THALVUE®

Turbidimetric Test for the screening of Beta Thalassemia trait in whole blood.

SUMMARY

Thalassemia trait is an inherited condition. The trait occurs due to the defect in the haemoglobin synthesis. There is insufficient production of one or more globin chains in the haemoglobin tetramer. There are two main types of thalassemias, alpha & beta depending upon the part of the hemoglobin molecule that is affected, the alpha chain or the beta chain.

Alpha thalassemia is caused by the insufficient production of the alpha chains. A person with one or two abnormal alpha globulin genes is said to have alpha thalassemia trait. The alpha thalassemia traits combine in different ways to produce a mild to a severe disorder in their effect on the person.

Beta thalassemia is caused by the insufficient production of the beta chains. If one of two beta genes is mutated, then the condition is known as beta thalassemia minor or trait. It causes mild anemia. In fact, the hereditary transmission of beta-thalassemia trait carriers in the heterozygous state i.e., from only one of the parents to the children may produce only a slight hematological abnormality in children (similar to those of carrier parent), without any symptoms of the disease. This fact leads to the diffusion of beta-thalassemia trait carriers among the population without any relevant damage. On the contrary the contemporaneous transmission of carriers from both the parents having microcythemia causes in 25% of the children severe anemia called beta thalassemia major or Cooley's anemia, which appears a few months after birth and seriously compromises the life of the patients, most of whom die young. Cooley's anemia has been incurable and therefore it must be prevented.

At present, the preventive measures are (a) health education intended to inform population mainly in areas in which the risk is greatest (b) pre-matrimonial diagnosis of the beta-thalassemia trait carriers (c) prenatal diagnosis of the risk in pregnancy.

For diagnosis of beta thalassemia trait, several different screening tests are being used. The prime objective of this method is to provide a diagnostic test method which overcomes the drawbacks of the screening tests used up to the present. Turbidimetry method being a quantitative method allows for measuring and recording of results unlike the routine screening methods which are subjective, therefore difficult to interpret.

PRESENTATION

REF REF	10840025
Tests	25 tests
L1	75 ml
L2	75 ml
L3	75 ml
Pack insert	1

REAGENT

Thalvue® comprises of:

a) Blank Reagent (L1): ready to use
b) Control Reagent (L2): ready to use
c) Test Reagent (L3): ready to use

PRINCIPLE

The test is based on a turbidimetric principle. Turbidity is produced by the resistance of the red blood cells to osmotic / lytic shock. The red blood cells of beta thalassemic individual (either major or minor) are more resistant to osmotic / lytic shock than a normal healthy person's red blood cells. These red blood cells do not get hemolysed easily in the reagent and can thus give rise to a higher amount of turbidity than the normal red blood cells. The extent of turbidity is then determined in comparison with a 100% turbid solution. Based on the percentage of turbidity, the individuals are screened as either positive or negative for beta thalassemia trait.

STORAGE / STABILITY

a) All the reagents are stable at room temperature
b) The shelf life of the reagents is as per the expiry date mentioned on the labels.

NOTES

a) *In-vitro* diagnostic reagents for laboratory and professional use only. Not for medicinal use.
b) Do not pipette the reagents by mouth.
c) As the reagents within lots have been matched, reagents from different lots must not be interchanged.
d) In case of multiple samples to be assayed simultaneously, only one Blank can be used for the entire series.

SPECIMEN COLLECTION AND PREPARATION

a) No special collection of the patient is necessary prior to specimen collection by approved techniques.
b) Use fresh anticoagulated (EDTA, Heparin, Sodium citrate or ACD) whole blood for testing.

c) Stored whole blood sample within 4 hours of collection can be used.
d) Do not use hemolysed or clotted specimens.

TEST PROCEDURE

Bring the samples to room temperature before use.

Assay protocol:

Wavelength : 490-510nm
Cuvette : 1cm path length
Temperature : R. T.

Method for testing:

a) Label 3 clean dry test tubes as Blank (B), Control (C), and Test (T).
b) Pipette the reagents into the labeled test tubes as follows:

Dispense	B	C	T
	(ml)	(ml)	(ml)
Blank Reagent (L1)	3.0	-	-
Control Reagent (L2)	-	3.0	-
Test Reagent (L3)	-	-	3.0
Sample (Whole blood)	-	0.01	0.01

c) Mix well and incubate at R.T. for 30 mins.
d) After incubation, thoroughly mix each tube again prior to measurement.
e) Measure the absorbances of the Control (Abs.C), and Test (Abs.T), against Blank (Abs.B).

CALCULATIONS

The degree of turbidity is calculated as follows:

$$\text{Turbidity (\%)} = \frac{\text{Abs. T}}{\text{Abs. C}} \times 100$$

INTERPRETATION OF RESULTS

Turbidity	Interpretation	Condition
< 30%	Negative	The subject is not affected by beta thalassemia trait
30-50%	Intermediate	May be a suspect carrier of beta thalassemia trait or "false positive" due to any pathological condition such as iron deficiency anemia or other types of thalassemia. This has to be ruled out clinically by other diagnostic test methods
> 50%	Positive	The subject is a carrier for beta thalassemia trait

PERFORMANCE CHARACTERISTICS

External Evaluation

Samples of eighteen suspected beta thalassemic trait (most of them were parents of transfusion dependent thalassemic children) and seventy-nine normal patients (negative for beta thalassemic trait) was evaluated with **Thalvue®** screening test at Rogi Kalyan Samiti, District Hospital, Neemuch - Bhopal. The results were further confirmed by HPLC (Biorad 'Variant') at SRL, Mumbai.

The summary of the evaluation are as follows:

Specimen data	Total No. of samples	Thalvue® (Screening test)	HPLC (Confirmatory test)
Total No. of specimens tested	97	97	97
Suspected beta thalassemic trait/carriers	18	18	18
Normal samples (negative for beta thalassemic trait)	79	71*	79

*Out of 79 Normal samples 8 samples showed intermediate results by **Thalvue®**.

Based on this evaluation:
Sensitivity of Thalvue®: 100%
Specificity of Thalvue®: 90.8%

REMARKS

a. **Thalvue**® is a sensitive and reliable screening test only, all positive samples from the screening should be further confirmed with reference methods such as electrophoresis or HPLC.
b. The test is not for neonatal screening.
c. RBC's of patients with iron deficiency anemia also resist osmotic / lytic shock and may give a false positive result.
d. Known thalassemic patients receiving regular blood transfusions have some normal red blood cells due to the same. In such cases they may show slightly lower ratios of turbidity, and hence give an erroneous result. The test is however not designed to screen such known cases.

WARRANTY

This product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

REFERENCES

a. Dacie & Lewis (2001) Practical Haematology, 9th Ed.
b. Data on File: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	Σ	Contains sufficient for <n> tests	L1	Blank Reagent
	Use by		Consult Instructions for use	LOT	Batch Number/ Lot Number	L2	Control Reagent
	Date of Manufacture	REF	Catalogue Number	IVD	*In vitro* Diagnostic Medical Device	L3	Test Reagent

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Production Site

Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

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