



Laboratory Procedure Manual

Analyte: **Tina-quant Soluble Transferrin Receptor**

Matrix: **Serum**

Method: **Particle enhanced immunoturbidimetric assay**

Method No: 4042.07

Revised: June 2020

as performed by: Nutritional Biomarkers Branch (NBB)
Division of Laboratory Sciences (DLS)
National Center for Environmental Health (NCEH)

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
TFR_J	LBXTFR	Transferrin receptor

1. Summary of Clinical Relevance and Principle

A. Clinical Relevance

The soluble transferrin receptor (sTfR) is the truncated form of the transmembrane receptor which mediates the cellular uptake of iron on most mammalian cells [1]. Its concentration in serum is affected by the cellular iron demands and the erythroid proliferation rate. Serum sTfR concentration increases in states of depletion of the iron functional pool and during activated erythropoiesis as seen in polycythemia, hemolytic anemia, thalassemia, hereditary spherocytosis, and vitamin B12 deficiency [2]. The measurement of sTfR is a powerful tool for the diagnosis of iron deficiency or for monitoring erythropoiesis [1,3]. In contrast to ferritin, sTfR concentrations are not affected by acute-phase reactions, acute liver function disorders, or malignant tumors [4]. sTfR can also be used to assess iron status in epidemiologic studies and was added to the battery of iron status measures included in the National Health and Nutrition Examination Survey (NHANES) in 2003 [5]. Because ferritin reflects iron stores and sTfR describes functional iron status after iron stores have been depleted, the ratio of sTfR to ferritin can be used to better describe the full range of iron status [6-9]. The ratio can also help to distinguish iron deficiency anemia from anemia of chronic disease [10].

B. Test Principle

Tina-quant soluble transferrin receptor (sTfR) is a particle enhanced immunoturbidimetric assay that uses Roche kits on the Cobas® c501 clinical analyzer. Latex particles coated with anti-sTfR antibodies react with the antigen in the sample to form an antigen/antibody complex. Following agglutination, the precipitate is determined photometrically [11].

2. Safety Precautions

Consider all plasma specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipet tips, autosampler vials, gloves, etc.) that contact plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution or similar disinfectant when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood. Reagents and solvents used in this study include those listed in Section 6. Safety data sheets (SDSs) for these chemicals are readily accessible as hard copies in the lab. If needed, SDSs for other chemicals can be viewed at <http://www.ilpi.com/msds/index.html> or at <http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html>.

3. Computerization; Data System Management

- (A) During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.
- (B) Calculation of sTfR concentration is accomplished with the software on the Cobas® 6000 and generated data are transferred to the DLS network where it is saved. The result file is imported into a database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See "SOP

Computerization and Data System Management" for a step-by-step description of data transfer, review and approval.

- (C) NHANES data is transmitted electronically on a regular basis (approximately weekly for 3-week turnaround analytes). Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file. NCHS makes arrangements for the abnormal report notifications to the NCHS Survey Physician.
- (D) The data file and results file from the instrument workstation are typically backed up daily to a USB and/or DVD for long-term storage. This is the responsibility of the analyst under the guidance of the project lead person. Files stored on the DLS network are automatically backed up nightly by ITSO support staff.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

- (A) Centrifuge samples containing precipitate before performing the assay.
- (B) Specimens for TFR analysis may be fresh or frozen serum or plasma. Serum specimens may be collected with regular red-top Vacutainers or tubes containing separating gel and plasma specimens may be collected with Li-heparin as an anticoagulant.
- (C) The appropriate amount of serum or plasma is dispensed into a Nalge cryovial or other plastic screw-capped vial labeled with the participant's ID. A 500- μ L sample of serum or plasma is preferable to allow for repeat analyses; a minimum volume of 150 μ L is required for pipetting into the sample cup.
- (D) Specimens collected in the field should be kept cold and protected from light. After processing, specimens should be frozen and shipped on dry ice by overnight mail. Once received, samples should be stored at ≤ -20 °C until analyzed. For long term storage, specimens should always be frozen at -70 °C. TFR is stable for up to 2 weeks at $2-8$ °C [12] or 4 weeks if stored at ≤ -20 °C [11]. Multiple freeze/thaw cycles are generally not recommended, however samples can withstand up to 3 freeze/thaw cycles [12,13].
- (E) Ensure patient samples, calibrators and QC are at ambient temperature ($20-25$ °C) before measurement. Once the samples, calibrators and controls are loaded on the analyzers, they should be measured within 2 hours because of possible evaporation effects.
- (F) Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The protocol discusses collection and transport of specimens and the special equipment required. If there is more than one test of interest in the specimen and it needs to be divided, the appropriate amount of blood, serum or plasma should be transferred into a sterile Nalgene cryovial labeled with the participant's ID; avoid cross contamination.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

