1.0 Synonyms

TRF, siderophilin

2.0 Clinical Significance

Transferrin (TRFN) is a plasma protein whose primary function is to transport absorbed iron or stored iron to sites in the body involved in erythropoiesis.

TRFN is synthesized in the liver and to a small extent in the reticuloendothelial system and in endocrine glands such as testes and ovaries. Its synthesis is regulated by iron stores. When hepatocyte iron is low or absent, transferrin levels rise in proportion to the deficiency. It is the earliest indicator of iron depletion and the last indicator to return to normal when iron deficiency is corrected. Our mean serum transferrin concentration is 270 mg/dL (36 µmol/L) [based upon a RMM of 75,000], making it the plasma protein with the third highest concentration. The site of catabolism is unknown, although some TRFN is lost from the body in exfoliated intestinal mucosal cells and other cells. It has a serum half-life of 7 days. About half of the extracellular TRFN exists outside the vascular compartment in body fluids such as lymph and CSF.

All cells in the body need some iron for cell maintenance, growth, or multiplication. The plasma membrane of every cell contains TRFN receptors, and the number of receptors is related to the iron needs of the cell. Active bone marrow normoblasts have the greatest need for iron. TRFN molecules are captured by the plasma membrane, internalized, acidified, stripped of their iron and extruded iron-free and intact to continue their iron transporting function.

TRFN has been demonstrated to be a vital part of the body's non-specific defense mechanisms. It has been shown to have bacteriostatic and fungistatic properties, both of which are abolished in vitro by saturation of the binding sites by the addition of iron. The mechanism for this effect is thought to be due to the ability of transferrin to deny the micro-organism's absolute requirement for iron. It has been suggested that the ability of a microbe to acquire iron from the host and the ability of the host to deny it to the microbe constitutes a "battle of chelating agents".

The major factors affecting synthesis rates of TRFN are the level of iron stores, nutritional status, liver function and estrogen levels. Transferrin catabolism is increased in patients with hemolytic anemias, infections, and nephrosis

.

EFFECTIVE DATE	09/18/15
SUPERSEDES	02/21/11
MEDICAL DIRECTOR	Dr. James Keefe
DEPARTMENT:	**Chemistry**
POLICY NUMBER	
SUBJECT:	**Transferrin**

3.0 Principle

3.1 TRFN reagent is used to measure the TRFN concentration by a turbidimetric method. In the reaction, TRFN combines with specific antibody to form insoluble antigen-antibody complexes.

3.2 The SYNCHRON LX or DxC System automatically dilutes sample and dispenses the appropriate sample and reagent volumes into a cuvette. The ratio used is one part diluted sample to 20 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is proportional to the concentration of transferrin in the sample and is used by the System to calculate and express the transferrin concentration based upon a single-point calibration.

3.3 Chemical Reaction Scheme

Transferrin (Antigen) + Anti-transferrin Antibody ⟶ Antigen-Antibody Complex

E015263L.EPS

4.0 Total Iron Binding Capacity (TIBC)

4.1 TRFN concentration are available for use in the calculation of TIBC. The conversion factor used is based on the molar ratio of iron to TRF, and depends on the molecular weight assigned to transferrin.

Based upon a molecular mass for TRFN of 79,600 Da, therefore, 1 g/L of TRFN = 12.56 □mol/L TRFN.
Knowing that 1 molecule of TRFN can bind two atoms of iron, therefore, 1 g/L TRFN = 12.56 □mol/L TRFN = 25
(FE) is 55.845, therefore, 1 g/L TRFN = 25.12 □mol/L TIBC = 1403.1 □g/L TIBC.

Therefore, 1 g/L TRFN = 100 mg/dL TRFN = 140.32 □g/dL TIBC, resul
conversion factor of 1.40 which can be used to calculate the TIBC. A sample calculation is shown in Table 2.

TABLE 2

Serum transferrin (mg/dL) x 1.40 = total iron binding capacity (TIBC) (µg/dL)

5.0 Specimen

4.1 Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum is the preferred specimens. Acceptable anticoagulants are ammonium heparin, lithium heparin and sodium heparin. EDTA, potassium oxalate and sodium fluoride are not compatible. Whole blood and urine are not recommended for use as a sample.

4.2 Specimen Storage and Stability

4.2.1 Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

4.2.2 Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

4.3 Sample Preparation

Sample preparation is not required. All samples are diluted automatically by the system using the DIL1 Cartridge.

4.4 Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample.

4.5 Criteria for unacceptable Specimen
Refer to the “Type of Specimen” and “Interference” section of this procedure for information on unacceptable specimens.

5.0 Reagents

5.1 Each kit contains the following items:

5.1.1 Two TRFN Reagent Cartridges (2 x 150 tests)

5.1.2 One lot-specific Parameter Card

5.2 Volumes per Test

Table 1

Sample Dilution Volumes		
	Sample Volume	10 µL
	Diluent Volume	190 µL
Diluted Sample Volume (1:20 dilution)		6 µL
Total Reagent Volume		225 µL
Cartridge Volumes		
	A	200 µL
	B	25 µL
	C	– –

5.3 Reactive Ingredients

Table 2

Reagent Constituents	
Reaction Buffer	43.0 mL
Antibody Monospecific for human Transferrin (Goat)	6.2 mL
Also non-reactive chemicals necessary for optimal system performance.	

