

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k080634

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Soluble Transferrin Receptor (sTfR)

sTfR / log Ferritin Index

**D. Type of Test:**

Automated chemiluminescent immunoassay

**E. Applicant:**

Beckman Coulter, Inc.

**F. Proprietary and Established Names:**

Access® sTfR, sTfR Calibrators, and sTfR QC

**G. Regulatory Information:**

1. Regulation section:

866.5880 Transferrin immunological test system

862.1150 Calibrator

862.1660 Quality control material (assayed and unassayed)

2. Classification:

Class II

3. Product code:

DDG Transferrin, antigen, antiserum, control

JIT Calibrator, Secondary

JJX Single (specified) analyte controls (assayed and unassayed)

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

**The Access sTfR assay** is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of soluble transferrin receptor (sTfR) levels in human serum and plasma (heparin) using the Access Immunoassay Systems. The assay is intended as an aid in the diagnosis of Iron Deficiency Anemia (IDA), and for the differential diagnosis of IDA and Anemia of Chronic Disease (ACD).

This assay may also be used in conjunction with an Access Ferritin measurement to provide a calculated **sTfR/log ferritin index**. This index is intended as an aid in the diagnosis of Iron Deficiency Anemia (IDA), and for the differential diagnosis of IDA and Anemia of Chronic Disease (ACD).

**The Access sTfR Calibrators** are intended to calibrate the Access sTfR assay for the quantitative determination of soluble transferrin receptor levels in human

serum and plasma (heparin) using the Access Immunoassay Systems.

**The Access sTfR QC** is intended for monitoring system performance of the Access sTfR assay.

2. Indication(s) for use: Same as intended use.
3. Special conditions for use statement(s):  
Prescription use only
4. Special instrument requirements:  
Access® Immunoassay Systems – Access, Access 2, SYNCHRON LXi 725, UniCel® DxI 800, UniCel® DxI 600i and UniCel® DxI 600

**I. Device Description:**

**Access sTfR:** The sTfR assay reagent pack consists of two specific reagents: (R1a) paramagnetic particles coated with streptavidin:biotinylated soluble transferrin receptor monoclonal antibody, proteins (mouse, goat, bovine), bovine serum albumin (BSA), 0.1% sodium azide, and 0.17% ProClin 300; and (R1b) Monoclonal mouse anti-human soluble transferrin receptor alkaline phosphatase (bovine) conjugate, BSA, 0.1% sodium azide and 0.17% ProClin 300. Two assay packs containing 50 tests per pack are provided for a total of 100 assay determinations.

**Access sTfR Calibrator:** The calibrator kit is provided at 6 levels: S0 (4 ml) zero calibrator, and S1-S5 (2.5 ml each) ~3, 10, 30, 80 and 150 nmol/L human soluble transferrin receptor in buffered BSA matrix with preservatives. The kit comes with 6 vials and one calibration card.

**Access sTfR QC:** The QC kit contains 2 vials for each of 3 levels different control levels of ~10, 25 and 90 nmol/L of human soluble transferrin receptor in a buffered BSA matrix with human plasma and preservatives. Each vial contains 2.5 ml. A QC card is provided with each kit and lists the mean value and standard deviation for each control level.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
R&D Systems, Inc Quantikine IVD sTfR ELISA, Standard set, Control set
2. Predicate K number(s):  
k970718
3. Comparison with predicate:

**Access sTfR**

Similarities		
Item	Device	Predicate
Intended Use	For the quantitative determination of soluble transferrin receptor	Same
Indications for Use	Intended for use as an aid	Same

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	in the diagnosis of iron deficiency anemia and for the differential diagnosis of iron deficiency anemia and anemia of chronic disease.	
Test principle	Two site, sandwich immunoassay	Same
Values in healthy population sTfR (2.5 <sup>th</sup> and 97.5 <sup>th</sup> percentile)	Mean 18.3 nmol/L (12.16 - 27.25 nmol/L)	Mean 18.4 nmol/L (8.7 – 28.1 nmol/L)
Capture antibody	Mouse anti-human sTfR	Same
Signal antibody	Mouse anti-human sTfR	Same
Storage	2-10°C	2-8°C
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	The Access sTfR assay may also be used in conjunction with a ferritin measurement to provide a calculated sTfR/log ferritin index	Not applicable
Test System	Automated; Paramagnetic particle-based	Manual; Polystyrene microplate
Matrices	Serum and plasma (heparin)	Serum and plasma (heparin, EDTA, and citrate)
Analytical range	3.0 to 150 nmol/L	3 to 80 nmol/L
Values in healthy population sTfR/log ferritin index	6.42 to 22.37 (using nmol/L sTfR values and ng/mL values for ferritin)	Not applicable
Detection System	Chemiluminescent	Chromogenic
Open stability	28 days, 2-10°C	Up to 1 month 2-8°C
Instrument System	Access Immunoassay Systems	Spectrophotometer

