Reactivos GPL

Barcelona, España

CE

- TRF -

TRANSFERRIN
Immunoturbidimetry

Store at: +2+8ºC.

Presentation:
Cod. IT200 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative measurement of Transferrin.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
Transferrin is a quantitative turbidimetric test for the measurement of transferrin in human serum or plasma.
Anti-transferrin antibodies when mixed with samples containing transferrin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the transferrin concentration of the patient sample, that can be quantified by comparison from a calibrator of know transferrin concentration.

REAGENT COMPOSITION

Diluent (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.3 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human transferrin, pH 7.5. Sodium azide 0.95 g/L.
Optional	General protein calibrator.

REAGENT PREPARATION AND STABILITY
Antibody and diluent are ready to use.
Signs of reagent deterioration:
- Particles and turbidity indicate contamination or reagents deterioration.

All the components are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC and contaminations are prevented during their use.
Do not freeze; frozen reagents could change the functionality of the test.

CALIBRATION
The assay has been standardized against the Reference Material ERMDA470k/IFCC. It must be used the PROT CAL to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of specifications, and when changing the reagent lot or the instrument settings.

SPECIMEN
Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged.
Do not use highly hemolized or lipemic samples.

Discard contaminated specimen

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 340 nm (320-360)
- Cuvettes with 1 cm light path.

General laboratory equipment

PROCEDURE
CALIBRATION CURVE:
Prepare the following PROT-CAL Calibrator dilutions in ClNa 9 g/L as diluent. Multiply the concentration of the transferrin calibrator by the corresponding factor stated in table bellow to obtain the transferrin concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	6.25	12.5	25	50	100
ClNa 9 g/L (µL)	100	93.75	87.5	75	50	--
Factor	0	0.0625	0.125	0.25	0.5	1.0

MANUAL PROCEDURE
1. Bring the working reagent and the photometer to 37ºC.
2. Assay conditions:
   - Wavelength: ..... 340 nm.
   - Temperature: 37ºC
   - Cuvette light path: ..... 1 cm.
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

R_1: Diluent (µL)	800
Sample or Calibrator (µL)	10

5. Mix and read the absorbance (A_1) of the sample addition.
6. Immediately pipette into the cuvette:

R_2: Antibody (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and samples exactly 2 minutes after the R_2 addition.

CALCULATIONS
Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the transferrin concentration of each dilution. transferrin concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL
GENERAL PROTEIN CONTROLS Ref.: IT220 is recommended to monitor the performance.

Serum Controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES
Between 200 and 360 mg/dL.[2]

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
Transferrin is a plasmatic protein that has a molecular weight of 77 000. It composed by a single polypeptide chain with approximately 6% carbohydrate. It is synthesized in the liver and carries iron in serum.
Evaluation of plasma transferring levels is useful for the differential diagnosis of anemia and for monitoring its treatment. In the very common disease iron deficiency or hypochromic anemia, the transferring level is increased. If the anemia is due to a failure to incorporate iron into erythrocytes, the transferring level is normal or low but the protein is highly saturated with iron. In iron overload, the transferring concentration is normal but saturation exceeds 55% and may be as great as 90%.
Transferrin levels may, in fact, be used for assessing nutritional status. In the congenital defect atransferrinemia, very low level of transferring is accompanied by iron overload and a severe hypochromic anemia that is resistant to iron therapy. High levels of transferring occur in pregnancy and during estrogen administration.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Linearity*: Up to calibrator value (approx. 800 mg/dL) under the described assay conditions (Notes 1)
- *Limit detection*: 0 mg/dL
- *Prozone effect*: No prozone effect was detected upon 2000 mg/dL.
- *Sensitivity*: Δ 3.0 mA / mg/dL. (94 mg/dL)
- *Precision:* The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

EP5	CV (%)		
	77.02 mg/dl.	206.99 mg/dl.	377 mg/dl.
Total	5.4 %	2.5 %	5.4 %
Within Run	1 %	0.8 %	1.2 %
Between Run	1.7 %	1.3 %	2.1 %
Between Day	5 %	2 %	4.9 %

- *Accuracy:* Results obtained using this reagent (y) were compared to those obtained using the Immage from Beckman. 100 samples ranging from 50 to 700 mg/dL of TRF were assayed. The correlation coefficient (r) was 0.95 and the regression equation y=1.046x + 3.843.
  The results of the performance characteristics depend on the used analyzer.

INTERFERING SUBSTANCES
Hemoglobin (20 g/L), bilirubin (20 mg/dL), Rheumatoid factors (300 IU/mL) and lipids (9 g/L.) do not interfere. Other substances may interfere[5,6].

NOTES
1. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L. and retested again. The linearity limit depends on the sample / reagent ratio, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. GPL have instructions for many automatic instruments, available on request.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34:517-520.
3. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
4. Kreutzer HJH. J Clin Chem Clin Biochem 1976; 14: 401-406
5. Young DS.Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

CHEMELEX, S.A.
Pol. Ind. Can Castells. C / Industria 113, Nave J
08420 Canovelles –BARCELONA-
Tel- 34 93 849 17 35 Fax- 34 93 846 78 75