MAYO CLINIC LABORATORIES

Test Definition: HITIG

Heparin-PF4 IgG Antibody, Serum

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, spontaneous heparin platelet-factor 4 IgG antibody, and thrombocytopenia and thrombosis occurring after SARS-CoV2 adenovirus vector vaccine

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Specimen Required

Patient Preparation:

Fasting: 8 hours, preferred but not required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)

Collection Container/Tube: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 1 mL

Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Stability Information: Frozen (preferred) 2 years/Refrigerate 7 days

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)		
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

There are established and emerging disorders that are collectively termed thrombocytopenia and thrombosis syndromes; the most commonly recognized is heparin-induced thrombocytopenia (HIT). Newer associations have also been recognized including adenovirus vector-based SARS-CoV-2 vaccine-induced thrombocytopenia and HIT-like syndromes that occur in the absence of exposure to heparin (currently termed spontaneous or autoimmune HIT). In this situation, the heparin platelet-factor 4 (PF4) IgG antibody typically develops after surgery or infection.

HIT is a serious immune-mediated syndrome (ie, type II HIT or immune HIT) that occurs in 1% to 5% of patients treated with unfractionated heparin and at a lower frequency in patients treated with low-molecular weight heparin.

The 4Ts score is a validated scoring system to estimate the pretest clinical probability of HIT. Scores are assigned to the degree and timing of onset of thrombocytopenia, and the presence or absence of thrombosis (arterial or venous) in the absence of other potential explanations for the thrombocytopenia. In HIT, typical onset of thrombocytopenia is between days 5 and 10 of heparin therapy, but thrombocytopenia can arise earlier (<5 days after heparin exposure, ie, rapid onset of HIT) or later (>4 weeks after heparin exposure, ie, delayed onset of HIT). The platelet count typically decreases by 40% to 50% from baseline or the postoperative peak (in surgical patients), even though the absolute count may remain normal and the thrombocytopenia resolves within 7 to 14 days of cessation of heparin therapy (unless there is another coexisting cause of thrombocytopenia). Development or progression of (venous or arterial) thrombosis is termed heparin-induced thrombocytopenia with thrombosis syndrome and can occur in 30% to 50% of patients, rarely even following discontinuation of heparin therapy.

Other Syndromes of Thrombocytopenia and Thrombosis:
There are an increasing number of reports of patients who develop thrombocytopenia and thrombosis after surgery, particularly after orthopedic surgery and after selected infections. The clinical course and laboratory characteristics of this group of patients are similar to the classical HIT occurring with heparin exposure except perhaps development of high titer antibodies against heparin/PF4 complexes. An emerging recognition is the development of thrombocytopenia and thrombosis occurring 3 to 4 weeks after adenovirus vector SARS-CoV-2 exposure. The clinical course is also similar to immune HIT.

Laboratory Characteristics of HIT:
HIT is caused, in at least 90% of cases, by antibodies to antigen complexes of heparinoid (heparin or similar glycosaminoglycans) and PF4. PF4 is a platelet-specific heparin-binding protein that is abundant in platelet alpha granules from which it is secreted following platelet stimulation. A reservoir of PF4 normally accumulates on 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 some

cases.

Functional assays for HIT antibody detection rely on antibody-mediated heparin-dependent platelet activation. The endpoint of platelet activation may be platelet aggregation or platelet secretion of serotonin or adenosine triphosphate (ATP) using patient serum or plasma supplemented with heparin and platelets from carefully normal selected donors. The sensitivity of functional assays for HIT ranges from 50% to 60% for heparin-dependent platelet aggregation 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 (ELISA) are available to detect HIT type 2 (HIT-II) antibodies and are based on the detection of human IgG 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.

Clinical Picture of Immune HIT or HIT-like Syndromes:
HIT in patients not previously exposed to heparin:
1. Decrease in platelet count (thrombocytopenia) of 50% from baseline or postoperative peak.
2. 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.

Spontaneous or Autoimmune HIT:
Patients typically present a week to 10 days after surgery or viral infections with symptoms of thrombosis (venous thromboembolism) or abdominal pain (suggesting adrenal infarction) and thrombocytopenia.

Vaccine Induced Thrombocytopenia and Thrombosis:
Patients typically present 4 days to 4 weeks after receiving the vaccine. Symptoms may include new onset of severe headache (suggesting cerebral venous sinus thrombosis), abdominal pain (suggesting mesenteric/portal vein thrombosis), or venous/arterial thromboembolism.

Reference Values

HIT ELISA:
<0.400

HIT Interpretation:
Negative

Interpretation

Results are reported as:
1. Heparin-induced thrombocytopenia (HIT) enzyme-linked immunosorbent assay (ELISA) optical density (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.

Table. Results and Interpretation

	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 HIT (HIT-II).

As up to 10% of patients with clinical 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 is little data about relative risk of HIT in various populations with positive tests for H/PF4 antibodies.

Cautions

Heparin-induced thrombocytopenia is a clinical diagnosis that is complemented by laboratory testing for antibodies to human platelet factor 4 (H/PF4) complexes and/or functional assays like the serotonin release assay. 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 (4T score) 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 heparin 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. Husseinzadeh HD, Gimotty PA, Pishko AM, Buckley M, Warkentin TE, Cuker A. Diagnostic accuracy of IgG-specific versus polyspecific enzyme-linked immunoassays in heparin-induced thrombocytopenia: a systematic review and meta-analysis. J Thromb Haemost. 2017;15(6):1203-1212. doi:10.1111/jth.13692
2. Warkentin TE, Greinacher A, eds. Heparin Induced Thrombocytopenia. Marcel Dekker; 2000:400
3. Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. J Thromb Haemost. 2008;6(8):1304-1312. doi:10.1111/j.1538-7836.2008.03025.x
4. Trossaert M, Gaillard A, Commin PL, Amiral J, Vissac AM, Fressinaud E. High incidence of anti-heparin/platelet factor 4 antibodies after cardiopulmonary bypass surgery. Br J Haematol. 1998;101(4):653-655. doi:10.1046/j.1365-2141.1998.00750.x

Performance

Method Description

Enzyme-linked immunosorbent assay (ELISA) testing is performed on the Janus G3 integrated liquid handling system and BioTek plate reader using the Immucor GTI Diagnostics, Inc PF4 IgG assay test kit. Patient serum is incubated in microwells precoated with an antigen complex of platelet factor 4 (PF4) and polyanionic heparinoid substitute (polyvinyl sulfonate; PVS). If antibodies to this complex are present in the patient serum, they will bind to the PF4:PVS antigen complex; all other antibodies are washed away. An anti-IgG reagent (conjugate) is added to the wells, incubated, and washed to remove any unbound conjugate. P-Nitrophenylphosphate is added and incubated; color is generated when bound conjugate cleaves a chromogenic phosphate substrate. The reaction is stopped, and the absorbance measured using a spectrophotometer 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 decreases absorbance (reactivity). This procedure is used to confirm 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; Rev D, 05/2015)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
1 to 3 days

Specimen Retention Time
7 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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 US 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	73818-7

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