#### **Access sTfR Calibrator**

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	For the quantitative determination of soluble	Same

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	transferrin receptor levels in human serum and plasma (heparin) using the Access Immunoassay Systems	
Measurand	Human soluble transferrin receptor	Same
Traceability	Manufacturers natural sTfR preparation designated Lot N016	Same
Composition	Buffered animal serum with preservative	Same
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Levels	6 levels: 0, 3, 10, 30, 80, 150 nmol/L	6 levels: 0, 8, 7, 20, 40, and 80 nmol/L
Storage	2-10°C	2-8°C

#### **Access sTfR QC**

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	For monitoring system performance of the Access sTfR assay.	Same
Measurand	Human soluble transferrin receptor	Same
Levels	10, 25, and 90 nmol/L	3 levels
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Preparation	Ready-to-use	Lyophilized
Storage	-20°C	2-8°C
Stability after opening	90 days, 2-10°C	1 month, 2-8°C

#### **K. Standard/Guidance Document referenced (if applicable):**

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline, 2<sup>nd</sup> edition. CLSI EP09-A2, Method Comparison and Bias Estimation using Patient Sample; Approved Guideline, 2<sup>nd</sup> edition. CLSI EP14-A2, Evaluation of Matrix Effects; Approved Guideline, 2<sup>nd</sup> edition.

#### **L. Test Principle:**

The Access sTfR assay is a sequential two-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with paramagnetic particles

coated with anti-sTfR antibody. During incubation, the sTfR antigen in the sample binds to the immobilized anti-sTfR antibody molecule on the solid phase. Alkaline phosphatase conjugated anti-sTfR antibody is then added and reacts with a different antigenic site on the sTfR molecule. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of sTfR in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

The calculation of the sTfR/log ferritin index is as follows where log refers to base-10 log and not to natural log:

$$\text{sTfR (nmol/L)} \div \log \text{ ferritin ng/mL}$$

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

(1) Precision of the sTfR assay was evaluated using 6 samples (pooled serum) in duplicate for 20 days, 2 runs per day on one Access 2 instrument. The mean of the replicates, standard deviation, and the %CV for within run and total precision were determined by analysis of variance. The acceptance criteria for total precision for sTfR levels > 9 nmol/L are %CV ≤ 8% and for levels ≤ 9 nmol/L are SD ≤ 1.00. The results of precision studies on 3 lots are presented below. Samples 3, 4, 5, and 6 were additionally spiked, and Samples 1 and 2 were diluted to achieve the desired concentrations.

SampleID	n	Mean (nmol/L)	Within Run		Total		95% CI of Total CV	
			SD	CV (%)	SD	CV (%)	95% LCL	95% UCL
1	80	0.93	0.04	4.72	0.08	8.59	7.43	10.17
2	80	8.45	0.18	2.09	0.26	3.03	2.62	3.58
3	80	41.60	0.86	2.08	1.22	2.93	2.53	3.47
4	80	75.63	1.24	1.64	2.13	2.82	2.44	3.34
5	80	109.74	2.15	1.96	3.62	3.29	2.85	3.90
6	80	132.60	2.29	1.73	3.73	2.81	2.44	3.33

SampleID	n	Mean (nmol/L)	Within Run		Total		95% CI of Total CV	
			SD	CV (%)	SD	CV (%)	95% LCL	95% UCL
1	84 <sup>(1)</sup>	0.95	0.04	3.82	0.08	8.12	7.05	9.57
2	83 <sup>(2)</sup>	8.27	0.22	2.65	0.38	4.56	3.96	5.39
3	83 <sup>(3)</sup>	42.72	1.37	3.21	1.65	3.86	3.35	4.55
4	82 <sup>(4)</sup>	74.55	1.24	1.67	1.90	2.55	2.21	3.02
5	84	108.60	2.16	1.99	3.67	3.38	2.93	3.98
6	82 <sup>(5)</sup>	134.12	6.93	5.16	7.20	5.37	4.65	6.34

Note: For all samples, only one run was completed on days 16 and 21. The study was extended for two additional days (4 runs) to replace these runs. All reported replicates (84) are included in the analyses, except as noted below.

- (1) Three replicates identified as outliers and excluded. Only 3 reps run on day 6. One additional day was run for sample 1.  
(2) One replicate identified as an outlier and excluded.  
(3) One replicate identified as an outlier and excluded.  
(4) One replicate identified as an outlier and excluded. One replicate missing due to human error.  
(5) Two replicates missing, due to insufficient sample quantity.

