MAYO CLINIC LABORATORIES

Test Definition: EEEV1

Red Blood Cell (RBC) Enzyme Evaluation, Blood

Overview

Useful For

Identifying defects of red blood cell enzyme metabolism

Evaluating patients with Coombs-negative hemolytic anemia

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
EEEVI	Erythrocyte Enzyme Interpretation	No	Yes
G6PDC	G6PD Enzyme Activity, B	Yes, (Order G6PD1)	Yes
PKC	PK Enzyme Activity, B	Yes, (Order PK1)	Yes
GPIC	Glucose Phosphate Isomerase, B	Yes, (Order GPI1)	Yes
HKC	Hexokinase, B	Yes, (Order HK1)	Yes
AKC	Adenylate Kinase, B	Yes, (Order AK1)	Yes
PFKC	Phosphofructokinase, B	Yes, (Order PFK1)	Yes
PGKC	Phosphoglycerate Kinase, B	Yes, (Order PGK1)	Yes
TPIC	Triosephosphate Isomerase, B	Yes, (Order TPI1)	Yes
GSH	Glutathione, B	Yes	Yes
P5NT	Pyrimidine 5' Nucleotidase, B	Yes	Yes

Testing Algorithm

This is a consultative evaluation in which the case will be evaluated at Mayo Clinic Laboratories.

For more information see Benign Hematology Evaluation Comparison

Special Instructions

- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison

Method Name

EEEVI: Medical Interpretation

G6PDC, GPIC, PKC, HKC, AKC, PFKC, PGKC, TPIC, GSH, P5NT: Kinetic Spectrophotometry (KS)

NY State Available

Yes

Specimen

Specimen Type
Whole Blood ACD-B

Necessary Information
Metabolic Hematology Patient Information **(T810) is strongly recommended** and should include clinical history. Testing may proceed without this information; however, if the requested information is received, it allows for a more complete interpretation.

Specimen Required
Container/Tube:
Preferred: Yellow top (ACD solution B)
Acceptable: Yellow top (ACD solution A)
Specimen Volume: 12 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Forms
1. Metabolic Hematology Patient Information (T810)
2. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

Specimen Minimum Volume
5 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood ACD-B	Refrigerated	11 days	

Clinical & Interpretive

Clinical Information
Erythrocyte (red blood cell) enzyme deficiencies are inherited causes of hemolytic anemia. Some are very common, such as glucose 6-phosphate dehydrogenase (G6PD) deficiency, and others are very rare, found in only a few families around the world. Most are autosomal in inheritance, but some are sex-linked and located on the X chromosome. Most enzyme deficiencies result in chronic nonspherocytic hemolytic anemia of variable severity; however, some, such as G6PD, can be hematologically normal with episodic acute hemolysis due to a trigger event such as medications, toxins, or some foods. The red blood cell (RBC) enzymopathies do not typically show recurrent pathognomonic changes on the

peripheral blood smear other than generic features of hemolytic anemia, although some such as pyruvate kinase deficiency can have echinocytes and pyrimidine 5' nucleotidase (P5NT) deficiency is associated with basophilic stippling. RBC enzyme activity levels are best evaluated as a panel as reticulocytosis can mask some deficient states and comparison to the background enzyme activity is useful.

This is a consultative evaluation of RBC enzyme activity as a potential cause of early RBC destruction.

Reference Values

Definitive results and an interpretive report will be provided.

Interpretation

A hematopathologist expert in these disorders evaluates the case. A detailed interpretation is given, including an overview of the results and their significance, as well as clinical research information regarding different enzyme deficiencies.

Cautions

Recent transfusion may mask the patient's intrinsic enzyme activity and cause unreliable results.

A very high white blood cell count can contribute to interference and falsely raise the activity of some enzymes.

Some enzyme deficiency disorders can be masked by reticulocytosis, and comparison of activities of other red blood cell enzymes in this panel can be useful.

Clinical Reference

1. Koralkova P, van Solinge WW, van Wijk R. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia - pathophysiology, clinical aspects, and laboratory diagnosis. Int J Lab Hematol. 2014;36(3):388-397
2. Bartels M, Beers E, Wijk R. Erythrocyte enzyme disorders. In: Kaushansky K, Prchal JT, Burns LJ, Lichtman MA, Levi M, Linch DC. eds. Williams Hematology, 10th ed. McGraw-Hill Education; 2021:chap 48
3. Beutler E. Glucose-6-phosphate dehydrogenase deficiency and other enzyme abnormalities. In: Beutler E, Lichtmann MA, Coller BS, Kipps TJ, eds. Hematology. 5th ed. McGraw-Hill Book Company; 1995:564-581

Performance

Method Description

Glucose-6-Phosphate Dehydrogenase:
Glucose-6-phosphate dehydrogenase (G6PD) in a hemolysate catalyzes the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconate. Concomitantly, nicotinamide adenine dinucleotide phosphate (NADP[+]) is changed to its reduced form, NADPH, and the reaction is measured spectrophotometrically on an automated chemistry analyzer.(Beutler E. Red cell metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)
Pyruvate Kinase:
Pyruvate kinase catalyzes the phosphorylation of adenine diphosphate (ADP) to adenine triphosphate (ATP) by converting phosphoenolpyruvate to pyruvate. The amount of pyruvate formed is quantitated by adding lactate

dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH) and measuring the rate of decrease in absorbance spectrophotometrically at 340 nm as the NADH is oxidized to NAD(+) on an automated chemistry analyzer.(Beutler E: Red Cell Metabolism. In: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; van Solinge WW, van Wijk: Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

