



IMPLEMENTATION OF HEREDITARY SPHEROCYTOSIS

April 22, 2013

Due to change in standard of care for diagnostic investigation of **Hereditary Spherocytosis (HS)**, the **Osmotic Fragility** test will be deleted from the test menu effective May 1, 2013.

A **flow cytometric measurement** of band 3 protein expression in red cell membrane will be offered to replace osmotic fragility, offering high sensitivity, specificity, and efficiency when screening for HS.

This method uses **eosin-5-maleimide (EMA)** for detection. EMA reacts covalently with lysine-430 on the first extracellular loop of band 3 protein on intact red cells. Band 3 interacts with ankyrin and protein 4.2, which interact with the spectrin-based cytoskeleton and stabilizes the membrane lipid bilayer. Absent or decreased expression of red blood cell membrane proteins found in HS cause a disruption of the cytoskeleton network and reduces normal expression of band 3 protein and its fluorescence emission. The EMA binding test serves two purposes:

1. To determine the presence of abnormal intact red cells in the patient's peripheral blood sample.
2. To distinguish hemolytic anemia associated with structural membrane protein abnormality (in particular, hereditary spherocytosis) from other types of hemolytic anemia.

Test name: Hereditary Spherocytosis testing by Flow Cytometry. See LIM.

Method Performance:

A range for mean channel fluorescence (mean fluorescence intensity) (MCF) for local normal red cells has been established.

The test is unaffected by a decrease in the size of red cells that do not have any known cytoskeletal protein deficiency (eg. red cells from an iron deficient patient with a lower MCV). On the other hand, an increase in fluorescence intensity is produced by red cells with higher MCV.

Membranopathies and other conditions may also produce abnormal results such as α -thalassaemia, cryohydrocytosis, or Southeast Asian ovalocytosis.

Sample requirements:

Adults: 4 mL of peripheral blood collected in an EDTA vacutainer tube.

Children: 0.5-1.0 mL of peripheral blood collected in an EDTA microtainer.

A copy of CBC report and stained peripheral blood slide must be attached.

Reference range:

13.6-16.0 MCF

The test can be requested by phoning the Flow Cytometry Laboratory at 204-787-1986

DSM Contact Information:

Dr. Carmen Morales, Medical Director, Hematology and Immunology Disciplines, 204-787-4682,

cmorales@dsmanitoba.ca

Sheila Ozamoto, Technical Director, Immunology, 204-787-3407, sozamoto@dsmanitoba.ca

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