

Overview

Useful For

Detection of IgG antibodies directed against heparin/platelet factor 4 complexes that are implicated in the pathogenesis of immune-mediated type II heparin-induced thrombocytopenia (HIT-II).

Clinical picture of HIT type II:

-In patients not previously exposed to heparin

-Decrease in platelet count (thrombocytopenia) of 50% or more from baseline or postoperative peak

-Onset of thrombocytopenia beginning approximately 5 to 10 days after initiation of heparin this may or may not be associated with new or progressive thrombosis in patients treated with heparin

Patients previously exposed to heparin (especially within the preceding 100 days), in addition to the above findings, the onset of thrombocytopenia could occur within 24 to 48 hours after reexposure to heparin

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Specimen Required

Collection Container/Tube:Red top

Submission Container/Tube:Plastic vial

Specimen Volume:1 mL

Forms

[If not ordering electronically, complete, print, and send a Coagulation Test Request \(T753\)](#) with the specimen.

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum Red	Frozen (preferred)	14 days	
	Refrigerated	48 hours	

Clinical and Interpretive

Clinical Information

Thrombocytopenia in patients treated with heparin is relatively common and has diverse, sometimes multifactorial, causes. Among the possible causes of thrombocytopenia in such patients, immune-mediated heparin-dependent thrombocytopenia (HIT) is clinically important because of its frequency and its associated risk of paradoxical new or progressive thrombosis.

HIT, also called heparin-associated thrombocytopenia (HAT), consists of 2 distinct clinicopathologic syndromes. The first, sometimes designated type I HIT (HIT-I) or nonimmune HAT, is a common benign condition that is not immunologically mediated. Type I HIT is characterized by a mild decrease of the platelet count (typically < or =30% decrease from baseline) occurring early (days) in the course of treatment with heparin, especially intravenous unfractionated heparin (UFH), and which does not progress and may resolve despite continuation of heparin therapy.

The second, more serious immune-mediated syndrome, sometimes designated type II heparin-induced thrombocytopenia (HIT-II), occurs in up to 1% to 5% of patients treated with UFH. It is typically characterized by onset of thrombocytopenia between days 5 and 10 of UFH therapy, but thrombocytopenia can arise earlier or later in association with continued heparin exposure. In patients recently exposed to heparin (eg, within the preceding 3-6 months), onset of thrombocytopenia can be rapid (within 24 hours) after heparin reexposure, probably reflecting persistence of heparin-dependent antiplatelet antibodies or anamnestic recall of them. Typically, during the course of HIT-II, the platelet count decreases by at least 40% to 50% from baseline or the postoperative peak (in surgical patients), even though the absolute count may remain normal, and thrombocytopenia resolves within 7 to 14 days of cessation of heparin therapy (unless there is another coexisting cause of thrombocytopenia). The risk of immune-mediated HIT-II is significantly greater with UFH exposure than with exposure to low-molecular-weight heparin (LMWH), although the latter can react with heparin-dependent antibodies induced by UFH. The risk is probably also associated with the dosage and route of heparin administration (eg, intravenous), as well as associated medical and surgical conditions.

HIT-II is clinically important, not because of the mild or moderate thrombocytopenia and minimal bleeding risk, but because of the high risk for development of paradoxical thrombosis (arterial or venous) that may be new or progressive. This evolution, termed heparin-induced thrombocytopenia with thrombosis (HITT) syndrome, can occur in up to 30% to 50% of patients with HIT-II, even following discontinuation of heparin therapy. Clinically, it is often difficult to distinguish between HIT-I and HIT-II and among other etiologies of thrombocytopenia occurring in patients receiving heparin. However, the development of new or progressive thrombosis is one defining clinical feature of HIT-II.