All reagents are supplied by Roche Diagnostics in liquid form ready for use in a cobas c pack. If the entire reagent pack is not used in one run, store the kit at 2-8 °C on the analyzer until the expiration date of the reagent is reached or the reagent has been on the analyzer for 12 weeks, whichever comes first.

B. Standards Preparation

Preciset sTfR, supplied by Roche Diagnostics, consists of 5 liquid ready-for-use calibrators based on a human serum/bovine serum albumin matrix. Water is used as the zero standard. The standard concentrations need to be installed on the instrument before use. Store the unopened standards at 2-8 °C until the expiration date of the kit. Once opened, calibrators are stable for 2 weeks at 2-8 °C.

C. Preparation of Quality Control Materials

1) Roche QC pools

Commercially prepared quality control material can be purchased from Roche Diagnostics in two levels (sTfR Control I and sTfR Control II). Controls are in liquid form and ready to use. Unopened controls are stable at 2-8 °C until the expiration date. Once opened, controls are stable for 4 weeks at 2-8 °C. Controls should be allowed to come to room temperature before analyzing. Control values are lot specific and values for each new lot should be entered into the Roche Cobas® 6000 before analyzing a new lot number of control.

2) CDC QC pools

Quality control materials for this assay are prepared in-house from blood products acquired from blood banks or from other volunteer blood donors. After screening the pools for sTfR, the serum is pooled to obtain the desired QC levels. All pools are filtered through gauze to remove debris before being dispensed. Serum (usually 750 µL) is aliquoted into labeled 2.0-mL Nalgene cryovials, capped, and stored at -70 °C. The QC pools are stable for at least 3 years.

The QC limits for all pools are established by analyzing duplicates of each pool for at least 20 consecutive runs.

D. Other Materials

The following materials are available from the manufacturer (Roche Diagnostics):

- (1) Sample racks
- (2) Sample cups (Standard or micro)
- (3) Reaction Cells
- (4) Wash solutions
- (5) Eco-D
- (6) Lamp

(7) Diluent, NaCl 9%

(8) PreciSet sTfR Calibrators (2-6) and Controls (sTfR 1 and sTfR 2)

E. Instrumentation

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.) a product listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed.

(1) Roche Cobas® 6000 system (Roche Diagnostics, Indianapolis, IN)

(2) Daigger Vortex Genie 2 (VWR, Suwanee, GA)

(3) Eppendorf micropipet and tips (Brinkmann Instruments Co., Westbury, NY)

7. Calibration and Calibration Verification Procedures

For commercial kit assays, calibration procedures recommended by the manufacturer are followed.

Roche Diagnostics standardized this method against an in-house reference preparation.

Six calibrators are required for sTfR calibration; Preciset sTfR (S2-S6) and H₂O (S1). Allow the calibrators to reach room temperature before use and mix carefully to ensure homogeneity. Avoid the formation of foam. The concentrations of the components are lot-specific and must be entered in the analyzer before use. The exact calibrator values are encoded in electronic files sent via the cobas link to the analyzers.

Preciset sTfR calibrator is stable unopened up to the stated expiration date at 2–8 °C. After opening, it is stable for 2 weeks when stored at 2–8 °C, provided that dispensing of the calibrator takes place without microbial contamination. Do not freeze.

Calibration is recommended as follows:

- Full calibration daily and after reagent lot change
- Full calibration following any dispenses system component replacement or any major maintenance performed on the instrument, i.e. lamp, reaction cells.

Please refer to the Roche *Cobas 6000® Operator's Manual* and the “**SOP Cobas 6000 Calibration and QC**” for additional details.

Calibration verification is conducted at least twice a year using international reference materials. For details, see “**4042_SOP Calibration and Calibration Verification sTfR**”.

