

HEALTH-CHEM DIAGNOSTICS LLC

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HealthTest Heparin/PF 4 Antibody Assay

For Laboratory Use Only

Intended Use

The HealthTest Heparin/PF 4 Antibody Assay is a qualitative *in vitro* diagnostic device designed for the detection of antibodies to Platelet Factor 4 complexed to polyanionic compounds such as polystyrene. 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. Currently available laboratory tests for HIT are classified 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 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.

Studies have determined that the antibodies associated with Type II HIT recognize sites on a platelet protein designated "Platelet Factor 4" (PF-4) that are created when PF-4 is complexed with heparin or another linear polyanionic compound.

Currently, there are three methods of identifying those with HIT: 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 HealthTest Heparin/PF 4 Antibody Assay is a rapid manual assay and can be easily performed in minutes in a moderate complexity laboratory.

Principle of Test:

The HealthTest Heparin/PF 4 Antibody Assay is based upon principles of the Particle ImmunoFiltration Assay. Dyed microparticles coated with purified Platelet Factor-4 (PF-4) 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 HealthTest Heparin/PF 4 Antibody Assay consists of different components: a Mini-reactor device containing 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 PF-4 protein as well as additional enhancing agents designed to promote rapid aggregation 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 Result window.

Materials Provided

- Kit containing:

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

Materials Required and available as a separate item

- Disposable Lab Gloves
- Timing Device
- Positive and Negative Controls
- Sample Transfer Pipets

Storage Conditions

- The tests should be stored refrigerated at 2-8°C (36-46°F).
- Do not freeze tests; if the tests are 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.

Specimen Collection and Preparation:

The HealthTest Heparin/PF 4 Antibody Assay should be performed using FRESH patient specimens of SERUM within 72 hours after draw only. DO NOT USE frozen or thawed specimens.

Important Pre-Test Preparation:

Before beginning the test procedures:

1. Remove the HealthTest Heparin/PF 4 Antibody Assay from refrigeration and allow to sit at an ambient temperature 18-27°C (64-81°F) for a minimum of 30 minutes. Note: DO NOT OPEN THE PACKAGE UNTIL THE 30 MINUTES HAS PASSED.
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. Pipette 20µl of the patients fresh Serum into the Sample Well on the top of the Tower.
3. Slide the device from side-to-side for 5 seconds, then keep it stationary for 1 minute.
4. Pull the Tower up to the stop position. Tilt the MiniReactor 45° so that the Tower portion is elevated and tap the device until a blue color appears in the Reagent Window.
5. Lay unit on the table and wait approximately 5 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 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

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

Quality Control:

Controls should be assayed routinely using the same procedure as the specimens. Use only confirmed Heparin PF-4 antibody positive and negative samples as controls.

Limitations of the Procedure:

Erroneous results can occur from bacterial contamination of specimens, frozen or thawed specimens, inadequate incubation periods, or omission of test reagents or steps. DO NOT USE frozen or thawed patient samples. DO NOT USE hemolyzed or icteric samples. The procedure outlined in the Assay Procedure section must be followed closely by the user.

Performance Characteristics:

Health-Chem Diagnostics has conducted a series of evaluations to determine the performance of the HealthTest Heparin/PF 4 Antibody 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 Heparin/PF4 Antibody 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

		<u>ELISA</u>	
		Positive	Negative
Health-Chem	Positive	21	3
	Negative	2	153

Specificity = 98.1% Sensitivity = 91.3%

Overall Agreement = 97.2%

Reproducibility:

A study was performed using 5 replicates each of positive and negative patient controls samples. These samples were tested with the HealthTest Heparin/PF4 Antibody Assay daily for 4 consecutive days. The results show the test is 100% reproducible.

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.

Manufactured in the USA by:

HEALTH-CHEM DIAGNOSTICS LLC

US FDA - ISO & CE Certified Facilities



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