MAYO CLINIC LABORATORIES

Test Definition: SRAU

Serotonin Release Assay, Unfractionated Heparin, Mass Spectrometry, Serum

Overview

Useful For

Detecting heparin-dependent platelet activating antibodies implicated in the pathogenesis of heparin-induced thrombocytopenia

Highlights

This test can be used to assess for the presence of heparin-induced thrombocytopenia antibodies, which may develop after unfractionated or low molecular weight heparin therapy.

Method Name

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Shipping Instructions

Specimen should be frozen when shipped or stored for longer than 48 hours.

Specimen Required

Patient Preparation:

1. Fasting: 8 hours, preferred but not required; Collect during an episode of suspected heparin-induced thrombocytopenia
2. Patient **should not** be on ticagrelor (Brilinta) as this may interfere with the assay, yielding a false-negative result.

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Red top (serum gel/SST are **not** acceptable)
Submission Container/Tube: 13-mm x 75-mm, 5-mL, plastic vial (polypropylene preferred)
Specimen Volume: 1 mL Serum
Collection Instructions:

1. After collection, allow the specimen to sit for a minimum of 1 hour at ambient temperature to ensure it clots completely.
2. Within 48 hours of collection, centrifuge and aliquot serum into a 13-mm x 75-mm, 5-mL, polypropylene vial

Forms

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

Specimen Minimum Volume
1 mL

Reject Due To

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); additional newer associations include 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.

Heparin-induced thrombocytopenia is a serious immune-mediated syndrome, (ie, type II HIT or immune HIT), which 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:
Heparin-induced thrombocytopenia 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 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 the microcirculation 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 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.

Reference Values

Low Heparin Serotonin Release: <20%
High Heparin Serotonin Release: <20%
Serotonin Release Assay Result: Negative

Interpretation

In a negative serotonin release assay (SRA), patient serum induced serotonin release of below 20% from normal donor platelets in presence of low concentration of unfractionated heparin (UFH) (0.1U/mL) regardless of the serotonin release with high concentration of UFH (100 U/mL).

In a positive SRA, patient serum induced serotonin release of 20% or more from normal donor platelets in presence of low concentration of UFH (0.1U/mL). This release is inhibited in presence of high concentration of UFH (100 U/mL) and falls below 20%.

In an indeterminate SRA, patient serum induced serotonin release of 20% or more from normal donor platelets in presence of low concentration of UFH (0.1U/mL) and high concentration of UFH (100 U/mL). Indeterminate results can occur due to non-heparin dependent antibodies (eg, human leukocyte antigen antibodies). Occasionally some indeterminate results may show greater than 50% inhibition of serotonin release from normal donor platelets in presence of high concentration of UFH (100 U/mL) compared to low concentration of UFH (0.1U/mL), which is suggestive for heparin-induced thrombocytopenia in the right clinical context.

Table. Results and Interpretation

Low heparin	High heparin	Interpretation

release %	release %	
<20%	<20%	Negative
<20%	> or =20%	Negative
> or =20%	<20%	Positive

Cautions

Ticagrelor (Brilinta) use by the patient may result in a false-negative serotonin release assay (SRA) result.

A negative SRA result makes the diagnosis of heparin-induced thrombocytopenia (HIT) less likely, however, SRA negative patients with bonafide clinical HIT in whom the SRA result may turn positive on subsequent testing have been reported in the literature.

Clinical Reference

1. Warkentin TE, Arnold DM, Nazi I, Kelton JG. The platelet serotonin-release assay. Am J Hemtol. 2015;90(6):564-572. doi:10.1002/ajh.24006
2. Minet V, Dogne JM, Mullier F. Functional assays in the diagnosis of heparin-induced thrombocytopenia: a review. Molecules. 2017;22(4):617. doi:10.3390/molecules22040617
3. Sono-Koree NK, Crist RA, Frank EL, Rodgers GM, Smock KJ. A high-performance liquid chromatography method for the serotonin release assay is equivalent to the radioactive method. Int. J Lab Hematol. 2016;38(1):72-80. doi:10.1111/ijlh.12442

Performance

Method Description

Patient serum is incubated with donor platelets and different concentrations of heparin. Anti-heparin platelet-factor 4/heparin antibodies present in the patient serum will bind to platelet Fc gamma receptors and activate donor platelets, releasing serotonin from the platelet granules. A derivative of released serotonin is measured utilizing liquid chromatography tandem mass spectrometry.(Carling RS, Degg TS, Allen KR, et al. Evaluation of whole blood serotonin and plasma and urine 5-hydroxyindole acetic acid in diagnosis of carcinoid disease. Ann Clin Biochem. 2002;39:577-582)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

1 to 4 days

Specimen Retention Time

7 days

Performing Laboratory Location

Test Definition: SRAU

Serotonin Release Assay, Unfractionated Heparin, Mass Spectrometry, Serum

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 was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

82542

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SRAU	Serotonin Release Assay, UFH, MS, S	50736-8

Result ID	Test Result Name	Result LOINC® Value
616230	Low Heparin Serotonin Release	50728-5
616231	High Heparin Serotonin Release	50727-7
616232	Serotonin Release Assay Result	66488-8
616233	Interpretation	50733-5
616234	Comment	77202-0
616235	Disclaimer	62364-5