NIBSC reference reagent 07/202, recombinant soluble transferrin receptor, was released in 2009 with an assigned value of 21.7 mg/L or 303 nmol/L when reconstituted with 0.50 mL distilled or deionized water [14]. These values apply to free rsTfR monomer. The Roche assay measures 60 mg/L for this material.

The laboratory performed in-house proficiency testing at least twice a year until December 2011.

In April 2012, the laboratory began participating in the College of American Pathologist (CAP) external proficiency testing survey:

- twice a year in the CAP sTfR survey

For general information on the handling, analysis, review, and reporting of proficiency testing materials, see “NBB_SOP Proficiency Testing Procedure”.

As this assay must be performed according to the manufacturer’s specifications, none of the parameters can be altered. Therefore, ruggedness testing cannot be performed for this assay.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

- (1) Allow frozen serum/plasma (patient samples) to reach ambient temperature.
- (2) Ensure that the amount of reagents, diluent, and wash solutions are adequate for the amount of samples to be run. You may place more than one bottle of reagent at a time on the analyzer; however, avoid using more than one lot number of reagent for a single run.
- (3) Make sure the analyzer and/or tests required are not masked.
- (4) Check to see if calibration is required for the tests that will be run.
- (5) If running the same tests on all samples, go to the “Start” global button and set the “default profile”.
- (6) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.
- (7) Perform the required maintenance on the c501 system

B. Instrument Maintenance

- (1) The c501 system maintenance consists of daily, weekly, monthly, quarterly and as needed maintenance.
- (2) Daily maintenance should be performed at the start of each 8-hour shift, or more frequently, if necessary.
- (3) For additional maintenance requirements, refer to the instrument maintenance logs. For detailed, step by step instructions, refer to the Roche Cobas® 6000 Operator’s Manual.

C. Preparing the Run

One run is defined as 100 samples or less.

Each run must contain sTfR QC at the beginning of the run before patient samples and at the end of each run.

When performing small runs or confirmation (repeat) runs, all levels of sTfR QC pools must be run in duplicate.

NOTE: Before starting a new run, backup all previous test results and clear the “data review” screen.

- (1) Thoroughly mix all calibrators, QC and patient samples before pipetting. Visually check for any unusual sample volume, specimen color or debris/precipitate.

- (2) Prior to loading samples on the instrument, ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles if necessary.
- (3) For a calibration run, use **black** calibrator racks. Nonbarcoded calibrators must be pipetted into sample cups and placed in their assigned positions in **black** calibrator racks. When calibration is completed, the results will be printed.
- (4) To run QC, use the **white** QC racks. For non-barcoded Roche QC and CDC QC, pipette 150 µL of each QC into a sample cup and place atop the tube in the assigned control position of the **white** QC racks. When the instrument is started, it will automatically run the correct tests on the preprogrammed QC and print the results.
- (5) To run patient samples, use the **gray** sample racks. Place empty sample cups in barcode labeled 13 X 75 tubes in **gray** sample racks and pipette 150 µL of the serum samples into the sample cup. Pipette 20-25 samples at a time and immediately place the racks on the input buffer tray. **Gray racks with yellow stickers are for urine samples only.** Patient results do not print until requested.

D. Initiating a Run

Do not load samples on the input tray if the green light is flashing.

When the instrument starts, it will run the default profile on all samples unless programmed differently prior to loading.

- (1) Once the calibrator, control or sample racks are loaded on the input tray, they should be measured within 2 hours because of possible evaporation effects. Calibration and QC checks must be completed before pipetting patient samples.
- (2) For detailed, step by step instructions, refer to “**SOP Cobas 6000 Operation**” or the Roche *Cobas 6000® Operator’s Manual*.

E. Processing and Reporting a Run

- (1) The Roche Cobas 6000 Control Module is used to review data and check for samples that need to be diluted or repeated for confirmation. The LIMS database is used for additional levels of data review by the analyst, project lead, QA officer, and supervisor and for data reporting.
- (2) For more detailed information, refer to Section 3 and the “SOP Computerization and Data System Management”.

F. Special Method Notes

The system can be turned off for the weekend or extended holidays or when indicated by maintenance procedure or error code. Refer to the Roche *Cobas 6000® Operator’s Manual* for instructions.

G. Calculations

All calculations are performed by the Cobas® 6000 software system using a machine-stored calibration curve.