SampleID	n	Mean (nmol/L)	Within Run		Total		95% CI of Total CV	
			SD	CV (%)	SD	CV (%)	95% LCL	95% UCL
1	79 <sup>(6)</sup>	1.33	0.05	3.57	0.16	12.35	10.68	14.64
2	79 <sup>(7)</sup>	8.73	0.22	2.57	0.27	3.11	2.69	3.69
3	80	43.48	0.89	2.05	1.24	2.84	2.46	3.37
4	80	78.13	1.91	2.44	2.49	3.19	2.76	3.78
5	80	114.68	3.25	2.84	4.39	3.82	3.31	4.53
6	84 <sup>(8)</sup>	142.15	2.35	1.65	4.43	3.11	2.70	3.67

- (6) One replicate was identified as an outlier and was excluded.  
(7) One replicate missing due to system error. Not replaced.  
(8) Four replicates recovered over range. Four additional runs (duplicates) completed.

- (2) Lot-to-Lot reproducibility was determined using 5 samples, 3 Access TfR controls (range 8.45 nmol/L to 132.60 nmol/L) and 3 lots. The %CV for this range was less than 3.76%.

Sample ID	Mean sTfR Concentration			Overall Mean (nmol/L)	%CV Between Lot
	Pilot Lot #1 (nmol/L)	Pilot Lot #2 (nmol/L)	Pilot Lot #3 (nmol/L)		
2	8.45	8.27	8.73	8.48	2.74%
3	41.60	42.72	43.48	42.60	2.22%
4	75.63	74.55	78.13	76.10	2.41%
5	109.74	108.60	114.68	111.00	2.91%
6	132.60	134.12	142.15	136.29	3.76%

- (3) Precision of controls across lots:

Sample ID	Mean sTfR Concentration			Overall Mean (nmol/L)	%CV Between Lot
	Pilot Lot #1 (nmol/L)	Pilot Lot #2 (nmol/L)	Pilot Lot #3 (nmol/L)		
Access sTfR QC1	9.78	9.98	10.08	9.95	1.49%
Access sTfR QC2	23.35	24.58	24.69	24.21	3.03%
Access sTfR QC3	93.29	93.48	96.93	94.57	2.11%

(4) sTfR/log ferritin index precision: The sTfR precision data from one lot (excluding Sample 1 which is below the claimed measuring range of the assay) were assayed using both the Access sTfR and Access Ferritin assays. The same methods and statistical analysis used for the sTfR precision study were used. The sTfR/log ferritin index was determined for all replicates with sTfR and Ferritin values. Within run precision for the sTfR /log ferritin index (index range 6.19 to 69.27) was less than 3% CV and the Total precision observed was less than 6%CV. Precision results for the Ferritin assay and sTfR/log ferritin assay are shown below (precision results for the sTfR assay are from Pilot 3 shown above).

Access Ferritin Precision Summary – Samples 2-6

SampleID	n	Mean	Within Run		Total		95% CI of Total CV	
		(ng/mL)	SD	CV (%)	SD	CV (%)	95% LCL	95% UCL
2	80	25.73	0.83	3.22	1.33	5.18	4.48	6.13
3	80	4.27	0.13	2.96	0.34	7.90	6.84	9.36
4	80	180.31	5.74	3.19	7.69	4.26	3.69	5.05
5	80	237.70	5.84	2.46	8.93	3.75	3.25	4.45
6	80	200.15	5.77	2.88	7.32	3.66	3.16	4.33

sTfR / log Ferritin Index Precision – Timepoints with sTfR and Ferritin Values

SampleID	n	Mean	Within Run		Total		95% CI of Total CV	
		-	SD	CV (%)	SD	CV (%)	95% LCL	95% UCL
2	79	6.19	0.16	2.56	0.23	3.67	3.17	4.35
3	80	69.27	1.73	2.50	3.54	5.11	4.42	6.05
4	80	34.64	0.84	2.44	1.03	2.98	2.58	3.53
5	80	48.27	1.35	2.79	1.83	3.80	3.29	4.50
6	76	61.76	0.99	1.60	1.78	2.88	2.48	3.43

(5) Precision of plasma samples: Four *plasma* samples were spiked or diluted to span to range from ~3 nmol/L to 100 nmol/L and run in replicates of 5 for 5 days. The within-run precision was < 1.7 %CV and the total imprecision was <2.2% CV.

b. Linearity/assay reportable range:

Dilution Recovery: Six serum and six plasma samples were spiked with known concentrations representing the middle and upper portions of the sTfR assay’s analytical range. At least 6 serial dilutions were prepared for each of these samples using the zero calibrator or wash buffer II as the diluent. The % Recovery was calculated as (Observed value/Expected value) x100. The Observed value represented the means of 4 replicates and the neat samples were replicates of 8. The results are summarized for serum and for plasma.