5.4 Caution

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

5.5 Materials needed but not supplied with Reagent Kit

5.5.1 SYNCHRON Cal 1

5.5.2 DIL 1 Cartridge

5.5.3 At least two levels of control material

5.6 Reagent Preparation

No preparation is required.

5.7 Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria.

5.8 Reagent Storage and Stability

5.8.1 TRFN reagent when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

5.8.2 DIL 1 stored unopened at room temperature is stable until the expiration date indicated on each cartridge. Once opened, DIL 1 is stable for 60 days on Instrument or until the expiration date, if sooner.

6.0 Instrument and Equipment

Beckman Coulter LXi or DxC.

7.0 Procedure

7.1 Calibration

7.1.1 Calibrator

SYNCHRON Cal 1

7.1.2 Calibrator Preparation

No preparation is required.

7.1.3 Calibrator Storage and Stability
SYNCHRON Cal 1 is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C. DO NOT FREEZE.

7.1.4 Caution

7.1.4.1 Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States

7.1.4.2 Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.

7.1.5 Calibration Information

7.1.5.1 The system must have a lot-specific parameter card and a valid calibration adjustment in memory before controls or patient samples can be run.

7.1.5.2 under typical operating conditions the TRFN reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the LXi or DxC Operations Manual. This assay has within-lot calibration available. Refer to the Operations Manual for information on this feature.

7.1.5.3 For detailed calibration instructions, refer to the Operations Manual.

7.1.5.4 The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the Diagnostics and Troubleshooting Manual.

7.2 Testing Procedures

7.2.1 If necessary, load the reagent onto the system as directed in the Operations Manual. A lot-specific parameter card must be loaded one time for each lot.

7.2.2 After reagent load is completed, calibration may be required. Refer to the

Operations Manual for details of the calibration procedure.

7.2.3 Program samples and controls for analysis as directed in the Operations Manual.

7.2.4 After loading samples and controls onto the system, follow the protocols for system operation as directed in the Operations Manual.

8.0 Quality Control

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the Operations Manual. At this facility, the Biorad Immunology Controls are used. The controls should be prepared and used in accordance with the package inserts.

9.0 Calculations

SYNCHRON LX or DxC System performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

10.0 Reporting Results

10.1 Reference Intervals (Ranges)

The reference intervals listed below were taken from literature and a Beckman study performed on SYNCHRON Systems and verified at this facility during validation.

Table 3: Reference Intervals

Intervals	Sample Type	Conventional Units
Literature	Serum or Plasma (Male)	215 – 365 mg/dL
	Serum or Plasma (Female)	250 – 380 mg/dL
SYNCHRON and this laboratory	Serum or Plasma (Male)	180 – 329 mg/dL
	Serum or Plasma (Female)	192 – 382 mg/dL

11.0 Limitations

None identified.

12.0 Interferences

The following substances were tested for interference with this methodology:

Table 4: Interferences

Substance	Source	Level tested	Observed Effect
Bilirubin (unconjugated)	Bovine	30 mg/dL	Not Significant (NSI)
Hemoglobin	RBC hemolysate	500 mg/dL	NSI
Lipemia	Intralipid[d]	400 mg/dL	NSI

13.0 Analytic Range (linearity)

The SYNCHRON Systems method for the determination of this analyte provides the following analytical ranges:

Table 5: Analytical Range

Sample Type	Conventional Units
Serum or Plasma	70 – 850 mg/dL

14.0 Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for TRFN determination is 70 mg/dL.

15.0 Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Table 6

Serum or Plasma (in the range of 98.6 to 502.0 mg/dL):		
	Y (SYNCHRON LX Systems)	= 1.041X - 20.7
	N	= 80
	Mean (SYNCHRON LX Systems)	= 216.9
	Mean (Array®)	= 228.2
	Correlation Coefficient (r)	= 0.9844

16.0 Precision

16.1 A properly operating SYNCHRON System should exhibit precision values less than or equal to the following:

Table 7: Precision Values

Type of Precision	Sample Type	1SD	Changeover value	% CV
		mg/dL	g/L	
Within-run	Serum or Plasma	5.0	1.00	5.0
Total	Serum or Plasma	7.5	1.00	7.5

16.2 These degrees of precision and equivalency were obtained in typical testing procedures on the SYNCHRON System and are not intended to represent the performance specifications for this reagent.

16.3 For details of precision studies done at this facility, refer to instrument validation data.

16.4 For more detailed information on SYNCHRON LX and DxC Systems, refer to the appropriate SYNCHRON System manual.

17.0 Backup Procedure

The LXi and DxC serve as backup for each other. For technical assistance, call Beckman Coulter at 800-854-3633.

18.0 References

18.1 Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", Textbook of Clinical Chemistry, 2nd Edition, W. B. Saunders, Philadelphia, PA.

18.2 Tietz, N. W., ed., Fundamentals of Clinical Chemistry, 5th Edition, W. B. Saunders, Philadelphia, PA (2001).

18.3 Beckman Coulter Synchron Chemistry Information Manual, Volume 3.

18.4 Beckman Coulter LXi Operations Manual.

18.5 Beckman Coulter DxC Operations Manual

Procedure Prepared by: Thomas Christopher Date: 09/18/15

Procedure Approved by: Jaffar Kermani Date: 9/18/2015