H. CDC Modifications

The method is run exactly as stipulated by Roche Diagnostics; CDC has introduced no modifications.

9. Reportable Range of Results (AMR – analytical measurement range)

The maximum reportable range is dependent on the concentration of the highest standard. For the purposes of CDC reporting, we will use a reportable range of 0.5-40 mg/L. Samples with values $>5.5 - \leq 40$ mg/L are automatically reanalyzed using routine (normal) sample volume. Samples >40 mg/L are diluted via automatic rerun and have an extended reportable range of 0.5-80 mg/L. There is no known maximum acceptable dilution. When possible, avoid small volume pipetting and minimize use of serial dilutions when generating diluted sample results.

10. Quality Control (QC) Procedures

A. Blind Quality Controls

Blind QC specimens can be inserted into the mix of patient specimens. These QC specimens are generally prepared at two levels that would be encountered in patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed.

Alternately, open label blind QC specimens can be used where the analyst knows that the sample is a blind QC, but they do not know what pool the sample is from. Open label blind QCs are only used if one can choose from at least 6 different pools and the analyte concentrations are similar to those found in patient samples.

B. Bench Quality Controls

Bench QC specimens are prepared from three serum pools, which represent low, medium, and high levels of sTfR. These pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

Three QC pools per run with two or more QC results (replicates) per pool:

- (1) If all three QC run means are within $2 S_m$ limits and individual results are within $2 S_i$ limits, accept the run
- (2) If 1 of the 3 QC run means is outside a $2 S_m$ limit – reject run if:
 - a) 1 3S Rule—Run mean is outside a $3 S_m$ limit or
 - b) 2 2S Rule—Two or more of the three run means are outside the same $2 S_m$ limit or
 - c) 10 X-bar Rule—Current and previous nine run means are on the same side of the characterization mean
- (3) If one of the six QC individual results is outside a $2 S_i$ limit – reject run if:
 - a) Outlier – One individual result is beyond the characterization mean $\pm 4 S_i$ or
 - b) R 4S Rule—Two or more of the within-run ranges in the same run exceed $4 S_w$ (i.e. 95 percent range limit)

S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

S_m = Standard deviation of the run means (the limits are shown on the chart).

S_w = Within-run standard deviation (the limits are not shown on the chart).

The QC results are checked after each run using of a multi-rule quality control program [15]. A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC. The initial limits are established by analyzing pool material in 20 consecutive runs and then are reevaluated periodically. When necessary, limits are updated to include more runs.

While a study is in progress, QC results are stored in a LIMS database. For runs that are not imported into the database (i.e., R&D, troubleshooting, research-type runs), QC results are stored electronically in the analyte-specific folder on the DLS network.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

- (A) Check to make sure that the hardware is functioning properly.
- (B) Recalibrate the instrument.
- (C) Analyze reference material.
- (D) If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.
- (E) Call the Roche "hotline" or service engineer.
- (F) Do not report analytical results for runs not in statistical control.

12. Limitations of Method; Interfering Substances and Conditions

- (A) Only one freeze/thaw cycle is recommended. Samples containing particulate matter should be centrifuged and the material removed before analysis.
- (B) Results are not known to be affected by icteric, hemolyzed or lipemic specimens. In very rare cases gammopathy, in particular type IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.
- (C) As for any assay employing mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes [11].
- (D) Special Wash Programming (Carryover evasion) is mandatory when certain test combinations are run together on Roche/Hitachi analyzers. Refer to the latest version of the Special Wash Programming document (located on MyLabOnline website) and the Roche *Cobas 6000*® *Operator's Manual* for special wash instructions.

13. Reference Ranges (Normal Values)

sTfR reference ranges are assay-specific. The expected ranges reported in the Roche Tina-quant sTfR package insert [11,16] are 2.2-5.0 mg/L for men and 1.9-4.4 mg/L for women.

Reference ranges for the U.S. population generated with the Tina-quant sTfR assay for NHANES 2003-2006 and published in the Second Nutrition Report [17] are shown below (also available by population subgroups):

Serum sTfR – children 1-5 y: 2.84-6.67 mg/L (2.5th -97.5th percentile; *n* = 1,375)

Serum sTfR – women 12-19 y: 2.12-6.47 mg/L (2.5th -97.5th percentile; *n* = 1,968)

Serum sTfR – women 20-39 y: 1.91-6.97 mg/L (2.5th -97.5th percentile; *n* = 1,761)

Serum sTfR – women 40-49 y: 1.91-7.96 mg/L (2.5th -97.5th percentile; *n* = 752)

14. Critical Call Results (“Panic Values”)

A sTfR result that is >5.5 mg/L is usually indicative of iron deficiency and requires follow-up (repeat analysis for confirmation of high sTfR level).