**Serum**

Dilution	Mean Expected Value (nmol/L) (Sample Range)	Mean % Recovery (%Recovery Range)
neat	132.35 (123.90 – 139.65)	–

1:2	66.17 (61.95 – 69.80)	98.8% (96 – 101%)
1:4	33.09 (30.98 – 34.90)	(102 – 109%)
1:8	16.49 (15.49 – 17.45)	(100 – 114%)
1:16	8.27 (7.74 – 8.73)	(101 – 112%)
1:32	4.13 (3.87 – 4.36)	(109 – 118%)
1:64	2.06 (1.93 – 2.18)	(110 – 131%)

**Plasma**

Dilution	Mean Expected Value (nmol/L) (Sample Range)	Mean % Recovery (%Recovery Range)
neat	136.68 (133.97 – 138.90)	–
1:2	68.35 (67.00 – 69.45)	97.3% (92 – 104%)
1:4	34.18 (33.50 – 34.73)	104% (99 – 110%)
1:8	17.09 (16.75 – 17.36)	106% (100 – 113%)
1:16	8.55 (8.38 – 8.68)	107% (99 – 116%)
1:32	4.27 (4.19 – 4.34)	114% (104 – 123%)
1:64	2.13 (2.09 – 2.17)	119% (112 – 129%)

An additional analysis of the dilution recovery was also performed using the methods outlined in CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. In this analysis, both linear and quadratic models were fit to the log transformed dilution data. For all sample dilutions, the % difference observed between the two models was less than 10%.

Spike Recovery: Test samples were prepared by adding sTfR into 6 serum and 6 plasma samples to obtain a final sTfR concentration of ~120nmol/L. Each high patient sample was then spiked into a low sample (~12nmol/L) to obtain samples consisting of 75%, 50%, and 25% of high sample. Four replicates were used to calculate the Average Observed Dose for each sample. %Recovery = (Average Observed Dose/Expected Dose) x 100. Mean recovery was between 96 and 104% in serum samples and between 97 and 114% in plasma samples.

- c. *Traceability and value assignment calibrator and controls:* Traceability to a highly characterized master lot (referred to as primary reference calibrator) prepared by contract manufacturer designated Lot N016 from normal human plasma and assigned values using a process based on International Standard EN ISO 17511:2003, Metrological Traceability of Values Assigned to Calibrator and Control Materials.

*Stability:* Stability data for the test reagent pack and calibrator was supplied for both open and closed stability. The data supported the claim of 28 days for open stability (acceptance criteria ±10%) and current shelf life up. Studies are ongoing to support a claim of 12 months. Stability studies for controls support claims and storage conditions (open 90 days; 6 freeze/thaw cycles).

d. *Detection limit:*

Limit of Blank (LoB): The Access sTfR zero (S0) calibrator was determined with a non-parametric estimate of the 95% percentile of 120 replicates, run in 6 runs of 20 replicates over 3 days. The LoB for the Access sTfR assay was determined to be 0.01 nmol/L.

Limit of Detection (LoD): Three serum and three plasma samples were treated to remove sTfR in the samples. In total, 20 replicates (2 runs of 10 replicates per sample) of each of the 6 samples (60 measurements per matrix) were measured. The LoD was determined to be 0.02 nmol/L.

e. *Analytical specificity:*

Interference: To evaluate potential interference, substances listed below were spiked individually into normal human serum and compared to the control sample (~ sTfR concentration 33nmol/L). The mean % interference was calculated as follows (mean observed value/mean expected value) x100. Means were calculated from 10 replicate measurements. The acceptance criteria of  $100 \pm 10\%$  were met.

### Biological Interferents and Common Medications

Substance Added	Concentration Added	Expected (nmol/L)	Observed (nmol/L)	Mean % Interference
Bilirubin (Conj.)	40 mg/dL	27.74	27.44	98.9
Bilirubin (Unconj)	40 mg/dL	32.88	32.77	99.7
Human Serum Albumin (HSA)	>9000 mg/dL	36.18	35.03	96.8
Human Serum Albumin (HSA)	<5000 mg/dL	35.28	35.18	99.7
Hemoglobin	500 mg/dL	29.73	32.12	108.0
Triglycerides (Triolein)	3000 mg/dL	29.89	32.27	108.0
Acetaminophen	20 mg/dL	36.31	36.44	100.4
Aspirin	50 mg/dL	35.38	35.56	100.5
Ibuprofen	40 mg/dl	33.99	34.40	101.2
Multi-vitamin	1:20 Dilution	32.15	33.39	103.9
Alpha-2-macroglobulin	400 mg/dL	29.47	32.22	109.3

Table 2: Therapeutic Drugs

Substance Added	Concentration Added	Expected (nmol/L)	Observed (nmol/L)	Mean % Interference
Folic Acid	20 mg/dL	34.10	34.94	102.4
Heparin	8000 U/dl	29.96	32.30	107.8
Methotrexate	2.0 mmol/L	33.93	34.53	101.8
Amoxicillin	343 umol/L	28.32	30.45	107.5
Epoetin Alpha	25 mIU/L	34.06	34.35	100.8
Levothyroxine	1.29 umol/L	28.59	30.62	107.1
Alendronate	2 ng/mL	31.49	30.62	97.2
Insulin	820 IU/mL	31.13	33.82	108.6
Hydrocodone	0.85 umol/L	31.78	31.93	100.5

An additional interference study evaluating the effects of the biological interferents on a serum sample below the clinical cut-off (~15.0 nmol/L) was

performed Results are shown below.

