TURBITEST AA LINE

TRF

For transferrin determination in serum or plasma

SUMMARY

Transferrin (TRF) is the main plasmatic iron transport protein. Each TRF molecule has two binding sites for iron, which only binds iron oxidized form (Fe^{3+}). Transferrin is synthesized in liver and plasmatic level is mainly regulated by iron availability.

TRF plasmatic level evaluation is useful in the differential diagnosis of anemia and to best monitoring treatment. In cases of hypochromic anemia caused by iron deficiency, TRF levels increase due to synthesis increase. However, TRF iron saturation decreases due to low iron levels. On the other hand, if anemia is caused by failure of iron incorporation into hemoglobin, TRF levels decrease but the protein is highly iron saturated. Transferrin is an acute phase protein and levels decrease during inflammatory processes and malign tumors. Decreased levels may also be encountered in hematological disorders, cirrhosis, renal diseases and malnutrition. Increased levels are found during pregnancy and estrogen administration.

PRINCIPLE

TRF reacts with the specific antibody yielding insoluble immunocomplexes. Turbidity caused by such immunocomplexes is proportional to the TRF concentration present in sample and may be spectrophotometrically measured.

PROVIDED REAGENTS

A. Reagent A: 50 mM Tris buffer, pH 7.5.
B. Reagent B: monospecific anti-human transferrin antibodies (goat).

NON-PROVIDED REAGENTS

- Saline solution.
- Wiener lab.'s **Control Inmunológico nivel 1 Turbitest AA** and **Calibrador Proteínas nivel alto Turbitest AA**.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
All patient samples should be handled as capable of transmitting infection.
Use the reagents according to the working procedures for clinical laboratories.
All reagents and samples should be discarded according to current regulations.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. Do not freeze.

SAMPLE

Serum or plasma
a) Sample collection: obtain in the usual way.
b) Additives: if plasma is used as sample, employ heparin as anticoagulant.
c) Known interfering substances: do not use hemolyzed, lipemic or contaminated samples. Samples containing particles should be centrifugate prior to use. No interference has been observed with triglycerides up to 1,600 mg/dl, hemoglobin up to 1,000 mg/dl, direct bilirubin up to 24 mg/dl, total bilirubin up to 40 mg/dl and rheumatoid factor up to 520 IU/ml.
d) Stability and storage instructions: sample could be stored at 2-10ºC for up to 7 days or at -20ºC for up to 6 months. Avoid repeated freezing and thawing.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Spectrophotometric square cuvettes
- Micropipettes and pipettes for measuring stated volumes
- Kahn or hemolysis tubes
- Water bath at 37ºC
- Stopwatch

ASSAY CONDITIONS

- Wavelength: 340 nm
- Reaction temperature: 37ºC
- Reaction time: 10 minutes
- Sample volume: 30 ul
- Final reaction volume: 1230 ul

Sample and reagent volumes may proportionally change without altering the calculation factors.

PROCEDURE

CALIBRATION CURVE
In Kahn tubes, perform the following **Calibrador Proteínas nivel alto Turbitest AA** dilutions in saline solution: 1/10, 1/20, 1/40, 1/80, 1/160. Use saline solution as zero point.

Reagent A	1000 ul
Diluted Calibrador Proteínas	30 ul

Homogenize and measure each dilution absorbance at 340 nm (OD_1) taking the instrument to zero with distilled water. Then add:

Reagent B	200 ul

Homogenize and incubate for 10 minutes at 37°C, measured absorbance at 340 nm (OD_2) within ten minutes taking the instrument to zero with distilled water.
Calculate the absorbance difference ($\Delta A = OD_2 - OD_1$) for each calibrator dilution, including zero point. Sketch the absorbance difference (ΔA) on millimeter paper based on the transferrin concentration in mg/dl in the calibrator.

