Transferrin Turbidimetric 5+1

IVD

Quantitative determination of Transferrin in human Serum and Plasma. Turbidimetric method.

REF TB1123 R1: 2x25 mL + R2: 1x10 mL

METHOD AND PRINCIPLE

Human Anti Transferrin (TRF) (Antibody) is added to samples containing TRF (Antigen). The developing antibody antigen reaction causes the formation of insoluble complexes that give turbidity to the solution which increases its absorbance at 340 nm. The measure of this increase is directly proportional to the amount of TRF present in the sample. Using a calibration curve with known values it is possible to determine the concentrations of TRF in the unknown samples.

CLINICAL SIGNIFICANCE

Transferrin (TFR) is a plasma protein that transports iron into the blood. Synthesized by the liver and the monocytic-macrophage system, the TFR is able to bind in a very stable, but reversible way, the iron coming from the degradation of the red blood cells and the alimentary one absorbed in the intestinal level. After having tied it to itself, the TFR carries it to the sites of use (in particular to the bone marrow) and storage (in particular to the liver). The determination of TFR in the blood evaluates the transport capacity of the iron. This test is often requested together with Sideremia and Ferritin, where iron metabolism abnormalities are suspected.

In the blood, the TFR can be found both in free form - not linked to iron (unsaturated TFR), or in form linked to iron (TFR saturated). The share of the latter coincides with the value of Sideremia.

In clinical practice, are detected Sideremia (part of TFR circulating saturated in iron), TFR, Total Iron-binding Capacity or TIBC (indirect measure of TFR's ability to bind iron), TFR Saturation (percentage of saturated TFR).

An increase in serum TFR concentrations occurs in all those situations that require an increased need for iron, for example in the presence of hemorrhages (including occult ones), sideropenic anemias, during growth and pregnancy, in hypoxemic states. TFR levels may increase following the use of hormonal contraceptives. Increases in values are also typical in the third trimester of pregnancy and in children between two and ten years.

A reduction in serum TFR concentrations occurs in cases of malnutrition, cachexia and protein deficiencies, liver disease (such as cirrhosis, hepatitis, liver failure) or renal (due to loss of protein in the urine), acute and chronic inflammatory states, hemochromatosis , repeated transfusions and in the martial overload. The concentration of TFR in the plasma varies inversely proportional to the level of the reserves. On the contrary, the saturation of TFR decreases in iron deficiencies and increases in excesses. Decreases in TFR can be observed during therapy with chloramphenicol or ACTH.

An almost total absence of TFR (<10 mg / dL) is typical of an extremely rare, autosomal recessive disorder called atransferrinemia.

REAGENT COMPOSITION

R1 (Buffer):

Tris Buffer, pH 7,5	100 mmol/L
Sodium Chloride	150 mmol/L

R2 (Antibody):

Tris Buffer, pH 7,5	100 mmol/L
Sodium Chloride	150 mmol/L
Anti-human TRF Antibody (Goat)	

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Absorbance of Blank Reagent >0.300 at 340 nm in cuvette 1 cm against water. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

Antibody Start (two-Reagents)

The Reagents are liquid and ready-to-use.

Sample Start (Mono-Reagent)

Mix 5 parts of **R1** + 1 part of **R2.** (e.g. 10 mL of R1 + 2 mL of R2) to obtain the Working Solution. Avoiding foaming, shake gently before to use.

Stability: 6 mounths at +2° to + 8°C; 5 days at +15° to + 25°C, 30 days on board (cooled rack).

SPECIMEN

Serum, Li-Heparin or EDTA Plasma.

Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly. Defrost only once. Stability in serum or plasma:

1 day at +15 to +25°C, 3 days at 2°- 8° C; 1 month at -20°C.

PROCEDURE

Wavelength:	340 nm
Temperature:	+37°C
Measurement:	against distilled water

Antisera Start procedure:

Reagent (R1)	700 µL
Sample / Calibrator /H_2O	6 µL

Mix and after 30" read Absorbance (Abs1). Then add:

Reagent (R2)	140 µL

Mix, after other 300" read Absorbance again (Abs 2).

Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

Sample Start procedure**:**

Working Solution	700 µL
Sample / Calibrator/H_2O	5 µL

Mix, read Absorbance (Abs 1) after 30".

After other 300" read Absorbance again (Abs 2).

Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

Use **MTD Diagnostics** Plasma Protein Multicalibrator

Plasma Protein Multicalibrator REF TUC1030 (3x1 mL)

Dilute calibrator in NaCl 9g/L as follows:

Dilution	1	2	3	4	5	6
CAL (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	--
Factor	0.0	0.1	0.25	0.5	0.75	1.0

Multiply the concentration of Protein Calibrator by the corresponding factor to obtain the concentration of each dilution.
To avoid to dilute the Multicalibrator, it is possible to use:

Plasma Protein Multicalibrator Set REF TUC1035 (5x1 mL)

a multipoint calibration curve in pre-filled vials, each with a specific concentration. The values are shown on the label of each vial.

CALCULATION

The analytical session can not be validated if the Δ Abs (Abs2 - Abs1) of the Blank Reagent is > 0.300 in a 1 cm cuvette of optical path at 340 nm against water.
Plot the different ΔAbs (Abs2-Abs1) absorbances against the concentration of each calibrator dilution. The concentration of the sample is calculated by interpolation of its ΔAbs (Abs2-Abs1) value on the calibration curve. For automatic calculation, use the SPLINE curve but other mathematical method can be used (Point-Point; Logit-Log 4P, etc..).

Conversion Factor: mg/dL x 10 = mg/L ; mg/L x 0,1 = mg/dL

Knowing the value of the Sideremia, it is possible to calculate the percentage of Saturation in iron of the TRF:
Saturation% = [Sideremia (µg / dL) / (TRF mg / dL x1.42)] x 100

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Plasma Protein Control Level 1 REF: TUC1040 (3 x 1 mL)
Plasma Protein Control Level 2 REF: TUC1050 (3 x 1 mL)

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user's manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

EXPECTED VALUES

Adults TFR 200 - 360 mg/dL
Adults Saturation TFR 15 – 45 %
Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples (n) = 20; Average = 304; S.D. = 5.82; CV = 1.91%
High Level: Samples (n) = 20; Average = 494; S.D. = 7.37; CV = 1.49%
ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y = 1.01x – 0.90 r = 0.998

SENSITIVITY: 3 mg/dL.
LINEARITY: 3 – 700 mg/dL.

SPECIFICITY / INTERFERENCES

No interferences was observed by Bilirubin up to 20 mg/dL, Haemoglobin up to 1000 mg/dL, Triglycerides up to 800 mg/dL.
Other substances may interfere.

PRECAUTIONS

R1 and R2 contain TRIS BUFFER 100 mmol/L – pH 7.5 - CAS 1185-53-1
H315: Causes skin irritation
H319: Causes serious eye damage
H335: May cause respiratory irritation
The antibody present in the preparation are of animal origin and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.
The products do not contain other dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.
FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

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