Substance Added	Concentration Added	Expected (nmol/L)	Observed (nmol/L)	Mean % Interference
Bilirubin (conj.)	40 mg/dL	15.65	15.44	98.7
Bilirubin (unconj.)	40 mg/dL	15.36	15.35	99.9
Hemoglobin	500 mg/dL	14.48	14.37	99.2
Triolein	3000 mg/dL	14.48	14.53	100.3

Interference by rheumatoid factor (RF) was evaluated using 10 serum samples positive for RF ranging from (284 to 872 IU/mL) in replicates of 4. Interference by RF was not detected in a low sTfR (~18 nmol/L) serum sample.

Cross-reactivity: Potential cross-reactants with sTfR were spiked individually into pooled human serum with sTfR ~ 35 nmol/L. The percent cross-reactivity was calculated as follows [(mean test value – mean control value)/cross reactant added (mg/mL)] x 100. The results are presented below and met design specifications of ≤ 0.5% cross-reactivity. Conversion factor: 1 nmol/L sTfR =  $7.4 \times 10^{-5}$  mg/mL sTfR. (% Interference 113% for Diferric(holo) transferrin and less 2.6% for the remaining substances).

Substance	Concentration Added	Cross-Reactivity (%)
Diferric(holo)transferrin	8.5 mg/mL	0.004
Apo-transferrin	8.5 mg/mL	0.001
Heart Ferritin	10 µg/mL	0.251
Liver Ferritin	10 µg/mL	-0.007
Spleen Ferritin	10 µg/mL	0.325

Hook Effect: A patient serum pool was spiked with recombinant sTfR to a concentration of ~6000 nmol/L (40 times the highest calibrator). The sample was then diluted in a 1:2 series to 6 nmol/L. All dilutions were assayed in duplicate. No Hook effect was seen to 6000 nmol/L.

f. *Assay cut-off:*  
See Clinical studies below.

2. Comparison studies:

a. *Method comparison with predicate device:*

(1) A correlation study was performed comparing the Access sTfR assay to a predicate device, the R&D Systems Quantikine IVDs TfR ELISA. A total of 125 samples (73 pooled serum samples, 30 native serum samples, 13 spiked to cover the high end of the range, and 9 diluted native samples to cover the low end of the range) were run to provide a distribution of samples with sTfR concentrations that span the predicate sTfR assay range (3-80 nmol/L). Samples were tested in duplicate on both the Access assay

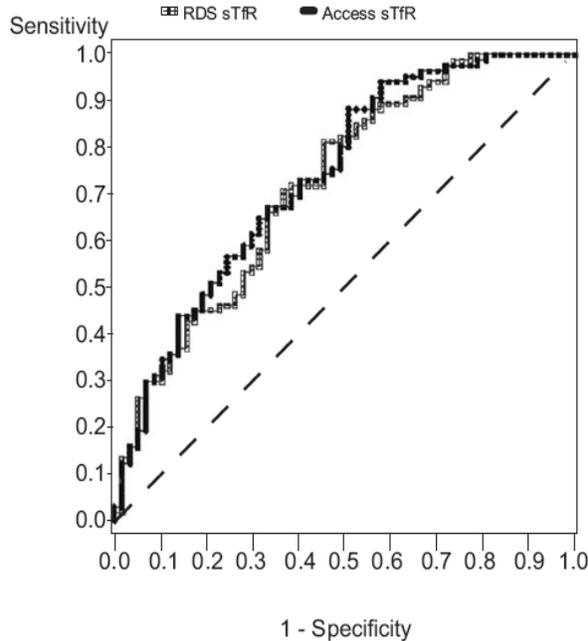
and the predicate. The first replicate from the Access assay and the mean of the duplicate results from the RDS sTfR assay were used for analysis. Deming regression analysis was used to calculate the results shown below. Acceptance criteria were met.

<b>Comparator Method (sample range)</b>	<b>Slope (95% CI)</b>	<b>Intercept nmol/L (95% CI)</b>	<b>Correlation coefficient (r)</b>	<b>n</b>
Quantikine sTfR ELISA (3.31 to 78.47 nmol/L)	0.87 (0.83 to 0.91)	1.79 ( 0.08 to 3.51)	0.96	125

- (2) An additional method comparison study was performed to compare the Access IMMUNOASSAY SYSTEMS sTfR Assay to the predicate R&D Systems Quantikine sTfR Assay. A total of 271 clinical samples from 5 geographically distinct sites were included in this analysis; sTfR and Ferritin concentrations for each sample were measured once on 1 lot of reagent for the Access 2 instrument and compared to the mean of 2 sTfR measurements on 1 lot of reagent for the reference ELISA assay. Passing-Bablok regression analysis was performed to determine the slope and intercept and Pearson’s correlation coefficient (r) was calculated. The results are shown below.