Since survey data are transmitted several times weekly to Westat, abnormal reports are automatically forwarded to the NCHS survey physician for follow-up. For smaller, non-NHANES studies, abnormal values are identified to the study principal investigator. Emails sent concerning abnormal results are maintained by the supervisor for the duration of the study. Most of these studies are epidemiological in nature.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach room temperature during preparation. After analysis, the unused portion of the patient specimen is then returned to frozen storage (typically ≤ -70 °C) as soon as possible.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

If the analytical system fails, we recommend that the specimens be stored at -20 °C until the analytical system is restored to functionality.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, generally through electronic mail or via ftp site.

For NHANES 1999+, all data are reported electronically weekly to Westat who then transfer the results to NCHS. For some smaller studies, hard copies of a data report are sent, as well as the results in electronic format.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

A LIMS database is used to keep records and track specimens for NHANES 1999+. If analyses are performed for smaller, non-NHANES studies, records may be kept in Excel files on the DLS network.

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies are retained for at least 1 year after results have been reported and may then be returned or discarded at the request of the principal investigator. Very little residual material will be available after NHANES analyses are completed, however residual serum is retained for at least 2 year after results have been publicly released; at that point, samples with sufficient volume (>0.2 mL) are returned to NHANES and samples with insufficient volume may be autoclaved.

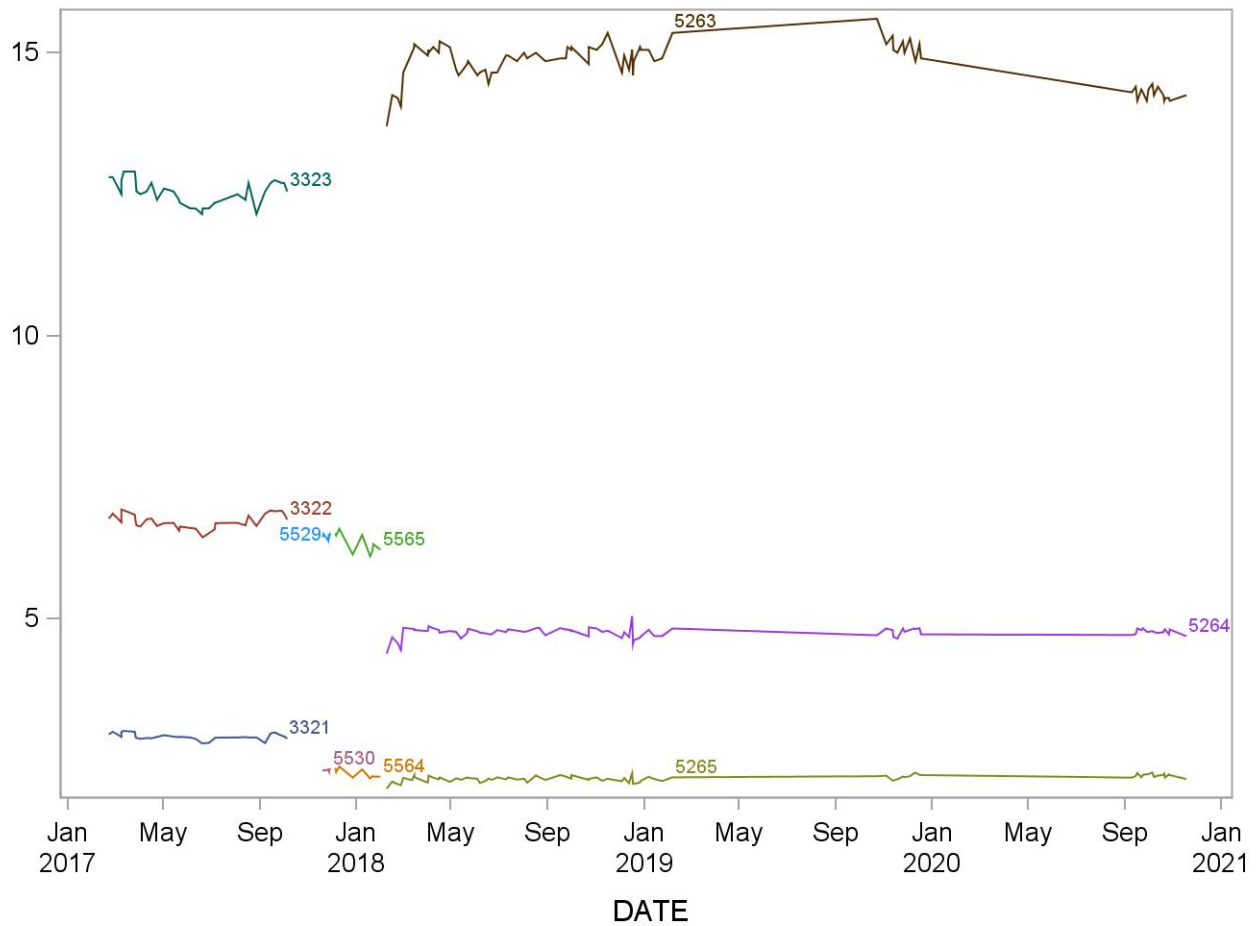
The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at -70 °C. The specimen ID is read off of the vial by a barcode reader used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the result file is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping records of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. In general, these are documented using codes in the LIMS.

