

Platelet Surface Glycoprotein by Flow Cytometry, Blood

### Overview

### **Useful For**

Identification of markedly decreased CD41 (GPIIb) and CD61 (GPIIIa) expression levels, which are diagnostic for Glanzmann thrombasthenia

Identification of markedly decreased CD42a (GPIX) and CD42b (GPIb-alpha) expression levels, which are diagnostic for Bernard-Soulier syndrome

Identification of decreased GPVI expression, which suggests collagen receptor deficiency

Identification of decreased CD49b (GPIa), which suggests collagen receptor deficiency

### **Special Instructions**

• Platelet Esoteric Testing Patient Information

### **Highlights**

This test serves as a confirmatory test for platelet aggregation studies.

Markedly decreased platelet surface glycoprotein expression levels are diagnostic for various hereditary or acquired platelet disorders.

# **Method Name**

Immunophenotyping

### **NY State Available**

Yes

# Specimen

### **Specimen Type**

Whole Blood ACD

### **Shipping Instructions**

Specimen must be shipped ambient and arrive within 4 days of draw.

Ship specimen overnight in an Ambient Shipping Box-Critical Specimens Only (T668) following the instructions in the mailer.

# **Necessary Information**

<u>Platelet Esoteric Testing Patient Information</u> is required. Testing may proceed without the patient information, however,



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the information aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.

### Specimen Required

Supplies: Ambient Shipping Box-Critical Specimens Only (T668)

Collection Container/Tube: ACD solution A or B

Specimen Volume: 6 mL Pediatric Volume: 1 mL

Collection Instructions: Do not transfer blood to other containers.

#### **Forms**

- 1. Platelet Esoteric Testing Patient Information is required.
- 2. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

### Specimen Minimum Volume

Adult: 1 mL Pediatric 200 mcL

### **Reject Due To**

Gross	Reject
hemolysis	
Fully Clotted	Reject
Gross lipemia	OK

## **Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Whole Blood ACD	Ambient	4 days	

## Clinical & Interpretive

# **Clinical Information**

Platelets have essential roles in primary hemostasis. Exposed collagen at a vascular damage site can activate platelets via collagen receptor GPVI and GPIa and bind shear-stretched multimeric VWF proteins, which subsequently interact with the platelet surface receptor, GPIb-V-IX. Upon full activation, platelets can aggregate by binding to fibrinogen through activated GPIIb-GPIIIa receptors. Deficiency of platelet surface glycoproteins can cause bleeding diathesis.

Platelet flow cytometric analysis is the preferred method to assess hereditary platelet disorders due to quantitative surface glycoprotein (GP) deficiencies. GP expression levels can be measured by using fluorescent-conjugated GP-specific antibodies and their fluorescent intensities can be compared to normal ranges of various glycoproteins.

CD Number	Glycoprotein Name	Integrin Name



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CD41	GPIIb	Alpha 2b
CD42a	GPIX	NA
CD42b	GPIb-alpha	NA
CD49b	GPIa	Alpha 2
CD61	GPIIIa	Beta 3
NA	GPVI	NA

### **Reference Values**

GPIIb CD41: > or =70.0% (Normal Range-Median)
GPIIIa CD61: > or =70.0% (Normal Range-Median)
GPIX CD42a: > or =70.0% (Normal Range-Median)
GPIb-alpha CD42b: > or =70.0% (Normal Range-Median)
GPIa CD49b: > or =60.0% (Normal Range-Median)

# Interpretation

	% Reference	
CD Markers	Range Median	Comments
CD41 and	50%-69%	Marginally decreased platelet surface receptors CD41
CD61	(Marginally)	(GPIIb) and CD61 (GPIIIa) are of uncertain clinical
		significance. This finding could be a laboratory artifact
		due to a suboptimal sample condition, benign
		polymorphisms, or a heterozygous state of Glanzmann
		thrombasthenia. Recommend correlation with patient's
		clinical findings and results of platelet functional studies,
		and consider repeating platelet glycoprotein profile
		studies by flow cytometry to verify the present finding if
		clinically indicated.
	30%-50%:	Platelet surface expression of CD41 (GPIIb) and CD61
	(Moderately)	(GPIIIa) are moderately or markedly decreased. This
		finding is suggestive of a variant of Glanzmann
	<30%: (Markedly)	thrombasthenia. Recommend correlation with patient's
		clinical findings and results of platelet functional studies,
		and consider repeating platelet glycoprotein profile
		studies by flow cytometry to verify the present finding if
		clinically indicated.
CD42a and	50%-69%	Marginally decreased platelet surface receptors CD42a
CD42b	(Marginally)	(GPIX) and CD42b (GPIb-alpha) are of uncertain clinical
		significance. This finding could be a laboratory artifact
		due to a suboptimal sample condition, benign
		polymorphisms, or a heterozygous state of
		Bernard-Soulier syndrome. Recommend correlation with
		patient's clinical findings and results of platelet functional
		studies, and consider repeating platelet glycoprotein