<b>Comparator Method (sample range)</b>	<b>Slope (95% CI)</b>	<b>Intercept nmol/L (95% CI)</b>	<b>Correlation coefficient (r)</b>	<b>n</b>
Quantikine sTfR ELISA (9.16 to 87.94 nmol/L)	0.89 (0.86 – 0.92)	0.69 ( -0.13 – 1.48)	0.96 (0.95 – 0.97)	271

- (3) An ROC curve analysis was performed of the prospective multicenter clinical trial samples tested with both Access sTfR and a commercially available immunoassay kit. This analysis showed that the AUCs are not statistically different for two sTfR assays (p=0.43). Access sTfR AUC = 0.74 (95% CI: 0.65- 0.82). RDS Quantikine sTfR AUC = 0.72 (95% CI: 0.64 -0.81).



**Access Platforms Equivalence:** To verify the equivalence of the Access platform family members with respect to the Access sTfR assay, method comparison, bias, and precision studies were performed. Representative platforms from the Access family of instruments were evaluated in paired comparisons using 8 samples that span the range of the assay 3 to 150 nmol/L. The acceptance criteria between the instruments require that the 95% confidence interval be within  $\pm 12\%$  for method comparison and bias, and  $\pm 10\%$  for precision. Acceptance criteria were met. Data contained in this submission were collected using the Access, Access 2 and UniCel DxI 800 Analyzers.

**b. Matrix comparison:**

(1) A matrix comparison study was performed using 20 matched serum (in both plain (Red Top) and gel (SST) tubes, and plasma (heparin) samples (sTfR range 11 to 48 nmol/L). Each set was spiked with sTfR to one of five approximate concentrations (40, 60, 80, 100, or 120 nmol/L). For each matched sample set, testing was performed on neat samples (n=20) and the spiked samples (n=20) for a total of 40 matched sample sets. The results are shown below (n=40, range ~10 to 130 nmol/L):

Comparison	Slope (95% CI)	Intercept (95% CI)	(r)	Estimated bias at 40 nmol/L (95% CI)
Serum (SST) vs. Plasma	1.0513 (1.03 to 1.07)	-1.5425 (-3.06 to -0.27)	1.0	1.32% (-0.04% to 2.70%)
Serum (Red Top) vs. Plasma	1.0176 (1.0 to 1.03)	-0.6389 (-1.82 to 0.54)	1.0	-0.05% (-1.17% to 1.09%)
Serum (SST) vs. Serum (Red Top)	1.0331 (1.02 to 1.05)	-0.8854 (-1.98 to 0.21)	1.0	1.34% (0.51% to 2.21%)

- (2) A total of 263 matched serum (SST) and plasma (Li-heparin PST) samples were collected. Sample range was ~ 12 to 83 nmol/L. Samples were assayed in replicates of 4 on two Access Instruments and two lots. Deming regression analysis was performed for both the sTfR and sTfR/log ferritin index and the results shown below:

Serum (SST) vs. Plasma	sTfR			(r) sTfR/log ferritin index		
	Slope	Intercept	r	Slope	Intercept	r
Lot 1	0.95	0.16	0.98	0.98	0.29	0.99
Lot 2	0.94	0.17	0.98	0.98	0.38	0.99

- (3) A matrix comparison study between Li-heparin plasma and Na-heparin plasma was conducted using 5 matched samples were tested in triplicate and evaluated across several time points. The %CV met the acceptance criteria of less than 10% difference between sample types.

### 3. Clinical studies:

#### a. *Clinical Sensitivity and Specificity:*

**Background:** Anemia of Chronic Disease (ACD) and iron deficiency anemia (IDA) are the most common forms of anemia, differentiated primarily by estimates of iron status. IDA is a condition that occurs when there is not enough iron in the body. A lack of iron in the body can come from blood loss, poor dietary intake, poor iron absorption, or when the body's need for iron is elevated, such as during pregnancy. When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in the bone marrow, liver, and spleen. ACD is a condition of impaired iron utilization. ACD is seen in a wide range of chronic malignant, autoimmune, leukemic, inflammatory, and infectious disease conditions. In some disease conditions, the two conditions co-exist (IDA+ACD).

*Soluble transferrin receptor (sTfR):* Transferrin receptor (TfR) is the major mediator of iron uptake by cells. When a cell needs iron, TfR expression is increased. Since the major use of iron is for hemoglobin synthesis, about 80% of total TfR is on erythroid progenitor cells. The sTfR arises from the extracellular proteolysis of TfR and can be measured in serum or plasma. A constant relationship exists between sTfR and TfR. The sTfR is increased in iron deficiency reflecting the cellular need for iron. The sTfR is elevated in iron deficiency but is not appreciably affected by chronic disease,

*Serum Ferritin* measurements aid in identifying conditions causing iron overload and iron deficiency anemia. Ferritin decreases in IDA patients, but increases in conditions associated with chronic inflammatory diseases. However, patients with anemia may have a falsely increased ferritin concentration as an acute phase reactant due to inflammation. Therefore, one

of the main difficulties encountered in the laboratory diagnosis of anemia is distinguishing IDA from ACD, especially when both disorders are concurrently present.