19. Summary Statistics and QC Chart

Please see following page.

2017-2018 Summary Statistics and QC Chart LBXTFR (Transferrin receptor (mg/L))

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3321	33	22FEB17	06OCT17	2.913	0.057	2.0
3322	33	22FEB17	06OCT17	6.716	0.136	2.0
3323	33	22FEB17	06OCT17	12.532	0.214	1.7
5529	7	20NOV17	30NOV17	6.464	0.045	0.7
5530	7	20NOV17	30NOV17	2.334	0.017	0.7
5564	9	06DEC17	01FEB18	2.268	0.080	3.5
5565	9	06DEC17	01FEB18	6.338	0.181	2.9
5263	84	09FEB18	18NOV20	14.780	0.373	2.5
5264	84	09FEB18	18NOV20	4.761	0.089	1.9
5265	84	09FEB18	18NOV20	2.185	0.056	2.5



20. Method Performance Documentation

Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in **Appendix A** of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

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Acknowledgements

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Appendix A: Method Performance Documentation

Accuracy compared to Reference Material - fill in yellow shaded cells												
Mean concentration should be within $\pm 15\%$ of the nominal value except at 3*LOD, where it should be within $\pm 20\%$												
Method name:	Soluble Transferrin Receptor											
Method #:	4042											
Matrix:	Serum											
Units:	mg/L											
Reference material:	NIBSC 07/202											
Analyte:	TFR											
Reference material	Replicate	Nominal value	Measured concentration									Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)		
Level 1	1	60.5	63.43	59.72	63.54	65.32	63.54	59.35	10.20	17.18	-1.9	
	2		60.48	64.25	34.55							
Level 2	1	30.25	28.99	29.76	29.91	30.99	29.91	30.04	0.70	2.32	-0.7	
	2		28.98	30.58	30.19	30.93	30.19					
Level 3	1	15.13	15.32	15.98	15.77	16.37	15.77	15.83	0.33	2.09	4.6	
	2		15.45	16.23	15.67	16.05	15.67					
Level 4	1	7.56	8.02	8.43	8.24	8.57	8.24	8.28	0.19	2.30	9.6	
	2		8.07	8.33	8.18	8.57	8.18					
Level 5	1	3.78	4.15	4.41	4.25	4.41	4.25	4.27	0.12	2.86	12.9	
	2		4.06	4.33	4.20	4.42	4.20					

