

CLIN-TECH LIMITED

OSMOTIC FRAGILITY

(Based on Parpart's Method)

IVD

Product Code 610400



INTENDED USE

The osmotic fragility test may be required to confirm the suspicion of spherocytosis in a blood film.

CONTENTS

Parpart's Buffered Saline 5 x 10 mL

PROTOCOL

1. Make one vial of Parpart's buffered saline to 100 mL in a volumetric flask. This Working Solution is osmotically equivalent to 1% (%v/v) or 10 g/L sodium chloride.
2. Using the pipetting chart as a guide, prepare a range of hypotonic solutions with purified water in suitable stoppered tubes.
3. Mix thoroughly.
4. Add 50 µL well mixed, whole blood collected in lithium heparin.
5. Mix gently by inversion.
6. Stand tubes at 20°C for 30 minutes.
7. Gently remix by inversion and centrifuge at 1200 xg for 10 minutes.
8. Read the optical density of the supernatants at 540 nm using tube 1 as the blank.
9. Calculate the degree of haemolysis at each concentration using,
$$\% \text{ haemolysis} = \frac{A_{540\text{nm}} \text{ Test supernatant}}{A_{540\text{nm}} \text{ Tube 12 supernatant}} \times 100$$
10. Plot a graph of percent haemolysis against concentration of NaCl.
11. The result of the osmotic fragility test may be expressed as the concentration of NaCl causing 50% haemolysis (median corpuscular fragility, MCF).

PIPETTING CHART

Tube N ^o	1% NaCl Working Solution (mL)	Purified Water (mL)	% NaCl	Normal Range of Haemolysis
1	4.25	0.75	0.85	0%
2	3.75	1.25	0.75	0%
3	3.25	1.75	0.65	0%
4	3.00	2.00	0.60	0%
5	2.75	2.25	0.55	0%
6	2.50	2.50	0.50	0-6%
7	2.25	2.75	0.45	5-45%
8	2.00	3.00	0.40	50-95%
9	1.75	3.25	0.35	90-99%
10	1.50	3.50	0.30	97-100%
11	1.00	4.00	0.20	100%
12	0.50	4.50	0.10	100%

EXPECTED RESULTS

The normal range of MCF at pH7.4 and 20°C is 4.0 to 4.45 g/L in fresh blood samples.

A sample from a known normal subject collected at the same time as the test sample should be run in parallel as a control and each laboratory should establish its own reference intervals.

REFERENCES

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Clin-Tech Ltd
Unit G Perram Works
Merrow Lane
GUILDFORD
GU4 7BN
United Kingdom

Telephone:
+44 (0)1483 301902
Fax:
+44 (0)1483 301907
Email:
info@clin-tech.co.uk

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