*sTfR/log ferritin index:* Because sTfR reflects the degree of tissue iron supply, and ferritin reflects stored iron levels, it has been demonstrated that the sTfR/log ferritin ratios (Index) is a good estimate of body iron. The index takes advantage of two variables influenced by iron deficiency: an increase in sTfR and a decrease in the ferritin concentration.

**Clinical Study:** To assess the clinical performance of the Access IMMUNOASSAY SYSTEMS sTfR assay and the sTfR/log ferritin index, two clinical utility trials were conducted. A retrospective clinical study was first performed to verify studies in the literature demonstrating efficacy of the sTfR and sTfR/log ferritin index and to identify cut-offs. Next, a prospective multicenter clinical trial was designed based on the results of the first trial. In this study, 145 patients from 4 geographically distinct regions were prospectively enrolled based on pre-specified inclusion and exclusion criteria. Anemia was detected and quantified by measurement of the RBC count, hemoglobin concentration, and hematocrit. All subjects had low hemoglobin levels. Subjects were classified into one of three categories of anemia: ACD (n = 57), IDA (n = 27), or ACD + IDA (n = 61) based on the current standard of care for diagnosis of anemia (low hemoglobin) and for classification of anemia, which included standardized testing and clinical evaluation. Standardized testing included Complete Blood Count (CBC) and differentials, iron profile (serum iron, total iron binding capacity (TIBC) or transferrin, transferrin saturation), serum ferritin, CRP and cytometric classification (e.g., hypochromic microcytic vs. normochromic normocytic). All patients with elevated CRP values ( $\geq 10$  mg/L) or WBC ( $\geq 10.5 \times 10^3/\mu\text{l}$ ) were classified as having chronic inflammation or infection.

To assess the clinical performance of the sTfR, a series of potential cutoffs were assessed using 2x2 tables and Fisher's Exact tests. Sensitivity in this clinical trial is defined as the percentage of patients with IDA or IDA+ACD correctly identified. Specificity is defined as the percentage of patients with ACD (without accompanying IDA) correctly identified. The sensitivity and specificity results for sTfR are shown below. A cutoff of 21nmol/L for sTfR, is recommended by Sponsor.

The sTfR assay is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and the patient's clinical presentation.

## **sTfR**

sTfR Cutoff Value $\geq$ (nmol/L)	sTfR Cutoff Value $\geq$ (mg/L)	Sensitivity % (95%CI) (% of patients with IDA or IDA+ACD correctly identified)	Specificity % (95%CI) (% of patients with ACD [without accompanying IDA] correctly identified)
19	1.40	95.5 (88.0–99.0)	36.8 (25.0–51.0)
20	1.48	90.9 (82.0–96.0)	42.1 (29.0–56.0)
<b>21</b>	<b>1.55</b>	<b>86.4</b> <b>(77.0–92.0)</b>	<b>49.1</b> <b>(36.0–63.0)</b>
22	1.62	79.6 (69.0–87.0)	50.9 (37.0–64.0)
23	1.70	73.9 (63.0–82.0)	59.7 (46.0–72.0)
24	1.77	68.2 (57.0–77.0)	61.4 (48.0–74.0)
25	1.85	67.1 (56.0–76.0)	66.7 (53.0–78.0)
26	1.92	62.5 (51.0–72.0)	70.2 (56.0–81.0)
27	1.99	60.2 (49.0–70.0)	71.9 (58.0–83.0)

To assess the clinical performance of the sTfR/log ferritin index, a series of potential cutoffs were assessed using 2x2 tables and Fisher’s Exact tests. Sensitivity in this clinical trial is defined as the percentage of patients with IDA or IDA+ACD correctly identified. Specificity is defined as the percentage of patients with ACD (without accompanying IDA) correctly identified. The sensitivity and specificity results for sTfR /log ferritin index are shown below. A cutoff of 14 for sTfR/log ferritin index is recommended by Sponsor.

#### **sTfR/log ferritin index**

sTfR Index Cutoff Value $\geq$ (using nmol/L for sTfR in Index calculations)	sTfR Index Cutoff Value $\geq$ (using mg/L for sTfR in Index calculations)	Sensitivity % (95%CI) (% of patients with IDA or IDA+ACD correctly identified)	Specificity % (95%CI) (% of patients with ACD [without accompanying IDA] correctly identified)
10	0.74	94.3 (87.0–98.0)	57.9 (44.0–71.0)
11	0.81	92.1 (84.0–97.0)	64.9 (51.0–77.0)
12	0.89	86.4 (77.0–92.0)	66.7 (53.0–78.0)
13	0.96	85.2 (76.0–92.0)	73.7 (60.0–84.0)
<b>14</b>	<b>1.03</b>	<b>80.7</b> <b>(71.0–88.0)</b>	<b>82.5</b> <b>(70.0–91.0)</b>
15	1.11	78.4 (68.0–86.0)	84.2 (72.0–92.0)
16	1.18	73.9 (63.0–82.0)	84.2 (72.0–92.0)
17	1.25	68.2 (57.0–77.0)	84.2 (72.0–92.0)
18	1.33	64.8 (54.0–74.0)	84.2 (72.0–92.0)
19	1.40	63.6 (53.0–73.0)	86.0 (74.0–93.0)
20	1.48	60.2 (49.0–70.0)	87.7 (76.0–94.0)
21	1.55	59.1 (48.0–69.0)	91.2 (80.0–97.0)
22	1.62	55.7 (45.0–66.0)	93.0 (82.0–98.0)