Precision - fill in yellow shaded cells						
Total relative standard deviation should be $\leq 15\%$ ($CV \leq 15\%$)						
Method name:	Soluble Transferrin Receptor					
Method #:	4042					
Matrix:	Serum					
Units:	mg/L					
Analyte:	TFR					
LS13460						
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12.60	12.60	12.60	0	0	317.52
2	12.5	12.3	12.40	0.01	0.01	307.52
3	12.4	12.4	12.40	0	0	307.52
4	12.6	12.8	12.70	0.01	0.01	322.58
5	12.8	12.3	12.55	0.0625	0.0625	315.005
6	12.5	12.5	12.50	0	0	312.5
7	12.7	12.4	12.55	0.0225	0.0225	315.005
8	12.7	12.8	12.75	0.0025	0.0025	325.125
9	13.10	12.70	12.90	0.04	0.04	332.82
10	12.8	13	12.90	0.01	0.01	332.82
Grand sum	252.5	Grand mean	12.625			
	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)		
Within Run	0.315	0.0315	0.177482393	1.41		
Between Run	0.6025	0.066944444	0.133124837	1.05		
Total	0.9175		0.221860817	1.76		
HS13462						
Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.94	2.95	2.95	2.5E-05	2.5E-05	17.34605
2	2.91	2.91	2.91	0	0	16.9362
3	2.93	2.90	2.92	0.000225	0.000225	16.99445
4	2.85	2.93	2.89	0.0016	0.0016	16.7042
5	2.91	2.88	2.90	0.000225	0.000225	16.76205
6	2.88	2.88	2.88	0	0	16.5888
7	2.87	2.92	2.90	0.000625	0.000625	16.76205
8	2.89	2.93	2.91	0.0004	0.0004	16.9362
9	3.00	3.01	3.01	2.5E-05	2.5E-05	18.06005
10	2.98	2.98	2.98	0	0	17.7608
Grand sum	58.45	Grand mean	2.9225			
	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)		
Within Run	0.00625	0.000625	0.025	0.86		
Between Run	0.030725	0.003413889	0.037342261	1.28		
Total	0.036975		0.044938229	1.54		

Stability - fill in yellow shaded cells										
The initial measurement can be from the same day for all stability experiments.										
Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions										
Describe condition: three times frozen at -70°C and three times thawed (4 hrs) at room temperature (3 freeze-thaw cycles)										
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)										
Describe condition: QC material stored at room temperature for 6 hrs, refrozen and thawed										
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler										
Describe condition: QC material aliquoted into sample cups and stored on instrument for 2 hrs at room temperature before analysis										
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis										
Describe condition: QC material stored at -70°C for 2 years and 4 years										
All stability sample results should be within ±15% of nominal concentration										
Method name: Soluble Transferrin Receptor										
Method #: 4042										
Matrix: Serum										
Units: mg/L										
Analyte: TFR										
Low QC material LS13460							2015	2017	2019	
8/1/2017 10/31/19	Initial measurement	Three freeze- thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	2015 measurement	Long-term stability	Long-term stability	
Replicate 1	12.34	11.93	12.34	11.87	12.34	12.51	12.52	12.34	12.38	
Replicate 2	11.85	12.06	11.85	11.97	11.85	12.96	12.75	11.85	12.3	
Replicate 3	12.4	12.08	12.4	12.08	12.4	12.83	12.39	12.4	12.47	
Mean	12.20	12.02	12.20	11.97	12.20	12.77	12.55	12.20	12.38	
% difference from initial measurement	--	-1.4	--	-1.8	--	4.7	--	-2.8	-1.40	
High QC material HS13462							2015	2017	2019	
8/1/2017 10/31/19	Initial measurement	Three freeze- thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	2015 measurement	Long-term stability	Long-term stability	
Replicate 1	2.99	2.86	2.99	2.76	2.99	2.8	2.83	2.99	2.49	
Replicate 2	2.71	2.79	2.71	2.78	2.71	2.99	2.91	2.71	2.58	
Replicate 3	2.98	2.86	2.98	2.8	2.98	2.93	2.88	2.98	2.55	
Mean	2.89	2.84	2.89	2.78	2.89	2.91	2.87	2.89	2.54	
% difference from initial measurement	--	-2.0	--	-3.9	--	0.5	--	0.7	-11.6	

LOD, specificity and fit for intended use - fill in yellow shaded cells			
Method name:	Soluble Transferrin Receptor		
Method #:	4042		
Matrix:	Serum		
Units:	mg/L		
Analyte:	TFR		
Kit assay is FDA approved- LOD and interference information are provided by the manufacturer			
Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
	TFR	0.5	Antibodies are specific for sTfR. There is no cross-reactivity with diferrotransferrin, apotransferrin or ferritin under the assay conditions. Erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies.