



One Tube Osmotic Fragility Test (OTOFT)

Thalassaemia carriers are identified by electrophoresis or HPLC. In a population based screening for thalassaemia electrophoresis or HPLC cannot be done on every individual because of the high cost. Therefore, an initial screening test is required to curtail the number of individuals requiring confirmatory tests. An ideal screening test for thalassaemia should be cheap as well as sensitive enough to detect maximum numbers of carriers. Low MCV and MCH found in thalassaemia, although not ideal, are the most commonly used red cell indices for this purpose.

The microcytic red cells, found in thalassaemia, can also be detected by a low ionic strength solution. We have developed a stable reagent for "One Tube Osmotic Fragility Test (OTOFT)" that can effectively discriminate between individuals with significant microcytosis or the ones who do not have it.

OTOFT is a very cheap and sensitive screening test that has a very high negative predictive value for thalassaemia carriers. The test is based on the principle that microcytic red cells are more resistant to osmotic lysis when placed in a hypotonic solution than the normocytic red cells.

Our OTOFT reagent can effectively distinguish between the microcytic and the normocytic red cells. The reagent has been tested on a very large number of healthy blood donors and known thalassaemia carriers. The results of our OTOFT reagent are almost comparable to the red cell indices.

Procedure:

1. Take 2.0 ml of OTOFT Reagent in as many labelled 10 x 75 mm plastic tubes as are required.
2. Collect 2.0 ml of venous blood from each individual in EDTA and centrifuge at 3000 rpm for 1-2 minutes. A known sample of thalassaemia trait and a sample with normal red cell indices may be included as a positive and a negative control respectively.
3. Carefully draw 25µl of packed red cells from the bottom of each sample and transfer it to the plastic tube with OTOFT Reagent. While drawing packed red cells avoid taking any plasma.
4. Gently mix the contents of each plastic tube and allow to stand at room temperature for three to five minutes.
5. Place the tubes with known positive and negative samples in the OTOFT Rack.
6. Place each unknown sample tube one by one in the OTOFT Rack and take readings.

7. Record results as “Positive”, “Negative”, and “Suspicious”.



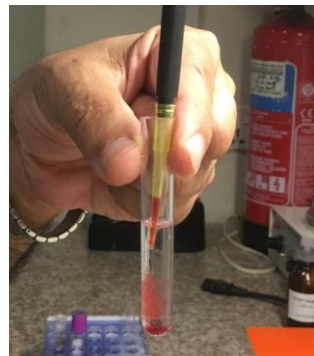
OTOFT Pack



Take 2.0 ml OTOFT Reagent



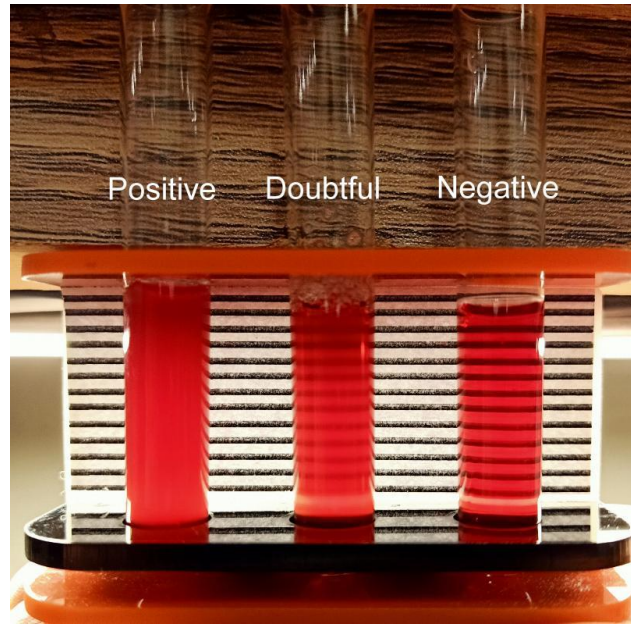
Take 25 μ l packed red cells



Add to the OTOFT Reagent



Wait for 5 minutes



Read results

Interpretation of OTOFT Results

Positive:

- A “Positive” result is completely opaque and the printed lines on the OTOFT Rack cannot be seen through the contents of the tube.
- Most likely the sample has microcytosis with MCV <75 fl. Approximately 5% of the individuals with normal MCV may also give “False Positive” OTOFT result. In any case all OTOFT Positives should go for haemoglobin electrophoresis.

Negative:

- A “Negative” result is completely transparent and the printed lines on the OTOFT Rack can be seen clearly through the contents of the tube.
- The sample does not have microcytosis with MCV in the normal range. A “False Negative” OTOFT result is very unlikely (<1%). However, individuals with silent thalassaemia alleles, like the Cap+1 mutation, usually have MCV in the normal range and may also give “False Negative” OTOFT result.

Suspicious:

- A “Suspicious” result is less clear than the “Negative” result.
- Individuals with borderline low MCV may give “Suspicious” OTOFT result. A “Suspicious” OTOFT result may also be seen as “False Positive” with MCV in the normal range. All individuals with “Suspicious” OTOFT result should go for haemoglobin electrophoresis.

OTOFT What next?