The sTfR/log ferritin index is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and the patient’s clinical presentation.

Comparison of sTfR, sTfR /log ferritin index, and ferritin measurements: the following table presents the sensitivity and specificity using each independently vs. all three.

Test	Cut-off	Sensitivity	Specificity
Ferritin	< 10	35% (31/88)	98% (56/57)
sTfR	$\geq$ 21	86% (76/88)	49% (38/57)
sTfR/log ferritin index	$\geq$ 14	81% (71/88)	83% (47/57)
ALL THREE		92% (81/88)	49% (28/57)

- c. Other clinical supportive data (when a. and b. are not applicable):

**Summary of patient characteristics from prospective trial:**

Age (years)	
Median	62
Mean $\pm$ SD	61 $\pm$ 16.7
Range (Minimum –Maximum)	24 - 98
Sex	
Male	33.1% (48/145)
Female	66.9% (97/145)

Race	
White	82.4% (103/125)
Black	15.2% (19/25)
Asian	0.8% (1/25)
Other	1.6% (2/125)

Gender: No statistically significant differences between males and females were found for the sTfR or the sTfR/log ferritin index in the intended use population.

Menopausal status: No statistically significant differences between pre-and post menopausal (>45 ears old) women were found for the sTfR or the sTfR/log ferritin index.

Age: To explore if there was an age-related trend in sTfR and Index results in the Prospective Pivotal Clinical Utility Trial, Pearson's correlation coefficients (r) were computed. No age related trends were observed for either sTfR or the sTfR/log ferritin index (r = -0.07 and r= -0.20, respectively). Age range for 145 subjects was 24 to 98 years old (mean  $\pm$  SD was  $61 \pm 16.7$ ; median 62 years old).

Race/Ethnicity: An analysis of trial data by race showed no statistically significant differences between blacks (19/125 subjects) and non blacks or for Hispanic/Latino (34/186 subjects) vs. non-Hispanic/Latino for the sTfR or the sTfR/log ferritin index.

Demographics: A subanalysis of the four geographically diverse sites used to enroll subjects for the study (Kansas, Arizona, California, and Finland) indicated that the sTfR or the sTfR/log ferritin index did not vary by site.

The Clinical utility trial included common disease conditions associated with ACD and IDA. The following table shows the underlying conditions of 145 subjects enrolled in the Prospective Clinical Utility Trial by anemia classification and site (1-4).

Underlying Conditions (Etiology of Anemia), by Anemia Classification and Site (1 - 4) \*

	ACD					ACD + IDA					IDA					TOTAL
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	
Iron Deficiency, Malabsorption, Blood Loss	1		1		2	2	24	2	2	30		16	2	7	25	57
Infections	15	12			27		14			14		1			1	42
Cancer	1	3			4		5			5		1			1	10
Autoimmune - Rheumatoid arthritis or Lupus	6		11		17	1		4		5		1	2		3	25
Inflammatory bowel disease						1	2		2	5				1	1	6
Other autoimmune or chronic inflammatory disease	6	4	1		11	1	8			9			1		1	21
Chronic kidney disease	3	5	2		10	2	9			11					0	21
Other **	1	5	2		8		16	2	2	20					0	28

\* Multiple etiologies of anemia were present in 34.5% (50/145) of patients. Two patients had both fungal and bacterial infections; three patients had two inflammatory conditions. For purposes of this table, all conditions are counted individually.

\*\* 'Other' etiologies included unknown etiologies, diabetes mellitus, osteoarthritis, pulmonary edema, COPD, liver disease, coronary artery disease, congestive heart failure, chronic epistaxis, alcoholism, seroma, hepatic adenoma, GERD, neuropathy

5. Expected values/Reference range:

Healthy Adults: A study in which 189 healthy, non-anemic subjects were enrolled demonstrated the following mean reference values  $\pm$  SD (and 2.5<sup>th</sup> to 97.5<sup>th</sup> percentiles). These numbers are for information purposes only and should not be used to establish medically relevant decision points:

sTfR: Mean  $18.33 \pm 4.16$  nmol/L (12.16 – 27.25 nmol/L)

sTfR/log ferritin index: Mean  $11.68 \pm 4.00$  (6.42 – 22.37 using nmol/L)

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.