Test Definition: RBCME

RBC Membrane Evaluation, B

Overview

Useful For

Investigation of suspected red cell membrane disorders such as hereditary spherocytosis or hereditary pyropoikilocytosis

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
FRAGO	Osmotic Fragility	Yes, (Order FRAG)	Yes
SCTRL	Shipping Control Vial	No	Yes
HSEV	Spherocytosis Interpretation	No	Yes
BND3	Band 3 Fluorescence Staining, RBC	No	Yes
SMPB	Peripheral Blood Smear Review	No	Yes

Testing Algorithm

Osmotic fragility and eosin-5-maleimide (EMA) binding (Band3) flow cytometry testing will always be performed. A normal shipping control is necessary to exclude false-positive results due to preanalytical artifact. Testing will be canceled if no shipping control is received or if the shipping control is abnormal. A consultative interpretation will be provided.

See Benign Hematology Evaluation Comparison in Special Instructions.

Special Instructions

- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison

Method Name

HSEV: Consultative Interpretation

FRAGO: Osmotic Lysis

BND3: Flow Cytometry

SMPB: Consultant Review

NY State Available

Yes

Specimen

Specimen Type

Control

Whole Blood EDTA
Whole Blood Slide

Shipping Instructions

Specimens must arrive within 72 hours of draw.

Necessary Information

Include recent transfusion information.

Include most recent CBC results.

Specimen Required

A whole blood EDTA specimen, an EDTA control specimen, and 2 well-made peripheral blood smears (Wright stained or fixed in absolute methanol) are required for testing.

Patient:

Specimen Type: Blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL

Collection Instructions:

1. Immediately refrigerate specimen after draw. Refrigerate at 0 to 4 degrees C. **Do not freeze.** Freezing causes sample lysis, and tests will not be performed on hemolyzed specimens.

2. Send specimen in original tube. **Do not aliquot.**

3. Rubber band patient specimen and control vial together. Control must accompany the patient sample at all times to ensure the reliability of testing results.

4. Be sure specimen and control are stored and transported together at refrigerate temperature, carefully following proper handling and shipping instructions.

Patient:

Specimen Type: Slides

Container/Tube: Blood smears

Specimen Volume: 2 well-made peripheral blood smears

Collection Instructions: Collect 2 well-made peripheral blood smears (Wright stained or fixed in absolute methanol).

Normal Shipping Control:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL

Collection Instructions:

1. Draw a control specimen from a normal (healthy), unrelated, nonsmoking person at the same time as the patient.

2. Label clearly on outermost label **normal control**.

3. Immediately refrigerate specimen after draw. Refrigerate at 0 to 4 degrees C. **Do not freeze.** Freezing causes sample lysis, and tests will not be performed on hemolyzed specimens.

4. Send specimen in original tube. **Do not aliquot.**

5. Rubber band patient specimen and control vial together. Control must accompany the patient sample at all times to ensure the reliability of testing results.

Forms

1. Metabolic Hematology Patient Information (T810) in Special Instruction

2. If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	Reject
Other	Clotted Frozen

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Control	Refrigerated	72 hours	PURPLE OR PINK TOP/EDTA
Whole Blood EDTA	Refrigerated	72 hours	
Whole Blood Slide	Refrigerated		CARTRIDGE

Clinical and Interpretive

Clinical Information

The functional red cell membrane is composed of a cholesterol and phospholipid bilayer anchored by integral proteins to an elastic cytoskeletal network. These interactions form the shape, deformability, and proper ion balance of the cell. Abnormalities in these moieties result in red blood cell membrane disorders.

Hereditary spherocytosis (HS) is a common membrane disorder that can be present in many ethnic groups. Its prevalence has been estimated at approximately 1 in 3,000 persons of Northern European ancestry. It is usually

associated with visible spherocytes on the peripheral blood smear and can be associated with variable clinical features of hemolysis ranging from completely compensated to mild to severe.

Hereditary elliptocytosis (HE) is another fairly common and clinically variable disorder that can range from normal RBC indices in the large majority of cases to rare patients with moderate to severe anemia.

Common hereditary elliptocytosis (CHE) is characterized by the presence of elliptocytes on the peripheral blood smear. Mutations associated with HE have been reported in widely variable ethnicities with greater prevalence in populations overlapping the malaria belt.

Hereditary pyropoikilocytosis (HPP) is best classified as a severe form of hereditary elliptocytosis. It is uncommon and presents in early childhood as a severe hemolytic anemia. These disorders are associated with marked poikilocytosis on the peripheral blood smear.(1,2) Red cell membrane disorders can result from abnormalities involving several red cell membrane proteins, such as band 3, alpha and beta spectrin, protein 4.1, protein 4.2, glycophorin C, and ankyrin.

Most often, red cell membrane disorders are diagnosed in childhood, adolescence, or early adult life. The diagnosis of HS is usually made by a combination of patient and family history, laboratory evidence of hemolysis, and review of a peripheral blood smear. The osmotic fragility (OF) test is usually markedly abnormal in HS cases. However, factors such as age, iron status, and medications can affect the OF test. The OF test is nonspecific and can be increased in acquired disorders such as autoimmune hemolytic anemia. Coombs testing should be negative prior to ordering this test.

