

Department:	Laboratory and Blood Bank (Haematology)		
Document:	Internal Policy and Procedure		
Title:	Red Cell Osmotic Fragility Test		
Applies To:	All Laboratory Staff		
Preparation Date:	January 07, 2025	Index No:	LB-IPP-064
Approval Date:	January 21, 2025	Version :	2
Effective Date:	February 21, 2025	Replacement No.:	LB-IPP-064 (1)
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1. PURPOSE:

- 1.1 A test to detect RBC that are more fragile than normal.

2. DEFINITONS:

N/A

3. POLICY:

- 3.1 Osmotic fragility is a special hematology test
- 3.2 OPD medical staff should discuss the case with the Hematology consultants or the section head prior to ordering the osmotic fragility.
- 3.3 Only heparinized venous blood sample
- 3.4 The test must be carried out immediately after specimen is received in the Hematology Laboratory.
- 3.5 The phlebotomist must send the specimen to Hematology as soon as it is collected.
- 3.6 A 24 hour osmotic fragility should also be performed routinely.
- 3.7 Spherocytes are osmotically fragile that rupture more easily in a hypotonic solution than normal RBCs

4. PROCEDURE:

4.1 Principle:

- 4.1.1 If red cells are placed in an isotonic solution (0.85% sodium chloride), fluid will enter the red blood cells until the cell either ruptures or equilibrium is reached
- 4.1.2 A spherocyte swells up in a hypotonic solution and ruptures much more quickly than a normal RBC or a red cell having a large surface area per volume e.g. target cells, sickle cells etc. The fragility is said to be increased or decreased when the rate of hemolysis is increased or decreased
- 4.1.3 Whole blood is added to varying concentrations of buffered sodium chloride solutions and allowed to incubate at room temperature and at 37°C. The amount of hemolysis in each saline concentration is then determined by reading the saline concentration in a spectrophotometer and compared to that of a normal control tested simultaneously

4.2 Specimen:

- 4.2.1 Specimen Type: Whole blood
- 4.2.2 Tube Type: Green Tube
- 4.2.3 Amount Required: 5ml
- 4.2.4 Delivery Arrangement: As soon as possible and should arrive in lab within 72 hours of draw
- 4.2.5 Temperature Restriction: Refrigerated
- 4.2.6 Stability: 72 hours when refrigerated
- 4.2.7 Unacceptable SP:
 - 4.2.7.1 Clerical Error
 - 4.2.7.2 Clotted

4.2.7.3 QNS

4.2.8 Time of the test: Daily morning

4.3 Procedure:

Tube No	1	2	3	4	5	6	7	8	9	10	11	12
%	0.9	0.75	0.65	0.6	0.55	0.5	0.45	0.4	0.35	0.3	0.2	0.1
Working solution (ml)	4.5	3.75	3.25	3.0	2.75	2.5	2.25	2.0	1.75	1.5	1.0	0.5
D. Water (ml)	0.5	1.25	1.75	2.0	2.25	2.5	2.75	3.0	3.25	3.5	4.0	4.5
Mixed Blood (uL)	50	50	50	50	50	50	50	50	50	50	50	50

4.3.1 Incubate the tubes at room temperature for 1 hour

4.3.2 Mix again.

4.3.3 Centrifuge for 5 minutes at 1200 rpm

4.3.4 Read and report where hemolysis began and the tube where hemolysis was complete

4.3.5 Note:

4.3.5.1 Peripheral smear should be examined before requesting Osmotic fragility test

4.3.5.2 Indicate patients date of birth on the request form

4.4 Results Interpretation

4.4.1 Normal Results:

4.4.1.1 Hemolysis began at 0.5 % NaCL

4.4.1.2 Hemolysis Complete at 0.3 % NaCl

4.4.2 Abnormal Results:

4.4.2.1 Increased Osmotic Fragility is found in

4.4.2.1.1 Hereditary Spherocytosis

4.4.2.1.2 Hemolytic Anemia (Acquired Immune)

4.4.2.1.3 Hemolytic Disease of the Newborn

4.4.2.1.4 Malaria

4.4.2.1.5 Severe Pyruvate kinase Deficiency

4.4.2.2 Decreased Osmotic Fragility occurs in

4.4.2.2.1 Iron Deficiency Anemia (Macrocytic Hypochromic)

4.4.2.2.2 Thalassemia

4.4.2.2.3 Asplenia (Postsplenectomy)

4.4.2.2.4 Liver Disease (Obstructive Jaundice)

4.4.2.2.5 Reticulocytosis

4.4.2.2.6 Hemoglobinopathy, especially Hb C, Hb S

5 MATERIALS AND EQUIPMENT:

5.1 Stock solution

5.1.1 NaCl 90 gm

5.1.2 Na₂HPO₄ 3.65 gm

5.1.3 KH₂PO₄: 2.34 gm

5.1.4 Dist. Water : complete to 1 liter

5.1.5 Store stock solutions at 2°-8° C. Stable for months

5.2 Working solution:

5.2.1 100 ml stock solution

5.2.2 + 900 ml Distilled water

6 RESPONSIBILITIES:

6.1 This policy applies to all Hematology technologists involved in this special Hematology test.

6.2 Doctor in charge

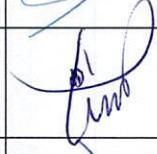
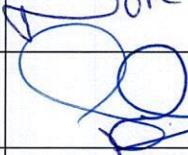
7 APPENDICES:

7.1 Table

8 REFERENCES:

- 8.1 Practical hematology ;Dacie Lewis, 7th edition 1991(p286)
- 8.2 Clinical Guide to Laboratory Tests;NW Tietz, 2ndedition 1990(94).
- 8.3 Hematology Principles & Procedures by RA. Brown. 6th Ed. 1993. Page 174-179. 3
- 8.4 Clinical Hematology & Fundamentals of Hemostasis, 2nd Ed. 1992 by F.A. Davis. Page 540-543.

9. APPROVALS:

	Name	Title	Signature	Date
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Appendix 7.

TABLE 1

Tube No	1	2	3	4	5	6	7	8	9	10	11	12
%	0.9	0.75	0.65	0.6	0.55	0.5	0.45	0.4	0.35	0.3	0.2	0.1
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D. Water (ml)	0.5	1.25	1.75	2.0	2.25	2.5	2.75	3.0	3.25	3.5	4.0	4.5
Mixed Blood (uL)	50	50	50	50	50	50	50	50	50	50	50	50