PROCEDURE FOR SAMPLES
Perform sample dilutions 1:10 in saline solution. In labeled Kahn tubes place:

Reagent A	1000 ul
Diluted sample	30 ul

Homogenize and measured absorbance for each dilution at 340 nm (OD_1) taking the instrument to zero with distilled water. Then add:

Reagent B	200 ul

Homogenize and incubate for 10 minutes at 37°C, measured absorbance at 340 nm (OD_2) within ten minutes taking the instrument to zero with distilled water.

CALCULATIONS
Calculate absorbance difference ($\Delta A = OD_2 - OD_1$) for each tested sample. Add this ΔA into the calibration curve to determine TRF concentration in mg/dl.
Samples with absorbance values higher than Calibrador Proteínas nivel alto's absorbance values should be diluted 1:2 with saline solution and retested. Multiply the obtained result by two.

QUALITY CONTROL METHOD
Wiener lab.'s **Control Inmunológico nivel 1** or **Control Inmunológico nivel 2 Turbitest AA**. Control is processed in the same way as samples.

REFERENCE VALUES
200 to 360 mg/dl (2.0 to 3.6 g/l)
It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION
TRF (mg/dl) x 0.01 = TRF (g/l)

PROCEDURE LIMITATIONS
See Known Interfering Substances under SAMPLE.
It is recommended to perform a complete recalibration when changing lot number reagent or when suggested by Quality Control.
Avoid contamination to preserve the integrity of the reagents. Use only thoroughly clean and dry micropipettes for measurements.

PERFORMANCE
a) Reproducibility: evaluated by a modification of protocol EP5-A from CLSI. Thus, samples from different TRF levels were tested. The intra-assay and total precision were calculated with the obtained data.

Intra-assay Precision

Mean	S.D.	C.V.
208 mg/dl	± 1.5 mg/dl	0.7%
238 mg/dl	± 2.0 mg/dl	0.9%
377 mg/dl	± 2.8 mg/dl	0.7%

Total Precision

Mean	S.D.	C.V.
208 mg/dl	± 3.8 mg/dl	1.8%
238 mg/dl	± 4.6 mg/dl	2.0%
377 mg/dl	± 6.3 mg/dl	1.7%

b) Detection limit: is the minimum analyte amount capable of being detected as sample, different than zero, and corresponds to the concentration of 3.8 mg/dl TRF.
c) Assay range: corresponds to the exactly quantifiable interval of values and ranges from 30 mg/dl 600 mg/dl transferrin.
d) Prozone effect: not detected up to TRF concentration of 3000 mg/l.

The performance results were obtained using Konelab 60i autoanalyzer. Therefore, such values may differ whenever another autoanalyzer or manual technique is used.

PARAMETERS FOR AUTOANALYZERS
Refer to the applications that are specific for each autoanalyzer.

WIENER LAB PROVIDES
60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1999703)

60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1009347)

60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1009220)

60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1009659)

REFERENCES

- Tietz Fundamentals of clinical chemistry - Burtis, C., Ashwood, E. (5th Edition) WB Saunders, 2001.
- Young, DS. - Effects of preanalytical variables on clinical laboratory test. AACC Press - Third Edition, 2007.
- Kasvosve, I; Delanghe, J. 2002 - Clin. Chem. Lab. Med. 40/10:1014, 2002.
- Bandi, ZL; Schoen, I; Bee DE. - Clin Chem. 31/10:1601, 1985.
- EP5-A Vol. 24 Nº 25 del CLSI - Evaluation of precision per-

formance of quantitative measurement methods (approved guideline - second edition).

- EP17-A2 Vol. 24 Nº 34 del CLSI - Protocols for determination of limits of detection and limits of quantitation; approved guideline.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.


This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices


Authorized representative in the European Community


"In vitro" diagnostic medical device


Contains sufficient for <n> tests


Use by

Temperature limitation (store at)


Do not freeze


Biological risks

Volume after reconstitution


Contents


Batch code


Manufactured by:


Harmful


Corrosive / Caustic


Irritant


Consult instructions for use


Calibrator


Control


Positive Control


Negative Control


Catalog number

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