Glucose Phosphate Isomerase:
Glucose phosphate isomerase (GPI) interconverts G6P and fructose 6-phosphate (F6P). In this assay, the F6P is then further converted to 6-phosphogluconate through the G6PD G6PD reaction resulting in the reduction of NADP(+) to NADPH. The reduction of NADP(+) is measured spectrophotometrically by the increase in absorbance at 340 nm on an automated chemistry analyzer.(Beutler E. Red cell metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984: 40-42; Rab MAE, van Wijk R. Enzymes of the red blood cell. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 78)

Hexokinase:
Hexokinase catalyzes the reaction of ATP and glucose to G6P and ADP. In this assay, the formation of G6P is measured by linking its further oxidation to 6-phosphogluconate to the reduction of NADP(+) through the G6PD reaction. The increase in absorbance, which occurs as NADP(+) is reduced to NADPH, is measured spectrophotometrically at 340 nm on an automated chemistry analyzer.(Beutler E: Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:38-40; Rab MAE, van Wijk R. Enzymes of the red blood cell. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 78)
Adenylate Kinase:
Adenylate kinase (myokinase) catalyzes the dismutation of ADP into adenosine monophosphate and ATP. In this assay, the reverse reaction is measured by following the formation of ADP with pyruvate kinase and lactate dehydrogenase reactions resulting in NADH being oxidized to NAD(+). The decrease in absorbance that occurs as NADH is oxidized is measured spectrophotometrically at 340 nm by an automated chemistry analyzer.(Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:93-95; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

Phosphofructokinase:
Phosphofructokinase (PFK) catalyzes the phosphorylation of F6P by ATP to fructose 1,6-diphosphate (FDP). FDP is then converted to dihydroxyacetone phosphate (DHAP) through subsequent aldolase and triosephosphate isomerase (TPI) catalyzed reactions. The rate of formation of DHAP is measured by linking its reduction to alpha-glycerophosphate by alpha-glycerophosphate dehydrogenase, which results in the oxidation of NADH to NAD(+). The decrease in absorbance at 340 nm is measured spectrophotometrically as the NADH is oxidized on an automated chemistry analyzer.(Beutler E: Red cell metabolism. A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; Rab MAE, van Wijk R. Enzymes of the red blood cell. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 78)

Phosphoglycerate Kinase:
Phosphoglycerate kinase (PGK) catalyzes the phosphorylation of ADP to ATP by conversion of 1,3-diphosphoglycerate (1,3-DPG) to 3,phosphoglyceric acid. In this assay, the reaction is driven in the reverse direction. The formation of 1,3-DPG is then measured through the glyceraldehyde phosphate dehydrogenase reaction as 1,3-DPG is converted to glyceraldehyde-3-phosphate (GAP) resulting in the oxidation of NADH to NAD(+). The decrease in absorbance, which

occurs as NADH is oxidized, is measured spectrophotometrically at 340 nm on an automated chemistry analyzer.(Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:53-55; Rab MAE, van Wijk R. Enzymes of the red blood cell. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 78)

Triosephosphate Isomerase:

Triosephosphate isomerase interconverts GAP and DHAP. The rate of DHAP formation is measured by further converting it to alpha-glycerophosphate by alpha-glycerophosphate dehydrogenase which results in the oxidation of NADH to NAD(+). The oxidation of NADH is measured spectrophotometrically by the decrease in absorbance at 340 nm on an automated chemistry analyzer.(Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods. Grune and Stratton; 1984; Rab MAE, van Wijk R. Enzymes of the red blood cell. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 78)

Glutathione:

Virtually all the nonprotein sulfhydryl of red blood cells is in the form of reduced glutathione (GSH). 5,5'-dithiobis (2-nitrobenzoic acid) is a disulfide compound that is readily reduced by sulfhydryl compounds, forming a highly colored yellow anion. The absorbance of this resultant yellow substance is measured by 412 nm and compared to that of a known standard.(Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984; Alisik M, Neselioglu S, Erel O. A colorimetric method to measure oxidized, reduced and total glutathione levels in erythrocytes. J Lab Med. 2019:43[5], 269-277. doi:10.1515/labmed-2019-0098)

Pyrimidine 5' Nucleotidase:

Pyrimidine nucleotides have a spectral absorption curve that is markedly different from that exhibited by (normally present) adenine nucleotides (eg, ATP). The former have a peak at about 270 nm; the latter at about 257 nm. Thus, pyrimidine 5' nucleotidase deficiency may be ascertained by demonstrating a very high spectral absorption maximum of 270 nm in erythrocyte extracts.(Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:100-102; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

PDF Report

No

Day(s) Performed

Tuesday, Thursday

Report Available

2 to 13 days

Specimen Retention Time

14 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 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

82955-G6PD Enzyme Activity
84087-Glucose Phosphate Isomerase
84220-Pyruvate Kinase Enzyme Activity
82657-Hexokinase
82657-Adenylate Kinase
82657-Phosphofructokinase
82657-Phosphoglycerate Kinase
82657-Triosephosphate Isomerase
82978-Glutathione
83915-Pyrimidine 5' Nucleotidase

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
EEEV1	RBC Enzyme Evaluation	72695-0

Result ID	Test Result Name	Result LOINC® Value
2734	Pyrimidine 5' Nucleotidase, B	2902-5
AKCL	Adenylate Kinase, B	44051-1
608087	Erythrocyte Enzyme Interpretation	59466-3
608109	Reviewed By	18771-6
608409	Glutathione, B	2383-8
TPICL	Triosephosphate Isomerase, B	44054-5
PKCL	PK Enzyme Activity, B	32552-2
PGKCL	Phosphoglycerate Kinase, B	44053-7
PFKCL	Phosphofructokinase, B	72664-6
HKCL	Hexokinase, B	49216-5
GPICL	Glucose Phosphate Isomerase, B	44050-3
G6PCL	G6PD Enzyme Activity, B	32546-4