Audit Diagnostics

CE

TRF

TRF (Transferrin)

Turbidimetry

Quantitative determination of Transferrin (TRF)
IVD

Store 2 - 8ºC.

INTENDEND USE
The Transferrin is a quantitative turbidimetric test for the measurement of transferrin in human serum or plasma.

PRINCIPLE OF THE METHOD
Anti-transferrin antibodies when mixed with samples containing TRF, form insoluble complexes. These complexes cause an absorbance change, dependent upon the TRF concentration of the patient sample, that can be quantified by comparison from a calibrator of know TRF concentration.

CLINICAL SIGNIFICANCE
Transferrin is a plasma protein formed by a single polypeptide chain. Carbohydrates are approximately the 6% of its total weight. It is synthesized by the liver and transfers iron trough the serum.
Evaluation of plasmatic TRF levels is useful for the differential diagnosis of anemia and for monitoring its treatment. In the hypochromic anemia (iron deficiency), the TRF level is increased. When anemia appears due to a failure at incorporating iron into erythrocytes, the TRF level could be normal or low but the protein is highly saturated with iron. In iron overload, the TRF concentration is normal but saturation exceeds 55% and may reach levels of 90%.
TRF concentration may be used for assessing nutritional status. In the congenital atransferrinemia, very low level of TRF is accompanied by iron overload and a severe hypochromic anemia.
High levels of TRF may also be detected during pregnancy and estrogen administration.

REAGENTS

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 azida 0.95 g/L.
Optional	Ref: 1102003 PROT CAL.

CALIBRATION
The assay has been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). 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.

PREPARATION
Reagents: Ready to use.

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)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.
Reagent deterioration: The presence of particles and turbidity.

Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT
- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter (320 – 360 nm).

SAMPLES
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 before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE
1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 340 nm
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	10

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

Reagent R2 (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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

QUALITY CONTROL
Control sera are recommended to monitor the performance of manual and automated assay procedures. Audit Diagnostics PROT CONTROL is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[2]
Between 200 - 360 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
1. *Measurement range:* Up to 750 mg/dL under the described assay conditions. 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. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. *Detection Limit:* Values less than 1 mg/dL give non-reproducible results.
3. *Prozone effect:* No prozone effect was detected upon 2000 mg/dL
4. *Sensitivity:* Δ 3.0 mA / mg/dL (94 mg/dL).
5. *Precision:* The reagent has been tested for 20 days, using three 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%

6. *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.

INTERFERENCES
Hemoglobin (10 g/L), bilirrubin (20 mg/dL), rheumatoid factors (300 IU/mL) and lipemia (5 g/L), do not interfere. Other substances may interfere [5,6].

NOTES
1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

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