Product Package Insert and/or Pictorial Guide provided with each sleeve of tests.

The HealthTest™ PIFA Heparin/PF4 Rapid Assay also includes an Internal/Procedural Control. When RED appears in the CONTROL Window, the device has functioned properly.

Q. Is the 1 minute incubation time, noted in Step 3 of the HealthTest™ PIFA Test Procedure, critical to the proper functioning of the assay?

A. Care should be taken to complete all 5 Steps of the Test Procedure according to the Package Insert and/or Pictorial Guide. However, since the HealthTest™ PIFA method is based on a rate reaction which occurs between serum and reagent mixture in Step 3, the Laboratory Technician must use a timing device to help ensure that exactly one minute has elapsed before pulling up the Tower in Step 4a.

Q. Should the HealthTest™ PIFA Heparin/PF4 Rapid Assay be included in Proficiency Testing?

A. It is the responsibility of the Laboratory to define Proficiency Testing procedures, as applicable, in accordance with good clinical and scientific laboratory practice. At present there is limited to no availability of proficiency panels for heparin/PF4 antibody assays.

Readability

Q. Why does the intensity of the BLUE color vary in the TEST Result Window?

A. Coloration variation is sample dependent. The rate reaction and aggregation of micro particles differ among samples causing more or less blue to appear in the TEST Result window.

Q. A negative result is read when ANY BLUE color appears in the TEST result window and RED appears in the Device CONTROL window. Does it matter that the Blue color is very faint?

A. No. The intensity of the BLUE color in the test window will vary. Any trace of BLUE in the TEST window (ranging from a darker, bright Blue to a very faint tint of Blue) along with a RED in the CONTROL window is considered a Negative result.

Q. What is the maximum time interval that I should wait for RED to appear in the Device CONTROL window?

A. Since flow rate is sample dependent, the time interval for RED to develop in the CONTROL window ranges from approximately 4 minutes to a maximum of 50 minutes; The latter time estimate often applies to strong positive specimens. If RED fails to appear in the CONTROL window beyond the 50minute mark, the test should be considered Invalid.

Q. How long is the Test result stable for once RED appears in the Device CONTROL Window?

A. One (1) hour.

Device Storage

Q. How should the test kits be stored?

A. Store the tests as packaged in their sealed pouch at 2- 8°C (36-46°F) until use. Do not freeze the tests. When a test device is removed from refrigeration, keep device within sealed pouch and allow it to equilibrate to Room Temperature 15 to 30°C - (59-86°F)), for at least 30 minutes, prior to use.

Q. If the pouch has been opened, should the test be used right away?

A. Because humidity in the air can affect the integrity of the test, it is recommended that the test be used immediately once the pouch has been opened. Likewise, if a pouch is found to be punctured, do not use the test inside.

Q. Once a HealthTest™ PIFA Heparin/PF4 Rapid Assay device is removed from refrigeration, can it be returned to refrigeration if it is not used?

A. If the device remains in the sealed pouch while equilibrating to room temperature, it can be returned to refrigeration within a maximum of 4hours.

Q. Can the device be used beyond the expiration date on the bottom of the device?

A. No. The device should not be used past its expiration date under any circumstances.

Q. What is the shelf life?

A. When stored as indicated in the package insert, the shelf life is 12 months from the date of manufacture. For your convenience, this expiration date is labeled on the bottom of each individual device, as well as on the outer packaging for the sleeve of 6 devices.