The addition of eosin-5-maleimide (EMA) binding (Band3) flow cytometry to this profile increases specificity if a typical moderately decreased pattern is present. Hereditary pyropoikilocytosis can have normal or only mildly increased OF results and often displays a very dim and sometimes dual peak pattern with EMA-binding testing. Common hereditary elliptocytosis cases are not discriminated from normal patients in either OF and EMA binding (band3) testing and this profile **does not** add confirmatory information for HE.

Reference Values

> or =12 months:

0.50 g/dL NaCl (unincubated): 3-53% hemolysis

0.60 g/dL NaCl (incubated): 14-74% hemolysis

0.65 g/dL NaCl (incubated): 4-40% hemolysis

0.75 g/dL NaCl (incubated): 1-11% hemolysis

An interpretive report will be provided.

Reference values have not been established for patients who are <12 months of age.

Interpretation

An interpretive report will be provided.

Cautions

This test is not useful for hereditary elliptocytosis.

Interfering factors:

-Recent transfusion

-Oral contraceptives

-H2 blockers

Infrequently, other hemolytic disorders may also be associated with positive osmotic fragility results, as in patients with congenital nonspherocytic hemolytic anemia due to glucose-6-phosphate dehydrogenase or pyruvate kinase deficiency. Eosin-5-maleimide (EMA) binding (band 3) results can be masked by reticulocytosis. Results must be interpreted within the clinical, familial, and peripheral blood smear findings.

Patients with an immunohemolytic anemia, or who have recently received a blood transfusion may also have increased RBC lysis.

The shipping control specimen is required to adequately interpret these cases, as temperature extremes can increase fragility of the specimen.

Resulting Cautions:

Osmotic fragility results will be reported if the shipping control is normal.

If the shipping control is abnormal and the osmotic fragility results are within normal range, the results will be reported; however, a comment will be added to the report indicating that the shipping control was not entirely satisfactory.

The test will be cancelled if the patient specimen and shipping control are both abnormal.

Clinical Reference

1. King MJ, Garcon L, Hoyer JD, et al: International Council for Standardization in Haematology. ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol 2015 Jun;37(3):304-325. PMID: 25790109

2. Lux SE, IV: Anatomy of the red cell membrane skeleton: unanswered questions. Blood 2016 Jan 14;127(2):187-199 DOI:10.1182/blood-2014-12-512772. PMID: 26537302

3. Gallagher PG: Abnormalities of the erythrocyte membrane. Pediatr Clin North Am 2013 Dec;60(6):1349-1362. PMID: 24237975

4. Bianchi P, Fermo E, Vercellati C, et al: Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. Haematologica 2012 Apr;97(4):516-523. PMID: 22058213

Performance

Method Description

Osmotic Fragility:

Specimens for erythrocyte osmotic fragility tests are anticoagulated with EDTA. Osmotic lysis is performed using sodium chloride (NaCl) solution, 0.50 g/dL. An incubated fragility test is performed following 24-hour incubation at 37 degrees C at the following NaCl concentrations: 0.60, 0.65, and 0.75 g/dL. Results are reported and interpreted.(Larson CJ, Scheidt R, Fairbanks VF: The osmotic fragility test for hereditary spherocytosis: use of EDTA-

anticoagulated blood stored at 4 degrees C for up to 96 hours. Am Soc Clin Pathol Meeting Abstract, 1988; Larson CJ, Scheidt R, Fairbanks VF: The osmotic fragility test for hereditary spherocytosis: objective criteria for test interpretation. Am Soc Clin Pathol Meeting Abstract, 1988)

Band 3:

Eosin-5-maleimide (EMA) is a fluorescent dye that binds to Lys-430 of the extracellular loop of the band 3 protein. Using a 1-color flow cytometry method (number of events plotted against fluorescence), the fluorescent intensity of EMA-stained RBC, is assessed and compared to normal-value patients.(King MJ, Behrens J, Rogers C, et al: Rapid flow cytometric test for the diagnosis of membrane cytoskeletal associated hemolytic anemia. Br J Haematol 2000;111:924-933)

Peripheral Blood Smear Review:
A hematopathologist who is an expert in these disorders evaluates the slides and an interpretive report is issued.

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Saturday

Analytic Time

4 days (not reported on Saturday or Sunday)

Maximum Laboratory Time

7 days

Specimen Retention Time

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 was developed and its performance characteristics 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

85557-Osmotic fragility

88184-Flow cytometry; first cell surface, cytoplasmic or nuclear marker x 1

85060-Morphology review

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
RBCME	RBC Membrane Evaluation, B	In Process

Result ID	Test Result Name	Result LOINC Value
83141	Band 3 Fluorescence Staining, RBC	In Process
9064	Osmotic Fragility, RBC	34964-7
SCTRL	Shipping Control Vial	40431-9
13065	Spherocytosis Interpretation	50595-8
37406	Peripheral Blood Smear Review	59465-5
37436	Reviewed By	18771-6
3306	Osmotic Fragility, 0.50 g/dL NaCl	23915-2
3307	Osmotic Fragility, 0.60 g/dL NaCl	23918-6
3308	Osmotic Fragility, 0.65 g/dL NaCl	23920-2
3309	Osmotic Fragility, 0.75 g/dL NaCl	23921-0
3310	Osmotic Fragility Comment	59466-3