MAYO CLINIC LABORATORIES

Test Definition: RBCME

Red Blood Cell Membrane Evaluation, Blood

Overview

Useful For

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

This test is **not useful for** hereditary elliptocytosis.

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 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.

For more information see Benign Hematology Evaluation Comparison.

Special Instructions

- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison
- Specimen Collection and Labeling Instructions for Osmotic Fragility Testing of Erythrocytes

Method Name

HSEV: Medical 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 collection.

Necessary Information
1. Include recent transfusion information and most recent complete blood cell count results.
2. Metabolic Hematology Patient Information (T810) is strongly recommended, but not required, to be filled out and sent with the specimen. This information aids in providing a more thorough interpretation of test results. Ordering providers are strongly encouraged to complete the form and send it with the specimen.

Specimen Required
Both patient and shipping control specimens are required.
For complete instructions, refer to Specimen Collection and Labeling Instructions for Osmotic Fragility Testing of Erythrocytes.

Shipping Control Specimen
Specimen Type: Whole blood (non-patient)
Container/Tube: Lavender top (EDTA)
Specimen Volume: 4 mL
Collection Instructions:
1. Collect a shipping control specimen from a healthy person unrelated to the patient at the same time (or within 4 hours) as the patient specimen.
Note: The shipping control specimen can be collected from a phlebotomist, volunteer or another heathy patient.
2. Clearly handwrite "CONTROL" on the outermost label of the tube.
3. Refrigerate (or place on cold gel pack/small amount of wet ice) immediately after collection.
4. Send shipping control in the original tube. **Do not aliquot**.
5. Keep the shipping control and the patient specimens **together**, either rubber banded or in a bag.
Additional Information:
1. **The shipping control and patient specimens must be handled identically** from the time of collection through receipt in the testing laboratory.
2. **If the shipping control is not sent with the patient specimen, test cancellation is likely.**
3. The shipping control specimen evaluates whether the patient result has been compromised by handling conditions such as temperature, motion, or other transportation interferences, as temperature and handling extremes can adversely impact the integrity of the specimen.

Patient Specimen
Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA)

Specimen Volume: 4 mL
Collection Instructions:
1. Collect and label patient specimen.
2. Refrigerate (or place on cold gel pack/small amount of wet ice) specimen immediately after collection.
3. Send whole blood specimen in the original tube. **Do not aliquot**.
4. Keep the shipping control and the patient specimens together, either rubber banded or in a bag.
Additional Information: The shipping control and patient specimens must be handled identically from the time of collection through receipt in the testing laboratory.

Patient Specimen
Specimen Type: Slides
Container/Tube: Blood smears
Specimen Volume: 2 Well-made peripheral blood smears
Collection Instructions:
1. Prepare 2 peripheral blood smears from the EDTA tube collected from the patient.
2. Either stain the smear with Wright stain or fix the smear with absolute methanol prior to shipping.
Additional Information: Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

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

Whole blood patient/Shipping control: 2 mL; Slides: See Specimen Required

Reject Due To

Gross hemolysis	Reject
Clotted	Reject
Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

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 & 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 cell membrane disorders.

Hereditary spherocytosis (HS) is a common membrane disorder, which can be present in many ethnic groups. Its prevalence has been estimated at approximately 1 in 3000 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 red blood cell indices in the large majority of cases to rare patients with moderate to severe anemia.

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

Hereditary pyropoikilocytosis 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. OF 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 (band 3) 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 testing, and this profile **does not** add confirmatory information for HE.

Reference Values

OSMOTIC FRAGILITY
<12 months: Not established
> 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
NaCl = sodium chloride

BAND 3 FLUORESCENCE STAINING RED BLOOD CELLS (RBC)
<12 months: Not established
> or =12 months: Normal (reported as normal, decreased, or equivocal)

An interpretive report will be provided.

Interpretation

A hematopathologist expert in these disorders evaluates the case and an interpretive report is issued summarizing the results from all appropriate testing performed.

Cautions

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 binding (band 3) results can be masked by reticulocytosis. Results must be interpreted within the clinical, familial, and peripheral blood smear findings.

Patients with immunohemolytic anemia or who have recently received a blood transfusion may also have increased red blood cell lysis.

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

Result Cautions:
Osmotic fragility results will be reported if the shipping control result 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 canceled if the patient specimen and shipping control are both abnormal.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

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;37(3):304-325
2. Lux SE 4th. Anatomy of the red cell membrane skeleton: unanswered questions. Blood. 2016;127(2):187-199. doi:10.1182/blood-2014-12-512772
3. Gallagher PG. Abnormalities of the erythrocyte membrane. Pediatr Clin North Am. 2013;60(6):1349-1362
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;97(4):516-523

Performance

Method Description

Osmotic Fragility:
Specimens for erythrocyte osmotic fragility tests are anticoagulated with EDTA. Osmotic lysis is performed using sodium chloride solution, 0.5 g/dL. An incubated fragility test is performed following 24-hour incubation at 37 degrees C at the following sodium chloride 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; King MJ, Zanella A. Hereditary red cell membrane disorders and laboratory diagnostic testing. Int J Lab Hematol. 2013;35[3]:237-243)

Band 3/Eosin-5-Maleimide Binding Assay:
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 red blood cells is assessed and compared to normal-value patients.(King MJ, Behrens J, Rogers C, Flynn C, Greenwood D, Chambers K. Rapid flow cytometric test for the diagnosis of membrane cytoskeleton-associated haemolytic anaemia. Br J Haematol. 2000;111[3]:924-933; King MJ, Zanella A. Hereditary red cell membrane disorders and laboratory diagnostic testing. Int J Lab Hematol. 2013;35[3]:237-243; Farias MG. Advances in laboratory diagnosis of hereditary spherocytosis. Clin Chem Lab Medi. 2017;55[7]:944-948)

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) Performed

Monday through Saturday

Report Available

4 to 7 days

Specimen Retention Time

28 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

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	98905-3

Result ID	Test Result Name	Result LOINC® Value
83141	Band 3 Fluorescence Staining, RBC	98906-1
9064	Osmotic Fragility, RBC	34964-7
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
SCTRL	Shipping Control Vial	40431-9
13065	Spherocytosis Interpretation	50595-8
37436	Reviewed By	18771-6
37406	Peripheral Blood Smear Review	59466-3