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		profile studies by flow cytometry to verify the present finding if clinically indicated.
	30%-50%: (Moderately) <30%: (Markedly)	Platelet surface expression of CD42a (GPIX) and CD42b (GPIb-alpha) are moderately or markedly decreased. This finding is suggestive of a variant of Bernard-Soulier syndrome. Recommend correlation with patient's clinical findings and results of platelet functional studies, and consider repeating platelet glycoprotein profile studies by flow cytometry to verify the present finding if clinically indicated.
CD49b	30%-59% (Marginally)	Marginally decreased platelet surface receptor CD49b (GPIa) is of uncertain clinical significance. This finding could be a laboratory artifact due to a suboptimal sample condition, a benign polymorphism, or a variant of platelet collagen receptor glycoprotein Ia/IIa deficiency.  Recommend correlation with patient's clinical findings and results of platelet functional studies, and consider repeating platelet glycoprotein profile studies by flow cytometry to verify the present finding if clinically indicated.
	10%-30% (moderately) <10% (Markedly)	Platelet surface expression of CD49b (GPIa) is moderately or markedly decreased. This finding is suggestive for a variant of a variant of platelet collagen receptor glycoprotein Ia/IIa deficiency. Recommend correlation with patient's clinical findings and results of platelet functional studies, and consider repeating platelet glycoprotein profile studies by flow cytometry to verify the present finding if clinically indicated.
GPVI	50%-69% (Marginally)	Marginally decreased platelet surface receptor glycoprotein VI (GPVI) is of uncertain clinical significance. This finding could be a laboratory artifact due to a suboptimal sample condition, a benign polymorphism or a variant of platelet collagen receptor GPVI deficiency. Recommend correlation with patient's clinical findings and results of platelet functional studies, and consider repeating platelet glycoprotein profile studies by flow cytometry to verify the present finding if clinically indicated.
	30%-50% (moderately) <30% (Markedly)	Platelet surface expression of glycoprotein VI (GPVI) is moderately or markedly decreased. This finding is suggestive of a variant of a variant of platelet collagen receptor GPVI deficiency. Recommend correlation with patient's clinical findings and results of platelet functional studies, and consider repeating platelet glycoprotein



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	profile studies by flow cytometry to verify the present	
	finding if clinically indicated.	

### **Cautions**

Suboptimal sample conditions due to improper blood draw, transportation, or storage may cause fluctuation of platelet surface receptors and consequently influence the results of platelet surface receptor measurement by flow cytometry.

### **Supportive Data**

The platelet glycoprotein flow cytometry method was established in the Mayo Clinic Special Coagulation Laboratory in 2009. Between the years of 2009 to 2014, a total of 155 clinical patients at Mayo Clinic were tested. The flow cytometry results were compared with the final impressions of platelet light transmission aggregation testing. There were 7 samples that had flow cytometric features of Glanzmann thrombasthenia, 2 samples that had flow cytometric features of Bernard-Soulier syndrome, and 3 samples that had flow cytometric features of May-Hegglin anomaly. All flow cytometric results were concordant with platelet light transmission aggregation results and other clinical findings.

#### **Clinical Reference**

- 1. Miller, JL. Glycoprotein analysis for the diagnostic evaluation of platelet disorders. Semin Thromb Hemost. 2009;35(2):224-232
- 2. Kannan M, Ahmad F, Yadav BK, et al. Carrier detection in Glanzmann thrombasthenia: comparison of flow cytometry and Western blot with respect to DNA mutation. Am J Clin Pathol. 2008;130(1):93-98
- 3. Savoia A, Pastore A, De Rocco D, et al: Clinical and genetic aspects of Bernard-Soulier syndrome: searching for genotype/phenotype correlations. Haematologica. 2011;96(3):417-423
- 4. Nurden AT, Freson K, Selifsohn U. Inherited platelet disorders. Haemophilia. 2012;18(4):154-160
- 5. Spurgeon BEJ, Naseem KM. Platelet Flow Cytometry: Instrument Setup, Controls, and Panel Performance. Cytometry B Clin Cytom. 2020;98(1):19-27
- 6. Frelinger AL, 3rd, Rivera J, Connor DE, et al. Consensus recommendations on flow cytometry for the assessment of inherited and acquired disorders of platelet number and function: Communication from the ISTH SSC Subcommittee on Platelet Physiology. J Thromb Haemost. 2021;19(12):3193-3202

### **Performance**

### **Method Description**

Flow cytometric immunophenotyping of peripheral blood platelets is performed using the following antibodies:

Panel: CD41 (IIb), CD42a (IX), CD42b (Ib-alpha), CD49b (GPIa), CD61 (GPIIIa), and GPVI. For sample quality purposes, CD62P is evaluated.

Using whole blood collected in ACD (A or B), platelet surface GPIa, Ib-alpha, IIb, IIIa, VI and IX expression levels are measured by flow cytometry method. Platelets in whole blood are stained with various fluorochrome-labeled primary antibodies and fixed. Then the platelet surface fluorescent intensities of various bound antibodies are measured by flow cytometers. Platelets are first gated by forward and side scatter. Mean fluorescent intensities are recorded and converted to percentage of a median fluorescent intensity of a normal donor study of 20 healthy donors. If the



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percentage of expression of a glycoprotein (GP) is lower than the corresponding normal range, a deficiency of a GP is detected. (Unpublished Mayo Method)

### **PDF Report**

No

### Day(s) Performed

Monday through Saturday

### Report Available

1 to 2 days

# **Specimen Retention Time**

14 days

# **Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

#### Fees & Codes

### Fees

- Authorized users can sign in to <u>Test Prices</u> for detailed fee information.
- Clients without access to Test Prices can contact <u>Customer Service</u> 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact <u>Customer Service</u>.

### **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**

88184-Flow cytometry; first cell surface, cytoplasmic or nuclear marker 88185-Flow cytometry; additional cell surface, cytoplasmic or nuclear marker (each) X5 88187-Flow cytometry interpretation, 2 to 8 markers

### **LOINC®** Information

Test ID	Test Order Name	Order LOINC® Value
PLAFL	Platelet Glycoprotein Flow, B	93320-0

Result ID	Test Result Name	Result LOINC® Value
CK111	GPIIb CD41	93319-2
CK112	GPIIIa CD61	93318-4
CK113	GPIX CD42a	93317-6



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CK114	GPIb-alpha CD42b	93316-8
CK115	GPIa CD49b	93315-0
CK116	GPVI	93314-3
CK117	Final Diagnosis	93313-5