Recent studies provide evidence that HIT-II is caused, in at least 90% of cases, by antibodies to antigen complexes of heparinoid (heparin or similar glycosaminoglycans) and platelet factor 4 (PF4). PF4 is a platelet-specific heparin-binding (neutralizing) protein that is abundant in platelet alpha granules from which it is secreted following platelet stimulation. A reservoir of PF4 normally accumulates upon vascular endothelium. Following heparin administration, immunogenic complexes of PF4 and heparin can provide an antigenic stimulus for antibody development in some patients. Antibodies bound to platelets that display complexes of PF4/heparin antigen can activate platelets via interaction of the Fc immunoglobulin tail of the IgG antibody with platelet Fc gamma IIA receptors, leading to perpetuation of the pathologic process that can cause platelet-rich thrombi in the microcirculation in some cases (eg,

HITT syndrome).

Functional assays for HIT-II antibody detection rely on antibody-mediated heparin-dependent platelet activation, as detected by platelet aggregation, or platelet secretion of serotonin or adenosine triphosphate (ATP) or other substances, using patient serum or plasma supplemented with heparin and normal test platelets from carefully selected donors. The sensitivity of functional assays for HIT-II ranges from 50% to 60% for heparin-dependent platelet aggregation (HDPa) assays, to 70% to 80% for serotonin release assays. The specificity of positive functional tests for HIT diagnosis is believed to be high (> or =90%). However, because of their complexity, functional tests for detecting HIT antibodies are not widely available.

Enzyme-linked immunosorbent assays (ELISAs) have recently been developed to detect HIT-II antibodies and are based on the detection of human antibodies that react with solid phase antigen complexes of heparinoid and human PF4 (H/PF4 complexes). The ELISA for H/PF4 antibodies is very sensitive for antibody detection, but relatively nonspecific for clinical HIT diagnosis.

Routine screening of all patients prior to, during, or following heparin use is currently not recommended. A positive H/PF4 ELISA result has relatively low and uncertain predictive value for the development of clinical HIT-II.

Reference Values

HIT ELISA:

<0.400

HIT Interpretation:

Negative

Interpretation

Results are reported as: 1) Heparin-induced thrombocytopenia (HIT) enzyme-linked immunosorbent assay (ELISA) OD; 2) Heparin inhibition (%); 3) Interpretation. Typical patterns of results and interpretations are depicted in the following table. Interpretive comments will also accompany test reports, when indicated.

	HIT ELISA OD	Heparin Inhibition	Interpretation
Normal Range	<0.400	Not done	Negative
Positive	> or =0.400	> or =50%	Positive
Equivocal	> or =0.400	<50%	Equivocal

A negative result of testing for human platelet factor 4 (H/PF4) antibodies has about a 90% negative predictive value for exclusion of clinical type II heparin-induced thrombocytopenia (HIT-II).

Because up to 10% of patients with clinical heparin-induced thrombocytopenia (HIT) may have a negative H/PF4 antibody ELISA result, a negative H/PF4 antibody ELISA result does not exclude the diagnosis of HIT when clinical suspicion remains high. A functional assay for HIT antibodies (eg, heparin-dependent platelet aggregation or serotonin release assay may be helpful in these circumstances). Call 800-533-1710 for ordering information.

A positive result is indicative of the presence of H/PF4 complex antibodies. However, this test's specificity is as low as 20% to 50% for clinical diagnosis of HIT, depending on the patient population studied. For example, up to 50% of

surgical patients and up to 20% of medical patients treated with heparin may develop H/PF4 antibodies as measured by ELISA, and only a small proportion (1%-5%) develop clinical HIT. Accordingly, this test does not confirm the diagnosis of HIT-II. The diagnosis must be made in conjunction with clinical findings, including evaluation for other potential causes of thrombocytopenia.

The presence of H/PF4 antibodies likely increases the risk of clinical HIT, with risk probably partly dependent on associated medical and surgical conditions, but currently there are few data about relative risk of HIT in various populations with positive tests for H/PF4 antibodies.

Cautions

Heparin-induced thrombocytopenia (HIT) is a clinical diagnosis that is complemented by laboratory testing for heparin-dependent platelet aggregation (HDPA) antibodies and/or antibodies to human platelet factor 4 (H/PF4) complexes. Assay results provide information on the presence or absence of H/PF4 antibodies, which are implicated in the pathogenesis of type II heparin-induced thrombocytopenia (HIT-II) with or without thrombosis. However, results of the H/PF4 antibody assay must be interpreted in conjunction with clinical findings and other pertinent tests to evaluate other causes of thrombocytopenia (eg, sepsis, intravascular coagulation and fibrinolysis, thrombotic thrombocytopenic purpura, post-transfusion purpura, malignancy, drug-induced thrombocytopenia, autoimmune thrombocytopenia) or to confirm the findings of this assay.

