Osmotic Fragility

Place Label Here

Test Condition	Saline Concentration	Patient Result (% Hemolysis)	Reference Range Male (% Hemolysis)	Reference Range Female (% Hemolysis)
Fresh, Unincubated	0.50%		0-14	0-10
Incubated 24 hours, 37C	0.55%		35-82	42-76
	0.60%		7-56	8-45
	0.65%		0-23	0-16

Osmotic fragility interpretations

OF01 No evidence for hereditary spherocytosis. There is no abnormal RBC hemolysis observed in fresh or incubated blood specimens, and therefore no abnormal osmotic fragility.

OF02 RBC hemolysis is increased in incubated blood specimens at saline concentrations (0.55%, 0.60%, 0.65% -- pick appropriate concentrations). This result is consistent with increased osmotic fragility, which is often observed with RBC membrane disorders such as hereditary spherocytosis. Acquired conditions such as autoimmune hemolytic anemia, pregnancy or severe burns may also result in increased osmotic fragility.

OF03: RBC hemolysis is decreased in incubated blood specimens at saline concentrations (0.55%, 0.60%, 0.65% -- pick appropriate concentrations). This result is consistent with decreased osmotic fragility, which may be observed with certain RBC membrane disorders as well as with hemoglobinopathies, thalassemia and other conditions in which microcytosis is present.

I. PRINCIPLE:

In this procedure, heparin-anticoagulated blood is added to increasingly hypotonic solutions of buffered sodium chloride (0.85% to 0.00%). The amount of hemolysis at each concentration is determined by measuring the supernatants spectrophotometrically. An increased osmotic fragility is associated with hemolytic anemias in which spherocytes are present. Spherocytes have decreased surface to volume ratios and have limited ability to expand in increasingly hypotonic solutions. A decreased osmotic fragility is associated with conditions where red blood cells have large surface to volume ratios, such as thalassemia, sickle cell anemia, and conditions with target cells. In order to enhance the differentiation between normal and abnormal fragilities, especially in cases with mild spherocytosis, the blood is tested after being incubated for 24 hours in a 37° water bath.

II. REAGENTS, REAGENT PEPARATION, AND REQUIRED MATERIALS:

A. Buffered hypotonic testing solutions:

Testing solutions are made in lots containing all necessary concentrations of buffered saline solutions. New preparations of testing solutions are tested on a known normal patient or in tandem with the previous lot to validate the preparation. When new preparations of 10% buffered NaCl stock solution or testing solutions are necessary, prepare as follows:

1. 10% buffered NaCl stock solution:

a. NaCl: **90 g**
b. Na_2HPO_4: **13.65 g**
c. $NaH_2PO_4 \cdot 2H_2O$: **2.34 g**

Dissolve these three reagents in distilled water and adjust final volume to 1000 mL. Utilizing the large blue conical tubes, add 50 mL of this working stock (10% NaCl) solution to each tube and freeze at -70 C. Label these tubes as 10% NaCl with the date made. Solutions are stable indefinitely at -70C.

2. Hypotonic testing solutions

To prepare testing solutions, thaw 1 tube of 10% buffered NaCl stock solution.

a. Dilute the buffered 10% NaCl stock solution 1:10
The pH of this 1 %solution should be 7.4. Discard solution if pH not 7.4.

b. Measure into a series of bottles, labeled as indicated, the following amounts of saline and deionized water:

Solution	0.50%	0.55%	0.60%	0.65%	0.85%
mL 1% Saline	50	55	60	65	85
mL Di H_20	50	45	40	35	15

c. Pipette 3 ml of the solutions into plastic tubes with caps and label. Testing solutions are saved and are stable indefinitely at -70C.

B. Spectrophotometer capable of reading optical densities at 540 nm

III. SPECIMEN REQUIREMENTS:

Heparin blood (lithium dark green top vacutainer) is the specimen of choice.

Reject samples in **non heparin tubes,** and samples received after 12 hours of collection. Samples that arrive before 3PM are processed immediately. Samples arriving after 3PM are processed at the discretion of the special hematology supervisor. Samples arriving after 5 PM are refrigerated until the next morning when they can be processed.

a. Specimens with a low hemoglobin.

If possible check patient's hemoglobin value prior to testing. If a specimen with a low hemoglobin (<6 gm/dl) content is to be tested, an aliquot of the sample is washed with saline to simulate a 50% hematocrit.

1. Prepare aliquot and centrifuge for 3000RPM for 10 minutes
2. Remove the plasma and replace with isotonic saline
3. Mix and centrifuge at 3000RPM for 10 minutes
4. Remove the supernatant, and replace with an equal amount of saline to simulate a 50% hematocrit.
5. Test preparation in parallel to the original sample.

IV. PROCEDURE:

A. Place Specimen label in Osmotic Fragility notebook

B. If time permits the processing of the unincubated specimen, half of the sample is aliquoted for immediate testing. The rest of the sample is placed in a 37C water bath for 24 hours, noting the time placed in the bath. After 5PM the specimen is refrigerated until the next morning when testing can be initiated.

C. Remove testing solutions from freezer and allow solutions to come to room temperature. The 0.0% solution is made fresh with 3 mL of Di-H_2O
1. Concentrations tested on unincubated samples: (0.0, 0.50, and 0.85%)
2. Concentrations tested on incubated samples: (0.0, 0.55, 0.60, 0.65, and 0.85%)

D. Pipette 20 µl of blood into each tube of known saline concentration.

E. Cap tubes and mix well by gentle inversion

F. Let blood/saline mixtures sit at room temperature for exactly 15 minutes

G. Centrifuge the mixtures at 3000 rpm for 10 minutes.

H. Zero the spectrophotometer with distilled water at 540 nm.

I. Transfer the supernatants into the spectrophotometer cuvettes.

J. Measure absorbance of all the tubes and record the results on the worksheet, See appendix A.

If visible hemolysis is present in 0.85% sodium chloride concentration, the test should be repeated

K. Calculate the degree of hemolysis in each tube.-See formula in results section.

V. RESULTS:

Determine percent hemolysis of each supernatant by substituting absorbance value or optical density (OD) for the desired concentration in the following formula.

$$\% \text{ hemolysis} = \frac{O.D._{x} - O.D._{0.85\%}}{O.D._{0\%} - O.D._{0.85\%}} \times 100$$

$O.D._x$= Absorbance of solution of any given osmotic strength of sodium chloride, i.e., 0.65%

% Hemolysis values greater than 100% or less than 0% are reported as 100% or 0%, respectively

VI. INTERPRETATION:

Reported by the attending and resident.

VII. TECHNICAL NOTES:

A. A split-sample comparison is completed every 6 months.

VIII. REFERENCES:

1. Beutler, E. Williams Hematology. Fifth Edition. McGraw-Hill, Inc. New York, New York, 1995. page L46-47

2. Mckenzie, Shirlyn. Clinical Laboratory Hematology. First Edition. Pearson Prentice Hall. Upper Saddle River, New Jersey, 2004. Pages 809-810

3. ARUP Osmotic Fragility Handling
< http://www.aruplab.com/guides/ug/tests/2002257.jsp>

IX. HISTORY:

H-1 This procedure was written by P. Morris on 07-02-07.
H-2 This procedure was revised by P. Morris on 10-5-2009.
H-3 This procedure was revised by P Morris on 12-1-2010.
H-4 This procedure was revised by S. Richardson on 3/9/12.
H-5 This procedure was revised by A. Link on 3/09/2013
H-6 This procedure was revised by A. Link on 6/24/2013