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-PF4 IgG antibody (HIT) and thrombocytopenia and thrombosis occurring after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) adenovirus vector vaccine

Method Name
Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available
Yes

Specimen

Specimen Type
Serum Red

Specimen Required

Patient Preparation: Fasting is preferred but not 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

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
</tr>
</tbody>
</table>

Specimen Stability Information

<table>
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<th>Specimen Type</th>
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<th>Time</th>
<th>Special Container</th>
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<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>48 hours</td>
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Clinical and Interpretive

Clinical Information

There are established and emerging disorders that are collectively termed thrombocytopenia and thrombosis syndromes, which most commonly include heparin induced thrombocytopenia, which occurs after exposure to heparin, unfractionated heparin (UFH) or low molecular weight heparin (LMWH). Additional newer associations include vaccine induced thrombocytopenia, which rarely occurs after exposure to adenovirus vector severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines, and spontaneous or autoimmune heparin platelet-factor 4 (PF4) IgG antibody, which occurs without exposure to UFH/LMWH and typically occurs after surgery or infection.

Heparin-induced thrombocytopenia (HIT) consists of 2 distinct clinicopathologic syndromes. The first, sometimes designated type I HIT (HIT-I) or nonimmune heparin-associated thrombocytopenia (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 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 HIT (immune HIT), occurs in 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 re-exposure, probably reflecting persistence of heparin-dependent antiplatelet antibodies or anamnestic recall of them. Typically, during the course of immune HIT, the platelet count decreases by 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 is significantly greater with UFH exposure than with exposure to 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.

Immune HIT 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 30% to 50% of patients with HIT-II, even following discontinuation of heparin therapy. Clinically, it is often difficult to distinguish between nonimmune HIT and immune HIT, 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 immune HIT.

Recent studies provide evidence that immune 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 (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 (ELISA) 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.

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 of this group of patients is similar to the classical HIT occurring with heparin exposure.

An emerging recognition is the development of thrombocytopenia and thrombosis occurring predominantly in young females within 3 to 4 weeks after adenovirus vector SARS-CoV-2 exposure. The clinical course is also similar to immune HIT.

Clinical picture of immune HIT

HIT: In patients not previously exposed to heparin: 1) decrease in platelet count (thrombocytopenia) of 50% or more 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**
Test Definition: HITIG
Heparin-PF4 IgG Ab (HIT), S

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.

<table>
<thead>
<tr>
<th>HIT ELISA OD</th>
<th>Heparin inhibition</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>&lt;0.400</td>
<td>Not done</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt; or =0.400</td>
<td>&gt; or =50%</td>
</tr>
<tr>
<td>Equivocal</td>
<td>&gt; or =0.400</td>
<td>&lt;50%</td>
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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. Warkentin TE, Greinacher A, eds: Heparin Induced Thrombocytopenia. Marcel Dekker; 2000:400

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; Rev D 05/2015)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
1 to 3 days

Specimen Retention Time
Test Definition: HITIG
Heparin-PF4 IgG Ab (HIT), S

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

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