Some low-titer, low-avidity antibodies and some antibodies that recognize sites on H/PF4 complex may not be detected using this assay.

Some patients may have naturally occurring antibodies to PF4 (no evidence of heparin dependence) of no known significance with respect to pathogenesis of HIT-II.

Supportive Data

The IgG human platelet factor 4 (H/PF4) enzyme-linked immunosorbent assay (ELISA) was compared with both the IgGAM H/PF4 ELISA (GTI Immucor) and the heparin-dependent platelet serotonin release assay (SRA) (Quest Diagnostics and Wisconsin Blood Center) in 208 patients.

Assuming the SRA as the gold standard, the data were analyzed to determine the sensitivity of the ELISA assays for a positive SRA. Of the 208 patients tested, 49 had a positive SRA. With the IgGAM H/PF4 ELISA, 47/49 were positive (sensitivity 96%); with the IgG H/PF4 ELISA, 45 were positive (sensitivity 92%).

Of those that tested negative with the SRA (n=159), 67 (42%) tested positive with the IgGAM H/PF4 ELISA, 37 (23%) tested positive with the IgG H/PF4 ELISA. (Mayo validation data)

In order to determine possible cross-reactivity between the target antigen and antibodies other than heparin-associated antibodies, 68 samples containing a variety of antibodies that included known antibodies to platelet alloantigens, platelet autoantibodies, antibodies to HLA class I and antirheumatoid factor were tested in this assay and none were found to cross react with the target antigen immobilized in the microwells.

Clinical Reference

1. Heparin Induced Thrombocytopenia. Edited by TE Warkentin, A Greinacher. New York, Marcel Dekker, 2000, p 400
2. Trossaert M, Gaillard A, Commin PL, et al: High incidence of anti-heparin/platelet factor 4 antibodies after cardiopulmonary bypass surgery. Br J Haematol 1998 June;101(4):653-655

Performance

Method Description

The human platelet factor 4 (H/PF4) IgG antibody immunoassay is an enzyme-linked immunosorbent assays (ELISA) using microwells precoated with an antigen complex of PF4 and polyanionic heparinoid substitute (polyvinyl sulfonate; PVS). Testing is performed on the Biomek FXP liquid handling system using the Immucor GTI Diagnostics, Inc. PF4 IgG assay test kit. Patient serum is incubated in the wells, and binding of antibodies to this complex is detected by binding of a second phosphatase-conjugated antihuman IgG antibody. Color is generated when this bound conjugate cleaves a chromogenic phosphate substrate, and is quantitated by light absorption at 405 nm.

Addition of excess heparin (100 U/mL) to patient serum prior to testing inhibits the reaction between heparin-dependent antibodies and the PF4:PVS complex, and produces a negative result. This procedure is used to confirm that a positive screening result is caused by heparin-dependent antibodies. Results are calculated as the percent heparin inhibition of the reactivity of the antibody. (Collins JL, Aster RH, Moghaddam M, et al: Diagnostic testing for heparin-induced thrombocytopenia [HIT]: An enhanced platelet factor 4 complex enzyme linked immunosorbent assay [PF4 ELISA]. Blood 1997 [Suppl 1] 90:461a; package insert: PF4 IgG assay. Immucor GTI Diagnostics, Inc. Waukesha, WI, Rev D 5/2015)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Sunday, Varies

Analytic Time

1 day

Maximum Laboratory Time

3 days

Specimen Retention Time

7 days

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

Test Classification

This test has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

86022

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HITIG	Heparin-PF4 IgG Ab (HIT), S	In Process

Result ID	Test Result Name	Result LOINC Value
46915	HIT ELISA	73818-7
21468	Heparin Inhibition	73817-9
21469	HIT Interpretation	73819-5
21470	HIT Comment	73816-1